



Responses of different physiological parameter thresholds to soil water availability in four plant species during prolonged drought



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ABSTRACT

Large-scale vegetation restoration on the Loess Plateau in China has been performed by the central government in recent decades; however, the planting of incompatible vegetation during these efforts has resulted in serious environmental problems, such as dry soil layers, that are widespread and difficult to ameliorate. To determine the proper evaluation indices for plant available soil water content (PASWC) in commonly reforested plants and crops, we examined the physiological responses of *Robinia pseudoacacia* (tree), *Amorpha fruticosa* (shrub), *Medicago sativa* (perennial leguminous herb) and *Zea mays* (crop) to prolonged drought; these plants were planted widely in the semiarid Loess Plateau region of China. Leaf water status, gas exchange and fluorescence parameters did not show marked changes at the beginning of the prolonged drought but changed rapidly as PASWC continued to decrease. These data were fitted with a sigmoid function ($P < 0.0001$). In addition, different physiological parameters showed different PASWC thresholds; the fluorescence parameters exhibited the lowest PASWC threshold among the four species, with an upper threshold that was less than 50% of the PASWC for all but *Z. mays*. In this study, the photosynthesis rate was a better indicator of the PASWC, and the upper and lower thresholds of PASWC of the normalized photosynthesis rate were 64.1% and 47.6%, 83.0% and 44.1%, 82.7% and 35.2%, 82.9% and 39.3% for *R. pseudoacacia*, *A. fruticosa*, *M. sativa* and *Z. mays*, respectively. The current study also suggests that *R. pseudoacacia* is a suitable afforestation species in areas with higher levels of rainfall. These results provide important information for determining the PASWC and the supply capacity of soil water on the Loess Plateau.

1. Introduction

Water availability is one of the principal factors limiting plant growth and development in ecosystems (Huxman et al., 2004), especially in semi-arid and arid areas, where precipitation is the main source of soil water due to climate changes (Hoerling and Kumar, 2003; Huxman et al., 2004). Severe drought could result in significant declines in net primary productivity and large-scale tree mortality events (Allen et al., 2010; Breshears et al., 2005; Hicke and Zeppel, 2013), which have received extensive attention from agroforestry and ecological researchers in recent years (Lacape et al., 1998; Lagergren and Lindroth, 2002; Sadras and Milroy, 1996; Sinclair et al., 2005).

Modeling plant responses to prolonged drought requires not only an understanding of but also quantitative relationships between soil water content and plant physiological traits (Sadras and Milroy, 1996; Soltani et al., 2000). To date, several physiological strategies of plants have been documented in response to drought, including the closure of leaf

stomata, decrease in water potential and changes in fluorescence (Bresson et al., 2015; Rouhi et al., 2007; Yan et al., 2016). The closure of leaf stomata may help to maintain a favorable leaf water level during drought, although this depends on the severity and duration of the stress and would reduce the movement of CO₂ and water vapor (Bresson et al., 2015). Therefore, the effects of a drought become evident in the stomata, which reduce their aperture to prevent desiccation (Flexas and Medrano, 2002). The photosynthesis rate is then affected by internal water deficiency following stomatal closure, and net photosynthesis is unavoidably reduced due to decreased CO₂ availability at the chloroplast level (Gallé et al., 2007). The decreases in mesophyll photosynthesis capacity and carboxylation efficiency also contribute to the decrease in photosynthesis under severe drought conditions (Galmés et al., 2007). Therefore, gas exchange responses to drought were used to determine drought conditions; a few studies have addressed the different thresholds of gas exchange response in different plants, under different experimental conditions and using different

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evaluation methods (Casadebaig et al., 2008; Sadras and Milroy, 1996; Sinclair et al., 2005; Soltani et al., 2000). Previous studies typically used single indicators to evaluate plant available soil water content (PASWC) based on type of plant; however, multi-index comprehensive research into different plants is rare.

Chlorophyll fluorescence has become a ubiquitous and powerful parameter used to track changes in the photosynthetic capacity of plants in response to abiotic factors (Bresson et al., 2015; Maxwell and Johnson, 2000). The closure of stomata promotes an imbalance between the photochemical activity of photosystem II (PSII) and the electron requirement for carbon fixation, leading to over-excitations and subsequent photoinhibitory damage to the PSII reaction centers under dry soil conditions (Bresson et al., 2015). Consequently, a substantial decline in the maximum quantum efficiency of PSII (Fv/Fm) in response to drought is observed in various plant species (Baker and Rosenqvist, 2004) and is closely related to decreased leaf water status (Woo et al., 2008), which could be used as an indicator of plant performance under drought conditions because chlorophyll fluorescence data is intuitive and easily comprehensible and could provide useful information on plant status (Calatayud et al., 2006). However, whether this parameter could be used to characterize and quantify the PASWC requires further research.

The Loess Plateau in China is well known for its severe soil erosion (Zhang et al., 2008), where features a dry climate and gullied topography and poses a major challenge for environmental restoration because of the aridity and severe soil erosion. Unfortunately, evapotranspiration is projected to increase in the future (Li et al., 2012). To improve the environmental quality and reduce water and soil losses in this region, the government has implemented vegetation restoration practices that include planting trees, shrubs and herbs; however, many of the selected plants were not ideal for extensive plant restoration, particularly given the limited soil water; this region often experiences low soil water potential for a majority of the year due to low annual precipitation, which is mostly concentrated in the months of July–September (Zhang et al., 2015). The planting of incompatible vegetation has led to some serious issues, such as stunted, old trees and dry soil layers, which are widespread and difficult to ameliorate on the Loess Plateau (Yan et al., 2015). Thus, there is an urgent need to better understand the thresholds for significant changes in the physiological properties of commonly used species in the region in response to low soil water, which could provide useful information for determining the soil water supply capacity, the sustainable utilization of soil reservoirs and the recovery of vegetation on the Loess Plateau.

To the best of our knowledge, the PASWC thresholds for physiological responses in the species planted in this area have not been studied. Thus, in this study, we characterize the response patterns of leaf water status, gas exchange and fluorescence parameters to prolonged drought in four species, including three large-scale vegetative restoration species, *Robinia pseudoacacia* L. (tree), *Amorpha fruticosa* L. (shrub), and *Medicago sativa* L. (herbage), and one crop (*Zea mays* L.), which is widely planted in this region. The primary aim of this study is to quantify how the leaf water status, gas exchange and fluorescence parameters depend on PASWC and to propose a robust methodology to characterize and quantify PASWC in the four different species. The results may assist in determining the PASWC and may provide information for the selection and management of reforestation species on the Loess Plateau.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was undertaken in an open-sided greenhouse with a glass roof at the Institute of Soil and Water Conservation in the Northwest A&F University in Yangling, Shaanxi (34°17'56"N, 108°04'07"E). The experimental site has a temperate, semi-humid

Table 1

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_2O)	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

climate with a mean annual temperature of 13 °C and a mean annual precipitation of 632 mm, of which approximately 60% falls from July–September.

The plants chosen in this study include two deciduous woody legume species (2-year-old seedlings), *R. pseudoacacia* and *A. fruticosa*, one perennial leguminous herb (*M. sativa*) and one crop (*Z. mays*). These plants have been planted widely on the Loess Plateau. Ninety days before the start of the experiment, *R. pseudoacacia* (40–60 cm tall and 3–5 mm in diameter at the stem base) and *A. fruticosa* (30–50 cm tall and 3–5 mm in diameter at the stem base) were transplanted from the field to pots; ninety and forty days before the experiment, the alfalfa and maize were planted as seeds, respectively. The pots were 400 L (980 × 760 × 680 cm, length × width × height) each; this size was chosen to ensure the reliability of the experimental results because small pots may change the experimental results and undermine the purpose of the experiment (Poorter et al., 2012). Four *A. fruticosa* and *R. pseudoacacia* plants, 30 alfalfa plants or 6 maize plants were planted in each pot. The soil used in the study was collected from the 0 to 20 cm soil layer; the physical and chemical properties of the soil are presented in Table 1.

The plants were subjected to two different treatments: well-watered (control saplings) and not watered (stressed saplings). These were arranged in a completely randomized design. The stressed and control saplings were identical during the drought. All plants were well irrigated until the onset of the experiments; the control plants were then continuously watered throughout the experimental period, while drought stress in the stressed saplings was induced by withholding water. Throughout the experiment, the predawn leaf water potential (PLWP), leaf relative water content (RWC), gas exchange and chlorophyll fluorescence were determined using sunny leaves from the upper crown of the selected plants in each treatment. A total of three duplicates per treatment were measured, and at least four measurements per duplicate were taken.

2.2. Measurements

Spot PLWP measurements from both the control and stressed plants were performed between 05:00 and 06:00 h using a PMS 600 pressure chamber (PMS Instruments Company, Albany, USA), with two repetitions for every plant. Gas exchange traits were measured in at least two leaves per plant selected from 09:00 to 11:00 h using the Li-Cor model 6400 system (Lincoln, NE, USA). Fully expanded, mature leaves from 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 for the PLWP measurements. The environmental conditions in the leaf chamber consisted of a saturating photosynthetic photon flux density between 1000 and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the measurement periods; the temperature and relative humidity inside the leaf cuvette were always close to ambient air values.

Chlorophyll fluorescence was recorded with a portable pulse

amplitude modulated fluorometer using the FMS-2.02 system (Hansatech, Norfolk, UK). The maximum and minimum fluorescence values (Fm and Fv) were measured after 20 min in the dark, and the maximal fluorescence level (Fm') from the leaves was recorded in the light. The following fluorescence parameters were identified and calculated: maximum quantum efficiency of PSII (Fv/Fm) and non-photochemical quenching (NPQ), which was calculated as (Fm-Fm')/Fm' (Baker and Rosenqvist, 2004).

After the gas exchange and chlorophyll fluorescence measurements, the leaves from the measured plants and leaf discs from the analyzed *Z. mays* leaves were sampled to determine the leaf RWC. The fresh weight was determined using an analytical balance, and the leaves were re-hydrated with distilled water at 4 °C in the dark until they reached a constant weight (12 h). Then, the saturated weight was measured, and the leaves were placed in a drying oven at 70 °C for 48 h to determine the dry weight. The RWC was calculated as follows: $RWC (\%) = (FW - DW)/(TW - DW)$, where FW is the fresh weight, TW is the turgid weight, and DW is the dry weight.

The soil water content (SWC) was observed using SWC reflectometer probes (CS650-L, Campbell Scientific, Australia), and the soil temperature and moisture were recorded at depths of 10 and 40 cm every 30 min. The average SWC was calculated. In this study, the SWC was represented by the mass soil water content, and the PASWC was calculated as the ratio of SWC to field capacity (FC).

2.3. Statistical analyses

Pearson correlation coefficients and a cluster analysis were used to test for associations between the physiological traits, which were performed using the R software package (version 3.1.1) (R Core Team, 2014). The results of cluster analysis indicated that the physiological traits were divided into five groups (Fig. 1); thus, one parameter was chosen from each group to determine the PASWC threshold. The gas exchange measurement data were normalized by dividing the data for each drought plant by the mean of the control plants measured on the same day; this was assumed to eliminate the error caused by different environmental conditions during the measurement days.

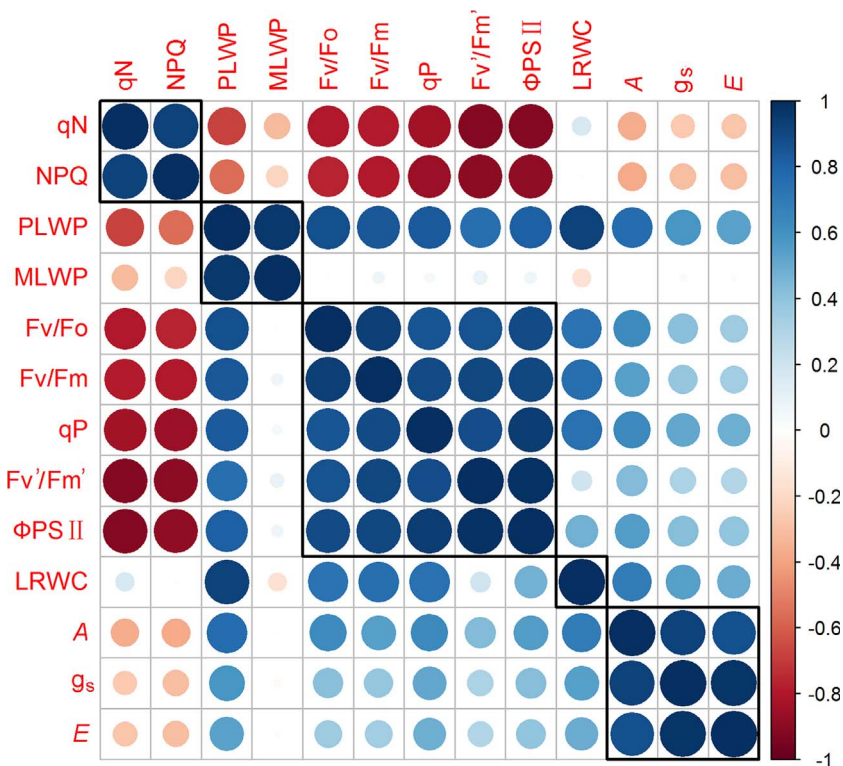


Fig. 1. Cluster analysis of physiological plant traits. The number stands for the correlation coefficient. A: photosynthesis rate; gs: stomatal conductance; E: transpiration rate; LRWC: leaf relative water content; PLWP: predawn leaf water potential; MLWP: midday leaf water potential; Fv/Fm: maximum quantum efficiency of photosystem II; NPQ: non-photochemical quenching; qN: non-photochemical quenching of variable fluorescence; qP: photochemical quenching; ΦPS II: quantum yield of PS II electron transport; Fv'/Fm': effective quantum yield of PS II photochemistry; Fv/Fo: ratio of variable fluorescence to original fluorescence.

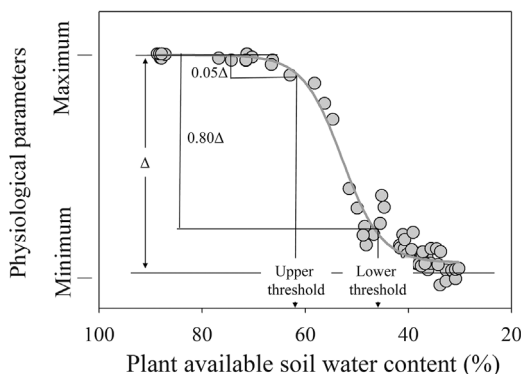


Fig. 2. Schematic diagram of the upper and lower thresholds of plant available soil water content.

The correlation was determined using regression-fitting software (SigmaPlot 10.0, Systat Software Inc., USA) to compare the physiological parameters collected for each sampling and the PASWC to one another; the regression coefficients and correlations were calculated. In the present study, the drought had a significant effect on the physiological parameters when the difference between the maximum and minimum value of the physiological parameters was greater than 5%. The PASWC for the same period was identified as the upper threshold for PASWC, and the lower threshold was defined as any variation in the physiological parameters that was greater than 80% (Fig. 2).

3. Results

3.1. Response of the gas exchange parameters to the drought

The normalized leaf gas exchange showed a significant correlation with the PASWC in the four species ($P < 0.0001$) (Figs. 3, S1 and S2). However, the PASWC threshold related to the leaf gas exchange was different for the four species. The upper PASWC threshold for the normalized photosynthesis rate for *R. pseudoacacia* was lower, while the

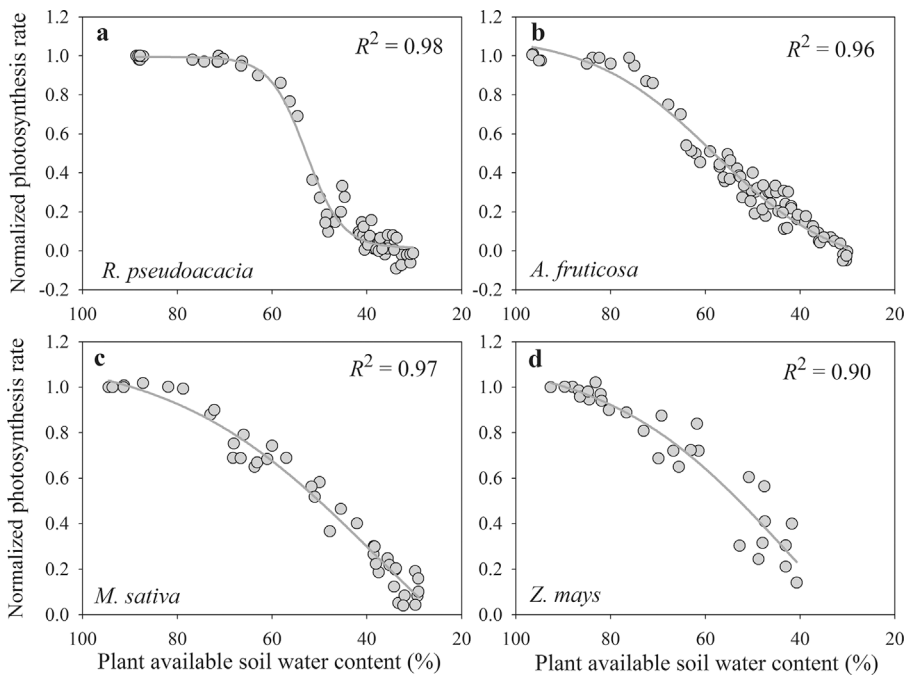


Fig. 3. Response of normalized photosynthesis rate to the plant available soil water content in all four species. All the fitted curves are statistically significant ($P < 0.001$).

Table 2

Upper and lower thresholds of plant availability soil water content (%) for different physiological parameters in the four species. Fv/Fm is the maximum quantum efficiency of photosystem II; NPQ is the non-photochemical quenching; and – indicates that the threshold was not measured during the drought.

Parameters	<i>R. pseudoacacia</i>		<i>A. fruticosa</i>		<i>M. sativa</i>		<i>Z. mays</i>	
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Leaf relative water content	54.8	31.5	66.0	40.1	78.5	42.2	83.6	41.0
Predawn leaf water potential	53.2	29.6	60.1	37.0	–	–	–	–
Normalized photosynthesis rate	64.1	47.6	83.0	44.1	82.7	35.2	82.9	39.3
Normalized stomatal conductance	79.9	50.8	93.2	50.2	88.0	41.7	87.1	60.7
Normalized transpiration rate	81.0	49.9	86.3	41.9	89.6	35.5	89.7	58.6
Fv/Fm	47.3	29.9	48.7	30.4	41.0	35.4	60.3	40.3
NPQ	41.4	32.2	48.6	30.1	40.5	28.6	67.2	33.5

minimum threshold was higher than that for the other three species at 64.1% and 47.6%, respectively. The upper and lower PASWC thresholds for the normalized photosynthesis rate for *A. fruticosa*, *M. sativa* and *Z. mays* were 83.0% and 44.1%, 82.7% and 35.2%, 82.9% and 39.3%, respectively. However, both the upper and lower PASWC thresholds for the normalized stomatal conductance were higher than those for the normalized photosynthesis rates in all four species (Table 2); these values were 79.9% and 50.8%, 93.2% and 50.2%, 88.0% and 41.7%, 87.1% and 60.7% for *R. pseudoacacia*, *A. fruticosa*, *M. sativa* and *Z. mays*, respectively. In addition, the upper and lower PASWC thresholds for the normalized transpiration rate were also higher than the normalized photosynthesis rate in all four species.

3.2. Response of leaf water status to the drought

The leaf water status thresholds at which plants responded to PASWC were estimated for each species; a significant correlation with PASWC was shown for all plants ($P < 0.0001$) (Figs. 4 and 5). The leaf RWC of the four species followed the same pattern (Fig. 4); no marked changes were identified until the PASWC decreased to 54.8%, 66.0%, 78.5% and 83.6% in *R. pseudoacacia*, *A. fruticosa*, *M. sativa* and *Z. mays* (Table 2), respectively; after this point, the values decreased sharply as PASWC continued to decline. PLWP exhibited a similar relationship to leaf RWC (Fig. 5); the upper PASWC thresholds were 53.2% and 60.1% in *R. pseudoacacia* and *A. fruticosa*, respectively. PLWP did not exhibit large variations between *R. pseudoacacia* and *A. fruticosa* during the

early stage, but leaf RWC differed between the species, with *M. sativa* and *Z. mays* exhibiting higher initial leaf RWC when the plants were well-watered.

3.3. Response of the chlorophyll fluorescence parameters to the drought

The Fv/Fm ratio is a useful and rapid parameter that reflects the maximum quantum efficiency of the PSII photochemistry, while the NPQ ratio has been used to quantify heat dissipation in plants. In this study, both Fv/Fm and NPQ also exhibited a significant correlation with the PASWC in the four species studied ($P < 0.0001$) (Figs. 6 and 7), with marked change identified when PASWC decreased to a lower level. Both the upper and lower PASWC thresholds for Fv/Fm and NPQ were lower than the leaf water status and gas exchange parameters (Table 2). The upper and lower PASWC thresholds associated with Fv/Fm were 47.3% and 29.9%, 48.7% and 30.4%, 41.0% and 35.4%, 60.3% and 40.3%, for *R. pseudoacacia*, *A. fruticosa*, *M. sativa* and *Z. mays*, respectively. The PASWC threshold for marked changes in NPQ was higher in *Z. mays* than in the other three species.

4. Discussion

Plant available soil water availability is one of the most important environmental factors in plant growth and development. Even in the humid tropics, plants are commonly exposed to drought periods of varying duration and intensity (Sadras and Milroy, 1996; Soltani et al.,

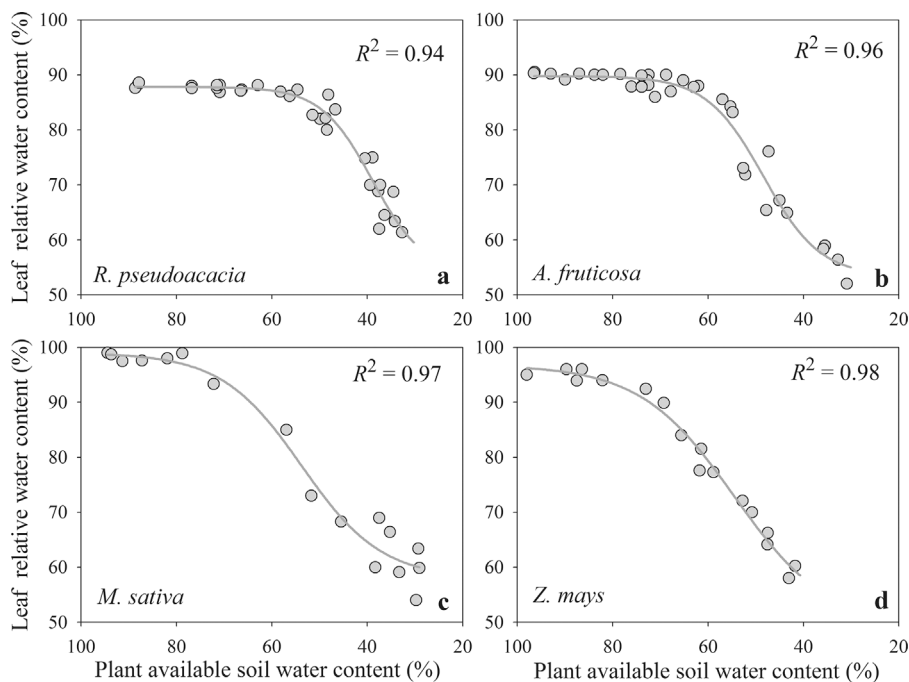


Fig. 4. Response of leaf relative water content to the plant available soil water content in all four species. All the fitted curves are statistically significant ($P < 0.001$).

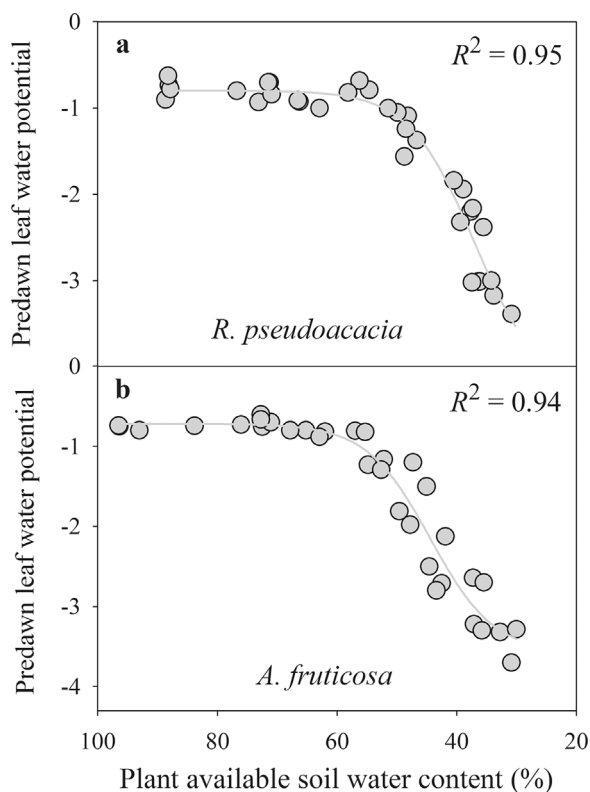


Fig. 5. Response of predawn leaf water potential for *R. pseudoacacia* (a) and *A. fruticosa* (b) to the plant available soil water content. All the nonlinear regression curves are statistically significant ($P < 0.001$).

2000). Plants consume significant amounts of water during the growing season, but the mechanism of soil water consumption differs by species; moreover, PASWC thresholds for different physiological parameters varied due to phenological timing, soil characteristics and environmental conditions. Thus, methods for the more efficient evaluation of PASWC attracted much attention from agroforestry researchers (Baker et al., 2007; Ortuño et al., 2006). Different parameters have been used

to quantify PASWC, including plant water potential, relative plant tissue water content and leaf and whole-canopy gas exchanges (Baker et al., 2007; Casadebaig et al., 2008; Jensen et al., 2000; Ortuño et al., 2006; Sadras and Milroy, 1996; Yan et al., 2010). Previous studies have reported a significant correlation between leaf water status and gas exchanges with the PASWC (Belko et al., 2012; Casadebaig et al., 2008; Yan et al., 2010). Thus, both leaf water status and gas exchanges have been used as indicators in the identification of drought, although the thresholds differ due to the different species, experimental conditions and evaluation methods (Casadebaig et al., 2008; Sinclair et al., 2005; Soltani et al., 2000). Moreover, the chlorophyll fluorescence parameters also exhibit changes in response to drought in various plant species (Baker and Rosenqvist, 2004; Bresson et al., 2015) and so can be used as a rapid, non-invasive way to track changes in plant performance; however, whether it could be used to characterize and quantify PASWC remains unclear.

A study of the PASWC on the Loess Plateau is necessary due to the extensive plant restoration and limited soil water resulting from low annual precipitation in this region; however, establishing an evaluation index under field conditions is difficult given the spatially heterogeneous nature of the environment and plant types. Thus, we conducted a greenhouse study in order to provide important information in the determination of the zone distribution of the PASWC in this area. In the study, many parameters were measured: first, a cluster analysis was used to determine the representative parameters. All physiological parameters were divided into five groups by this analysis (Fig. 1), and one parameter was chosen for each group to determine the value of the PASWC threshold; thus, leaf RWC, PLWP, photosynthetic rate, F_v/F_m and NPQ were selected in the present study. All of these parameters correlated significantly with the PASWC when the sigmoid function was used (Figs. 3–7), which is consistent with previous studies (Belko et al., 2012; Casadebaig et al., 2008; Yan et al., 2010).

In recent years, numerous works have focused on plant gas exchanges' response to drought and have shown that drought decreases gas exchanges. Thus, these parameters have often been used as indicators to predict droughts (Baker et al., 2007; Casadebaig et al., 2008; Lipiec et al., 2013; Sadras and Milroy, 1996; Yan et al., 2017). In this study, the PASWC threshold for gas exchanges was higher than that for the leaf water status, F_v/F_m and NPQ parameters in all four species (Table 2), indicating that gas exchange is most sensitive to drought

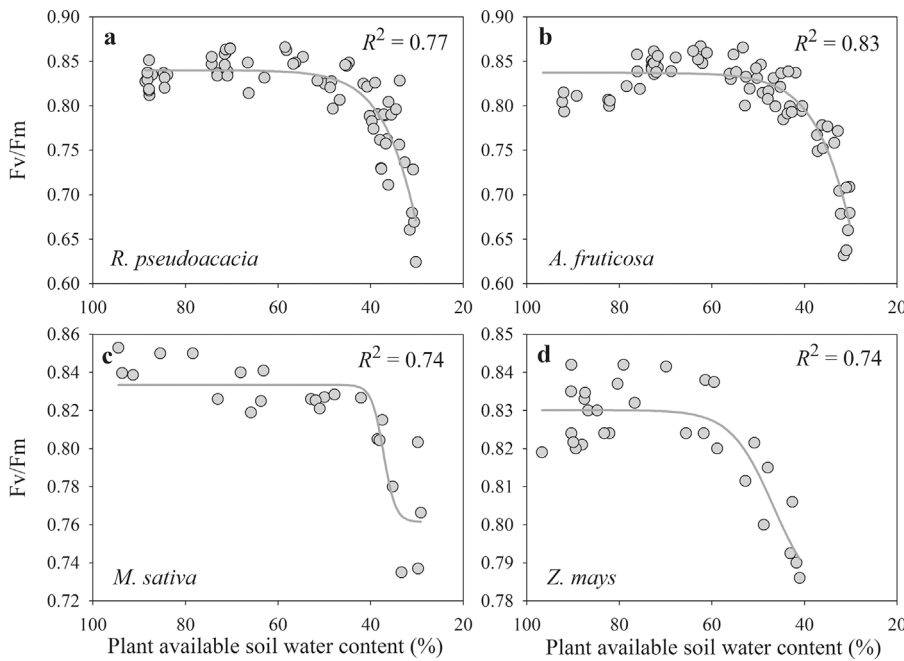


Fig. 6. Relationship between the maximum quantum efficiency of photosystem II (Fv/Fm) and the plant available soil water content in all four species. All the nonlinear regression curves are statistically significant ($P < 0.001$).

conditions. This is consistent with the works of Medrano et al. (2002) and Socias et al. (1997). A high degree of co-regulation of stomatal conductance with photosynthesis rate and transpiration rates is usually observed (Yan et al., 2016). The results of the study (Figs. S3 and S4) indicated that a change in the stomatal closure was the dominant driver of the decreases in photosynthesis and transpiration in response to drought; it is generally accepted that stomatal closures are the primary determining factor in decreased photosynthesis under mild to moderate drought (Medrano et al., 2002; Yan et al., 2016). Stomatal conductance is responsive to the external (soil water availability, VPD) and internal (ABA, leaf water status) factors related to drought, and it represents a more integrative basis for identifying the overall effects of drought. However, whether stomatal conductance is dominated by the available soil water or meteorological conditions remains controversial, and reports suggesting that drought causes stomatal closure supports the

control of the stomatal mechanism by the abscisic acid signal produced by the roots (Yan et al., 2010). Other reports suggest a relationship between leaf water status and stomatal conductance (Whitehead et al., 1996). It is difficult to reject either of these explanations, as drought was observed to cause declines in both leaf water status and gas exchange in this study (Figs. 3–5). In the study, the results showed that the threshold of normalized stomatal conductance was higher than that of the normalized photosynthesis rate, indicating that stomatal conductance was often more sensitive to drought than photosynthesis rate (James et al., 2002); this result occurs because the earliest response of the plants to prevent desiccation under drought was stomatal closure (Hura et al., 2007). In addition, stomatal conductance was affected by many factors, while photosynthesis rate did not decrease when the stomatal conductance began to decrease; thus, of the gas exchange parameters, photosynthesis rate is likely a better indicator of drought.

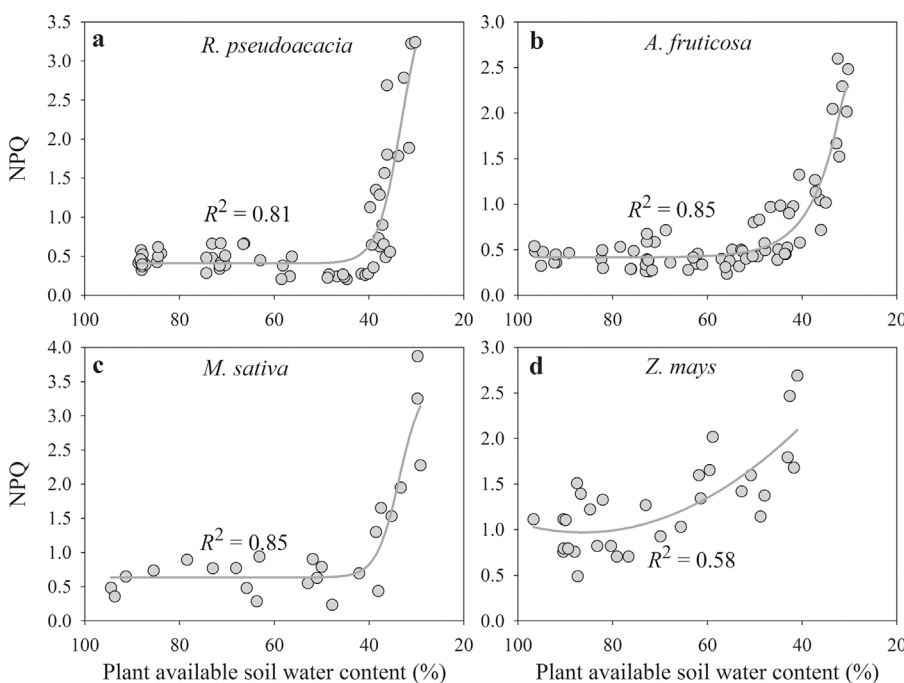


Fig. 7. Relationship between the non-photochemical quenching (NPQ) and the plant available soil water content in all four species. All the nonlinear regression curves are statistically significant ($P < 0.001$).

Leaf water status depends on PASWC and exhibits a rapid decrease when PASWC decreases to a certain threshold; therefore, this parameter is often regarded as a stressor (Jones, 2007). Leaf water potential could reflect water transport, and leaf RWC could reflect the metabolic activity in plant tissues. In this study, the leaf water status of plants decreased slightly at the beginning of the drought, followed by a large decrease due to the decline in PASWC; this is consistent with the results of Galmés et al. (2007). Moreover, the PASWC threshold for leaf water status in *R. pseudoacacia* and *A. fruticosa* lagged behind that of the other two species, *M. sativa* and *Z. mays* (Table 2), indicating that woody plants are more drought resistant. The larger and differently structured root systems of the two woody plant species were able to absorb the maximum available water inside the pots even when the soil water content was low (Lipiec et al., 2013; Yan et al., 2010). In addition, the abscission of leaves also explained the lower PASWC threshold in woody plants, which may have improved the leaf water status (Yan et al., 2010). Although leaf water status could be used as an indicator of drought, the normalized photosynthesis rate, stomatal conductance and transpiration rate showed significant decreases when leaf RWC began to decline significantly in the woody plants, and the normalized photosynthesis rate decreased more than 40% (Fig. 8); thus, photosynthesis rate may be a better indicator of PASWC than leaf water status under

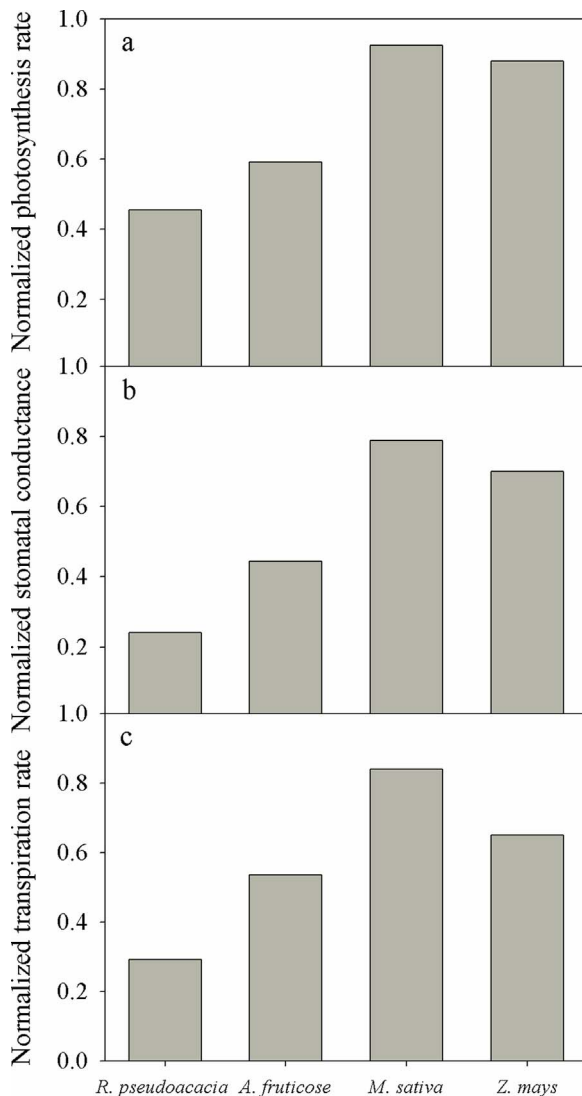


Fig. 8. Normalized photosynthesis rate (a), stomatal conductance (b) and transpiration rate (c) at the upper threshold of plant available soil water content of leaf relative water content for all four species.

drought.

Stomata closure rates support a favorable leaf water status and promote an imbalance between the photochemical activity of PSII and the electron requirements for carbon fixation under drought, which leads to over-excitation and subsequent photoinhibitory damage to PSII reaction centers (Krause, 1988). Chlorophyll fluorescence parameters are often used to rapidly and non-invasively estimate the photosynthetic performance of the leaves of plants in drought conditions (Baker and Rosenqvist, 2004; Bresson et al., 2015). In this study, we chose Fv/Fm and NPQ to estimate the PASWC of the four species due to the strong correlations between the chlorophyll fluorescence parameters. The Fv/Fm ratio has been widely used to detect stress-induced perturbations in the photosynthetic apparatus, and changes in NPQ likely reflect heat dissipation rates, which protect the leaf from light-induced damage under drought (Maxwell and Johnson, 2000). The results showed a close relationship between PASWC and Fv/Fm with NPQ, and a lower threshold was observed for these parameters than leaf water status and gas exchange parameters among the four species (Table 2). These results indicate that Fv/Fm and NPQ are less sensitive indicators than leaf water status and gas exchange when plants are exposed to drought. Fv/Fm has been shown to be a robust indicator of plant health, and most plant species exhibit a mean Fv/Fm of 0.83 in healthy photosynthetic tissues, which is close to the results in this study (Fig. 6), whereas lower values are indicative of an impaired physiological status (Bresson et al., 2015; Maxwell and Johnson, 2000; Woo et al., 2008). Although the Fv/Fm and NPQ could be used to detect plant performance under drought, the normalized photosynthesis rate, stomatal conductance and transpiration rate showed significant decreases when the Fv/Fm and NPQ began to decline significantly in the woody plants, and the normalized photosynthesis rate declined by more than 70% (Fig. 9) in the woody plants; thus, Fv/Fm and NPQ are not suitable indicators of PASWC.

The Loess Plateau in China has experienced severe soil erosion, vegetation degradation, and desertification in recent decades (Jian et al., 2015). Precipitation is the only source of soil water, which limits vegetation restoration (Zheng et al., 2014). Thus, there is an urgent need to better understand the PASWC thresholds of commonly applied reforestation species that have high water consumption rates and cause desiccation of soils in this region (Chen et al., 2008). In the present study, we found that leaf water status, gas exchange and fluorescence parameters were significantly related to PASWC in all four species using the sigmoid function; these parameters decreased slightly when PASWC began to decrease and then decreased more rapidly with further decreases in the PASWC values. In addition, different species were associated with different PASWC thresholds, with the woody plants showing a lower threshold than the herbaceous plants. The Fv/Fm and NPQ parameters showed the lowest PASWC thresholds of all the physiological parameters studied for the four species. In the herbaceous plants, leaf RWC could be used to characterize and quantify PASWC; however, in woody plants, photosynthesis rate was a better indicator than the leaf water status and fluorescence parameters. These results provide important information for the determination of the PASWC under field conditions, which could guide vegetation restoration strategies in this region.

5. Conclusions

The current study reveals that the physiological responses of four investigated plant species to drought are similar; parameter levels decrease slightly at the beginning of the drought and then decrease more rapidly with further decreases in soil water. Leaf RWC could be used to characterize and quantify PASWC in herbaceous plants, whereas photosynthesis rate is suitable for all plant species. Moreover, although *R. pseudoacacia* showed a lower PASWC threshold of photosynthesis rates than other species, these values declined faster in a narrow range of PASWC, suggesting that the exotic *R. pseudoacacia* is more sensitive to

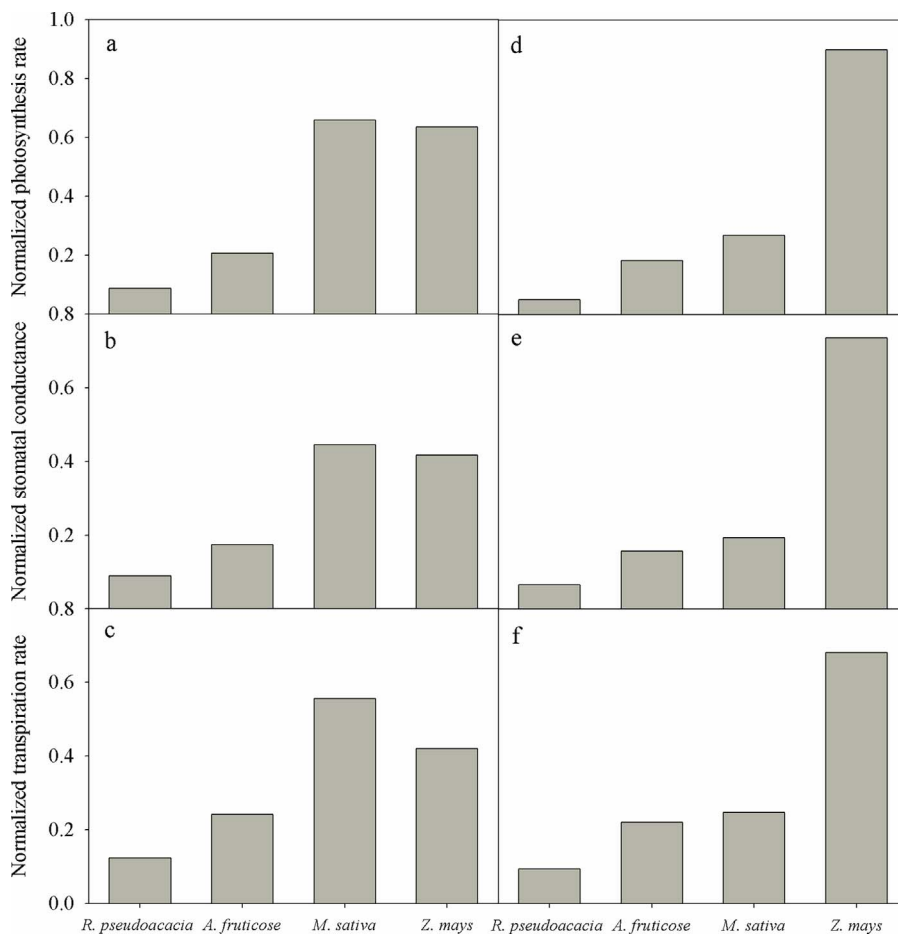


Fig. 9. Normalized photosynthesis rate (a, d), stomatal conductance (b, e) and transpiration rate (c, f) at the upper threshold of plant available soil water content for maximum quantum efficiency of photosystem II (Fv/Fm) (a–c) and the non-photochemical quenching (NPQ) (d–f) for all four species.

lower soil water content and should be considered with caution when restoring vegetation species in this area.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agrformet.2017.08.017>.

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