Response of Leaf-Level Gas Exchange and Hydraulic Conductance on a Typical Subtropical Tree Species (*Osmanthus fragrans*) to a Severe Summer Drought

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**Abstract**: Increasing drought frequency and intensity are considered to be a driven factor for recent declines in terrestrial ecosystem productivity. Therefore, knowledge of how tree species respond to drought is critical for modeling and predicting the
impacts of climate change on forest and woodland ecosystems. The aim of this study was to evaluate the effects of a severe summer drought on the performance of a subtropical evergreen tree species (*Osmanthus fragrans*) based on field measurements, including stem water potential, sap flow, and leaf gas exchange. The results show that drought stress posed a significant consequence on leaf gas exchange, and this influence come from combined effect of decreased soil water potential and enhanced vapor pressure deficit (*VPD*) during the drought period. Leaf transpiration shows a similar behavior to the carbon assimilation in terms of its response to soil moisture condition, but effect of *VPD* are opposite. Daily maximum water use efficiency shows a parabolic curve in response to soil water potential *Ψpd*. The threshold of *Ψpd* for the daily maximum *WUE* is around -1.5 MPa for *WUE*. These results provide useful information for understanding ecosystem responses to seasonal droughts in humid climate zones.

**Key Words:** droughts; subtropical humid climate; soil water potential; leaf carbon assimilation; stomatal conductance; water use efficiency; plant hydraulic conductance
1. Introduction

Research into the plant in response to drought stress is becoming increasingly important, as most climate change scenarios showing an increase in aridity in many areas of the globe [1, 2]. On a globe basis, drought, in conjunction with high temperature, pose most important environmental constraints to plant growth and productivity in most terrestrial plant communities [3, 4]. A well-known example is that the 2005 and 2010 droughts across Amazon have caused large-scale vegetation motility in the basin [5, 6]. Besides, heat waves and droughts induced a 30% reduction in gross primary productivity over Europe during 2003 [7]. With climate change, drought will likely become more intensified and more frequent [8, 9]. Knowledge of how tree species respond to drought is therefore critical for modeling and predicting the impacts of climate change on forest and woodland ecosystems [10, 11].

Impacts of water availability on photosynthetic carbon assimilation largely stem from the intrinsic link between plant photosynthesis and transpiration processes, through plant stomatal functions, mediated by environmental factors, such as root zone soil water availability [12] and vapor pressure deficit (VPD) [13, 14]. Previous studies have demonstrated that under drought conditions, plants tend to balance the trade-off between carbon assimilation and water consumption [15-21]. When the water availability declines during drought periods, plants typically reduce their stomatal aperture to reduce transpiration rates and maintain a stable leaf water potential to avoid hydraulic failure [22]. Meanwhile, the decrease of stomatal
conductance leads to reduction in supply of CO₂ into leaves, and eventually slow down photosynthetic rate. The degree of stomatal closure is found to be closely linked with the critical soil water potential causing hydraulic failure, demonstrating close coordination between stomatal behavior and xylem hydraulic traits [23, 24]. As soil moisture depletes, increasing tension leads to an increasing risk of xylem cavitations, leading to a loss of hydraulic conductance and a further decline in xylem water potential [22]. Specifically, xylem hydraulic conductance (\(K_s\)) provides a measure of the hydraulic capacity of xylem tissue to transport water from root to leaves, supplying water for transpired water losses during the process of carbon assimilation [25-27].

Drought stress usually co-occurs with high temperature and increasing evaporative demand, which in turn lead to more intensified drought stress [28, 29]. High temperature is also has great impact on metabolic activity in leaves, such as down regulation of leaf photochemistry [8, 28, 30]. Persistent drought stress in combination with high temperature may cause photosynthetic enzyme irreversibly damage [31].

Leaf gas exchange is fundamental to ecosystem- and global-level fluxes of water and CO₂, in turn affecting soil moisture condition and atmospheric CO₂ concentration [32]. Many previous studies on leaf gas exchange response to drought stress has been conducted in many tree species across habitats including Mediterranean climate [18, 33-36], temperate continental climates [37], and in Karst regions [38]. However, little effort has been made for areas of periodic seasonal droughts in humid climatic zones,
such as subtropical monsoon areas in southern China, where the ecosystem comprises subtropical evergreen tree species and has a good potential capacity for carbon sequestration [39]. In this study, we examined the effects of soil drought on and subsequent recovery of the physiologic performance of a 10-year-old subtropical tree species (*Osmanthus fragrans*). *O. fragrans* widely distributes in natural ecosystems in the central and southern China, and is commonly planted in urban streets and parklands. In some areas, such as upper montane forest in Northern Thailand, *Osmanthus fragrans* occurs more commonly in natural ecosystems [40]. Vegetation in subtropical monsoon areas, such as centre and southern China, suffers frequent water stress during summer period due to anomalous precipitation with high evapotranspiration demand. The frequency of drought occurrence in this area is 8.7 times per decade over past 40 years, with a drought duration up to 100 days [41]. A severe drought in company of frequent high temperatures persisted from late June to the end of August, 2013 of this region. Rainfall amount of this year summer period was much lower than a normal year in this region [42], which has triggered many of tree seedlings morality in this region (investigation data). The primary objective of this study was to examine how does tree physiological performance including carbon assimilation, transpiration, water use efficiency, responds to an extreme summer drought event.

2. Materials and Methods

2.1 Site description

The experimental site is an *Osmanthus fragrans* plantation stand, located in the
countryside of Changsha city (112°53′20″E, 28°09′46″N, elevation 70 m above sea level) (Fig.1). The area is characterized with a subtropical monsoon climate, with a mean annual temperature of 17.2 °C and mean annual precipitation of 1360 mm. Most of precipitation occurs in late spring and early summer. The plantation was established in 2003, covering an area of 1500 m². It was surrounded by subtropical evergreen forest and crop land. Two *Osmanthus fragrans* trees were selected for leaf gas exchange measurements in this study. Measurements were conducted from July-October 2012 on tree B and June - September 2013 on tree A. Predawn leaf water potential measurements for tree B and stem and leaf water potential measurements for tree A were simultaneously conducted during the leaf gas exchange measurement periods. The studied trees were about 10-year old with a height of 4.2 m and, 3.8 m for tree A and tree B, respectively. Sap flow and micrometeorological measurements were also conducted at the site during the experiment periods.

### 2.2 Stem water potential and predawn leaf water potential measurement

Stem Psychrometers (PSY, ICT International Pty Ltd., NSW, Australia) developed by Dixon and Tyree [43] were used to measure stem water potential. The device measures water potential in a closed chamber which is installed on a tree stem so that the chamber air is sealed and in contact with sapwood surface. The measurement range of PSY is from -0.01 to -10 MPa, with an accuracy of +/-0.01 MPa and a resolution of 0.002 MPa. Water potential measurements were calibrated using sodium chloride solution[44] before each installation. Studies have shown that water potential in the soil-plant continuum is most likely in equilibrium with each
other before sunrise [45, 46]. Therefore, it is reasonable to use predawn stem water potential \((\Psi_{pd})\) as surrogate for root zone soil water potential. This assumption was cross-checked with predawn leaf water potential measurements using a standard Scholander-type pressure chamber (PMS 1000, PMS Inst., Corvallis, OR, USA) before sunrise to evaluate the accuracy of stem psychrometer measurements. Midday stem water potential at 13:00 local time, which selected from 30-min interval logged stem water potential data. Average daily plant water potential was the calculation of mean of diurnal stem water potential.

Both stem water potential and predawn leaf water potential measurements were performed for the experiment in 2013, but only predawn leaf water potential was measured in 2012.

### 2.3 Sap flow measurement and estimation of xylem hydraulic conductance

Compensation heat-pulse technique [47] was used to measure sap flow in trees with 30mm long radial sap flow meters and 1.8 mm in diameter installed at a height of 1.1m in the tree trunk. Two sets of sap flow probes (HRM, ICT International Pty Ltd., NSW, Australia) were installed at north-face side and south face side of the trunk, respectively and data was logged at 30min intervals,. Detailed description of this instrument can be found in Wang, et al. [48]. The daily volumetric sap flow amount was calculated from the heat transport velocity and corrected for wounding, sapwood area, volume fraction of wood and water using Sap Flow Tool provided by ICT International Corporation. Daily transpiration amount will be equal to daily
volumetric sap flow amount if the change of water storage in xylem be neglected. Xylem hydraulic conductance ($K_s$, kg MPa$^{-1}$ m$^{-2}$ s$^{-1}$) was calculated using the following relationship (1) based on Darcy’s law [23, 49] as follows:

$$K_s = \frac{ET}{(\Psi_{pd} - \Psi_{pl})}$$ (1)

$ET$ is whole tree average daily transpiration rate (kg H$_2$O m$^{-2}$ s$^{-1}$); $\Psi_{pd}$ is predawn stem water potential and representing root zone soil water potential (MPa); $\Psi_{pl}$ is average daily plant water potential, which is calculated from the logged data at 30min intervals. For calculations of $K_s$, measurements with a water potential gradient of less than 0.5 MPa difference between $\Psi_{pd}$ and $\Psi_{pl}$ and rainy days data were removed in the analysis.

2.4 Micrometeorological data

An automatic weather station (WeatherHawk-232, USA) was installed about 20m away from the leaf measurements. Air temperature (°C), relative humidity(%), global radiation (W/s), and rainfall (mm) data were collected from 1st September 2012 to 10th September 2013. Data was recorded at a 1-min interval and stored at a 30-min interval. Micrometeorological data during the measurement period (1st June to 1st September of 2013) was selected and aggregated into daily values for this study.

2.5 Leaf gas exchange measurement

Leaf gas exchange was measured using the cuvette method (Li-6400XT, 6400-08) with a portable gas exchange analyzer (LI-6400XT, Li-COR Inc., Lincoln, Nebraska, USA) on clear days, very 1.5–2 h from 8 am to 6 pm from 26th July to 2th October 2012 on tree B and from 1st June to 1st September 2013 on tree A. Measurements were
made *in situ* under natural conditions on sunlit leaves as well as shaded leaves of the *O. fragrans* tree. Before each run of leaf measurements (about half an hour), gas exchange analyzer was checked and calibrated. Readings such as carbon assimilation rate ($A_n$, $\mu$mol m$^{-2}$ s$^{-1}$), transpiration ($E$, mmol m$^{-2}$ s$^{-1}$), stomatal conductance ($g_s$, $\mu$mol m$^{-2}$ s$^{-1}$), leaf temperature ($T$, $^\circ$C), vapor pressure deficit ($VPD$, kPa), photosynthetic active radiation ($PAR$, $\mu$mol m$^{-2}$ s$^{-1}$) etc, were recorded once the parameters showed no obvious change. A 2.5 m ladder was used in order to get full access to the canopy. All the sunlit measurements were conducted on leaves in the outer part of the tree crown and shaded leaves were measured on leaves of internal canopy and shaded face, about 3.5 m above ground level. About 20 (10 for sunlit leaves and 10 for shade leaves) fully expanded and health leaves were selected for leaf gas exchange measurement per measurement time point. For sunlit leaves, leaf orientation was adjust to reach light saturating ($PAR$ over 800$\mu$mol m$^{-2}$ s$^{-1}$) and shaded leaves were conducted under natural $PAR$. Temperature, humidity and CO$_2$ concentration in the cuvette were kept the same with ambient environment during measurement period.

In addition, light response curves were measured across drying seasons at 10 days intervals. Relative humidity and temperature in the chamber were kept close to ambient air values during each response curve measurement and the $PAR$ was controlled using a red-blue LED light source (Li-Cor 6400-02B). This information was used to determine the light saturation level.
2.6 Estimation of leaf-level WUE and iWUE

It has been proposed[15] that plants control stomata to optimally satisfy the trade-off between the amount of carbon assimilated and the amount of water transpired. Water use efficiency ($WUE$), defined as the amount of carbon gained per unit of water loss, is used to quantify this trade-off. Stomatal conductance determines both diffusion of CO₂ into the leaf and diffusion water out of it [50]. Intrinsic water use efficiency ($iWUE$), defined as the ratio between assimilation rate and stomatal conductance, is useful for characterizing different physiological responses of plant functional types to environmental changes. Their expression can be found as follows

$$WUE = \frac{A_n}{E}$$  \hspace{1cm} (2)

$$iWUE = \frac{A_n}{g_s}$$  \hspace{1cm} (3)

where $A_n$ is leaf carbon assimilation rate, $E$ is leaf transpiration rate, $g_s$ is stomatal conductance.

2.7 Curve fitting

Apart from the soil water status, photosynthetic and transpiration rates were also controlled by other environmental factors. The limitations can be expressed by Jarvis function[51]:

$$A_n = A_{\text{max}}(0) f(T) f(VPD) f(C_a) f(PAR) f(\Psi)$$ \hspace{1cm} (4)

in which $A_n$ is the leaf carbon assimilation rate, $A_{\text{max}}(0)$ is the maximum leaf carbon assimilation rate under a non-stress condition, $T$ is leaf temperature, $VPD$ is vapor pressure deficit surrounding the leaf, $C_a$ is CO₂ concentration in the ambient
environment, \( PAR \) is the photosynthetic active radiation and \( \Psi \) is root zone water potential.

In order to evaluate the effects of changes in soil moisture condition on photosynthetic and transpiration performances, we need to minimize the impacts of other factors. Thus, daily maximum value of carbon assimilation, stomatal conductance, transpiration and water use efficiency were selected to study the effect of soil moisture condition on gas exchange rates. Daily maximum \( A_n \), \( g_s \) and \( E \) response to soil water potential during the drought is fitted by an S-shape water stress function proposed by Van Genuchten [52] and \( WUE \) and \( iWUE \) is fitted using parabolic curve. The expression of S-shape water stress function is shown as following

\[
y = \frac{a}{1 + \left( \frac{x}{\Psi_{50}} \right)^p}
\]

(5)

where \( a \) is maximum value under light saturation free from water stress, \( \Psi_{50} \) is soil water potential at which the value reduced by 50% from \( a \), and \( p \) is an empirical coefficient. Changes in \( CO_2 \) concentration between days are very small and its effect on leaf gas exchange can be neglected in this study. Meanwhile, all the measurement data were used to study the response of leaf gas exchange to various microclimatic conditions which originated from measurements at the adjacent in Li-6400XT.
Irrigation was applied on 9th August 2013, the amount water equal to 20mm rainfall at specific area, which provided an opportunity to examine how leaf carbon assimilation recovered from the drought.  

2.8 Statistical analyses

In order to examine $K_s$, carbon assimilation and stomatal conductance can completely recovery from the drought stress. Paired-Samples $t$ test was conducted to detect the significance of $K_s$, carbon assimilation rate and stomatal conductance between pre-drought period and after drought period. Firstly, the pre-drought period data were fitted with a curve and then the data of the post-drought period were compared with those of curve derived values at the same soil water condition. All statistical analyses were performed in SPSS 17.0 (SPSS Inc. Chicago, USA).

3. Results and Discussion

3.1 Micrometeorological condition throughout the measurement period

Micrometeorological conditions during the leaf gas exchange measurement period for tree A are shown in Fig.2. The drought period occurred from the end of June (DOY: 181) to the end of August (DOY: 236) when a large natural rainfall event happened. Most of days average daily temperature was over 30°C during the drought period (Fig.2a), and the temperature seems to increase with the enhanced drought stress, with the maximum value appeared at the end period of drought. There was only 15 mm amount of rainfall happened between 30th June (DOY: 181) and 23rd August (DOY: 236) of 2013 (Fig.2b), which was much less than a normal year level (248 mm, average over 1980-2010). Average daily $VPD$ was 3.4 kPa, with majority of its values
over 4 kPa during the drought period (Fig.2c). The maximum value of daily $VPD$ was 5.2kPa which occurred at the end of drought in 11th August, resulting from a high air temperature and low relative humidity. Therefore, low rainfall in company with high temperature and strong solar radiation (Fig.2d) resulted in a severe drought stress during 2013 summer.

3.2 Stem water potential and volumetric soil water content

The response of stem water potential to precipitation are significant (Fig.3). Predawn stem water potential ($\Psi_{pd}$) decreased from 11th June (DOY 162) till 24th June (DOY 175) when a large rainfall event occurred. $\Psi_{pd}$ returned to nearly zero in 28th June (DOY 179) and decreased till an irrigation applied on 9th August (DOY 223). The minimum value of $\Psi_{pd}$ was -2.53 MPa occurred right before the irrigation (DOY223). Predawn leaf water potential was also measured to compare with predawn stem water potential (Fig.3). It shows that the predawn stem water potential corresponds very well with the predawn leaf water potential, confirming a hydro-equilibrium condition within the plant has reached at this time. The midday water potential $\Psi_m$ and average daily xylem water potential $\Psi_{pl}$ exhibited a similar trend with $\Psi_{pd}$. Whereas, $\Psi_m$ and $\Psi_{pl}$ show obviously more negative values than $\Psi_{pd}$ due to plant transpiration caused water loss with exception in rainy and cloudy days in which no significantly different gradient was existed among them. The difference between $\Psi_{pd}$ and $\Psi_m$, or $\Delta \Psi = (\Psi_{pd} - \Psi_m)$, performed a slightly increased with the enhanced drought stress. Our findings is contrast to the results for a Mediterranean species of *Quercus robur* [34].
3.3 Carbon assimilation and stomatal conductance in response to $\Psi_{pd}$ and VPD

Relationships between root zone water potential $\Psi_{pd}$ and leaf carbon assimilation rates ($A_n$) for sunlit leaves ($A_1$) and shaded leaves ($A_2$) are presented in Fig.4a. $A_1$ are response sensitively to both $\Psi_{pd}$ and VPD ($p<0.001$), and declines as $\Psi_{pd}$ become more negative or as enhanced in VPD. daily maximum $A_1$ (here we call $A_{max}$) experiences a sharp decrease with soil water potential from near 0 MPa to moderate soil water stress ($\Psi_{pd} = -1 \sim -1.5$ MPa), followed by a weak decrease with soil water potential. $A_1$ shows a higher value with a lower VPD under the same soil water potential. The weaker effect of VPD on $A_1$ as the drought persisting. These results suggest that the impacts of drought on carbon assimilation come from a combined consequence of root zone soil moisture and vapor pressure deficit around leaves. Due to light limitation, $A_2$ performed a much lower rates than $A_{max}$ throughout the whole drought period in 2013. Owing to varying light intensities, it is difficult to examine the response of $A_2$ to $\Psi_{pd}$ in Fig.4a.

The response of daily maximum stomatal conductance (here we call maximum stomatal conductance $g_{smax}$) to decreasing $\Psi_{pd}$ is in a nonlinear manner similar to the response of $A_{max}$ (Fig.4b). These patterns of $g_{smax}$ response to $\Psi_{pd}$ are in good agreement with previous studies on other species [12, 17, 37, 53-55]. Stomatal conductance becomes low for days with high VPD, corresponding well with carbon assimilation rate respond to VPD. This result supports that the effects of drought stress (low soil moisture and high VPD) on carbon assimilation are partly due to its effects on reducing stomatal conductance. Decreasing leaf stomatal conductance is
one important mechanism to reduce the loss of water to the atmosphere in order to prevent leaf desiccation from severe water stress or high VPD. Simultaneously, it reduces CO₂ concentration in leaves resulting in a decrease in carbon assimilation rates [37]. To its extreme, a complete stomatal closure protects further water loss and irreversible cell dehydration under severe droughts [18].

As carbon assimilation rate $A_n$ decreases in parallel with stomatal conductance $g_s$ from high water availability ($\Psi_{pd}$ close to 0 MPa) to moderate water stress ($\Psi_{pd} = -1$ MPa) (Fig.4), stomatal limitations seem to account primarily for the reduction of assimilation rates. However, when soil water potential decreases from -1 MPa to -2.5 MPa, $g_s$ remains unchanged while $A_n$ is still experiencing a gradual decrease (Fig.4a, Fig.4b). This suggests that drought-induced reduction in photosynthesis capacity may include an increased mesophyll resistance [56], reduced Rubisco activity [57, 58], and/or reduced electron transport capacity [37, 59]. This possibility is also reported at the ecosystem level [60].

The shaded leaf carbon assimilation rate ($A_2$) is lower than $A_{max}$ (Fig.4a), implying that light limitation is a dominant constraint on carbon assimilation rate of shaded leaves. However, $VPD$ and soil water potential are also two important factors influencing $A_2$ during the drought period as shown in (Fig.5a, 5b). At the same $PAR$, $A_2$ shows a larger value with a lower $VPD$ and a higher soil water potential. However, when $PAR$ is extreme low (< 50 $\mu$molm$^{-2}$s$^{-1}$), the impact of these two factors on $A_2$ can be neglected. An interesting finding is showed in our result that the effect of light limitation on shaded leaves becomes weaker as drought stress enhanced (Fig.5a).
It is generally agreed that sunlight is the dominant limiting factor to vegetation growth in the humid central and southern China [61]. However, our results demonstrate that water shortage can become an important environmental stress on vegetation growth and carbon assimilation in midsummer when seasonal droughts occur at this time of the year owing to the control of subtropical high over the area.

3.4 Leaf transpiration in response to $\Psi_{pd}$ and VPD

Both soil water potential and VPD are shown to be two dominant factor limiting leaf transpiration rates similar to carbon assimilation. The patterns of transpiration rates of sunlit leaves ($E_1$) and those of shaded leaves ($E_2$) in response to $\Psi_{pd}$ and VPD are shows in Fig.7. $E_1$ displays a significant change in response to $\Psi_{pd}$. Initially, $E_1$ shows a linear decrease with $\Psi_{pd}$ from 0 MPa to -1 MPa, and then remains constant when $\Psi_{pd}$ decreases from -1 MPa until -2.5 MPa, while $E_2$ does not appear to be dependent of $\Psi_{pd}$. Different from carbon assimilation, $E_1$ with larger VPDs perform higher values than that with smaller VPDs (Fig.7). Most of $E_2$ appear to perform a lower value than that of $E_1$ during the whole drought period, especially in high water availability.

Atmospheric condition and soil water status are two main influencing factors on leaf transpiration rates [62]. As shown in Fig.7, greater VPD likely occurring in the midday and afternoon, transpiration occurs at higher rates than those with a low VPD likely occurred in early morning. During the period of high water availability, sunlit leaves receive larger solar radiation than shaded leaves, resulting in higher transpiration rates in sunlit leaves. However, as drought progresses, plant starts to
suffer water stress and leaf transpiration rate is restricted by soil and leaf water status. In this situation, shaded leaves have a better water status, thus likely to have higher leaf water potential to facilitate transpiration.

### 3.5 Water use efficiency and intrinsic water use efficiency

As can be seen from Fig.7a, $WUE$ varies from 0 to 4 $\mu$mol CO$_2$ mmol$^{-1}$ H$_2$O; and $iWUE$ varies from 0 to 60$\mu$mol CO$_2$ mol$^{-1}$ H$_2$O throughout the whole experimental period. The relationship between daily maximum leaf $(i)WUE$ (here we call $(i)WUE_{max}$ ) and $\psi_{pd}$ shows a biphasic pattern, with an initial increase of $(i)WUE_{max}$ with decreasing $\psi_{pd}$, followed by a decrease of $(i)WUE_{max}$ as $\psi_{pd}$ became more negative (Fig.7). The threshold of $\psi_{pd}$ dividing the two phases of the relationship is around -1.2 MPa and -1.5MPa for $WUE_{max}$ and $iWUE_{max}$, respectively. No clear relationship with $\psi_{pd}$ is found for sunlit leaves measured at other time periods of day.

The biphasic pattern in $(i)WUE_{max}$ and $\psi_{pd}$, can be explained by the changes in $A_n$ and $g_s$ as soil becomes progressively depleted with water. The initial progressive increase in $(i)WUE_{max}$ is likely associated with a reduction in stomatal conductance and, therefore, resulting in a decrease in water loss, which only causes a smaller reduction in photosynthesis [63]. Metabolic impairment of the photosynthetic apparatus under severe drought condition [64, 65] can account for the subsequent gradually decline in $iWUE_1$ and $WUE_1$. The decrease in $WUE_1$ might be also associated with slight increase in transpiration due to a larger $VPD$ in dry conditions.

The result of $(i)WUE_{max}$ parabolic relationship with $\psi_{pd}$, is consistent with Mediterranean species. Apart from $\psi_{pd}$, variations in $VPD$ also contribute to those in
WUE and iWUE. As shown in Fig.7, leaves subjected to high VPD would have lower WUE and iWUE than those with low VPD under the same soil moisture condition. Previous studies have demonstrated that an increasing VPD cause a decreasing WUE [14, 66, 67]. A higher VPD tends to increase transpiration due to a larger atmospheric demand, which may interpret the observed lower WUE values. In this regard, Beer, Ciais, Reichstein, Baldocchi, Law, Papale, Soussana, Ammann, Buchmann and Frank [50] reported that correlation coefficient between gross primary production(GPP) and evapotranspiration(ET) is improved when the effect of VPD is taken into account at the ecosystem level.

It should be noted that high VPD in this study were generally accompanied with high temperature observed at noon and afternoon in summer (data not shown). Physiological studies show that high temperatures may decrease assimilation rates by reducing photosystem II (PSII) activity [37, 68, 69]. Damage to PSII as a result of high temperature can be more severe when plants are in drought stress [31]. This mechanism may have some contribution to the apparent low WUE (and some iWUE) under high VPD conditions (Fig.8). However, the data collected in this study are not sufficient to confirm this mechanism. Future work is required in this regard.

The average iWUE of all measured leaves for the Osmanthus fragrans tree (35μmol CO2 mmol⁻¹ H2O) is much lower than evergreen trees in Mediterranean (73μmol CO2 mmol⁻¹ H2O under good water condition, and 106μmol CO2 mmol⁻¹ H2O under drought stress) [36]. This difference may reflect plant acclimation to moisture availability in its environment.
3.6 Response of plant hydraulic conductance $K_s$ to drought

The main limitation to transpiration during drought period is soil water availability, and accordingly $K_s$ showed a significant and positive relationship with $\psi_{pd}$ (Fig.8). This relationship is particularly clear when at good soil water condition (0-1MPa). As soil become much drier, the decline in $K_s$ become slower. After drought, $K_s$, is lower in comparison to pre-drought and during drought periods at the same soil water condition (Fig.8) (p<0.001), indicating that $K_s$ had not fully recovered from the drought stress. We compare $K_s$ estimated from sap-flow measurement to leaf-level gas exchange rates measured in years 2013 (Fig.9a, 9b). $A_{max}$ and $g_{s,max}$ significantly decrease with a decreasing plant hydraulic conductance. This result indicates that a close coordination exists between stomatal behavior and xylem hydraulic traits.

3.7 Leaf gas exchange rate after irrigation and rainfall

Data with $VPD$ less than 1.5kPa are used to study how leaf gas exchange rates recover from the drought after irrigation and natural rainfall. To do this, we first find a relationship between $A_n$, $g_s$, and $WUE$ and $\psi_{pd}$, which is then used to evaluate the $A_n$, $g_s$, and $WUE$ recovery from the drought. The results of these relationships are presented in Fig.9. After irrigation in 10$^{th}$ August, $\psi_{pd}$ increased from -2.53 MPa to -1.52 MPa. However, carbon assimilation performed slightly lower than the reference level under this root zone water potential (p=0.094). In the first, second, third and fourth day after irrigation was applied, carbon assimilation was 66%, 77%, 85% and 87%) of the predicted value, respectively. Similarly, after rainfall events in 20$^{th}$-24$^{th}$ August, the carbon assimilation rate was slightly below the reference value (Fig.9a)
(p=0.04). The response of stomatal conductance has a similar pattern (Fig.9b), and significant lower than reference value (p=0.008).

This result indicates that a lower carbon assimilation than the reference values after wetting from irrigation and rainfall was associated with stomatal response. This could be either owing to that the plant has not recovered from drought or to that the micrometeorological condition has changed from the reference. The weather after the irrigation had a large VPD and temperature, and lower solar radiation than the reference value (Fig.10). This larger VPD might explain the slightly lower stomatal conductance as evident in Fig.5. If this is the case, the lower carbon assimilation is not associated with drought impact, but the change of weather condition. After the rainfall event, both temperature and solar radiation were lower than the reference values, which might also account for the slightly lower stomatal conductance than the reference values.

Leaf-level WUE shows a very interesting pattern after the wetting events. It fell below reference line after the irrigation, and above the line after rain events (Fig.9c). This result is most likely owing to the response of transpiration to the change in micrometeorological conditions (Fig.10). A higher VPD than the reference enhanced transpiration, which accounts for the lower WUE after very local irrigation wetting. After rainfall, the micrometeorological condition has changed over a large area. Lower solar radiation (and temperature) led to a lower transpiration, while didn’t reduce photosynthesis because of light saturation. This explains a higher WUE than the reference.
4. Conclusions

In this paper, leaf-level gas exchange of *Osmanthus fragrans* in response to a severe summer drought was examined based on field observations. Main results are summarized in the following. Drought stress on leaf carbon assimilation comes from the lumped effects of both a decrease in soil water potential and an increase in VPD on stomatal conductance. Daily maximum leaf carbon assimilation rates ($A_{max}$) decline with a decrease of root zone water potential in a nonlinear manner, with an initial sharp decrease followed by a gradual change. An S-shape water stress function is suitable to represent the relationship between $A_{max}$ and $\Psi_{pd}$. The effect of VPD on leaf carbon assimilation is that high VPD tends to reduce the assimilation rate. Leaf transpiration behaves similarly with carbon assimilation in terms of their response to soil water potential, but the effect of VPD on transpiration rate is opposite to carbon assimilation rate, with high VPD enhancing transpiration. Water use efficiency and intrinsic water use efficiency with a low VPD shows a biphasic pattern in response to soil water potential. The threshold of root zone water potential dividing the two phases of the relationship is around -1.2 and -1.5 MPa for $WUE$ and $iWUE$, respectively. When a moist root zone gets drier, an initial increase in $WUE$ and $iWUE$ is likely due to a decline in stomatal conductance. A decrease of $WUE$ ($iWUE$) with the progressive drought is likely due to drought-induced decline in the photosynthetic capacity. Plant hydraulic conductance $K_s$ follows a similar pattern with $A_{max}$ in term of response to $\Psi_{pd}$ and shows a significant positive relationship with $A_{max}$ and $g_{max}$. 
Osmanthus fragrans is chosen for this study to investigate the behavior of one component species in this environment, rather than to use it as representative for the whole ecosystem. Obviously, investigation on other species in this environment is required in the future to gain a better picture of the ecosystem response to environmental changes.

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References


20. Galmés, J.; Flexas, J.; Savé, R.; Medrano, H., Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress


34. Damesin, C.; Rambal, S., Field study of leaf photosynthetic performance by a Mediterranean deciduous oak tree (Quercus pubescens) during a severe summer drought. *New Phytologist* 1995, 131, (2), 159-167.


36. Medrano, H.; Flexas, J.; Galmés, J., Variability in water use efficiency at the leaf level among


Figure 1 Location of the study site and the experimental set-up showing the tree being monitored with a stem psychrometer.

Figure 2
Micrometeorological data at the study site. a: average daily temperature (°C), b: daily rainfall $P$ (mm), c: average daily $VPD$ (kPa), d: daily radiation $Ra$ (MJ).
Figure 3: Predawn stem water potential (green squares), predawn leaf water potential (red triangles), midday stem water potential (orange squares), average daily stem potential (magenta squares) and precipitation (blue bars) during the measurement period. The red bar is irrigation applied date and irrigation amount.

Figure 4: Average leaf carbon assimilation rates $A_n$ ($\mu$mol m$^{-2}$ s$^{-1}$) at light saturation measured in tree A (dots), measures in tree B (triangles), and the assimilation rates of shaded leaves (+) vs. predawn stem water potential (MPa) of tree A, with VPD shown in color. b: Leaf stomatal conductance for water vapor $g_s$ (mmol m$^{-2}$ s$^{-1}$) vs. root zone water potential, symbols representing the same type of measurements as in panel (a).
Figure 5a: Shaded leaf carbon assimilation of tree A as a function of vapor pressure deficit and photosynthetic active radiation (a), and a function of root zone water potential and photosynthetic active radiation (b). Color shows the corresponding value of $A_s$ (μmol m$^{-2}$ s$^{-1}$).

Figure 6: Average leaf-level transpiration rates $E$ (mmol m$^{-2}$ s$^{-1}$) at light saturation (dots) for tree A, at light saturation for tree B (triangles), and of shaded leaves for tree A (+) vs. root zone water potential (MPa).
Figure 7  

**a**: Leaf water use efficiency $WUE$ ($\mu$mol CO$_2$ mmol$^{-1}$ H$_2$O) and root zone water potential (MPa): average $WUE$ at light saturation for tree A (dots), average $WUE$ for tree B (triangles). The color curve fitted with the data of daily maximum $WUE$.  

**b**: The same as the top panel except that they are for leaf intrinsic water use efficiency $iWUE$ ($\mu$mol CO$_2$ mol$^{-1}$ H$_2$O).

Figure 8  

Relationship between xylem hydraulic conductance $K_s$ (kg MPa$^{-1}$ m$^2$ s$^{-1}$) and root zone water potential $\Psi_{pd}$ (MPa) prior to drought (red dots), during drought (blue squares) and after drought periods (magenta triangles). The curve is fitted with Eq.(5).
Figure 9 a: Relationship between maximum carbon assimilation rate ($A_{\text{max}}$) and whole-plant $K_s$ during years 2013. b: Relationship between maximum stomatal conductance ($g_{s\text{max}}$) and whole plant $K_s$ during years 2013. Data points are mean values of measurements with $VPD$ less than 2kPa on a particular measurement date. All data represent dates when concurrent data from both leaf gas exchange measurements and stem sap-flow measurements (for $K_s$) were available.
Figure 10  

Figure 10: a: leaf carbon assimilation rates \( A_n \) (μmol m\(^{-2}\) s\(^{-1}\)), b: stomatal conductance \( g_s \) (mol m\(^{-2}\) s\(^{-1}\)), c: water use efficiency \( WUE \) (μmol CO\(_2\)/mmol H\(_2\)O) as a function of root zone water potential (MPa). Symbols: leaf gas exchange were measured after irrigation (filled triangle), after natural rainfall event (filled circles). The number in the figures is days after irrigation and natural rainfall event. The curve is fitted with Eq. (3) data with \( VPD \) less than 1.5kPa. Color bar shows the corresponding value of \( VPD \) (kPa).

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