Stomatal and non-stomatal limitations to photosynthesis of Populus euphratica Oliv. leaves in an extremely arid area of northwestern China

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Abstract
On the basis of successive measurements of leaf gas exchange during the main growing seasons of Populus euphratica Oliv. in 2013 and 2014, respectively, we analyzed the stomatal and non-stomatal limitations to photosynthesis under natural conditions in an extremely arid region of northwestern China. Our results showed that (1) the distribution patterns of net photosynthesis (Pn) and stomatal conductance (gs) were similar, both of which increased in the morning, peaked at around noon, and then decreased. This contrasted with the observed changes in sub-stomatal CO2 concentrations (Ci). (2) The phenomenon of midday depression of photosynthesis (MDP) was obvious from July to September during the two years. At the beginning of MDP, the stomatal limitation to photosynthesis (Ls) peaked, where its predominance was supported by Ci being at a minimum. Thereafter, Ls decreased and Ci/gs increased sharply, indicating that the non-stomatal limitation to photosynthesis predominated. (3) Both the Ls and relative stomatal limitation to photosynthesis (RLs) increased in the morning, and then decreased, whereas Ci/gs presented contrary changes. (4) The RLs values were greater than the Ls values, which was mainly due to the nonlinearity of the Pn/Ci curve, which often leads to large overestimations. (5) The Ls values in our study were much greater than those from other studies under natural conditions. The most probable reason was that the extremely high temperature and scarce water resource caused the stomata to close to reduce transpiration, resulting in the stomatal limitation to photosynthesis being more intense.

Background
Arid and semi-arid lands cover approximately 40 % of Earth’s terrestrial surface and are expanding globally (Reynolds 2000) and the arid regions are expected to become drier as a result of climate change (Dai 2011, Sheffield et al. 2012, McDowell et al. 2015). The ecosystems in arid and semi-arid lands represent a dynamic but poorly understood component of the global carbon, water, and energy cycles (Naithani et al. 2012). The Ejin oasis, which is located in the lower reaches of the Heihe River Basin of northwestern China, is one of the most arid regions in the world, with annual rainfalls of less than 50 mm (Si et al. 2009). Populus euphratica Oliv. is the constructive species of the desert riparian forest in this region, where its regular growth is important for the formation of a natural barrier to
support the existence of the Ejin oasis. Owing to the lack of water resources, however, the *P. euphratica* forests have degenerated in this region in the last century.

Drought stress is an important environmental factor inhibiting the growth and reducing the yield of plants worldwide (Li et al. 2000, Li and Wang 2003, Zhang et al. 2005). The limitation of plant growth due to low water availability is mainly caused by reductions in the plant carbon balance, which is largely dependent on photosynthesis (Flexas et al. 2009). Photosynthesis, the basic manufacturing process required for the existence of life on this planet, contributes to more than 90% of crop biomass (Makino 2011). It can increase the carbon gain of crops and, thus, improve their yield and quality (Xie et al. 2017). Previous studies have shown that photosynthesis is one of the first physiological processes to be affected under drought (Lawlor 1995, Munns 2002). According to Farquhar and Sharkey (1982), there are two aspects to the restrictions in photosynthesis: (1) the intercellular CO₂ concentration (*Cᵢ*) cannot meet the needs of photosynthesis owing to the limitation of stomatal conductance (stomatal limitation); and (2) the chloroplast and Rubisco activities and ribulose bisphosphate (RuBP) regeneration are decreased (non-stomatal limitation). Thus far, it is still unclear and under debate on whether drought limits photosynthesis mainly through stomatal or non-stomatal limitations (Noormets et al. 2001, Flexas and Medrano 2002).

Previous studies on stomatal and non-stomatal limitations of photosynthesis were conducted mostly under various controlled conditions, such as water stress (Ni and Pallardy 1992, Signarbieux and Feller 2011, Varone et al. 2012, Campos et al. 2014, Anev et al. 2016), saline-alkali stress (Steduto et al. 2000, Centritto et al. 2003, Meloni et al. 2003, Yang et al. 2008, Li et al. 2010), multifactor stress (Correia et al. 2005), and other stressors (Pereira et al. 2013, Maggard et al. 2016), whereas studies conducted under natural conditions were rare. In particular, in the extremely arid regions of northwestern China where the natural environments are harsh, studies on stomatal and non-stomatal limitations to photosynthesis have seldom been carried out. For *P. euphratica* grown in the Ejin oasis, researchers have focused mainly on the aspects of hydraulic redistribution (Yu et al. 2013), measuring and modeling of evapotranspiration and stomatal conductance (Hou et al. 2010, Zhu et al. 2011, Gao et al. 2016a, 2016b, 2017), and so on, whereas little research effort has been devoted to
the stomatal and non-stomatal limitations to photosynthesis. Furthermore, drought can decrease the leaf photosynthetic rate (Zhou et al. 2015), which consequently limits the ecosystem productivity of *P. euphratica*. Therefore, the study of stomatal and non-stomatal limitations to photosynthesis of *P. euphratica* leaves in extremely arid areas is of particular importance.

The objectives of this study were to (1) analyze the diurnal changes in the environmental conditions and physiological factors of *P. euphratica* leaves and (2) study the stomatal and non-stomatal limitations to photosynthesis of *P. euphratica* leaves under natural conditions in an extremely arid region. Our research should be of great significance, as the analysis of stomatal and non-stomatal limitations to photosynthesis of *P. euphratica* leaves under natural conditions in the Ejin oasis has not yet been reported.

Methods

2.1. Experimental site

Measurements of leaf gas exchange in *P. euphratica* leaves were recorded at the Qidaoqiao *P. euphratica* Forest Reserve in Ejin County (Inner Mongolia, China) from June to September in 2013 and 2014, respectively (Fig. 1, 42°21′N, 101°15′E, altitude 920.5 m a.s.l.). In the forest reserve, the average tree age is 32 years, with good growth status. The trees have an average height of 10.2 m, an average diameter at breast height of 24.67 cm, and an average crown breadth of 442 cm – 450 cm. The soil is sandy loam of approximately 2 m in depth and has a volumetric water content of 0.35 m$^3$ m$^{-3}$. The bulk density of the soil is 1.53 g cm$^{-3}$.

2.2. Measurements and data processing

We selected three individual *P. euphratica* trees in 2013 and 2014, respectively, and measured the leaf gas exchange in three fully expanded leaves of each tree hourly from the east and west, on four or five sunny days every month. An LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) was used for the measurements. We measured all the available parameters; namely, net photosynthesis (*P*$_n$, µmol CO$_2$ m$^{-2}$ s$^{-1}$), stomatal conductance (*g*$_s$, mol H$_2$O m$^{-2}$ s$^{-1}$), sub-stomatal and ambient CO$_2$ concentrations (*C*$_i$ and *C*$_a$, µmol CO$_2$ mol$^{-1}$), photosynthetically active radiation (PAR,
µmol m⁻² s⁻¹), air temperature ($T_a$, °C), relative humidity ($h_s$, %), and vapor pressure deficit (VPD, kPa). The measurements were conducted from 8:00 to 20:00, where the observation session was adjusted by advancing or postponing it by 1 h according to the specific circumstances of sunrise and sunset. We also used a sky lift to reach the canopy when measuring the leaves. The sample sizes in 2013 and 2014 were 103 averages based on 927 measurements and 183 averages based on 1647 measurements, respectively.

2.3. Formulae

Stomatal limitation to photosynthesis can be quantified by the parameter $L_s$, which can be calculated according to the following formula described by Farquhar and Sharkey (1982):

Due to technical limitations, Equation 1 has been placed in the Supplementary Files section.

where $G$ is the CO₂ compensation point of assimilation in the presence of dark respiration. $G$ can always be neglected, whereupon the formula is then expressed as follows:

Due to technical limitations, Equation 2 has been placed in the Supplementary Files section.

Another way to quantify the relative importance of the stomata in controlling the processes of photosynthesis is the resistance-based method described by Jones (Jones 1998), who proposed the relative stomatal limitation to photosynthesis ($RL_s$). The value can be calculated by the following formula:

Due to technical limitations, Equation 3 has been placed in the Supplementary Files section.

where $r_s$ is the stomatal resistance, $r_a$ is the boundary layer resistance, and $r^*$ is the slope of the tangent to the $P_n/C_i$ curve at the operating point.
and $r_a$ can be calculated by the following formulae:

\[ r_s \text{ and } r_a \text{ can be calculated by the following formulae:} \]

Due to technical limitations, Equation 4 has been placed in the Supplementary Files section.

where $l_w$ is the leaf width, $u_h$ is the wind speed at the top of the canopy, and $h$ is the mean height of the canopy.

The non-stomatal limitation to photosynthesis can be indicated by $C_i/g_s$.

Discussion

3.1 Diurnal changes in the environmental factors

Detailed information on the key environmental variables is essential to assess diurnal changes in the stomatal and non-stomatal limitations to photosynthesis. We chose one sunny day per month to analyze the diurnal change patterns of the environmental factors (PAR, $T_a$, VPD, and $h_s$) during the main growing seasons of *P. euphratica* in 2013 and 2014, respectively (Fig. 2). Generally, the distribution patterns of PAR, $T_a$, and VPD all presented single-peak curves. For the most part, PAR, $T_a$, and VPD increased in the morning, peaked between 13:00 and 15:00, and then decreased. During the study period, the daily average PAR was 941.74 W m$^{-2}$, varying from 11.62 to 1689.33 W m$^{-2}$. The daily average $T_a$ was 31.60 °C, which was very close to those of the previous years (data not shown). The $h_s$ value decreased gradually after 10:00, mainly due to the increase in $T_a$, which in turn caused the enhancement of evaporation and water loss to the air. The daily average $h_s$ was 46.63 %, varying from 17.54 % to 75.21 %. It was obvious that the values for all four parameters did not show significant differences between 2013 and 2014.

3.2 Diurnal changes in the physiological factors

We selected the same days to analyze the diurnal change patterns of the physiological factors ($P_n$, $g_s$, and $C_i$) during the main growing seasons of *P. euphratica* in 2013 and 2014, respectively (Fig. 3). We can see from Figure 3 that the distribution patterns of $P_n$ and $g_s$ were similar. $P_n$ increased in the morning, peaked at around 14:00, and then decreased. In August and September, the values of $P_n$
were much greater than those in June and July. The $g_s$ value increased rapidly in the morning, peaked between 10:00 and 12:00, and then decreased gradually in the afternoon. The peak values of $g_s$ in the different months were fairly close to one another (0.40–0.46 mol H$_2$O m$^{-2}$ s$^{-1}$). We can also see that there were fluctuations in $P_n$ and $g_s$ between 11:00 and 14:00 from July to September, which indicated the obvious phenomenon of midday depression of photosynthesis (MDP) (e.g., July and September 2013 and August 2014, respectively). $C_i$ presented a contrasting pattern of diurnal changes to those of $P_n$ and $g_s$ (Fig. 3), where the minimums appeared between 14:00 and 16:00. The ranges of $g_s$ and $C_i$ values in the different months were similar, where $g_s$ ranged from 0.02 to 0.47 mol H$_2$O m$^{-2}$ s$^{-1}$ and $C_i$ ranged from 126.78 to 401.21 µmol CO$_2$ mol$^{-1}$.

3.3 Diurnal changes in $L_s$, $RL_s$, and $C_i/g_s$

The diurnal changes in $L_s$, $RL_s$, and $C_i/g_s$ during the main growing seasons of $P.$ euphratica in 2013 and 2014 are shown in Figure 4. We can see that both $L_s$ and $RL_s$ increased in the morning and then decreased. The $L_s$ values in the afternoon were significantly greater than those in the morning. Generally, the $RL_s$ values were greater than the $L_s$ values. $C_i/g_s$ presented a contrasting pattern of diurnal changes to those of $L_s$ and $RL_s$. The $C_i/g_s$ values were generally low in the morning and increased sharply after 17:00. The highest values of these parameters in 2013 and 2014 were 0.51 and 0.70 for $L_s$, 0.44 and 0.58 for $RL_s$, and 7.30 and 4.83 for $C_i/g_s$, respectively.

Conclusions

The Ejin oasis is one of the most arid regions in the world, and less precipitation can be expected to be available for plants there. Drought can be considered as one of the main environmental factors limiting photosynthesis and plant productivity. Nevertheless, whether water deficit affects plant photosynthesis via stomatal or non-stomatal limitation is still unclear and under debate. In order to clarify this question, we analyzed stomatal and non-stomatal limitations to photosynthesis under natural conditions in an extremely arid region of northwestern China through successive measurements of leaf gas exchange in $P.$ euphratica during its main growing seasons in 2013 and
2014, respectively.

The diurnal changes in $C_i$ presented “V-shaped” curves (greater $C_i$ values in the morning and evening, and lower values at noon), which was basically consistent with the conclusions made in studies of Wheat (Jiang et al. 2001) and *Haloxylon ammodendron* (Yang et al. 2015) in arid regions. The similarity in the distribution patterns of $P_n$ and $g_s$ indicated that $g_s$ was to some extent the main factor influencing $P_n$ under extremely arid conditions.

During the study periods, especially from July to September, the MDP phenomenon was evident. This was mainly owing to the high transpiration rates caused by the high VPD values, which led to an intense water deficit in the leaves. On the one hand, $g_s$ decreased and limited the entry of $C_a$; on the other hand, the water deficit caused a decrease in mesophyll conductance ($g_m$), which decreased the CO$_2$ concentration in the chloroplast to a very low level, resulting in the decrease in $P_n$. For *P. euphratica* growing in extremely arid regions from July to September, where the $T_a$ at around noon is the highest and the transpiration rate is the fastest, MDP can protect the tree from losing too much water.

Although the $L_s$ increased gradually in the morning and then decreased, it still remained at high levels and was the main reason for the decrease in $P_n$ in the afternoon. During the MDP periods, $L_s$ peaked, and $C_i$ was at a minimum, showing the apparent stomatal limitation to photosynthesis. This was consistent with the viewpoint of Farquhar and Sharkey (1982), who proposed the simultaneous increase in $L_s$ and decrease in $C_i$ as being the criterion for determining stomatal limitation. The low soil moisture content limited the water uptake by *P. euphratica*, and the intense solar radiation and high VPD values increased the water loss from the trees. Owing to a combination of transpiration and photosynthesis, the stomata closed to maintain water balance inside the plant and limit CO$_2$ entry into the leaves. Therefore, stomatal limitation to photosynthesis was predominant. Afterward, $L_s$ decreased and $C_i/g_s$ increased sharply, and the non-stomatal limitation to photosynthesis predominated. Several studies have shown $g_m$ to be the main factor controlling non-stomatal
limitation to photosynthesis, especially under stress (Grassi and Magnani 2005, Niinemets et al. 2005, Warren 2008). The structure of leaves determined the maximum value of \( g_m \) (Nobel 1977), and \( g_m \) also changed rapidly according to the environment conditions (Flexas et al. 2008). In extremely arid regions (like our study area, Ejin oasis), the soil moisture content is very low, and water stress is the main factor limiting \( g_m \) (Scartazza et al. 1998, Delfine et al. 2001, Monti et al. 2006). Because a greater VPD usually leads to lower \( g_m \) (Bongi and Loreto, 1989), we can infer that the \( g_m \) values in the morning were greater than those in the afternoon, as the VPD changed inversely (Fig. 2). It was much easier for \( C_i \) to enter into the chloroplast through the cytomembrane and cytoplasm and supply the CO\(_2\) that would be consumed by photosynthesis, leading subsequently to low \( C_i/g_s \) values in the morning. In the afternoon, \( g_m \) decreased and hindered the diffusion of CO\(_2\) into the chloroplast, and \( C_i/g_s \) subsequently increased.

We can also see from Figure 4 that the values of \( RL_s \) were greater than those of \( L_s \), and this was mainly due to the nonlinearity of the \( P_n/C_i \) curve. Such occurrence often leads to a large overestimation of the importance of the stomata in controlling photosynthesis (Jones 1998). Consequently, \( L_s \) is more applicable than \( RL_s \) for representing stomatal limitation to photosynthesis.

We have summarized the maximum \( L_s \) values of different species from previous research studies and this study in Table 1. We can see that the maximum \( L_s \) values from Yang et al. (2015) (0.64) and this study (0.70 in 2013 and 0.51 in 2014, respectively) were much greater than those from other studies, probably because both experiments were conducted under conditions of extremely intense water stress in northwestern China. At our study site specifically, \( P. euphratica \) grew in an environment with high temperatures (mean \( T_a \) values at 31.6 °C in 2013 and 29.9 °C in 2014, respectively) and scarce water resources (annual precipitation of 25.6 mm and 27.0 mm, and mean \( h_s \) values of 54.2 % and 43.0 % in 2013 and 2014, respectively) during the study periods, and therefore, the stomata closed to reduce transpiration, resulting in the predominance of stomatal limitation to photosynthesis.

As our data were obtained using the LI-6400 portable photosynthesis system, there was a lack of
parameters (chloroplast structure, photosynthetic pigment content, Rubisco activity, RuBP regeneration capacity) measured to represent the non-stomatal limitation to photosynthesis. We will make up for this in our future work.

Declarations

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Authors’ contributions

X.L. and Q.F. designed and G.G. performed the experiments; all authors analyzed the data; G.G. wrote the manuscript; Q.F. provided the guidance on the whole manuscript and all authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.
Consent for publication

Not applicable.

Competing interests

The authors have no conflict of interest to declare.

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Tables

Table 1 Maximum values of stomatal limitation to photosynthesis (Ls) under natural conditions for different species from previous research studies and this study
| Species                        | Maximum $L_s$ | Location     | Literature resources                      |
|-------------------------------|---------------|--------------|-------------------------------------------|
| *Dacrydium cupressinum* Lamb. | 0.29          | New Zealand  | Tissue et al. (2005)                      |
| *Metrosideros umbellate* Cav. | 0.41          |              |                                           |
| *Weinmannia racemosa* L.f.    | 0.42          |              |                                           |
| *Quintinia acutifolia* Kirk.  | 0.41          |              |                                           |
| *Quercus robur* L.            | 0.17          | Italy        | Grassi and Magnani (2005)                 |
| *Fraxinus oxyphylla* Bieb.    | 0.21          |              |                                           |
| Spring wheat                  | 0.64          | China        | Yang et al. (2015)                        |
| *Populus euphratica* Oliv.    | 0.70 (2013)   | China        | This study                                |
|                               | 0.51 (2014)   |              |                                           |

**Figures**
Figure 1

Location map of the study area. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Diurnal change patterns of photosynthetically active radiation (PAR), vapor pressure deficit (VPD), air temperature (Ta) and relative humidity (hs) during the main growing seasons of *Populus euphratica* in 2013 and 2014, respectively.
Diurnal change patterns of the net photosynthetic rate (Pn), stomatal conductance (gs), and intercellular CO2 concentration (Ci) during the main growing seasons of Populus euphratica in 2013 and 2014, respectively.
Figure 4

Diurnal change patterns of (relative) stomatal and non-stomatal limitations to photosynthesis of Populus euphratica leaves during the main growing seasons in 2013 and 2014, respectively. Rls, relative stomatal limitation to photosynthesis; Ls, stomatal limitation to photosynthesis; Ci/gs, non-stomatal limitation to photosynthesis.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

Equation 4.png
Equation 3.png
Equation 1.png
Equation 2.png