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## Hierarchy of plasticity traits in responses of *Quercus aliena* to light conditions and water availability

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**Abstract:** This study aimed to investigate plasticity of different plant traits to varied light and water availability. A greenhouse experiment was conducted with *Quercus aliena* seedlings with two light and four soil water levels. Plant traits related to leaf physiology, morphology, anatomy, and biomass production were determined. The results showed that plant size had significant effects on leaf area, leaf number, shoot height, basal diameter and crown area. After excluding the influence of plant size, water treatment had stronger effects on plants compared to light levels, and their interaction effect was significant. The limited water supply significantly inhibited leaf photosynthetic rate and the fluorescence efficiency under high light. However, leaves submitted to moderate drought stress showed enhanced fluorescence activity under shade condition. Grand plasticity of leaf physiology and growth was the highest, followed by biomass allocation and leaf morphology, and lastly anatomy, and this ranking did not change as resources considered. Among the variables, leaf petiole length, chlorophyll content and leaf area could be selected as candidates for estimation of species' plasticity to water, light and their interaction, respectively. Therefore, our results suggested that there was a hierarchy existing among traits plasticity in *Q. aliena*, and supported the above-ground facilitation hypothesis that shade could alleviate the adverse effect of drought.

**Additional key words:** Above-ground hypothesis, grand plasticity, leaf anatomy, light-water interaction

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### Introduction

Plants can adjust to a broad variety of environmental conditions through plastic responses. These responses involve at the leaf level, such as morphology, physiology, and anatomy, and changes in biomass allocation and growth at the whole-plant level (Jimenez et al. 2009; Craven et al. 2010; Mielke and Schaffer 2010). Plasticity, once considered as noise,

is now recognized to be of great importance to species adaptability. To date, plasticity has been realized to be crucial for plant survival, species distributions, and community (Valladares et al. 2006). However, there are still a few essential questions that need to be addressed about plant plasticity, one of which is whether and how the adaptive plasticity regularly varies among different traits (e.g. anatomy versus physiology versus allocation) (Nicotra et al. 2010).

In recent years, there has been an increase in studies on plastic changes among different properties and to different resources. Zunzunegui et al. (2009) reported that physiological traits of a semi-deciduous shrub were more plastic than allocation traits. According to the results of Lei et al. (2006), growth of *Populus przewalskii* is more plastic than allocation in responses to water availability, but its physiological plasticity varies between populations. Callaway et al. (2003) claimed that individuals may vary by orders of magnitude in size, growth rate, allocation, reproduction and chemical contents, whereas Navas and Garnier (2002) reported a comparable plasticity between whole plant and metamer traits. The question on rankings of the plasticity of plant traits within species is still ambiguous and needs further investigation. If a hierarchy of plasticity among traits does exist, we can postulate that some plant traits would be more useful than others for plant adaptation to changing climate.

The combined effect of light and water conditions is controversial in the field of ecological study, which affects plant growth and survival in various ways (Quero et al. 2006; Huang et al. 2008; Puértolas et al. 2008). The influential trade-off hypothesis predicts that deep shade aggravates drought-induced damage, because plants allocate more photosynthate to shoots under low light conditions for maximization of light capture, compared to the root allocation, consequently resulting in less water absorption (Smith and Huston 1989). However, contrary evidence obtained from natural forests and controlled experiments demonstrates that upper canopy shading helps to alleviate drought impacts by maintaining the water status of plants. This is a result of decreased leaf and air temperatures, as well as lowered vapor pressure deficit under shaded conditions, which contribute to less oxidative stress (Holmgren 2000). Canham C.D. et al. (1996) argued that plants under light limited conditions are less affected by water shortage than those in full light. However, the facilitation effects could be not true when there is water competition at roots level among plants, which may overcome the alleviation of canopy shade (Esquivias et al. 2015). Additionally, some authors report an orthogonal function between drought and shade (Sack and Grubb 2002).

These diverse conclusions may be due to the involvement of plant size, thus it is necessary to test results excluding the influence of plant size. Exploring mechanisms of plants' plastic responses to simultaneous light and water limitation is essential for understanding and explaining plants' capacity to adaptation in variable environments (Valladares et al. 2006; Arend et al. 2013), and improves the efficiency of the Grain to Green Projects in China. Water deficit is often the key factor adversely affecting reforesta-

tion practices of the Grain to Green Projects in arid and semi-arid areas (Du et al. 2013). In addition, plants' adaptive response to heterogeneous forest light conditions is closely associated with successful restoration under limited soil water conditions.

Oak species that are widely distributed all over the world have important significance for the stability of ecosystems, and conservation of soil and water. *Quercus aliena* Bl. is a dominant species that widely distributes in the warm temperate regions and the northern subtropical regions of China. It is photophilous, and usually found in open areas of sunny slopes. *Q. aliena* is a pioneer species, easy to form one single stand, and occupies the favorable upper space niche, but it has low conversion rate from seedlings to young trees in nature. Because of limited knowledge on the species' response mechanisms in relation to light and water availability, *Q. aliena* was not successfully regenerated during "Grain to Green Projects" in China. In the experiment, we selected seedlings of *Q. aliena* in the first year of growth to determine 1) the plastic degrees among different traits in response to varied resources, 2) which one could explain the responsive mechanisms in relation to combined light and water stress, with regard to the trade-off, aboveground facilitation, interplay, and independent hypotheses.

## Methods

### Study site and plant materials

The study was conducted at Fanggan Research Station of Shandong University (36°26'N, 117°27'E), China. This site has a typical warm temperate monsoon climate with mean annual temperature of  $13\pm 1^{\circ}\text{C}$  and annual precipitation of 600–850 mm. Seeds of *Q. aliena* were collected from Kunyu Mountain, Shandong Province in November 2009. They were germinated and transplanted into pots containing haydite (Shang Dao Biotech Co., Ltd, Shandong, China) in spring 2010. Uniformly sized saplings were then carefully selected and planted in 9-L pots (32 cm high  $\times$  29 cm diameter) containing 7 kg of mixed substrate (sieved soil and humus, 2:1, v/v). The composition of the substrate was as follows: organic matter 20.61 g kg<sup>-1</sup>, pH 5.60, total N 0.77 g kg<sup>-1</sup>, total P 1.56 g kg<sup>-1</sup>, and total K 20.85 g kg<sup>-1</sup>, and its field capacity (FC) was 31% by mass.

### Experimental design

The experiment was randomly arranged as two-factorial layouts with two light levels: 66% (achieved using neutral film) and 10% (achieved by black nylon woven net in the greenhouse) of sunlight, and four

soil water levels: 15, 40, 65 and 90% of FC in a greenhouse built with metal frames and neutral polyethylene roof to prevent rainfall disturbance. The watering regimes severed as severe, moderate, mild water deficit and well water conditions, respectively. The 10% sunlight was selected to simulate the irradiance of forest understory after measuring the irradiance of forest understory in local areas (Du et al. 2013), and the 66% sunlight equaled to irradiance of forest gaps, which served as control here. Ten seedlings were used as replication in each water and light treatment combination. The PPFD of the two light treatments were approximately  $593 \pm 13.7 \mu\text{mol m}^{-2} \text{s}^{-1}$  (66% sunlight) and  $89.5 \pm 5.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  (10% sunlight) on sunny days monitored by a Mini-PAM micro-quantum sensor (Walz GmbH, Effeltrich, Germany).

All seedlings were cultivated for 1 month before treatment. Then, watering was ceased to reach the water levels by soil drying. After two weeks, when the driest level was achieved, half of the seedlings were transferred into shading rooms for the shade treatment. Water was compensated by weighing the pots every afternoon. The experiment lasted for three months until 28 September 2010.

## Growth measurements

Seedling height (H), basal diameter (BD, 1 cm above the ground), and crown area (CA,  $CA = 0.25 \times 3.14 \times \text{crown length} \times \text{crown width}$ ) were recorded every 15 days. At the end of the experiment, four seedlings per treatment were harvested, and separated into roots, stems, and leaves. Then, the samples were oven-dried (80°C for 48 h, followed by 30 min at 105°C) and weighed. Leaf mass ratio (LMF), stem mass ratio (SMF), root mass ratio (RMF), root/shoot ratio (R/S) and total biomass (TB) were calculated.

## Leaf morphological and physiological traits

Before harvesting, leaf number was recorded, and total leaf area (TLA) was determined by a CID-203 laser area meter (CID Inc., Washington, USA). The specific leaf area (SLA, ratio of leaf area to leaf dry mass), leaf area ratio (LAR, ratio of leaf area to total biomass), and mean leaf area (MLA) of individuals were calculated. Upper crown leaves were selected for leaf morphology determination, including leaf length (LL), leaf width (LW), leaf petiole length (LPL), leaf length to leaf width (LL/LW), and leaf length to leaf petiole length (LL/LPL) with a digital caliper.

On sunny days, nine healthy leaves from three or four individuals were selected for measurements of gas exchange parameters with a portable gas ex-

change system (GFS-3000, Walz GmbH, Effeltrich, Germany) in mid-September. These measurements included net photosynthetic rate ( $P_n$ ), transpiration rate ( $T_r$ ), stomatal conductance ( $G_s$ ), and intercellular  $\text{CO}_2$  concentration ( $C_i$ ) based on leaf area. The measurements were conducted at  $800 \mu\text{mol m}^{-2} \text{m}^{-1}$  PAR supplied by an external LED light, which was close to the light saturation point for seedlings. Instantaneous water use efficiency ( $\text{WUE}_i$ ) was calculated as the ratio of  $P_n$  to  $T_r$ . The maximal quantum yield ( $F_v/F_m$ ,  $F_v = F_m - F_0$ ) of photosystem II (PSII) was determined on the same leaves after 30 min of dark adaptation, with a pulse amplitude modulation chlorophyll fluorometer (Mini-PAM, Walz GmbH, Effeltrich, Germany). Effective quantum yield (Yield) and relative rate of electron transport (ETR) were calculated after the actinic light (about  $402 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) applied for 1 min. Photosynthetic pigments including chlorophyll (Chl) and carotenoids (Car) were extracted with ethanol and quantified spectrophotometrically as described by Lichtenthaler (1987).

## Leaf anatomy

Anatomical tissue was determined on nine healthy and fully-expanded leaves from three or four individuals after physiological measurement at the end of the experiment. The thickness of the palisade (PP) and spongy (SP) parenchyma, upper (UE) and lower (LE) epidermis, diameter of the largest vessel (DV), and xylem width (XW) and area (XA) were recorded by Image-Pro Plus software (IPP, Media cybernetics, USA) attached to light microscope. Leaf sections were taken from the 1/3 fraction of leaves, and stored in mixed formalin, alcohol, and glacial acetic acid solution (FAA) until measurements.

## Statistical analysis

### Plasticity Index

Grand plasticity ( $P_i$ ) was calculated as the difference between the maximum value and the minimum value divided by the maximum value over eight treatments. Plasticity to light or water was separately calculated as well, as  $P_{Ti} = (\text{the maximum value}_i - \text{the minimum value}_i) / \text{the maximum value}_i$ , where  $P_{Ti}$  is the plasticity of one trait to resource T under the  $i^{\text{th}}$  supply level of the other resource.  $P_{L1}$  or  $P_{W2}$  corresponds to the plasticity of certain trait to variation of light under the lowest water supply level (15% FC) or that of water under the high light level (66% sunlight), respectively. The maximum plasticity index among levels was regarded as the plasticity of the traits to light or water.

Table 1. Results of the ANCOVA analysis (with biomass being the covariate) that examining variations in the response of traits to light (L) intensity, water (W) availability and their interaction (W×L), and overall experimental treatment (T)

| Traits  | F and its significance |                   |                   |                   |          |
|---|------------------------|-------------------|-------------------|-------------------|----------|
|   | Biomass                | W                 | L                 | W×L               | T        |
| TB/g  | –                      | 64.7**            | 273.8**           | 61.0**            | 93.0**   |
| R/S   | 1.9 <sup>ns</sup>      | 30.4**            | 7.7*              | 12.5**            | 144.3**  |
| LMR   | 0.4 <sup>ns</sup>      | 8.3**             | 1.8 <sup>ns</sup> | 3.6*              | 140.2**  |
| SMR   | 1.7 <sup>ns</sup>      | 6.5**             | 2.9 <sup>ns</sup> | 0.9 <sup>ns</sup> | 48.5**   |
| RMR   | 1.5 <sup>ns</sup>      | 12.8**            | 4.0 <sup>ns</sup> | 3.1*              | 654.2**  |
| H/cm  | 1.6 <sup>ns</sup>      | 5.2**             | 1.4 <sup>ns</sup> | 6.9**             | 138.2**  |
| BD/mm   | 25.7**                 | 12.3**            | 0.3 <sup>ns</sup> | 2.7 <sup>ns</sup> | 7.1**    |
| CA/cm <sup>2</sup>  | 11.8**                 | 10.0**            | 0.4 <sup>ns</sup> | 3.4*              | 6.6**    |
| LN  | 4.6*                   | 3.4*              | 0.3 <sup>ns</sup> | 0.3 <sup>ns</sup> | 17.4*    |
| TLA/cm <sup>2</sup>   | 42.4**                 | 4.4*              | 11.1**            | 3.3*              | 4.1**    |
| MLA/cm <sup>2</sup>   | 12.0**                 | 1.1 <sup>ns</sup> | 3.8 <sup>ns</sup> | 1.4 <sup>ns</sup> | 23.3**   |
| SLA/cm <sup>2</sup> g <sup>-1</sup>                                   | 0.7 <sup>ns</sup>      | 7.7*              | 3.4*              | 1.5 <sup>ns</sup> | 181.7**  |
| LAR   | 1.8 <sup>ns</sup>      | 16.4**            | 18.8**            | 5.4**             | 204.4**  |
| LL/cm   | 0.5 <sup>ns</sup>      | 5.9**             | 10.2**            | 4.9**             | 139.2**  |
| LW/cm   | 1.2 <sup>ns</sup>      | 3.6*              | 8.5**             | 4.4*              | 96.8**   |
| LPL/cm  | 0.0 <sup>ns</sup>      | 3.5*              | 0.2 <sup>ns</sup> | 1.7 <sup>ns</sup> | 52.4**   |
| LL/LW   | 1.0 <sup>ns</sup>      | 0.8 <sup>ns</sup> | 0.7 <sup>ns</sup> | 0.8 <sup>ns</sup> | 375.4**  |
| LL/LPL  | 0.1 <sup>ns</sup>      | 1.9 <sup>ns</sup> | 1.8 <sup>ns</sup> | 0.1 <sup>ns</sup> | 71.2**   |
| P <sub>n</sub> /mol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup>   | –                      | 108.0**           | 230.7**           | 42.4**            | 99.0**   |
| G <sub>s</sub> /mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> | –                      | 74.0**            | 66.5**            | 5.0**             | 44.1**   |
| T <sub>r</sub> /mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> | –                      | 41.8**            | 80.9**            | 2.1 <sup>ns</sup> | 301**    |
| C <sub>i</sub> /mol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup>   | –                      | 5.7**             | 0.0 <sup>ns</sup> | 24.3**            | 13.2**   |
| WUE <sub>v</sub> /mmol mol <sup>-1</sup>                              | –                      | 4.93**            | 0.2 <sup>ns</sup> | 25.7**            | 13.2**   |
| F <sub>v</sub> /F <sub>m</sub>  | –                      | 5.7*              | 60.7**            | 13.2**            | 16.8**   |
| Yield   | –                      | 13.9**            | 22.5**            | 3.3*              | 10.6**   |
| ETR   | –                      | 13.9**            | 22.5**            | 3.3*              | 10.6**   |
| Chl/mg g <sup>-1</sup>  | –                      | 2.5 <sup>ns</sup> | 19.9**            | 0.9 <sup>ns</sup> | 3.4*     |
| Car/mg g <sup>-1</sup>  | –                      | 1.0 <sup>ns</sup> | 14.3*             | 2.3 <sup>ns</sup> | 3.2*     |
| UE/μm   | 0.7 <sup>ns</sup>      | 7.2**             | 1.8 <sup>ns</sup> | 6.6**             | 7.1**    |
| PP/μm   | 1.2 <sup>ns</sup>      | 16.9**            | 26.1**            | 18.2**            | 33.8**   |
| SP/μm   | 3.7 <sup>ns</sup>      | 51.8**            | 0.3 <sup>ns</sup> | 6.4**             | 506.0**  |
| LE/μm   | 2.3 <sup>ns</sup>      | 5.0**             | 1.3 <sup>ns</sup> | 1.6 <sup>ns</sup> | 519.4**  |
| LT/μm   | 0.0 <sup>ns</sup>      | 4.2**             | 2.5 <sup>ns</sup> | 0.4 <sup>ns</sup> | 1014.4** |
| DV/μm   | 2.4 <sup>ns</sup>      | 5.5**             | 7.1**             | 3.4*              | 1051.6** |
| XW/μm   | 0.1 <sup>ns</sup>      | 15.0**            | 7.4**             | 9.8**             | 430.1**  |
| XA/μm   | 0.0 <sup>ns</sup>      | 45.7**            | 15.4**            | 18.9**            | 191.6**  |

Total biomass was used as a covariate when ANCOVA was performed, except for physiological traits. ns:  $p > 0.05$ ; \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ . TB total biomass; R/S root to shoot mass ratio; LMR leaf mass ratio; SMR stem mass ratio; RMR root mass ratio; H height; BD basal diameter; CA crown area; LN leaf number; TLA total leaf area; MLA mean leaf area; SLA specific leaf area; LAR leaf area ratio; LL leaf length; LW leaf width; LPL leaf petiole length; LL/LW, leaf length to leaf width ratio; P<sub>n</sub> net photosynthetic rate; G<sub>s</sub> stomata conductance; T<sub>r</sub>, transpiration rate; C<sub>i</sub> intercellular CO<sub>2</sub> concentration; F<sub>v</sub>/F<sub>m</sub> maximal quantum yield of PSII; Yield effective quantum yield of PSII; ETR, electron transportation rate; Chl chlorophyll content; Car carotenoids content; UE upper epidermis; PP palisade parenchyma; SP spongy parenchyma; LE lower epidermis; LT leaf thickness; DV diameter of the largest vessel; XW xylem width; XA xylem area.

## Ranking trait in relation to plasticity

All traits were defined into five categories: whole-plant growth (H, BD, CA, TB, LN and TLA), leaf morphology (LW, LL, LPL, LL/LPL, LL/LW, MLA and SLA), physiology (P<sub>n</sub>, T<sub>r</sub>, G<sub>s</sub>, C<sub>i</sub>, WUE<sub>v</sub>, F<sub>v</sub>/F<sub>m</sub>, Chl, Car, Yield and ETR), anatomy (LT, UE, LE, PP, SP, DV, XW and XA) and allocation (LMR, SMR, RMR, R/S and LAR). As there was no difference in LL/LW among treatments, its plasticity was not considered. The Kruskal-Wallis non-parametric test was used to determine whether a ranking of grand plasticity among trait groups exists or not.

## Data analysis

The effects of light, water and their interaction on traits were analyzed by univariate process of general linear model (GLM), with plant biomass as covariant. When the effect of biomass was significant as covariate, adjusted means were calculated, and used for comparisons of the trait plasticity. LSD multiple comparison was used to estimate differences among treatments after ANCOVA. Physiological characteristics, such as photosynthesis parameters, fluorescence yield and photosynthetic pigment contents were not included in ANCOVA following the previous study (Funk 2008). All statistical analyses were conducted using SPSS software (Version 13.0; SPSS, IL, USA). Figures were drawn with Origin 8.0 software (OriginLab Co., Northampton, MA, USA).

## Results

### The effects of light and water treatments on *Q. aliena* seedlings, with biomass as a corrector

The effect of experimental treatment was significant for all measured parameters, as shown in Table 1. Plant size had significant effect on some of the parameters, including BD, CA, LN, TLA, and MLA. Drought stress depressed plant performance in most aspects after excluding the influence of plant size, but not MLA, LL/LW, LL/LPL, Chl and Car. The effect of light on plant performance was less influential compared to water treatment, with higher proportion of parameters showing no responses to it after excluding the influence of plant size ( $F_{\text{water}} > F_{\text{light}}$ , Table 1).

Plants were larger under 65% FC and 66% sunlight, and had higher biomass production, with relatively low SLA (Fig. 1–3). Drought stress increased RMR and R/S in 40% FC at 66% sunlight, thus LMR was inhibited accordingly. Then, RMR and R/S showed decline under the severest water limitation (15% FC), and LMR increased accordingly (Fig. 2).

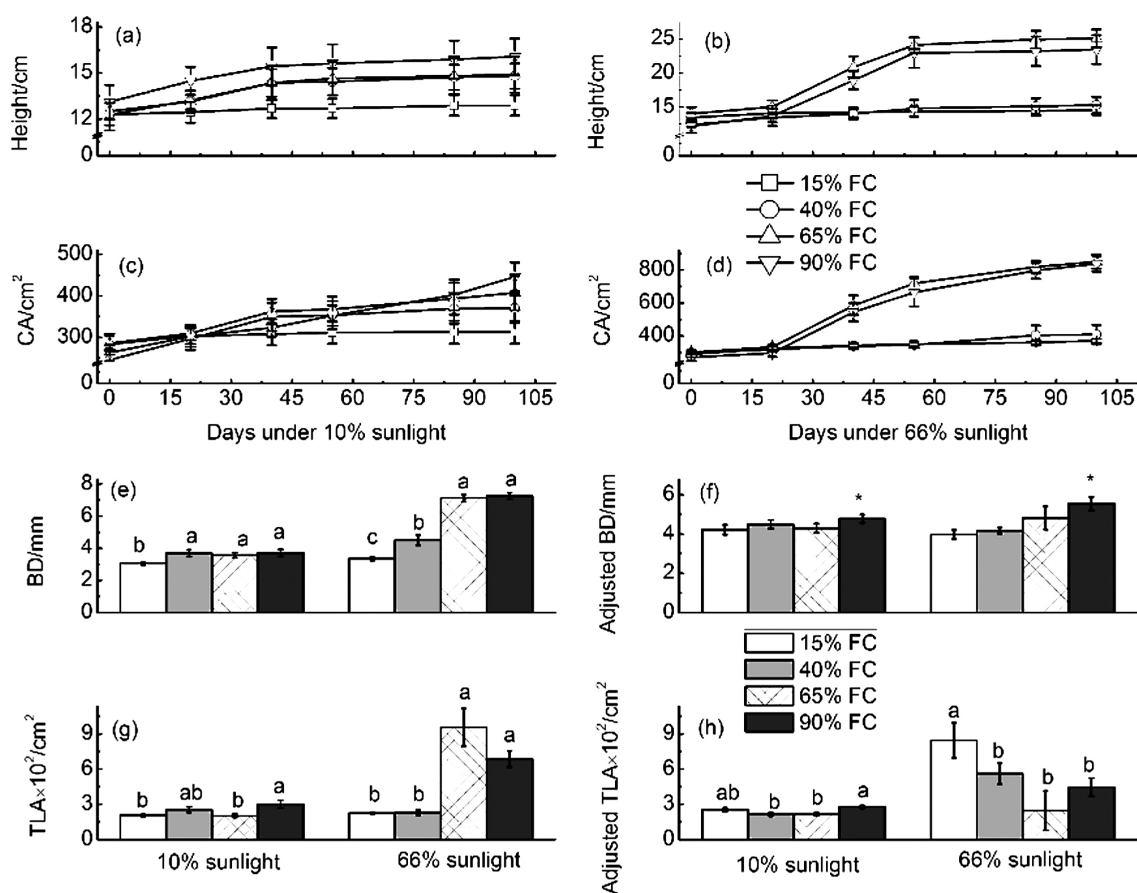


Fig. 1. Variations of plant height and crown area growth over the whole experimental periods, and of final basal diameter and total leaf area among treatments. (a) and (c) Height and crown area under 10% of sunlight; (b) and (d) Height and crown area under 66% of sunlight; (e) and (g) True means for basal diameter and total leaf area; (f) and (h) Adjusted means correcting for biomass effects (see Table 1). Means ( $\pm$ S.E.) followed by different letters differed at  $p < 0.05$  (Turkey's HSD test for true means). Treatments are: 10% and 66% of sunlight; 15%, 40%, 65% and 90% FC

In 10% sunlight, the biomass ratios showed similar trend across water treatments, but differences were not significant. Drought stress decreased parameters of LPL and MLA of seedlings in 66% sunlight, but the trend was not significant in 10% sunlight (Fig. 3). The parameters of SLA, LAR, and LL/LPL significantly increased in 15% FC conditions at 66% sunlight, and also the adjusted means of TLA and MLA were higher after adjusting by plant size (Fig. 1 and 3).

Light, water and their interaction had significant effects on photosynthetic gas exchange and Chl fluorescence parameters (Fig. 4). At 66% sunlight, remarkable reductions in  $P_n$ ,  $T_i$ , Gs, WUE<sub>i</sub>,  $F_v/F_m$ , Yield, and ETR were observed under the severest drought stress of 15% FC. However,  $C_i$  under 15% FC conditions increased in contrast to the decreasing trend in  $P_n$ , Gs, and Tr. At 10% sunlight,  $P_n$ , Yield and ETR decreased only at 15% FC, and no opposite tendency occurred between  $C_i$  and other gas exchange parameters. Parameters of  $F_v/F_m$  and Chl of shade-seedlings showed insignificant decline in 15% FC, and WUE<sub>i</sub> increased.

Water availability significantly influenced UE, LE, and SP, but irradiance had no significant effect

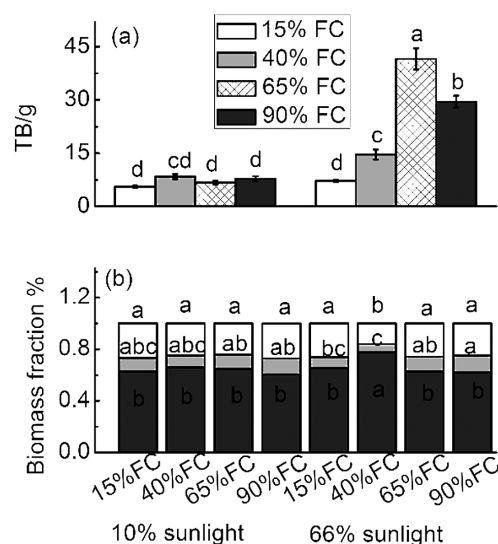


Fig. 2. Variations of (a) plant biomass and (b) biomass allocation among treatments. The bars indicate means  $\pm$  S.E. Means ( $\pm$ S.E.) followed by different letters differed at  $p < 0.05$  (Turkey's HSD test). Leaf mass ratio is shown in blank, stem mass ratio in grey and root mass ratio in dark grey. Treatments are: 10% and 66% of sunlight; 15%, 40%, 65% and 90% of field capacity (FC)

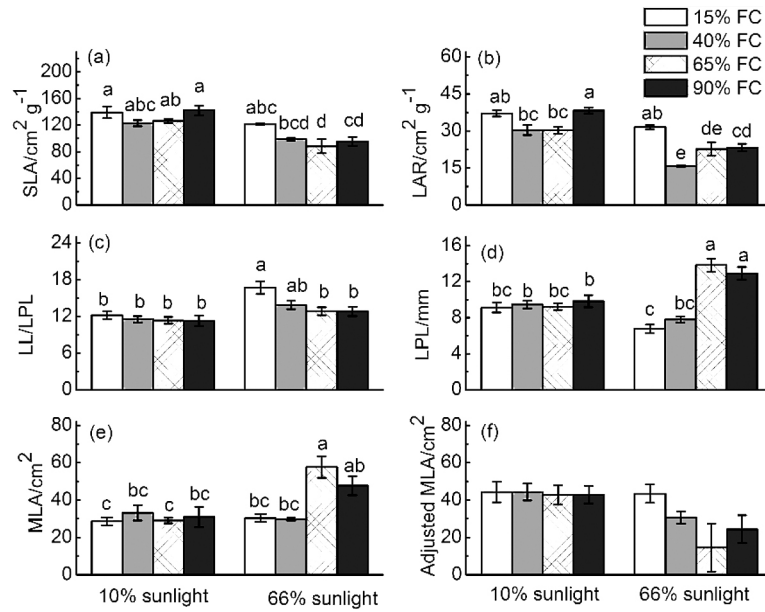


Fig. 3. Variations of leaf morphological traits among treatments. (a) SLA (b) LAR (c) LL/LPL (d) LPL (e) MLA, true means (f) Mean leaf area, adjusted means correcting for biomass effect (see Table 1). Means ( $\pm$ S.E.) followed by different letters differed at  $p < 0.05$  (Turkey's HSD test for true means). Treatments are: 10% and 66% of sunlight; 15%, 40%, 65% and 90% FC. Abbreviations seen in Table 1

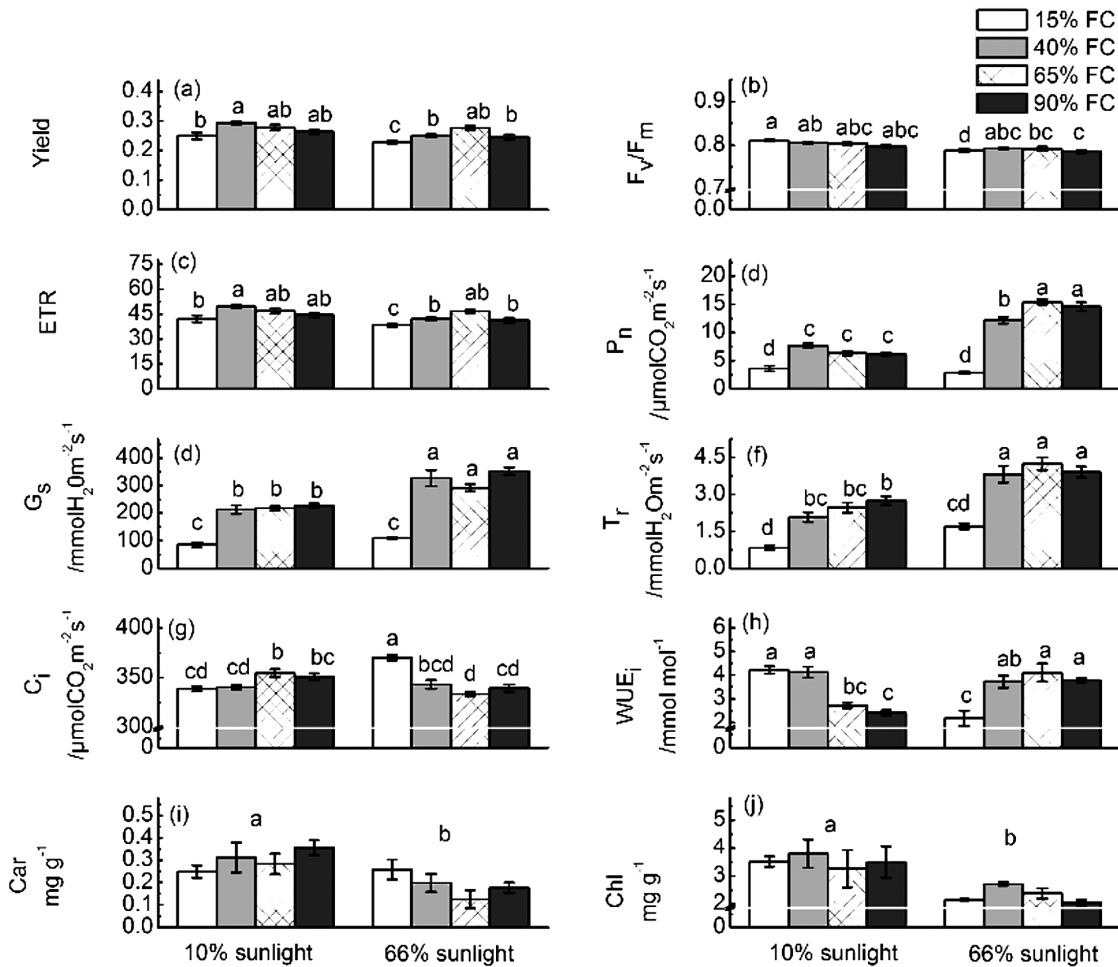


Fig. 4. Variations of leaf physiological traits among treatments. (a) Yield (b)  $F_v/F_m$  (c) ETR (d)  $P_n$  (e)  $G_s$  (f)  $T_r$  (g)  $C_i$  (h)  $WUE_i$  (i) Car (j) Chl. Means ( $\pm$ S.E.) followed by different letters differed at  $p < 0.05$  (Turkey's HSD test for true mean). Treatments are: 10% and 66% of sunlight; 15%, 40%, 65% and 90% FC. Abbreviations seen in Table 1

on these parameters (Table 1, 2). Both light and water influenced the levels of PP and xylem dimension. XW and XA significantly increased with soil moisture under both light levels. The mesophyll tissues became thick with decreased soil water under high light, mainly because leaves formed compact palisade cells in 1–2 layers. Shade-leaf had thicker PP under 15% FC compared to these growing in other water

Table 2. Variations of leaf anatomical characteristics among treatments

|   | 10% sunlight                 |                               |                                |                               | 66% sunlight                   |                               |                               |                                |
|---|------------------------------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|--------------------------------|
|   | 15% FC                       | 40% FC                        | 65% FC                         | 90% FC                        | 15% FC                         | 40% FC                        | 65% FC                        | 90% FC                         |
| UE/ $\mu\text{m}$                           | 89.0 $\pm$ 2.8 <sup>c</sup>  | 107.3 $\pm$ 2.8 <sup>a</sup>  | 100.1 $\pm$ 1.9 <sup>abc</sup> | 89.2 $\pm$ 2.3 <sup>c</sup>   | 105.7 $\pm$ 3.7 <sup>ab</sup>  | 96.8 $\pm$ 2.8 <sup>abc</sup> | 104.5 $\pm$ 2.3 <sup>ab</sup> | 94.3 $\pm$ 2.0 <sup>bc</sup>   |
| PP/ $\mu\text{m}$                           | 360.6 $\pm$ 7.2 <sup>c</sup> | 317.0 $\pm$ 6.6 <sup>d</sup>  | 323.1 $\pm$ 3.2 <sup>d</sup>   | 312.3 $\pm$ 8.0 <sup>d</sup>  | 361.6 $\pm$ 4.1 <sup>c</sup>   | 393.6 $\pm$ 3.3 <sup>b</sup>  | 415.0 $\pm$ 3.2 <sup>a</sup>  | 366.3 $\pm$ 2.7 <sup>c</sup>   |
| SP/ $\mu\text{m}$                           | 378.7 $\pm$ 8.7 <sup>d</sup> | 555.2 $\pm$ 16.9 <sup>a</sup> | 464.8 $\pm$ 9.6 <sup>c</sup>   | 407.5 $\pm$ 8.6 <sup>d</sup>  | 468.5 $\pm$ 20.4 <sup>bc</sup> | 557.5 $\pm$ 13.8 <sup>a</sup> | 523.4 $\pm$ 9.6 <sup>ab</sup> | 412.6 $\pm$ 19.0 <sup>cd</sup> |
| XA $\times$ 10 <sup>5</sup> / $\mu\text{m}$ | 9.6 $\pm$ 0.5 <sup>de</sup>  | 8.6 $\pm$ 0.3 <sup>e</sup>    | 10.1 $\pm$ 0.1 <sup>cde</sup>  | 11.4 $\pm$ 0.3 <sup>c</sup>   | 9.0 $\pm$ 0.1 <sup>e</sup>     | 11.0 $\pm$ 0.8 <sup>cd</sup>  | 16.5 $\pm$ 0.2 <sup>b</sup>   | 19.8 $\pm$ 0.2 <sup>a</sup>    |
| XW $\times$ 10 <sup>2</sup> / $\mu\text{m}$ | 41.1 $\pm$ 1.5 <sup>de</sup> | 37.4 $\pm$ 0.6 <sup>e</sup>   | 43.5 $\pm$ 0.4 <sup>cd</sup>   | 44.6 $\pm$ 0.7 <sup>cd</sup>  | 41.3 $\pm$ 1.0 <sup>de</sup>   | 47.8 $\pm$ 2.4 <sup>e</sup>   | 53.8 $\pm$ 0.6 <sup>b</sup>   | 60.7 $\pm$ 0.8 <sup>a</sup>    |
| LE/ $\mu\text{m}$                           | 70.8 $\pm$ 2.66 <sup>b</sup> | 80.3 $\pm$ 3.0 <sup>a</sup>   | 69.4 $\pm$ 2.3 <sup>b</sup>    | 65.6 $\pm$ 1.6 <sup>b</sup>   | 63.6 $\pm$ 1.9 <sup>b</sup>    | 67.3 $\pm$ 2.5 <sup>b</sup>   | 63.2 $\pm$ 1.1 <sup>b</sup>   | 62.5 $\pm$ 1.5 <sup>b</sup>    |
| DV/ $\mu\text{m}$                           | 213.4 $\pm$ 4.9 <sup>c</sup> | 210.7 $\pm$ 4.6 <sup>c</sup>  | 209.7 $\pm$ 4.1 <sup>c</sup>   | 220.4 $\pm$ 3.9 <sup>bc</sup> | 207.5 $\pm$ 4.4 <sup>c</sup>   | 214.5 $\pm$ 3.3 <sup>c</sup>  | 240.6 $\pm$ 4.2 <sup>a</sup>  | 237.5 $\pm$ 2.8 <sup>ab</sup>  |

Means ( $\pm$ S.E.) followed by different letters differed at  $p < 0.05$  (Turkey's HSD test). Treatments are: 10% and 66% of sunlight; 15%, 40%, 65% and 90% of field capacity (FC). Abbreviations seen in Table 1.

Table 3. Grand plasticity ( $P_i$ ) of traits and plasticity to light ( $P_l$ ) and water ( $P_w$ ) under different water and light levels

| Trait               | $P_i$ | $P_l$  |        |        |        | $P_w$     |           |
|---------------------|-------|--------|--------|--------|--------|-----------|-----------|
|                     |       | 15% FC | 40% FC | 65% FC | 90% FC | 10% light | 66% light |
| Whole-plant growth  |       |        |        |        |        |           |           |
| TB 1                | 0.87  | 0.22   | 0.43   | 0.84   | 0.74   | 0.33      | 0.83      |
| H 2                 | 0.49  | 0.12   | 0.04   | 0.41   | 0.31   | 0.21      | 0.42      |
| BD 3                | 0.28  | 0.06   | 0.07   | 0.11   | 0.14   | 0.12      | 0.28      |
| CA 4                | 0.52  | 0.35   | 0.20   | 0.42   | 0.39   | 0.22      | 0.38      |
| TLA 5               | 0.75  | 0.70   | 0.62   | 0.13   | 0.37   | 0.23      | 0.71      |
| Allocation          |       |        |        |        |        |           |           |
| LMR 6               | 0.41  | 0.03   | 0.35   | 0.07   | 0.08   | 0.11      | 0.39      |
| SMR 7               | 0.53  | 0.20   | 0.32   | 0.02   | 0.08   | 0.24      | 0.53      |
| RMR 8               | 0.22  | 0.04   | 0.15   | 0.03   | 0.02   | 0.08      | 0.20      |
| R/S 9               | 0.55  | 0.12   | 0.44   | 0.07   | 0.07   | 0.20      | 0.52      |
| LAR 10              | 0.59  | 0.15   | 0.48   | 0.25   | 0.39   | 0.21      | 0.50      |
| Leaf morphology     |       |        |        |        |        |           |           |
| LL/LPL 11           | 0.33  | 0.27   | 0.17   | 0.12   | 0.12   | 0.08      | 0.23      |
| LPL 12              | 0.51  | 0.26   | 0.18   | 0.34   | 0.24   | 0.07      | 0.51      |
| MLA 13              | 0.67  | 0.02   | 0.31   | 0.66   | 0.43   | 0.03      | 0.66      |
| LL 14               | 0.41  | 0.01   | 0.02   | 0.41   | 0.34   | 0.05      | 0.39      |
| LW 15               | 0.40  | 0.04   | 0.04   | 0.40   | 0.36   | 0.09      | 0.37      |
| SLA 16              | 0.38  | 0.13   | 0.20   | 0.30   | 0.33   | 0.14      | 0.27      |
| Physiology          |       |        |        |        |        |           |           |
| $P_n$ 17            | 0.81  | 0.21   | 0.37   | 0.59   | 0.57   | 0.53      | 0.81      |
| $T_r$ 18            | 0.80  | 0.51   | 0.46   | 0.42   | 0.30   | 0.70      | 0.60      |
| $G_s$ 19            | 0.76  | 0.22   | 0.35   | 0.25   | 0.36   | 0.62      | 0.69      |
| WUE <sub>i</sub> 20 | 0.49  | 0.49   | 0.10   | 0.34   | 0.36   | 0.43      | 0.47      |
| Yield 21            | 0.30  | 0.09   | 0.15   | 0.01   | 0.07   | 0.15      | 0.18      |
| Chl 22              | 0.46  | 0.39   | 0.29   | 0.27   | 0.42   | 0.14      | 0.25      |
| Car 23              | 0.65  | 0.04   | 0.37   | 0.56   | 0.50   | 0.30      | 0.51      |
| Anatomy             |       |        |        |        |        |           |           |
| UE 24               | 0.17  | 0.16   | 0.10   | 0.04   | 0.05   | 0.17      | 0.11      |
| PP 25               | 0.25  | 0.02   | 0.20   | 0.22   | 0.13   | 0.13      | 0.15      |
| SP 26               | 0.32  | 0.19   | 0.00   | 0.11   | 0.01   | 0.32      | 0.26      |
| LE 27               | 0.22  | 0.10   | 0.16   | 0.09   | 0.05   | 0.18      | 0.07      |
| LT 28               | 0.17  | 0.07   | 0.04   | 0.12   | 0.08   | 0.07      | 0.11      |
| XA 29               | 0.57  | 0.07   | 0.22   | 0.39   | 0.42   | 0.25      | 0.55      |
| XW 30               | 0.39  | 0.00   | 0.22   | 0.19   | 0.27   | 0.16      | 0.32      |
| DV 31               | 0.14  | 0.03   | 0.02   | 0.13   | 0.07   | 0.05      | 0.14      |

Treatments are: 10% and 66% of sunlight; 15%, 40%, 65% and 90% of field capacity (FC). Abbreviations seen in Table 1.

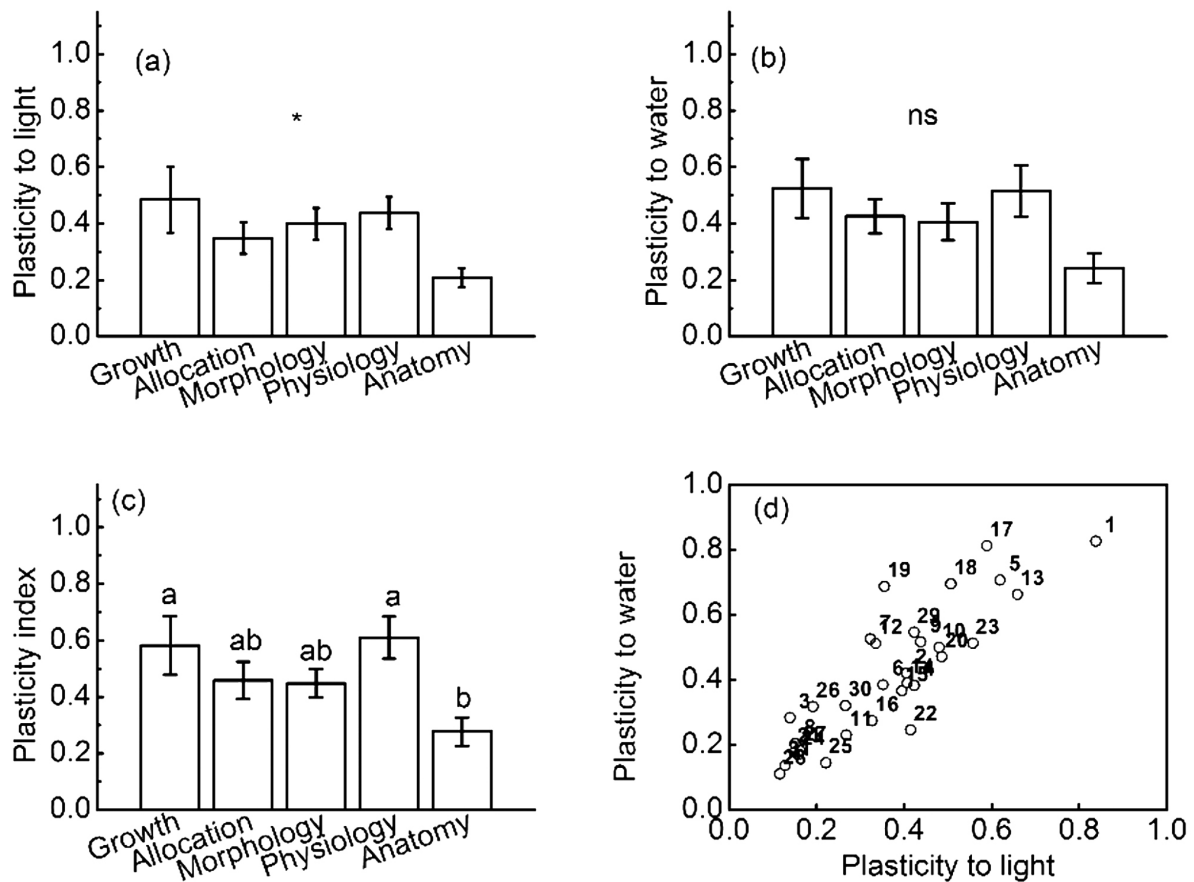


Fig. 5. Whole-plant growth, allocation, leaf morphology, physiology and anatomy plasticity index, and relationship between plasticity of traits (d) to light and water levels. (a) Plasticity to light (b) Plasticity to water (c) Grand plasticity index among trait categories. Traits are numbered in (d) according to the sequence shown in Table 3. Means ( $\pm$ S.E.) followed by different letters differed at  $p < 0.05$  (Kruskal-Wallis test)

treatments, but the spongy mesophyll showed similar trend as under high light.

### Grand plasticity among traits of *Q. aliena* seedlings

The grand plasticity of different traits greatly varied (Table 3). A clear difference in plasticity was found across different categories, with photosynthetic parameters being the most plastic, whereas anatomy the least plastic traits. Allocation and leaf morphology were similar, both having intermediate plasticity. Among the five categories, total leaf area per plant, mean leaf area per leaf, net photosynthetic rate and xylem area were the most plastic traits within their categories.

### Plasticity among traits of *Q. aliena* seedlings to water and light

Plasticity varied in response to different resources, and among different traits (Table 3, Fig. 5). All of the traits showed divergent plasticity under different resources levels. Consequently, plasticity of traits

sequenced differently between light and water over treatments. Traits exhibiting the highest plasticity were TLA (plants under the lowest water supply level) and MLA (in well-watered plants) in response to light, and  $P_n$  and TLA (under the high light level) in response to water. While the lowest values of plasticity index were registered for LL and XW (under the lowest water supply level) in response to light, and DV (under the low light level) in response to water.

Allocation showed stronger response to light under 40% FC, whereas they varied little under the other three water conditions. Leaf morphology responded mostly to light under well water (above 65% FC, Table 3). Similarly, plant growth showed such a plastic tendency among different water levels as well. Almost all traits responded strongly to water supply at the high light level. Traits could be grouped according to their responses degree to resource. A significant difference in grand plasticity was found among trait-categories but plasticity did not change in response to light or water conditions (Fig. 5).



## Discussion

Our study found that drought stress had stronger influence on *Q. aliena* seedlings than low light did when the interference of plant size is excluded. Seedlings could adapt to unfavorable environment through modifying leaf phenotype, and changing biomass partitioning strategy. However, the lowest water deficit (15%FC) threw great challenge on plant survival, and was more likely to be the key factor affecting *Q. aliena* seedling performance in silviculture and restoration practice.

### Which hypothesis to support?

Plant traits showed strongly interactive responses to light and water treatments, and the difference of biomass parameters and plant growth between 10% and 66% sunlight decreased with the decreasing of soil moisture. Reduced SLA under high light suggested that the lower vapor-pressure deficit under shade compensated for high transpiration load from high SLA and drought (Quero et al. 2006; Du et al. 2013). Therefore, these results did not support predictions of the trade-off hypothesis that shade stress aggravated drought-induced damages (Smith and Huston 1989).

The efficiency and stability of PSII ( $F_v/F_m$ ) was lower significantly in seedlings submitted to the lowest water supply under high irradiance. This was somehow alleviated when seedlings growing in shade environment, although the value of  $F_v/F_m$  was consistently close to 0.8 and over 0.75 and could be regarded as typical healthy leaves (Maxwell and Johnson 2000; Aranda et al. 2005). In addition, Yield and ETR of light-adapted leaves exhibited negative responses to drought under high light, but was also efficiently improved when leaves were subjected to low irradiance. Therefore, these responses supported the above-ground facilitation hypothesis that shading helped to alleviate drought impacts.

Additionally, the effect of drought on photosynthesis parameters under shade was much weaker than that in high level of light. As a consequence, plant growth and biomass production were relatively comparable across water treatments. This could be explained by the interplay hypothesis or the primary limitation hypothesis stating that the influence of drought is weaker in moderate light and stronger in high light, which has been put forward as part of the above-ground facilitation hypothesis. Additionally, the conclusion was drawn from testing seedlings, which may be changed and turning into competition when seedlings grew up (Armas and Pugnaire et al. 2009).

## Plasticity among different traits

Our study indicated that the plastic variations of leaf phenotypes among individual may be mainly due to environmental variation in oak species, but not of the sole consequences of plant biomass differences (Rice and Bazzaz 1989). Likewise, changes in leaf morphology and physiology that more closely associated with environmental conditions than with plant size was also reported in other oak and tree species (Delagrangue et al. 2004; Paquette et al. 2007; Funk 2008). This means that the effect of total biomass could be excluded in evaluation of plastic changes at the leaf-level of *Q. aliena* seedlings, for both the original and adjusted values. Plants generally allocate more to root early in development when they are small and young but more to shoot as they are getting larger, thus it is not sure that differences in ontogenetic stages would not cause any difference in plastic responses of biomass allocation (Weiner 2004). Studies have shown that most plants have a constant reproduction allocation compared to vegetative allocation after achieving a minimum size, which may later be interfered by individual competition (Weiner 2004). Some studies even concluded that plasticity in allocation is simply the result of the plant size (Wright and McConnaughay 2002). However, our study showed that environmental changes were the main reasons for differences in biomass partitioning in tree seedlings and similar conclusions have been observed by Zhang et al (2006). Such diverse allocation strategies are often considered to be genetically determined, i.e. species- or genotype-specific (Weiner 2004). Additionally, it should be noticed that our study was carried out in seedlings, which may be differed from larger trees. Therefore, long-term investigation on different woody populations should be conducted to clarify the biomass partitioning mechanism in relation to environmental changes as plant grows over time.

The plasticity changes of traits due to plant biomass are defined as passive plasticity (Nicotra et al. 2010). In our study, passive plasticity was positive and greater than the effect of treatment on TLA, LN, CA and BD. Therefore, plant size should be taken into consideration when detecting variations in these properties among plants from different environments (Semchenko and Zobel 2005).

Our study showed that leaf physiological responses were very plastic to environmental conditions, while the plasticity of leaf anatomy was observed to be the lowest among the studied traits. Thicker upper epidermis and compact palisade parenchyma under drought stress improved leaf water balance, and protected inner tissues from damage of high light (Oguchi et al. 2003; Guerfel et al. 2009; Wyka and Oleksyn 2014). Formation of xylem at midrib with

smaller vessel diameter could enhance the structural strength, and reduce risks of embolism in wood subjected to water deficit (Woodruff et al. 2008). It is suggested that leaf physiology could sensitively react even on a scale of minutes or hours, such as photosynthetic chemistry (Callaway et al. 2003; Alongi 2008). Our study found that the plasticity of allocation and leaf morphology were quite similar among plant functional types in consistent with conclusions of other studies (Navas and Garnier 2002; Zunzunegui et al. 2009). Therefore, a hierarchy of plasticity was outlined in the sequence of leaf physiology, whole-plant growth, leaf morphology and biomass ratios, and lastly the anatomical traits on the basis of their grand plasticity. Moreover, the ranking of traits did not change as resources considered, but changed under different resource availability in some of them, such as higher allocation plasticity to varied irradiance under limited water supply compared to that under sufficient water supply. That means that the ranking of traits plasticity to one kind resource depends on supply of other resources. This ranking of traits plasticity was also partly summarized on shrub seedlings by Zunzunegui et al (2009). Plant plasticity differs among traits and levels of resources (Valladares et al. 2006). Traits that are closely related to utility of particular resources could be selected as candidate to estimate the plasticity of a species (Navas and Garnier 2002). In our study, LPL showed a marked plasticity to water availability under high light, which could be considered to estimate plant plastic response to water, but not to light. In the latter case, other traits such as chlorophyll content may be more appropriate. Characterization of the plasticity of a species to combined resource variations among habitats is relatively complicated, since environmental conditions are heterogeneous in availability and correlated to more than one functional changes (Callaway et al. 2003). It was reported that allocation traits are more suitable than metamer traits for assessing the plasticity because of its consistent responsiveness to different resources (Navas and Garnier 2002). However, our results indicated that allocation plasticity to light was quite low under different soil conditions, except that under 40% FC. In such cases, TLA and MLA are of more general values than others to evaluate the plasticity of a species to environment, due to the consistently sensitive plastic capacity to treatment when the other one is relatively sufficient.

In conclusion, drought stress had stronger influence on *Q. aliena* seedlings than light intensity did when the interference of plant size was excluded. The response of seedling traits to combined light and water availability supported the above-ground facilitation hypothesis. For young seedlings, environmental changes acted as the main element influ-

encing biomass partitioning relative to plant size. A hierarchy of plasticity was outlined in the sequence of leaf physiology, whole-plant growth, leaf morphology and biomass ratios, and anatomical traits, which could not be changed by resource types considered, but its availability. Leaf petiole length, chlorophyll content, and leaf area could be recommended as candidates for estimation of species' plasticity to water, light and their interaction, respectively.

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