



Original article

Phenotypic variation and water selection potential in the stem structure of invasive alligator weed

Leshan Du ^{a, b, c}, Beifen Yang ^{b, c}, Wenbin Guan ^{a, *}, Junmin Li ^{b, c, **}^a College of Nature Reserve, Beijing Forestry University, Beijing 100083, China^b Zhejiang Provincial Key Laboratory of Evolutionary Ecology and Conservation, Taizhou University, Taizhou 318000, China^c Institute of Ecology, Taizhou University, Taizhou 318000, China

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ABSTRACT

The morphological and anatomical characteristics of stems have been found to be related to drought resistance in plants. Testing the phenotypic selection of water availability on stem anatomical traits would be useful for exploring the evolutionary potential of the stem in response to water availability. To test the phenotypic variation of the stem anatomical traits of an invasive plant in response to water availability, we collected a total of 320 individuals of *Alternanthera philoxeroides* from 16 populations from terrestrial and aquatic habitats in 8 plots in China and then analyzed the variation, differentiation, plasticity and selection potential of water availability on the stem anatomical traits. We found that except for the thickness of the cortex, all of the examined phenotypic parameters of the *A. philoxeroides* stem were significantly and positively correlated with soil water availability. The phenotypic differentiation coefficient for all of the anatomical structural parameters indicated that most of the variation existed between habitats within the same plot, whereas there was little variation among plots or among individuals within the same habitat except for variation in the thickness of the cortex. A significant phenotypic plasticity response to water availability was found for all of the anatomical traits of *A. philoxeroides* stem except for the thickness of the cortex. The associations between fitness and some of the anatomical traits, such as the stem diameter, the cortex area-to-stem area ratio, the pith cavity area-to-stem area ratio and the density of vascular bundles, differed with heterogeneous water availability. In both the aquatic and terrestrial habitats, no significant directional selection gradient was found for the stem diameter, the cortex area-to-stem area ratio or the density of vascular bundles. These results indicated that the anatomical structure of the *A. philoxeroides* stem may play an important role in the adaptation to changes in water availability.

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1. Introduction

Phenotypic plasticity, i.e., the capacity of an organism to express variable phenotypes in different environments, is a widespread phenomenon among many animals, plants and other organisms (Agrawal, 2001; Engel et al., 2011; Nicotra et al., 2010). Phenotypic plasticity plays an important role in the adaptation of species to heterogeneous environments and is a prerequisite for evolution by natural selection (Agrawal, 2001; Stanton et al., 2000). Functionally

adaptive plasticity, i.e., the capacity for specific appropriate environmental responses, can play major roles in both the ecological distribution of organisms and their patterns of evolutionary diversification from individual to population to species (Sultan and Spencer, 2002; Sultan, 2003). Adaptive plasticity may also contribute specifically to species invasiveness by allowing rapid colonization of diverse new habitats without the need to undergo local selection (Williams et al., 1995), which might allow a species to exhibit broader ecological amplitude and greater environmental tolerance than is required in their native regions (Williamson, 1996) and facilitate successful invasions in the introduced range (Buswell et al., 2011). Understanding how invasive plants adaptively evolve in response to environmental stress during the invasive process may help us predict the invasiveness of plants in the context of global climate change.

* Corresponding author. College of Nature Reserve, Beijing Forestry University, No. 35 Qinghua Dong Road, Beijing 100083, China.

** Corresponding author. Institute of Ecology, Taizhou University, No. 1139 Shifu Road, Taizhou 318000, China.

E-mail addresses: swlab@bjfu.edu.cn (W. Guan), lijmtzc@126.com (J. Li).

Phenotypic selection, known as the differences in fitness (or its components) associated with phenotypic variation among individuals, is used to estimate the strength of natural selection (Munguia-Rosas et al., 2011; Kingsolver et al., 2012). Some studies have shown that phenotypic selection in natural populations is strong (Kingsolver et al., 2001; Kingsolver and Pfennig, 2007), whereas other studies show that it is weak (Kingsolver et al., 2012). The strength of phenotypic selection can be influenced by selection-driven force and environmental factors (Munguia-Rosas et al., 2011; Bartkowska and Johnston, 2012). Bartkowska and Johnston (2012) found that pollinators cause a stronger selection than herbivores on floral traits in *Lobelia cardinalis* (Lobeliaceae). Based on meta-analysis data, Munguia-Rosas et al. (2011) found that phenotypic selection on flowering phenology is influenced by latitude.

To date, selection has been detected in hundreds of populations in nature, with thousands of estimates of the strength and form of selection on numerous phenotypic traits (Kingsolver et al., 2001; Siepielski et al., 2009). Kingsolver et al. (2012) proposed that phenotypic selection on physiological and behavioral traits from natural populations is a promising area for expanding our understanding of selection in the wild (Kingsolver et al., 2012). Most studies in this area have focused on ecophysiological traits (Dudley, 1996), particularly leaf traits (Donovan et al., 2007, 2009) and flower traits (Beans and Roach, 2015). For example, Donovan et al. (2007) documented phenotypic selection on leaf water-use efficiency and related ecophysiological traits in natural populations of desert sunflowers. Donovan et al. (2009) examined spatial and temporal variation in phenotypic selection on leaf ecophysiological traits for 10 *Helianthus* populations. However, little attention has been paid to the anatomical structure of plants, particularly that of the stem. The stem is an important organ that exhibits high plasticity in adapting to variable environments (Ackerly et al., 2000). For example, Dudley and Schmitt (1996) found that the stem elongation response to density contributes to the fitness differences and presents adaptive properties. In particular, the morphological and anatomical characteristics of stems have been found to be related to drought resistance in plants (De Micco and Aronne, 2012). Therefore, testing the phenotypic selection on stem anatomical traits would be useful for exploring the evolutionary potential of the stem in response to water stress.

Alternanthera philoxeroides (Martius) Grisebach (Amaranthaceae) originated in the Parana River region of South America and is now widely distributed around the world (Li and Ye, 2006). *A. philoxeroides* was introduced into Shanghai, China, in the 1930s and occurs in most regions of southern China (Ye et al., 2003). *A. philoxeroides* causes severe damage to sectors such as agriculture, forestry, fisheries and irrigation and causes serious economic losses and ecological disasters (Ye et al., 2003). This species has been listed as one of the 12 most harmful alien invasive species in China (Li and Xie, 2002). *A. philoxeroides* is an aquatic plant in its native range but is widely distributed in different habitats in its introduced range, including terrestrial, aquatic and semi-aquatic habitats (Li and Ye, 2006).

A. philoxeroides is a stoloniferous and rhizomatous perennial plant that uses asexual reproduction as an important strategy for the colonization of new habitats (Li and Ye, 2006). Pan et al. (2006a) indicated that its high degree of plasticity may be an alternative strategy that *A. philoxeroides* uses to adapt to new environments. The anatomical structure of the *A. philoxeroides* stem has both terrestrial and aquatic characteristics (Tao et al., 2009). The phenotypic variation observed in the *A. philoxeroides* stem has been shown to be shaped by the water conditions in its habitat (Tao et al., 2009). In this study, *A. philoxeroides* was employed as the experimental material, and phenotypic selection was used to analyze the

selection potential of water availability on the anatomical phenotypic traits of *A. philoxeroides*. We sought to determine 1) the variation, differentiation and plasticity of the stem anatomical structure among and within populations of *A. philoxeroides* under different water availability levels and 2) the strength of the selection potential of environmental conditions (water availability) on the phenotypic variation of invasive *A. philoxeroides*. These results will help elucidate the mechanisms underlying the rapid evolution of *A. philoxeroides* in adapting to habitats with differing water availability levels and will provide an important reference for the management of this species.

2. Materials and methods

2.1. Study area and sampling

This study was conducted in Linhai City, Zhejiang Province, China, which is located at 28°40′–29°04′ N, and 120°49′–121°41′ E. This area has a typical subtropical monsoon climate with abundant illumination and rainfall. The annual average temperature is 17.1 °C, rainfall is 1522.4 mm, relative humidity is 82%, and the amount of radiation is 1009.5 kcal/cm². Although *A. philoxeroides* is distributed widely in China, we chose only one location for this study to exclude the phenotypic plasticity caused by climate, soil and other abiotic or biotic factors.

In July 2012, 8 sampling plots were selected for this study. All eight plots were located in farmland or abandoned land with similar soil types and physical and chemical properties (Table 1), and the vegetation was dominated by *A. philoxeroides*. Among every plot, two populations of *A. philoxeroides* were collected from two different habitats: aquatic habitat (*A. philoxeroides* plants growing in ditches) terrestrial habitat (*A. philoxeroides* plants growing on dry land near ditches). The water regimes of all 16 populations were determined at a depth of 3 cm using an HH2 Handheld Moisture Meter (Version 2.0, Delta-T Devices Ltd., Cambridgeshire, UK). *A. philoxeroides* weeds showing good growth with low levels of infestation by pests and diseases and seldom consumption by herbivores were collected for further analysis. In every population, twenty individuals of *A. philoxeroides* separated by a distance at least 5 m were randomly collected and transported to the laboratory immediately in cooler bags at 4 °C. The plants were stored at 4 °C and used to measure the stem traits within 12 h. The experiment was continued in one week, i.e. from July 12 to July 18, 2013. A total of 320 individuals from 16 populations, 8 plots were analyzed in this study. No specific permits were required for collection in the localities sampled in this study, and none of the populations were privately owned or protected in any way. Additionally, we verified that the field studies did not involve endangered or protected species.

2.2. Measurements

The third fully developed stem of *A. philoxeroides* from the top of every individual was used for further analysis. The diameter of the stem (DS) was measured with a Vernier Caliper with a precision of 0.002 cm. Then, the third stem from the top was cut, and three free-hand cross-section slices of the stem were prepared using a single blade. Anatomical structural features of the *A. philoxeroides* stem, including the thickness of the cortex (TCx), the diameter of the pith cavity (DPC), the number of vascular bundles (NVB), the diameter of vascular bundles (DVB) and the density of vascular bundles (DsVB), were measured under a microscope (Leica DM/LS, Leica Microsystems Ltd., Wetzlar, Germany) using an objective micrometer with a precision of 10 μm. The cortex area-to-stem area ratio (Cx/S) was calculated as the cortical area/total area of stem cross-sections. The

Table 1
Information regarding the eight plots in this study.

No.	Sampling plots	Latitude	Longitude	Description of sites	Soil water content in different plots	
					Aquatic plot	Terrestrial plot
1	Sanjiang Nature reserve	28°53.202N	121°03.146E	Abandoned land	57.1	20.4
2	Taizhou University	28°52.739N	121°09.472E	Farmland	68.8	10.4
3	Houshan Village	28°52.825N	121°05.374E	Farmland	68.6	8.7
4	Children's Hall	28°51.791N	121°10.952E	Abandoned land	67.4	11.1
5	Datian Port	28°51.102N	121°10.027E	Farmland	67.9	10.4
6	Taizhou Middle school	28°51.805N	121°10.950E	Abandoned land	53.1	17.8
7	Daluwang Village	28°51.812N	121°10.947E	Farmland	65.9	21.8
8	Huipu Middle School	28°52.625N	121°09.194E	Farmland	67.9	9.4

pith cavity area-to-stem area ratio (PC/S) was calculated as the pith cavity area/total area of stem cross-sections. The vascular bundle area-to-stem area ratio (VB/S) was calculated as the vascular bundle area/total area of stem cross-sections (Pan et al., 2006b).

2.3. Statistical analysis

All of the data are shown as means \pm standard deviations. The phenotypic plasticity index, ranging from zero to one, was calculated for each trait as the difference between the maximum and minimum mean values among the two habitats divided by the maximum mean value (Cheplick, 1995; Valladares et al., 2000, 2006). The effects of plot and habitat on the anatomical structure parameters were tested using two-way ANOVAs. Pearson correlation analysis was performed to determine the relationships between the anatomical structure parameters and the soil water content. Where a significant correlation was found, a simple linear regression model ($y = a + bx$) was used to find the line that best predicted the structural trait from the soil water content (Feng et al., 2009; Xu et al., 2010). To determine whether variation existed within or between habitats or among plots, the phenotypic differentiation coefficient (Vst) of each anatomical structure trait was calculated as follows (Ge et al., 1988): $Vst_{habitat} = \sigma_{T,habitat}^2 / (\sigma_{T,habitat}^2 + \sigma_T^2 + \sigma_S^2) \times 100\%$, where $\sigma_{T,habitat}^2$ is the variance component between habitats in the same plot, $\sigma_{T,plot}^2$ is the variance component among plots, and σ_S^2 is the variance component among individuals within a habitat; $Vst_{plot} = (\sigma_{T,plot}^2 / \sigma_{T,habitat}^2 + \sigma_{T,plot}^2 + \sigma_S^2) \times 100\%$.

A simple linear regression model ($y = a+bx$) was used to fit a straight line to the relationship between fitness (biomass) and each of the measured parameters in aquatic and terrestrial habitats, respectively, quantitatively reflecting the relationship between fitness and the examined parameters in the aquatic and terrestrial habitats. To determine whether the slopes of the regression lines were significant in the aquatic and terrestrial habitats, the homogeneity of the slopes (parallelism) was tested via one-way ANCOVA (Li et al., 2001). Regression lines for relationship between the parameters and biomass that were significantly nonparallel indicated that the selection differentials were significant between aquatic and terrestrial habitats (Li et al., 2001), and these parameters were subjected to further phenotypic selection analysis. Multiple regression was used to estimate directional gradients (β_i) as well as quadratic selection gradients (γ_{ii} and γ_{ij}) to reveal which parameters were undergoing selection (Gómez, 2000; Maad, 2000). Selection gradients were measured as the deviations from their respective means based on the regression coefficients of first and second order in a regression of fitness on characters: $\omega = \alpha + \sum \beta_i X_i + \sum \gamma_{ii} (X_i^2) + \sum \gamma_{ij} (X_i X_j) + \epsilon$, where ω is relative fitness, α

is a constant (the intercept), β_i is the directional selection gradient for character i , γ_{ii} is the quadratic selection gradient for character i (indicating concave/convex selection), γ_{ij} is the quadratic selection gradient for characters i and j (indicating correlational selection), X_i and X_j are trait values of characters i and j , and ϵ is an error term (Maad, 2000). Relative fitness was measured based on the total biomass of *A. philoxeroides* because in the invaded area, this invasive species exclusively employs asexual reproduction (Jia et al., 2008), and at the stem nodes can produce new individuals through asexual reproduction. The significance of the regression coefficients in this model in aquatic and terrestrial habitats was assessed using a Z-test.

All analyses were done with R (Version 15.0) and figures were drawn with Origin (Version 8.0).

3. Results

3.1. Phenotypic variation in the anatomical structure of the *A. philoxeroides* stem

The stem anatomical structure varied between aquatic and terrestrial habitats (Fig. 1). With the exception of the thickness of the cortex, all of the parameters related to the anatomical structure of the *A. philoxeroides* stem differed significantly between the aquatic and terrestrial habitats among the eight sampling plots (Fig. 2). The two-way ANOVA showed that the sampling plots had no significant effect on any of the parameters, whereas water availability significantly affected 8 parameters of *A. philoxeroides*, with the thickness of the cortex being the exception. The diameter of the stem, the diameter of the pith cavity, the pith cavity area-to-stem area ratio, the number of vascular bundles and the diameter of vascular bundles of *A. philoxeroides* showed significantly higher values in aquatic habitats than in terrestrial habitats, whereas the density of vascular bundles, the cortex area-to-stem area ratio and the vascular bundle area-to-stem area ratio did not (Fig. 2).

A Pearson correlation analysis showed that all of the parameters with the exception of the thickness of the cortex were significantly correlated with soil water availability (Fig. 3). Linear regression analysis suggested that the density of vascular bundles, the vascular bundle area-to-stem area ratio and the cortex area-to-stem area ratio significantly decreased with increasing soil water availability, whereas the other parameters and ratios significantly increased with increasing soil water availability (Fig. 3).

The phenotypic differentiation coefficient (Vst) of each of the examined anatomical structural parameters indicated that most of the variation existed between habitats in the same plot, whereas little variation existed among plots or among individuals in the same habitat (Table 2). However, for the thickness of the cortex,

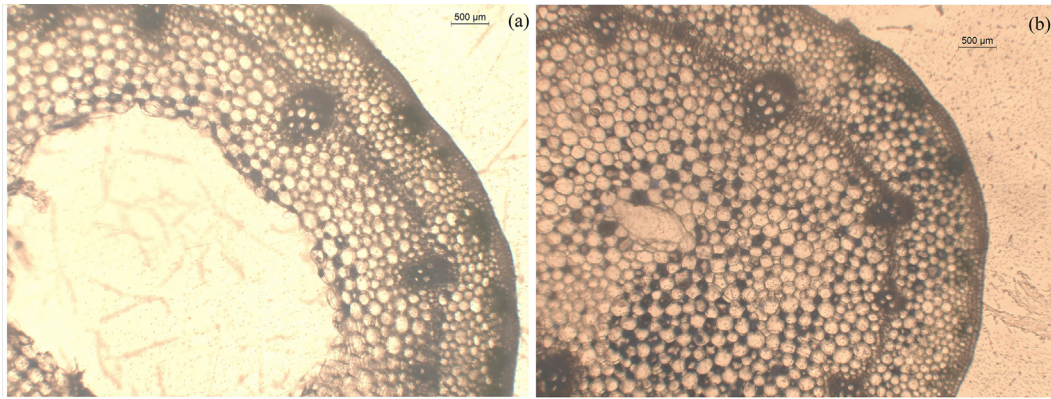


Fig. 1. Freehand cross sections of *Alternanthera philoxeroides* stems from aquatic habitat (a) and terrestrial habitat (b) in Sanjiang Nature Reserve, Linhai City, Zhejiang Province, China.

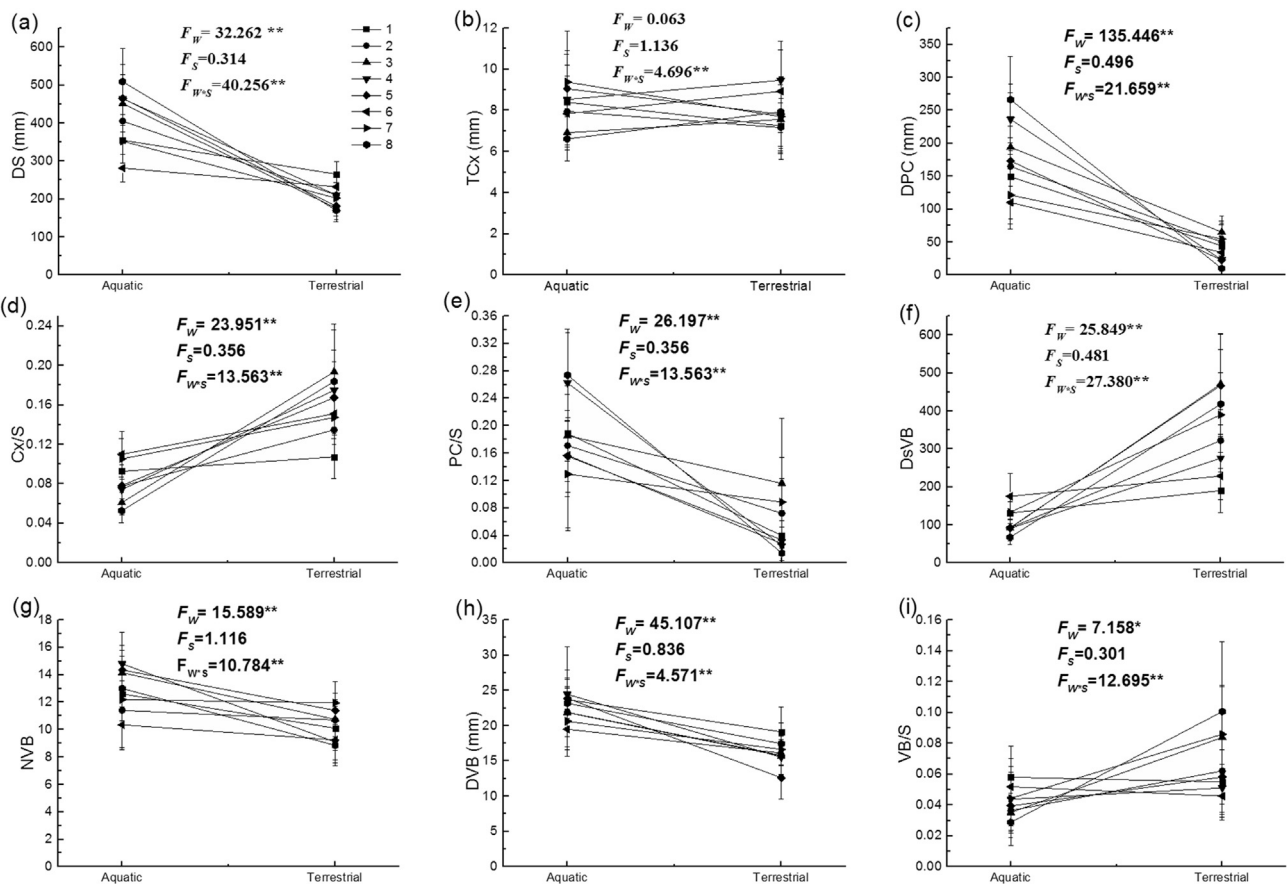


Fig. 2. Anatomical structure parameters of the *Alternanthera philoxeroides* stem in two different habitat types among eight plots. The data are presented as the mean \pm SD; the two habitats at each sampling site are connected by a line. (a) Diameter of the stem (DS); (b) thickness of the cortex (TCx); (c) diameter of the pith cavity (DPC); (d) cortex area-to-stem area ratio (Cx/S); (e) pith cavity area-to-stem area ratio (PCx/S); (f) density of vascular bundles (DsVB); (g) number of vascular bundles (NVB); (h) diameter of vascular bundles (DVB); (i) vascular bundle area-to-stem area ratio (VB/S). F represents the results of a two-way ANOVA with the habitat as a fixed factor and the plot as a random factor; F_W represents the effect of water on the anatomical structural parameters; F_S represents the effect of sampling; and $F_{W \times S}$ represents the interactive effect of water and sampling.

most of the variance was observed among plots and individuals, whereas little variance existed between habitats.

A significant phenotypic plasticity response to water availability was found for all of the evaluated traits and ratios related to the anatomical structure of the *A. philoxeroides* stem except for the thickness of the cortex (Fig. 2 and Fig. 4). Among the examined parameters, the diameter of the pith cavity (0.7690),

the pith cavity area-to-stem area ratio (0.7075), the density of vascular bundles (0.6291), the cortex area-to-stem area ratio (0.4551), the vascular bundle area-to-stem area ratio (0.4403) and the diameter of the stem (0.4774) were more plastic, and the number of vascular bundles (0.3334), the thickness of the cortex (0.1543) and the diameter of vascular bundles (0.2818) were less plastic.

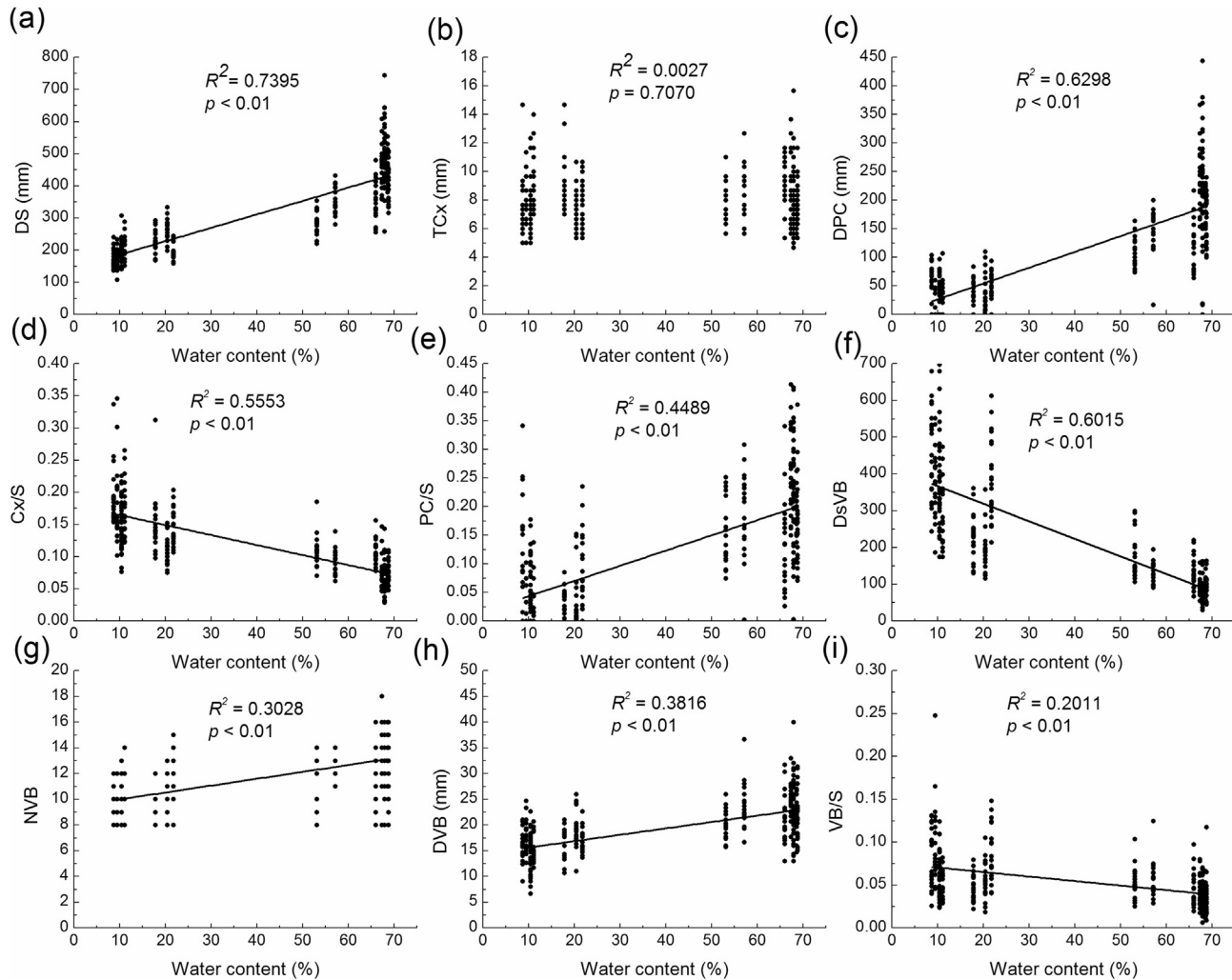


Fig. 3. Correlation between the anatomical structure parameters of the *Alternanthera philoxeroides* stem and the water content. The linear regression curve is shown in the figure if the correlation is positive ($n = 320$). (a) Diameter of the stem (DS); (b) thickness of the cortex (TCx); (c) diameter of the pith cavity (DPC); (d) cortex area-to-stem area ratio (Cx/S); (e) pith cavity area-to-stem area ratio (PC/S); (f) density of vascular bundles (DsVB); (g) number of vascular bundles (NVB); (h) diameter of vascular bundles (DVB); (i) vascular bundle area-to-stem area ratio (VB/S). R^2 represents the degree of fitting of the straight line, and p indicates the significance of the linear fit.

Table 2

The phenotypic differentiation coefficients for the anatomical structure parameters of the *Alternanthera philoxeroides* stem.

Traits	Habitats	Plots	Individuals
Diameter of the stem	0.9133	0.0246	0.0621
Thickness of the cortex	0.0045	0.4331	0.5624
Diameter of the pith cavity	0.8831	0.0308	0.0861
Cortex area-to-stem area ratio	0.8863	0.0506	0.0631
Pith cavity area-to-stem area ratio	0.8622	0.0558	0.0820
Density of vascular bundles	0.8602	0.1120	0.0278
Number of vascular bundles	0.6180	0.0723	0.3097
Diameter of vascular bundles	0.8026	0.0933	0.1041
Vascular bundle area-to-stem area ratio	0.5767	0.2535	0.1699

3.2. Selection differentials of the anatomical structure of the *A. philoxeroides* stem in response to water availability

Linear regression models were used to predict the relationships between fitness and the parameters of the anatomical structure of the *A. philoxeroides* stem in the aquatic and terrestrial habitats (Fig. 5). Although the obtained R^2 values were all very low, fitness was shown to be significantly related to the diameter of the stem

($p < 0.01$), the density of vascular bundles ($p < 0.01$), the number of vascular bundles ($p < 0.05$) and the diameter of vascular bundles ($p < 0.05$) in aquatic habitats (Fig. 5 a, f, g, h) and to the diameter of the stem ($p < 0.01$), the thickness of the cortex ($p < 0.05$), Cx/S ($p < 0.01$), PC/S ($p < 0.05$), and DsVB ($p < 0.001$) in terrestrial habitats (Fig. 5 a, b, d, e, f). The slopes of the regression lines were significantly different between the aquatic and terrestrial habitats for the parameters DS ($p < 0.001$), cortex area-to-stem area ratio ($p < 0.01$), pith cavity area-to-stem area ratio ($p < 0.05$) and density of vascular bundles ($p < 0.001$, Fig. 5 a, d, e, f), indicating that the associations between these stem anatomical structural parameters and fitness differed with differences in water availability.

Parameters showing very significant differences ($p < 0.01$), including the diameter of the stem, the cortex area-to-stem area ratio and the density of vascular bundles, were then subjected to multiple regression analysis to reveal the parameters undergoing selection due to water availability (Table 3). In both the aquatic and terrestrial habitats, no significant directional selection gradient was found for the diameter of the stem, the cortex area-to-stem area ratio or the density of vascular bundles. In the aquatic habitats, no significant correlational selection was found among the diameter of the stem, the cortex area-to-stem area ratio and the density of vascular bundles. In the terrestrial habitats, weak but significantly

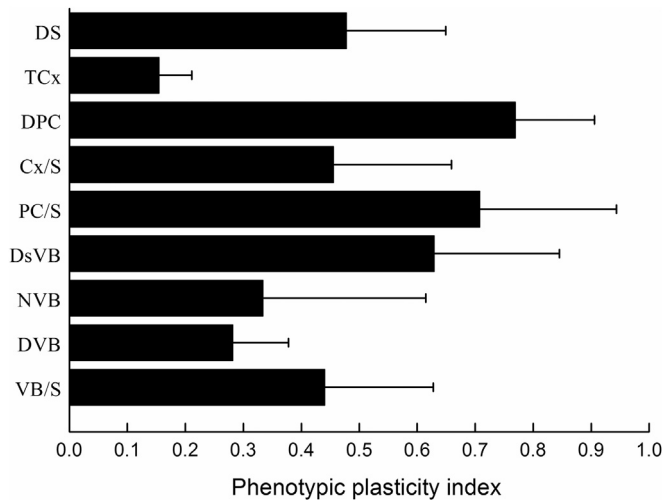


Fig. 4. Phenotypic plasticity index of the anatomical structure parameters of the *Alternanthera philoxeroides* stem. Diameter of the stem (DS), thickness of the cortex (TCx), diameter of the pith cavity (DPC), cortex area-to-stem area ratio (Cx/S), pith cavity area-to-stem area ratio (PC/S), density of vascular bundles (DsVB), number of vascular bundles (NVB), diameter of vascular bundles (DVB), and vascular bundle area-to-stem area ratio (VB/S).

positive correlational selection was observed only between the cortex area-to-stem area ratio and the density of vascular bundles. All of the results regarding directional selection and correlational selection showed significant differences between the aquatic and terrestrial habitats except for the correlational selection between the diameter of the stem and the cortex area-to-stem area ratio (Table 3).

4. Discussion

In this study, we found that all of the parameters with the exception of the thickness of the cortex were significantly correlated with soil water availability. However, no significant directional or nondirectional selection gradients were found for and among stem anatomical structure traits in both the aquatic and terrestrial habitats, except for the significantly positive correlational selection between the cortex area-to-stem area ratio and the density of vascular bundles in the terrestrial habitats. These results indicate that the stem anatomical structure of *A. philoxeroides* plays an important role in the adaptation to changing water availability but that the selection potential of water availability on the stem anatomical structure of *A. philoxeroides* is very weak. There are two reasons underlying the weak selection potential. One is the shorter invasion history of *A. philoxeroides* in these habitats in Linhai City (no more than 20 years estimated based on the city's history of expansion). The phenotype differentiation coefficient (V_{st}) showed that with the exception of the thickness of the cortex (0.4331), the variance component mainly existed between different habitats in the same plot rather than among individuals or populations, which provides strong support for our findings concerning the weak selection of water availability on the phenotypic parameters of *A. philoxeroides* individuals. The other is the low genetic variation of *A. philoxeroides*. The evolutionary response to stress depends on the existence of genetically based phenotypic variation that is associated with variation in fitness (Stanton et al., 2000). The genetic diversity of *A. philoxeroides* in China is extremely low according to RAPD and ISSR data (Wang et al., 2005; Ye et al., 2003), which might be due to the clonal propagation of this weed. Xu et al. (2003)

speculated that the *A. philoxeroides* plants in China might have originated from a small number of clones. It has been demonstrated that low genetic variation may weaken the strength of natural selection (Conner and Hartl, 2004). The extremely low genetic diversity of *A. philoxeroides* in China (Wang et al., 2005; Ye et al., 2003) might contribute to the low selection potential acting on the phenotypic plasticity of *A. philoxeroides*.

Strong phenotypic plasticity plays an important role in the colonizing of invasive plants in the novel introduced region (Parker et al., 2003; Geng et al., 2006). It has been well documented that *A. philoxeroides* has high phenotypic plasticity in response to varied environmental factors, such as the availability of water, light, and nutrients (Geng et al., 2006; Zhang et al., 2015; Xu et al., 2005; Pan et al., 2006a), which may facilitate the success of the invasion of *A. philoxeroides* (Pan et al., 2006a). In this study, we found a significant phenotypic plasticity response to water availability for the anatomical traits of the *A. philoxeroides* stem with the exception of the thickness of the cortex. Although our study did not compare the phenotypic plasticity of native congeners or co-occurring native species, several studies have compared the reaction norms of invasive species to those of the alien non-invasive or co-occurring native species and support the hypothesis that invaders are more plastic than non-invasive species (see review by Daehler, 2003). Geng et al. (2006) found that *A. philoxeroides* showed greater plasticity than did *Alternanthera sessilis* in many traits examined during the switch from wet conditions to drought treatment. Thus, our results support the previous hypothesis that the high phenotypic plasticity in response to water availability may contribute to the successful invasion of *A. philoxeroides* (Geng et al., 2006; Pan et al., 2006a).

The anatomical structure of *A. philoxeroides* stem performed differently in response to different water availability conditions (Geng et al., 2006; Tao et al., 2009; Tao and Jiang, 2004). Here, linear regression analyses revealed that the density of vascular bundles, the vascular bundle area-to-stem area ratio and the cortex area-to-stem area ratio significantly decreased with increasing soil water availability, whereas the other parameters and ratios significantly increased with increasing soil water availability. The pith cavity, which is an important tissue in water distribution and aeration in response to soil anoxia (Jackson and Armstrong, 1999), enables gases to diffuse or ventilate from aerial parts to below-ground parts and thereby helps to maintain aerobic respiration and rhizosphere oxygenation (Blom and Voeselek, 1996; Jackson and Armstrong, 1999). In the present study, we found that the diameter of the pith cavity and the pith cavity area-to-stem area ratio of *A. philoxeroides* were significantly higher in aquatic habitats than in terrestrial habitats. Similar results have been found in *Paspalum dilatatum* in response to flooding (Vasellati et al., 2001). The increasing ratio of the pith cavity associated with an increased water regime might contribute to the ability of the aquatic ecotype of *A. philoxeroides* to float on water and grow additional branches and leaves (Tao et al., 2009; Zuo et al., 2012). These characteristics are essential for the survival of plants that grow in flood-prone soils (Vasellati et al., 2001). The parenchyma cells distributed in the cortex could form aerenchyma in the stem as an adaptation to submergence to facilitate gas exchange (Steffens et al., 2011). Here, we found that the cortex area-to-stem area ratio decreased with increasing soil water availability, indicating that aerenchyma in the stem of *A. philoxeroides* might not contribute to the tolerance to flooding. Similarly, Núñez-Elisea et al. (1999) also verified that flood tolerance did not involve aerenchyma formation in the stem of *Annona* species. In addition, we also found that the number of vascular bundles and the diameter of vascular bundles were significantly smaller in terrestrial habitats than in aquatic habitats, whereas the density of vascular bundles and the vascular bundle

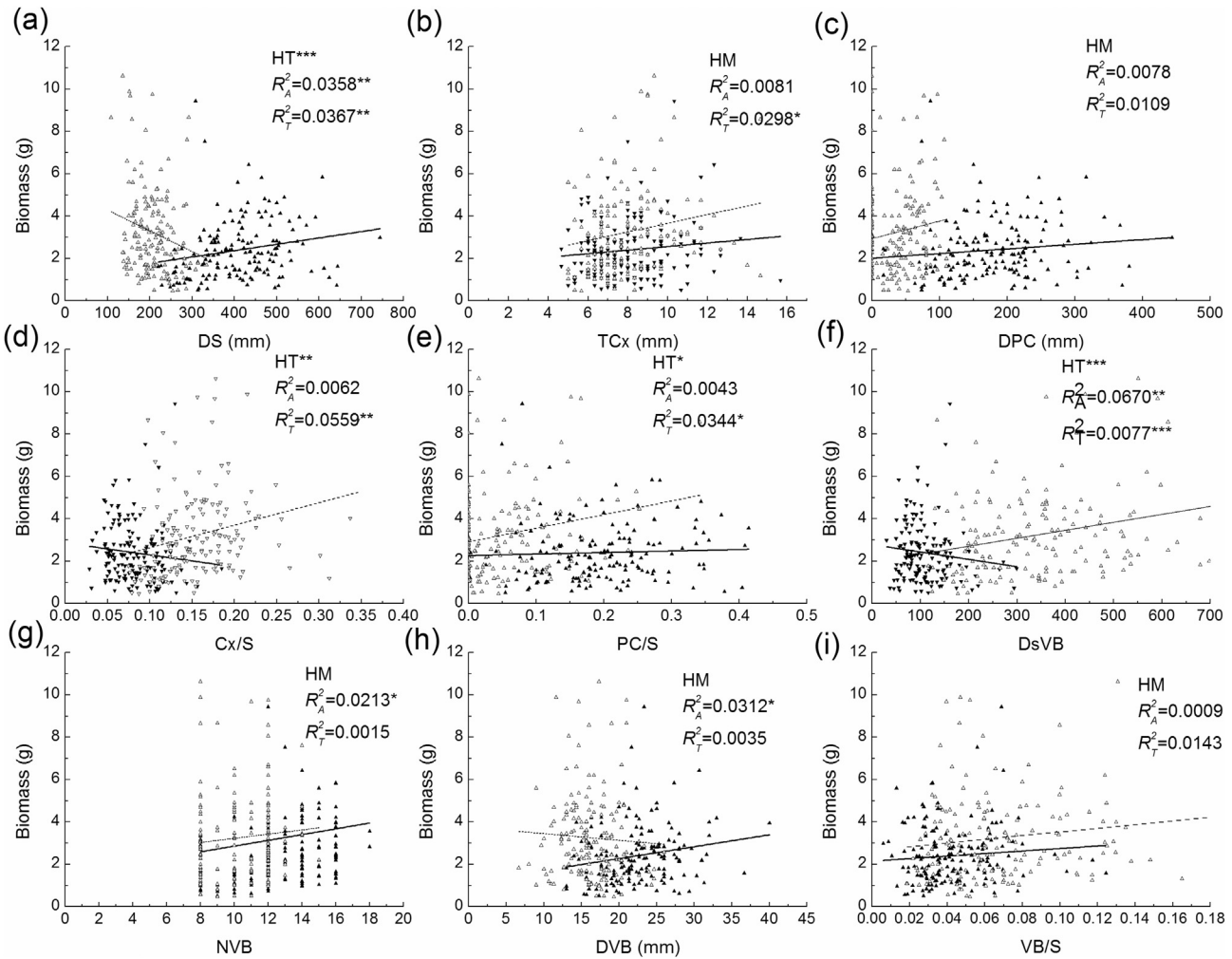


Fig. 5. Phenotypic correlations between biomass and the anatomical structure parameters of the *Alternanthera philoxeroides* stem in aquatic (solid triangle) and terrestrial (empty triangle) habitats. (a) Diameter of the stem (DS); (b) thickness of the cortex (TCx); (c) diameter of the pith cavity (DPC); (d) cortex area-to-stem area ratio (Cx/S); (e) pith cavity area-to-stem area ratio (PC/S); (f) density of vascular bundles (DsVB); (g) number of vascular bundles (NVB); (h) diameter of vascular bundles (DVB); (i) vascular bundle area-to-stem area ratio (VB/S). Regression analyses were performed separately for aquatic (solid line) and terrestrial (dashed line) habitats. The parallelism of the regression lines was examined through one-way ANCOVA and marked as HM (parallel, $p > 0.05$) and HT (unparallel, $p < 0.05$). R^2_A and R^2_T are the correlation coefficients of the regressions for dry and wet conditions, respectively. The significances of the regression are marked as ns $p > 0.05$ (non-significant), * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Table 3

Analysis of phenotypic selection through the water availability on the anatomical structure parameters of the *Alternanthera philoxeroides* stem. Directional selection (β_i), concave/convex selection (γ_{ii}) and correlational selection (γ_{ij}) gradients \pm standard errors are listed. The Z-test values are used to assess the significance of the regression coefficients in this model in aquatic and terrestrial habitats. *, significant at $p < 0.05$, **, very significant at $p < 0.01$.

	Aquatic	Terrestrial	Z-test
Intercept	$-1.0E^{-15} \pm 0.07$	$4.9E^{-15} \pm 0.075$	$1.933E^{-13}$
Linear selection gradient			
Directional selection (β_i)			
DS	-0.731 ± 3.193	1.387 ± 4.328	-9.769^{**}
Cx/S	-0.733 ± 1.564	0.247 ± 1.76	-6.799^{**}
DsVB	-0.882 ± 2.833	1.446 ± 4.132	-11.158^{**}
Nonlinear selection gradient			
Concave/convex selection (γ_{ii})			
DS \times DS	0.413 ± 2.105	-1.014 ± 2.921	8.051^{**}
Cx/S \times Cx/S	-0.346 ± 0.756	-1.265 ± 0.707	9.611^{**}
DsVB \times DsVB	-0.661 ± 1.223	-1.293 ± 1.543	4.807^{**}
Correlational selection (γ_{ij})			
DS \times Cx/S	0.544 ± 0.718	0.690 ± 0.802	-1.498
DS \times DsVB	0.844 ± 1.079	-0.102 ± 1.618	7.286^{**}
Cx/S \times DsVB	1.612 ± 1.436	$2.843 \pm 1.355^*$	-9.320^{**}
DS \times Cx/S \times DsVB	-0.513 ± 1.19	-1.626 ± 1.129	9.245^{**}

Note: DS indicates the diameter of the stem; Cx/S indicates the cortex area-to-stem area ratio; DsVB indicates the density of vascular bundles.

area-to-stem area ratio were significantly higher in terrestrial habitats than aquatic ones. These results indicate that the mechanical tissues of *A. philoxeroides* increased under decreasing water availability during the dispersal from aquatic to terrestrial habitats; i.e., terrestrial habitats favored plants with mechanically strong tissue. Similar conclusions have been reached for other plant species, such as *Annona* species and some fruit crops (Núñez-Elisea et al., 1999).

In conclusion, although the results of the present study are limited to the phenotypic traits of the anatomical structure of the *A. philoxeroides* stem, our study first found that the stem anatomical structure of *A. philoxeroides* plays an important role in the adaptation to changing water availability. In addition, our results are consistent with the viewpoint that high phenotypic plasticity has likely played an important role in the successful expansion of *A. philoxeroides* in China, particularly in its survival in both aquatic and terrestrial habitats (Tao et al., 2009; Geng et al., 2006). However, our results also revealed that the selection potential of water availability on the anatomical structure of the *A. philoxeroides* stem is very weak. A further study should focus on the selection potential of water availability on other physiological and morphological traits of *A. philoxeroides*.

Contributors

DL and YB conducted the experiment, WG and JL designed the experiment, DL, WG and JL wrote the manuscript.

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