

Research Article

The effects of changing sedimentation disturbance on the invasiveness of *Alternanthera philoxeroides* are trait dependent

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Abstract

Tolerance and resistance to environmental stresses determine the invasion success of alien plants under disturbed conditions. Colonization time and environmental heterogeneity have been thought to contribute to the invasiveness of alien plants. However, few previous studies have synthesized disturbance, environmental heterogeneity and colonization time in the exploration of the invasion mechanisms of invasive clonal plants. In the present study, the performance of the invasive clonal wetland plant *Alternanthera philoxeroides* was compared under undisturbed (UD), homogeneous burial (HoD) and temporal heterogeneous burial (HeD) conditions at the early and late phases of colonization. Using functional traits including biomass allocation and plant physiology as indicators of invasive performance, we found that in the early phase *A. philoxeroides* showed phenotypic maintenance in stolon mass ratio (SMR), root mass ratio (RMR), root-shoot ratio and non-structural carbohydrate content of stolon (NSSC_{stolon}) after either HoD or HeD; leaf mass ratio (LMR), specific leaf area (SLA), the maximal fluorescence (F_v/F_m), nitrogen content per leaf mass (N_{mass}) and leaf carbon/nitrogen (C/N) ratio after HoD; chlorophyll content (Ch_t) and leaf construction cost per leaf area (CC_{area}) after HeD. We found that CC_{area} varied HoD while LMR, SLA, F_v/F_m , N_{mass} and C/N ratio varied after HeD in the early phase. In the late phase, *A. philoxeroides* showed phenotypic maintenance in LMR, SMR, RMR, root-shoot ratio and F_v/F_m after either HoD or HeD; Ch_t and NSSC_{stolon} after HoD. Moreover, we found that SLA, N_{mass} , C/N ratio and CC_{area} varied after either HoD or HeD while Ch_t and NSSC_{stolon} after HeD in the late phase. Both tolerance (phenotypic maintenance) and resistance (phenotypic variation) to the burial disturbance are trait dependent and facilitate the invasion of *A. philoxeroides*. In addition, the heterogeneity of the disturbance exerted facilitative or insignificant impacts on its invasive performance. These mechanisms may explain the broad invasion of *A. philoxeroides* into diverse habitats.

Key words: disturbance heterogeneity, colonization time, functional traits, tolerance, resistance, invasion mechanism, invasive clonal wetland plant

Introduction

The invasion success of alien plants is commonly associated with disturbance (Jauni et al. 2015; Uyà et al. 2018). Disturbance influences the invasibility

of introduced habitats by altering resource availability and/or stress status (Davis et al. 2000; Li and Shipley 2018). Hence, the effects of disturbance on alien plant invasion can be complex (Lockwood et al. 2007; Moles et al. 2012; Jauni et al. 2015). On the one hand, disturbance may improve the resource availability of recipient habitats by creating space (e.g., trampling and fire), adding nutrients (e.g., eutrophication) and limiting the resource assimilation of native neighbours (e.g., grazing and herbivory on native plants) (Davis et al. 2000; Shea and Chesson 2002; Moles et al. 2012; Jauni et al. 2015; Mallon et al. 2015), which likely improve the hospitability of invasion sites and thus promote invasion by alien plants (Davis et al. 2000; Jauni et al. 2015). On the other hand, disturbances such as submergence and burial likely create environmental stress (Moles et al. 2012; Chen et al. 2013; Jauni et al. 2015), which may exert negative impacts on alien plant invasions (Alpert et al. 2000). Tolerance and resistance, which are two principal strategies by which plants cope with environmental stress and maximize their fitness, likely determine the invasion success of alien plants under such disturbed conditions (Chen et al. 2013; Hammann et al. 2016; Winkler et al. 2016; Wang et al. 2018). For instance, Zhang et al. (2011) reported that the tolerance of two congeneric invasive thistles contributes to their survival after multiple mowing events. Dong et al. (2011) found that alligator weed *Alternanthera philoxeroides* (Mart.) Griseb. survived burial by a resistant response, including changing its biomass allocation and stolon length. From a functional trait perspective, *A. philoxeroides* showed both tolerance and resistance to submergence through the respective approaches of phenotypic maintenance and phenotypic variation, which enabled this invasive species to establish superiority over its competitors (Wang et al. 2018).

Functional traits are associated with plant invasiveness (Pyšek and Richardson 2007; Violle et al. 2007; Drenovsky et al. 2012; Adler et al. 2014). Biomass allocation and physiological traits are two common functional trait representatives of plant life strategies (Wright and Westoby 2001). For instance, biomass allocations to leaves, stems and roots determine the plant functional assignment to photosynthesis (carbon gain), support and nutrient assimilation, respectively, to a certain extent (Givnish 1995; Wang et al. 2017a, b). Leaf physiological traits such as specific leaf area, nutrient concentration, carbon:nutrient ratio and construction cost reflect the resource use efficiency of plants and correlate with the plant growth rate (Wright et al. 2004; Leishman et al. 2007; Wang et al. 2017a, b). Non-structural carbohydrates indicate the capacity of plants to counteract environmental stress (Bailey-Serres and Voesenek 2008; Dietze et al. 2014; Wang et al. 2017a, 2018). Variations in trait values can demonstrate alterations in plant life strategies in response to disturbance (Pyšek and Richardson 2007; Violle et al. 2007; Drenovsky et al. 2012; Adler et al. 2014). Phenotypic maintenance and variation separately contribute to the

tolerance and resistance strategies of plants and help them cope with disturbance (Brown 1997; Perumal and Maun 2006; Li and Xie 2009; Dong et al. 2011; Pan et al. 2012; Chen et al. 2013; Fan et al. 2015; Hammann et al. 2016; Winkler et al. 2016; Wang et al. 2018). Thus, tolerance and resistance are likely advantageous for the successful invasion of alien plants in stressful habitats (Wang et al. 2018).

Environmental heterogeneity and colonization time are two important factors influencing the effects of disturbance on the performance of alien plant invaders (Pyšek et al. 2009; Parepa et al. 2013). Several previous studies have suggested that environmental heterogeneity facilitates the invasion success of alien species (Davis et al. 2000; Melbourne et al. 2007). Environmental heterogeneity may induce the fluctuation and uneven distribution of resources and create opportunities for the invasion of alien plant invaders (Davis et al. 2000; Melbourne et al. 2007), which often perform better under heterogeneous disturbances. In particular, environmental heterogeneity favours clonal plants (Price and Marshall 1999; Wang et al. 2009; You et al. 2016; Wang et al. 2017c). Phenotypic plasticity helps clonal plant invaders exploit fertile patches, enhance their resource use efficiency and promote their invasive performance in heterogeneous environments (Price and Marshall 1999; Wang et al. 2009; You et al. 2016; Wang et al. 2017c). Colonization time is closely correlated with the developmental status and invasion phase of alien plant invaders (Theoharides and Dukes 2007). Generally, the invasiveness of alien species is enhanced as a consequence of increasing colonization time (Pyšek et al. 2009). In addition, invasive clonal plants usually construct extensive clonal stands in a short colonization time (Francis 2012). Thus, if disturbances induce environmental stress to invasive clonal plants, large stands in the late invasion phase may show better phenotypic performance in tolerating and/or resisting the stress than small stands in the early invasion phase. Nevertheless, the previous researchers did not integrate environmental heterogeneity and colonization time together in their explorations of the role of disturbance in alien plant invasions.

Sedimentation is a common environmental disturbance confronted by wetland plants (Maun and Perumal 1999; Li and Xie 2009; Dong et al. 2011; Pan et al. 2012; Ceccherelli et al. 2018; Pattison et al. 2018). Sedimentation usually brings about three facets of perturbation to plants: a reduction in light penetrating the soil, increased pressure from soil and an increase in nutrient availability (Li and Xie 2009). To adapt to burial, plant species generally alter their biomass allocation (Brown 1997), stem elongation (Li and Xie 2009), photosynthetic capacity, water use efficiency and chlorophyll content in emergent leaves (Perumal and Maun 2006). However, previous studies merely focused on the effects of the intensity and/or frequency of sedimentation on plant performance. Few studies have examined the effects of heterogeneous sedimentation on plant performance in

invasive clonal species at different colonization times. It is noteworthy that prior experiments on clonal plant ecology focused more on the spatial heterogeneity of sediments, for instance, different combinations of nutrient patches with identical total nutrient levels (Wang et al. 2017c). However, few studies have examined temporal heterogeneity, that is changing disturbances (e.g., sedimentation and inundation) at different time intervals with identical disturbance intensities. In the present study, the globally invasive clonal plant species *A. philoxeroides* was used to explore the effects of heterogeneous disturbance, i.e., the temporal heterogeneity of sedimentation, on plant performance for two different colonization times. Considering colonization time, two different developmental stages were simulated during the plant growing period. The following hypotheses were postulated:

1. *A. philoxeroides* shows tolerance and resistance to sedimentation in the measurement of functional trait variations;
2. *A. philoxeroides* tolerates and resists sedimentation disturbance better in the late phase of colonization than in the early phase;
3. *A. philoxeroides* tolerates and resists sedimentation disturbance better under heterogeneous disturbance conditions than under homogeneous disturbance conditions.

Materials and methods

Plant material

Alternanthera philoxeroides is a cosmopolitan noxious invasive amphibious weed that originated from South America (Francis 2012). With over 80 years of invasion history in China, this species has thrived in terrestrial, semi-aquatic and aquatic habitats (Francis 2012). Asexual propagation (e.g., ramet production) dominates its reproductive system (Francis 2012). Previous studies have reported that this species has low genetic diversity in China, strong clonal growth and high phenotypic plasticity (Wang et al. 2005; Geng et al. 2007; Wang et al. 2009).

Experimental design

One hundred ramets of *A. philoxeroides* were collected from the littoral zone of Nansi Lake Wetland Park, Shandong Province (34°27'–35°20'N; 116°34'–117°21'E) on April 26th, 2017. Then, these ramets were immediately delivered to Fanggan Experimental Station of Shandong University (36°26'N; 117°27'E). Sixty eight-cm-long leafless stolons with one node each were selected to be separately cultivated in the centre of 60 pots (30 cm in height, 20 cm in diameter) with 20 cm-thick sandy loam in a greenhouse. Tap water was added to the pots every two days to maintain a 1 cm depth of water above the surface of the sandy loam. After 50 days of growth, forty-two ramets with similar morphology (approximately 12 cm in length, 6–8 leaves)

were cut from the cultured plants. Then, the fresh weights of all 42 selected ramets were determined. Six selected ramets were oven dried at 70 °C for 72 h and then used to calculate the water content based on the fresh weight and dry mass. The initial biomass of the remaining 36 ramets was calculated according to the fresh weight and the water content. Then, the remaining 36 ramets (initial biomass, mean \pm SE, 0.2418 \pm 0.0030 g) were planted into the centres of 36 mesocosms (round tubs, 60 cm in height, 56 cm in diameter) with 15 cm-thick sediments of sandy loam on June 13th, 2017, and the experiment started.

Different disturbance regimes were applied at different phases of the colonization time in our experiment. Two colonization time treatments were set: 1) early phase–burial disturbance was applied after 15 days of growth and 2) late phase–burial disturbance was applied after 45 days of growth. Hence, two sets of plants at different growth stages were selected to set up the experiment. Three disturbance treatments were provided as follows: 1) undisturbed condition (UD) – control group, in which no disturbance was implemented; 2) homogeneous disturbance (HoD), in which 8 cm of sandy loam was placed in the mesocosms to entirely bury the plants; 3) heterogeneous disturbance (HeD), in which 4 cm of sandy loam placed in the mesocosms twice to entirely bury the plants, and the time interval between the two burial treatments was 15 days. The burial treatments were designed based on Dong et al. (2011) and Li and Xie (2009). The sandy loam used for the substrate was identical to that used for the burial treatments. Each treatment was replicated 6 times. The detailed process of burial treatment was as follows: on June 28th, 2017, eighteen replicates were randomly selected after 15 days of growth, *i.e.* the early colonization phase. Six replicates were set as the control undisturbed group, 6 replicates were treated with homogeneous disturbance, and the remaining 6 replicates were treated with heterogeneous disturbance. These 18 replicates were harvested on July 28th, 2017. On July 28th, 2017, the UD, HoD and HeD treatments were implemented for the remaining 18 replicates after 45 days of growth, *i.e.* the late colonization phase. On August 28th, 2017, the remaining 18 replicates of plant materials were harvested.

Harvest and measurement

One leaf on the third leaf node at the apical end of the longest stolon was selected for the measurement of maximum fluorescence (F_v/F_m) *in situ* using a LI-6800 portable photosynthesis system (LICOR. Inc., Lincoln, Nebraska, USA) after at least 20 minutes of dark adaptation of the leaf chamber, which is sufficient for the photosystem II (PS II) reaction centres to open (Schreiber et al. 1998). Then, the leaves used for the measurement of maximum fluorescence were harvested and scanned using an Epson Perfection V800 photo scanner (Seiko Epson Corp., Japan) to generate 1:1 high-definition images (tiff format, 400 dpi). ImageJ 1.46 (National Institute

of Health, Bethesda, Maryland, USA) was used to analyse the leaf area per single leaf. The leaves used for leaf area measurement were subsequently oven-dried at 70 °C for 72 h. Then, the dry mass per single leaf was determined. The specific leaf area (SLA) was calculated as follows:

$$\text{SLA} = \text{leaf area per single leaf} / \text{dry mass per single leaf}$$

Four leaves on the first two leaf nodes at the apical end of the longest stolon were harvested to test chlorophyll content. The chlorophyll content of fresh leaves was measured using the ethanol method. Approximately 0.1 g of sheared fresh leaf tissue was ground in 10 ml of 95% ethanol with calcium carbonate and quartz sand until all the leaf tissue dissolved in the solution. After dark treatment for 24 h, the absorbances of the supernatant were determined at 649 nm (A_{649}) and 665 nm (A_{665}) using a UV-2100 spectrophotometer (UNICO, USA). Chlorophyll *a* content (Ch_a), chlorophyll *b* content (Ch_b) and total chlorophyll content (Ch_t) were calculated separately as Lichtenthaler (1987):

$$Ch_a = (13.95 \times A_{665} - 6.88 \times A_{649}) \times 0.01 / \text{fresh weight of leaf tissue}$$

$$Ch_b = (24.96 \times A_{649} - 7.32 \times A_{665}) \times 0.01 / \text{fresh weight of leaf tissue}$$

$$Ch_t = Ch_a + Ch_b$$

The remaining plant materials were harvested and then separated into leaves, stolons and roots. All plant parts were oven-dried at 70 °C for 72 h. The biomass of each part was determined. Biomass allocation parameters, including leaf mass ratio (LMR), stolon mass ratio (SMR), root mass ratio (RMR) to total biomass, and root-shoot ratio, were calculated as follows:

$$\text{LMR} = \text{leaf mass} / \text{total biomass}$$

$$\text{SMR} = \text{stolon mass} / \text{total biomass}$$

$$\text{RMR} = \text{root mass} / \text{total biomass}$$

$$\text{Root-shoot ratio} = \text{root mass} / (\text{leaf mass} + \text{stolon mass})$$

One of the oven-dried leaves for specific leaf area (SLA) determination was used for elemental analysis. The carbon content per leaf mass (C_{mass}) and nitrogen content per leaf mass (N_{mass}) for each treatment and replicate were measured using a EuroEA3000 CHNS-O analyser (EuroVector, Italy). The leaf carbon/nitrogen (C/N) ratio, leaf construction cost per leaf mass (CC_{mass}), and leaf construction cost per leaf area (CC_{area}) were calculated as follows:

$$\text{C/N ratio} = C_{\text{mass}} / N_{\text{mass}}$$

$$CC_{\text{mass}} = (5.39 \times C_{\text{mass}} - 1191) / 1000 \text{ (McDowell 2002)}$$

$$CC_{\text{area}} = CC_{\text{mass}} / \text{SLA}$$

Leaf construction cost is the amount of glucose needed to construct carbon skeletons, reductants and ATP for synthesizing the organic compounds in tissue via standard biochemical pathways. The leaf construction cost reflects the fundamental energy invested in leaf carbon

gain and is relevant to processes driven by light interception or limited by light diffusion to the plant surface (Williams et al. 1987).

Six replicates of each treatment were used to measure the non-soluble sugar content in stolons (NSSC_{stolon}). The non-soluble sugar content indicates the energy storage in plants, and its variation correlates with plant growth, such as shoot elongation (Bailey-Serres and Voesenek 2008). Oven-dried stolons of each replicate were ground into powder and stored in separate 2 ml centrifuge tubes at -20°C . Approximately 20 mg of powder was used for analysis. The perchloric acid/anthrone method was used to determine NSSC_{stolon} (Chen et al. 2013).

Statistical analysis

LMR was square root(x) transformed. The C/N ratio and NSSC_{stolon} were log(x) transformed so that all data met the assumptions of variance normality and homogeneity prior to analysis. One-way ANOVA was performed to determine the effects of the disturbance regime on plant functional traits at an early and at a late colonization phase. If a significant treatment effect was detected, post hoc pairwise comparisons of means were made to examine the differences between treatments using Tukey's HSD for multiple comparisons. All data analyses were conducted using SPSS 22.0 (SPSS, Chicago, IL, USA).

Results

Early phase

No significant differences in biomass allocation, including LMR, SMR, RMR and root-shoot ratio, were found between HoD and UD (Figure 1a–d). Significantly lower values of Ch_t and CC_{area} were observed in HoD than in UD (Figure 2b, f), while no significant differences in SLA, F_v/F_m , N_{mass} , C/N ratio or NSSC_{stolon} were observed between HoD and UD (Figure 2a, c, d, e, g).

No significant differences in biomass allocation, including SMR, RMR and root-shoot ratio, were found between HeD and UD (Figure 1b–d). Significantly higher values of LMR, SLA, F_v/F_m and N_{mass} were observed in HeD than in UD (Figures 1a, 2a, c, d). No significant differences in Ch_t , CC_{area} or NSSC_{stolon} were observed between HeD and UD (Figure 2b, f, g). A significantly lower C/N ratio was found in HeD than in UD (Figure 2e).

No significant differences in LMR, SMR, RMR, root-shoot ratio, SLA, Ch_t , F_v/F_m , CC_{area} and NSSC_{stolon}, were found between HoD and HeD (Figures 1a–d, 2a, b, c, f, g). A significantly higher N_{mass} value was found in HeD than in HoD (Figure 2d), while a significantly lower C/N ratio was found in HeD than in HoD (Figure 2e).

Late phase

No significant differences in biomass allocation, including LMR, SMR, RMR and root-shoot ratio, were observed between HoD and UD (Figure 1a–d).

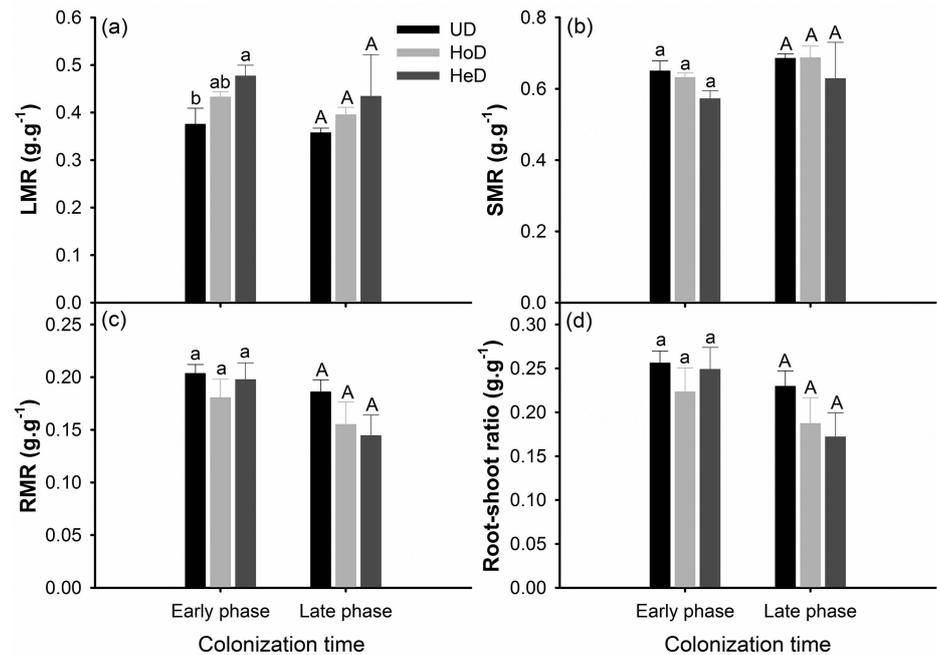


Figure 1. Variation of biomass allocation: (a) leaf mass ratio (LMR), (b) stolon mass ratio (SMR), (c) root mass ratio (RMR) and (d) root-shoot ratio under different disturbance regimes in the early and late phases of colonization. Values are mean \pm SE. Means with the different letters are significantly different at $P < 0.05$ in different treatments.

Significantly higher values of SLA and N_{mass} were found in HoD than in UD (Figure 2a, d). No significant differences in Ch_t , F_v/F_m or $NSSC_{\text{stolon}}$ were observed between HoD and UD (Figure 2b, c, g). Significantly lower values of the C/N ratio and CC_{area} were observed in HoD than in UD (Figure 2e, f).

No significant differences in biomass allocation, including LMR, SMR, RMR and root-shoot ratio, were observed between HeD and UD (Figure 1a–d). Significantly higher values of SLA, Ch_t and N_{mass} were observed in HeD than in UD (Figure 2a, b, d). No significant difference in F_v/F_m was found between HeD and UD (Figure 2c). Significantly lower values of the C/N ratio, CC_{area} and $NSSC_{\text{stolon}}$ were observed in HeD than in UD (Figure 2e, f, g).

No significant differences in LMR, SMR, RMR, root-shoot ratio and F_v/F_m , were observed between HoD and HeD (Figures 1a–d, 2c). Significantly higher values of SLA, Ch_t , N_{mass} and CC_{area} were found in HeD than in HoD (Figure 2a, b, d, f), while significantly lower values of C/N ratio and $NSSC_{\text{stolon}}$ were observed in HeD than in HoD (Figure 2e, g).

Discussion

Tolerance and resistance to disturbance favoured the invasion of A. philoxeroides

Sedimentation disturbance induces environmental stress to the growth of riparian aquatic macrophytes (Maun and Perumal 1999; Li and Xie 2009; Dong et al. 2011; Pan et al. 2012; Ceccherelli et al. 2018; Pattison et al. 2018). In consideration of phenotypic responses, separate strategies of tolerance

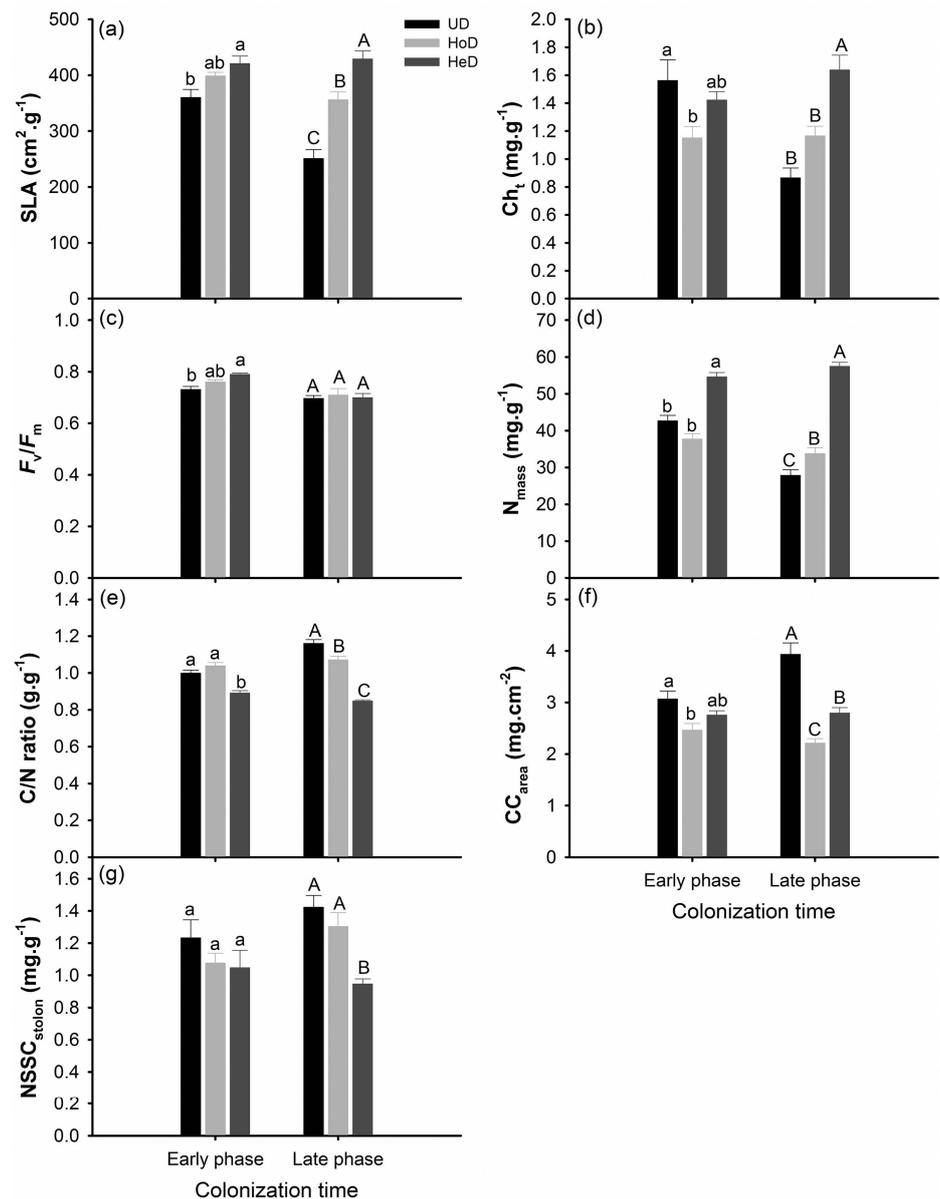


Figure 2. Variation of physiological traits: (a) specific leaf area (SLA), (b) total chlorophyll content (Ch_t), (c) maximum PS II efficiency (F_v/F_m), (d) nitrogen content per leaf mass (N_{mass}), (e) leaf C/N ratio, (f) leaf construction cost per leaf area (CC_{area}) and (g) the non-soluble sugar content of stolon (NSSC_{stolon}) under different disturbance regimes in the early and late phases of colonization. Values are mean ± SE. Means with the different letters are significantly different at $P < 0.05$ in different treatments.

and resistance maximize plant performance and thus optimize plant fitness under stressful conditions (Li and Xie 2009; Chen et al. 2013; Fan et al. 2015; Hammann et al. 2016; Winkler et al. 2016; Wang et al. 2018). Thus, tolerance and resistance to disturbance can be used to evaluate the invasiveness of alien invaders.

In our experiment, the phenotypic maintenance of biomass allocation traits, including SMR, RMR and root-shoot ratio, was observed after sedimentation disturbance. Phenotypic maintenance in growth is probably advantageous for fitness maintenance when facing disturbance (Zhang et al. 2011; Wang et al. 2018). Therefore, tolerance helped *A. philoxeroides*

cope with sedimentation disturbance to a large extent (Zhang et al. 2011). However, *A. philoxeroides* allocated more biomass to leaves after HeD in the early phase. This result indicates that *A. philoxeroides* grew more leaves to escape the insufficiency of light and CO₂ resources resulting from burial and searched for light and CO₂ when facing sedimentation (Brown 1997; Pan et al. 2012). Deng et al. (2008) found that another invasive clonal wetland plant, *Spartina alterniflora* Loisel., invested more resources in aboveground parts after burial and showed morphological resistance to burial, which is partly concordant with our finding in the early phase of colonization. Thus, *A. philoxeroides* also showed a degree of resistance to sedimentation disturbance after repeated burial (HeD) in the early phase, which may have helped maximize its fitness.

In terms of physiological traits, overall no significant variation in the values of considered traits was found, mainly in HoD. In one aspect, phenotypic maintenance of several traits under certain conditions helps *A. philoxeroides* tolerate the sedimentation disturbance to a certain degree (Zhang et al. 2011; Wang et al. 2018). In another aspect, phenotypic variation can be treated as a resistance strategy to the sedimentation disturbance. Resource use traits are correlated with the photosynthetic capacity and carbon gain strategy of plants (Leishman et al. 2007). Significant differences were found for several traits in HeD. Previous studies have suggested that increases in SLA, Ch_t, F_v/F_m and N_{mass} and decreases in the C/N ratio and CC_{area} contribute to the enhancement of photosynthetic efficiency, which also positively influences plant growth (Wang et al. 2017a, b, 2018). In our study, a significant decrease in NSSC_{stolon} was shown after HeD. The consumption of non-structural carbohydrates has been considered to correlate with the elongation of stolons in clonal plants (Wang et al. 2017a, 2018). Stolon elongation may prevent clonal plants from suffering the light and CO₂ shortages caused by burial (Li and Xie 2009; Dong et al. 2011; Fan et al. 2015). Therefore, variation in these physiological traits, as an indicator of compensatory growth, likely facilitated the regeneration and elongation of *A. philoxeroides* and thus helped the plant to “escape” burial. In addition, a decrease in the value of Ch_t was shown after HoD in the early phase. This result indicated that severe sedimentation at one time (burial in 8 cm of sandy loam in the present experiment) might reduce light availability and thus restrict the synthesis of photosynthetic pigments. However, the overall plant performance was maintained and enhanced, which contributed to the invasion success of *A. philoxeroides* under disturbance conditions (Wang et al. 2018).

Colonization time made a difference in the response of A. philoxeroides to disturbance

Invasiveness of alien species increases as the colonization time continues (Pyšek et al. 2009). Thus, invasive alien plants are predicted to perform

better at tolerating and/or resisting disturbance in the late phase than in the early phase.

In the present study, an increased biomass allocation to leaves was present after HeD in the early phase, while the leaf mass fraction was maintained after HeD in the late phase. This compensatory growth to leaves enabled *A. philoxeroides* to escape the unfavourable environments, such as the shortage of light and CO₂, caused by burial, while the maintenance of LMR in the late stage prevented the photosynthetic system from the interference of burial (Brown 1997; Pan et al. 2012). The clonal stand was more mature in the late phase than in the early phase before the exertion of the sedimentation disturbance, since the colonization time for the plant was longer in the late phase than in the early phase. This implies that a more mature clonal stand tends to maintain a steady state of biomass allocation when facing disturbance, while a less developmentally mature clonal stand tends to vary, in this phenotype. Considering physiological traits, including SLA, Ch_t, F_v/F_m , N_{mass}, C/N ratio, CC_{area} and NSSC_{stolon}, *A. philoxeroides* performed more variance in phenotypic response after the exertion of sedimentation disturbance. Although the HeD treatment enhanced the performance of F_v/F_m in the early phase and the maintenance of F_v/F_m existed after HeD in the late phase, our results generally indicate that photosynthesis in a less developed clonal stand tends to be insignificantly or negatively affected by the disturbance compared to that in a more mature clonal stand (Erskine-Ogden et al. 2016; Wang et al. 2017a, b). Burial with 8 cm of sandy loam at one time created stresses, such as physical pressure from the sediments and light attenuation, that the photosynthetic system of a less developed clonal stand might not be able to form a positive response. The maintenance of NSSC_{stolon} existed after HeD in the early phase while a decrease of NSSC_{stolon} was shown after HeD in the late phase, which implies an occurrence of fast stolon elongation and clonal expansion after HeD in the late phase (Wang et al. 2017a, 2018). Thus, colonization time—the early and late phases—made a difference in the trait response of *A. philoxeroides* to disturbance and an advantage appeared to be provided in the late phase.

The effects of temporal disturbance heterogeneity on the invasiveness of A. philoxeroides were trait- and context- specific

Previous studies have suggested that environmental heterogeneity likely promotes the invasion of alien species (Davis et al. 2000; Melbourne et al. 2007). Thus, heterogeneous disturbance, such as temporally heterogeneous sedimentation, may promote the invasive performance of alien plants more than homogeneous disturbance.

Based on these results, the HeD treatment led to significant increases in LMR, SLA, F_v/F_m and N_{mass} and a significant decrease in the C/N ratio compared to those in UD in the early phase, while phenotypic maintenance

was observed in these traits under the HoD treatment in the early phase. LMR reflects the construction of photosynthetic system as a “leafier” system may indicate a higher photosynthetic efficiency, while the maximal fluorescence (F_v/F_m) reveals the photosynthetic capacity (Wang et al. 2018). SLA has been considered to be positively correlated with the relative growth rate of plants (Leishman et al. 2007). Thus, higher SLA likely contributes to the faster colonization and establishment of alien invasive plants (Leishman et al. 2007; Pyšek and Richardson 2007). The “leaf economics spectrum” proposes that SLA is likely positively correlated with the leaf nitrogen concentration and negatively correlated with the leaf C/N ratio (Wright et al. 2004; Leishman et al. 2007). Thus, higher N_{mass} and lower C/N ratio may induce faster plant growth, which likely improves the invasion potential of alien invaders. The increase in chlorophyll content in the late phase of HeD likely indicates an increase in photosynthetic capacity, which is representative of invasiveness (Erskine-Ogden et al. 2016; Wang et al. 2017a, b). Additionally, the HeD treatment induced a decrease in the $\text{NSSC}_{\text{stolon}}$ value compared to that in UD in the late phase, while maintenance of the $\text{NSSC}_{\text{stolon}}$ value was shown after HoD in the early phase. The rapid depletion of non-structural carbon may account for the fast stolon growth of clonal plants, which likely helps them escape stressful environments and promotes their expansion into introduced habitats (Wang et al. 2017a, 2018). Therefore, temporal heterogeneous disturbance may promote the invasiveness of *A. philoxeroides* more than homogeneous disturbance since better photosynthesis indicated by LMR, Ch_t and F_v/F_m and faster growth indicated by SLA, N_{mass} , C/N ratio and $\text{NSSC}_{\text{stolon}}$ were realized under heterogeneous conditions (Davis et al. 2000; Melbourne et al. 2007). In addition, a higher N_{mass} and a lower C/N ratio were shown in HeD than in HoD in the early phase, while in the late phase, higher values in SLA, Ch_t and N_{mass} , and lower values in C/N ratio and $\text{NSSC}_{\text{stolon}}$ were shown in HeD than in HoD. Since SLA and N_{mass} are positively correlated with the plant growth (Wright et al. 2004; Leishman et al. 2007; Pyšek and Richardson 2007), Ch_t is positively correlated with the photosynthetic capacity (Erskine-Ogden et al. 2016; Wang et al. 2017a, b), and C/N ratio and $\text{NSSC}_{\text{stolon}}$ are negatively correlated with the plant growth (Wright et al. 2004; Leishman et al. 2007; Wang et al. 2017a, 2018), the effects of disturbance heterogeneity on invasiveness were facilitative from this perspective (Davis et al. 2000; Melbourne et al. 2007). However, compared to UD, the HeD treatment led to similar variations as the HoD treatment for certain traits and colonization times, including LMR, SLA, F_v/F_m , N_{mass} , C/N ratio and CC_{area} in the late phase, SMR, RMR and root-shoot ratio in both phases and $\text{NSSC}_{\text{stolon}}$ in the early phase. This implied that clonality (e.g., the division of labour) may promote the homogenization of environmental heterogeneity (Price and Marshall 1999; Wang et al. 2009; You et al. 2016; Wang et al. 2017c). In this view, the effects of disturbance heterogeneity on invasiveness were insignificant.

Hence, the effects of environmental heterogeneity on the invasion of alien species are trait- and context- specific to a great extent.

Conclusions

Tolerance and resistance to burial disturbance promoted the invasion of *A. philoxeroides*. However, colonization time made a difference in the invasion success of *A. philoxeroides*, as an advantage seemed to be provided in the late phase. The effects of environmental heterogeneity on the invasive performance of *A. philoxeroides* were trait- and context- specific, while facilitative and insignificant effects were observed. The above mechanisms may explain the invasion of *A. philoxeroides* into vast areas of disturbed habitats. Future studies on the effects of disturbance on alien invasion should concern the neighbouring non-invasive competitors of invasive species (i.e., comparative studies of invasive alien, non-invasive alien and native species under disturbed conditions), since the effects of disturbance on non-invasive competitors may indirectly alter the invasion status of the invasive species.

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