Short-time response in root morphology of alien invasive plant *Amaranthus retroflexus* to water level gradient

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**ABSTRACT**

Investigating the response of alien invasive plants to water level gradient in wetlands is important for developing strategies to prevent invasions by alien plants in these ecosystems. Controlled experiments were conducted to investigate the response of root morphology in the alien invasive plant *Amaranthus retroflexus* to water level gradient. The plants were planted in three water levels from low to high that resulted in drought, wet and flooded conditions. The results showed that biomass and allocation of *A. retroflexus* under flooded conditions were significantly lower than the other two treatments (\(p < .05\)). Root morphological parameters (root mean diameter, root length, root surface area and root volume) were maximum under wet condition, followed by dry and flooded conditions, and the differences were significant among the treatments (\(p < .05\)). Special root length and special root surface area were also significantly greater under wet condition than under dry condition (\(p < .05\)). The results indicate that water level gradient in wetlands affects root biomass and morphology of *A. retroflexus*, which has the highest growth potential under wet condition. The roots of the plant also exhibited plasticity to water level gradient. Therefore, maintaining a high water level in wetlands could restrict the growth of *A. retroflexus* and prevent its successful invasion. Moreover, a high water level is beneficial to the growth of wetland plants and further decreases the invasibility of wetland ecosystems.

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*Amaranthus retroflexus*; water level gradient; biomass allocation; root morphology; wetland

**Introduction**

Biological invasion is usually defined as the transportation of a few organisms to new ranges where their descendants proliferate, spread and persist (Elton 1958; Wang et al. 2012). It is a global environmental problem today, which has garnered much attention. The invasion mechanism of alien species is the key focus among researchers of biological invasion, and community invasibility is one of the important aspects of these studies. Community invasibility is a measure of whether the community can be easily invaded or...
not. It can be used to evaluate the complexity of the community or region invaded by alien invasive species (Lonsdale 1999; Mack et al. 2000). Experience indicates that it is easier to determine the invasibility of different communities than the invasiveness of alien species. Community characteristics are more important than alien species to determine the invasion occurrence, because the community involves many mechanisms such as the interaction between biotic and abiotic factors (Zheng and Ma 2010). Studies on alien plant invasion dominate biological invasion studies. With the aggravation of global climate change and rapid economic development, the frequency and degree of damage from alien plant invasions increases quickly (Ding et al. 2008; Weber and Li 2008) and it further severely threatens biological diversity, the economy and the environment (Xu et al. 2006).

China suffers from biological invasion severely. In the four lists of alien invasive species in China that have been published, alien invasive plants, such as redroot pigweed (Amaranthus retroflexus), ragweed (Ambrosia artemisifolia), crofton weed (Eupatorium adenophora), water hyacinth (Eichhornia crassipes), Canadian goldenrod (Solidago canadensis), are predominant. Among the alien invasive plants, A. retroflexus is an important species in the family of Amaranthaceae. It is an erect, annual broadleaf herb with taproot system with plant height ranging from 20 cm to 80 cm (maximum of 100 cm) in China. Investigation shows that A. retroflexus is adaptable to all kinds of environments and can invade and establish in different communities (Gao et al. 2011), such as farmland, orchards, parks, roadsides, intertidal zone and wasteland and has negative impacts on them.

As one of the three most common ecosystems in the world, wetland ecosystems are also invaded by A. retroflexus. The plant has resulted in moderate damage to Dongting Lake wetland in Hunan province, China (Hou et al. 2011). Studies on A. retroflexus have mainly focused on farmland ecosystems (Saberali et al. 2012; Gholamhoseini et al. 2013; Amini et al. 2014) and there is little information about the plant in wetland ecosystems, especially in the context of the invasibility of wetland ecosystems. As an important environmental factor in wetlands, water level gradient affects the growth, species composition, distribution and community structure and succession of wetland plants. Response of plants to water level gradient is an important research field within wetland ecology (Keddy and Ellis 1985; Wang et al. 2001). The composition and structure of wetland communities are determined by the growth of wetland plants, which relates to the stability of wetland ecosystem and influences its invasibility. Although A. retroflexus is a xerophyte, its distribution in wetlands indicates that it has a certain adaptability to wetland environments. Up to now, there is little information about the response of A. retroflexus growth to water level gradients in wetlands, resulting in the lack of systematic understanding of the A. retroflexus invasive mechanism in wetland ecosystems and of strategies for inhibiting invasions. Water level gradient may influence the growth of A. retroflexus and affect the invasibility of the wetland ecosystem to the plant. Therefore, controlled experiments were conducted to investigate the response in root morphology of A. retroflexus to water level gradient. The results can reveal the response in root growth to an environmental gradient in the wetland and further provide useful information for understanding the ecosystem’s invasibility and the invasive mechanism of A. retroflexus. Results will also be useful in the prevention of plant invasions in wetlands, providing scientific references for wetland ecosystem management and conservation.

Materials and methods

Materials and experimental design

Seeds of A. retroflexus were collected on the last autumn in farmland near Hebei University of Environmental Engineering (39°52'05"N, 119°26'50"E), Qinhuangdao, Hebei
province, China. In early June, 60 full seeds were selected and planted uniformly in a plant slot 40 cm by 15 cm in length and width, respectively. After germination and growth, 30 plants with the same growth potential (plant height was about 10 cm) were selected and planted in plant pots. The pots were 15 cm in diameter and 20 cm in height with drain holes in the bottom. Each pot contained one experimental plant and 18 cm sediment in depth. The sediment was collected from the campus of the university and sieved. It was clay with pH 8.13 ± 0.04, the content of organic matter, total nitrogen and total phosphorus were 6.93 ± 0.32 mg-g⁻¹, 3.08 ± 0.17 mg-g⁻¹ and 0.38 ± 0.08 mg-g⁻¹, respectively.

After seedling recovery for 5 days, the experimental plants grew normally with the same growth potential. Then, the 30 plants were divided into three groups in three plastic tanks. The tanks contained tap water that had set for 5 days at different depths for three treatments: no water, 8 cm water in depth and 3 cm water above soil surface. The three treatments low, medium and high wetland water level, represented drought condition (DC), wet condition (WC) and flooded condition (FC), respectively. There were 10 experimental plants in each treatment. Water in WC and FC was replenished to its specified level immediately to keep the stability of soil water condition. Due to the weaker adaptability of A. retroflexus to flooding and the comparability among the treatments, the experiment was ended at 30 days.

**Plant harvest and analysis**

At the end of the experiment, plants were harvested and analyzed. After they were removed from the planting pots, sediment adhering to roots was carefully rinsed off in water. Plants in each treatment were divided into two groups for biomass determination and root morphology measurement. There were five plants in each group. Plant biomass (shoot and root biomass) was determined after oven drying at 65°C for 72 h. Root to shoot ratio was the ratio of root to shoot biomass. A root scanner was used to scan the treated roots, and images were saved for root morphology analysis by WinRHIZO. The root morphological parameters included root mean diameter, root length, root surface area and root volume. After analyzing, roots were dried and weighed to calculate special root length and special root surface area. The two terms were the ratio of root length and root surface area to root biomass, respectively.

**Statistical analysis**

Data analysis was performed with SPSS 16.0. One-way analysis of Variance (ANOVA) with water level gradient as the factor was used to test the significance of biomass and root morphology after variances testing for normality. Multicomparison was performed to determine the significant difference between individual means at the level of $\alpha = 0.05$. Origin 8.5 was used to create figures.

**Results**

**Biomass allocation**

Both total biomass and root biomass of A. retroflexus varied with the increasing water level (Figure 1). The maximum total biomass and root biomass occurred under wet condition. Under drought and flooded condition, total biomass decreased by 4.53% and
28.96%, respectively, while root biomass decreased by 2.16% and 37.73%, respectively. Results of the multicomparison test showed that differences among individual means was the same between total biomass and root biomass. There was no significant difference between wet and drought conditions, whereas the wet condition biomasses were significantly higher than those under flooded condition ($p < .05$). However, root to shoot ratio of *A. retroflexus* decreased with increasing water levels. Results of multicomparison tests showed the same with total biomass and root biomass: root to shoot ratio under flooded condition was significantly lower than that under wet and drought conditions ($p < .05$) between which no significant differences existed.

**Root morphology**

Root morphological parameters (root mean diameter, root length, root surface area and root volume) of *A. retroflexus* varied with increasing water levels (Figure 2). Here, also maximum values were observed under wet condition. Under drought condition, root mean diameter, root length, root surface area and root volume decreased by 14.64%, 15.76%, 18.42% and 18.34%, while decreasing 44.51%, 42.54%, 40.88% and 49.27% under flooded condition, respectively. There were significant differences of root morphological parameters among treatments ($p < .05$). Root mean diameter, root length, root surface area and root volume had the same differences among treatments that they were significantly different from each other ($p < .05$).

Special root length and special root surface area of *A. retroflexus* also exhibited maximum values under wet condition (Figure 3). Different from other root morphological parameters, the two parameters showed the minimum values under drought condition, (decreased by 13.62% and 16.58%, respectively), while decreasing only 7.50% and 4.89% under flooded condition, respectively. There were also significant differences among the treatments ($p < .05$). Special root length under wet condition was significantly different from that under drought condition ($p < .05$), while no significant differences were observed between flooded condition and the other two treatments. For special root surface area, there were no significant differences between wet and flooded condition, while they were significantly higher than that under drought condition ($p < .05$).
Correlation between root biomass and root morphology

The coefficient and significance of root biomass and root morphology are listed in Table 1. There were significant linear positive correlations among the root morphological parameters (root biomass, root mean diameter, root length, root surface area and root volume) \( r > 0.857, p < .01 \). However, there was no significant correlation between special root length, special root surface area and other root morphological parameters. The two parameters also showed no significant correlation between themselves.
Discussion

Water level gradient is one of the important components of the water regime in wetlands. Wetland plants have evolved special adaptive characteristics for dealing with environmental gradients. The alien invasive plant *A. retroflexus* is well-adapted to xeric environment due to its special xeromorphic structures (Liu et al. 2013). The results of this experiment showed that simulating water level gradient in wetland could affect the biomass allocation and root morphology of the plant.

Compared to wet condition in moderate water level, drought and flooded condition resulting from low and high water levels inhibit biomass accumulation and root morphology development of *A. retroflexus*, especially under flooded condition. Soil water condition has been proved as one of the important environmental factors affecting plant growth in terms of photosynthesis and photosynthetic physiological process, and water stress can significantly decrease photosynthetic efficiency and inhibit plant growth consequently (Wang et al. 2017). Although *A. retroflexus* is an alien invasive xerophyte, its growth is inhibited when suffers from xeric stress to some extent. However, when suffering from flooding stress, root is under anaerobic condition resulting in different extent inhibition to normal physiological metabolism, and then has negative effects on shoot growth. Among the parameters of plant growth potential, biomass is a main measurable indicator, while biomass allocation reflects the general characteristics of resource or capacity allocation in plant growth (Bostock and Benton 1979). The increase of root to shoot ratio (biomass allocation is inclined to root) enhances the absorption and utilization of root to nutrient and water. Meanwhile, it can also strengthen the adaptation of plant to adverse environment (Song et al. 2017). In this study, biomass of *A. retroflexus* decreased under drought and flooded condition (especially the latter) comparing to wet condition, indicating that plant growth was inhibited under the two conditions. However, *A. retroflexus* had relatively higher root to shoot ratio under drought condition, which further indicated that the plant had strong adaptation to xeric environment in certain growth stage. Root to shoot ratio under flooded condition was significantly lower than that in other two treatments, indicating that not only is the biomass accumulation of *A. retroflexus* inhibited by flooding, but also the biomass allocation to root.

Root growth concerns with the absorption of nutrient and water, the synthesis of hormone, organic acid and amino acid and plant anchoring, which is the important constituent and the functional organs for adapting to different environmental conditions (Yang et al. 2004). Root morphology is one of the important mechanism to determine root function. The effective absorption of nutrient and water from soil by plant, biomass formation and exertion of ecological function are all determined by root morphology largely (Zhu et al. 2002; Hong et al. 2013). Each plant has its special root morphology, and the differences among root morphology reflect the differences of plant adapting to different

|        | RBM | RD   | RL   | RSA  | RV   | SRL  | SRS  |
|--------|-----|------|------|------|------|------|------|
| RBM    | 1   |      |      |      |      |      |      |
| RD     | 0.887 ** | 1   |      |      |      |      |      |
| RL     | 0.901 ** | 0.950 ** | 1 |      |      |      |      |
| RSA    | 0.879 ** | 0.893 ** | 0.914 ** | 1 |      |      |      |
| RV     | 0.857 ** | 0.892 ** | 0.879 ** | 0.917 ** | 1 |      |      |
| SRL    | -0.006 ns | 0.344 ns | 0.422 ns | 0.237 ns | 0.225 ns | 1 |      |
| SRS    | -0.131 ns | 0.092 ns | 0.093 ns | 0.351 ns | 0.220 ns | 0.399 ns | 1 |

RBM: root biomass; RD: root mean diameter; RL: root length; RS: root surface area; RV: root volume; SRL: special root length; SRS: special root surface area.

*ns p > .05; **p < .01.*
environments (Vartapetian and Jackson 1997). Plasticity of plant root morphology is considered as one of the adaptive mechanisms for resource acquisition.

Root morphological parameters reflect different root functions. Root mean diameter is the indicator for root architectural feature affecting the morphology and function of fine root. Comparing to thick and short, plant with fine and long root has higher nutrition acquisition efficiency generally (Rosolem et al. 1999; Xie and Yu 2003). Root length reflects the elongation growth of root in soil, which affects nutrient and water absorption. It is an important feature of root growth and development (Garcia and Gonzalez 1997). Higher root length is beneficial for plants to acquire nutrient and water. Root surface area reflects the contact area between root and soil. Higher root surface area increases the contact area, which is more favorable for plant to acquire nutrient and water. Special root length and special root surface area relate to root physiological vitality, such as physiological metabolism, respiration and utilization of water and nutrient. The former mainly represents the relationship between root yield and input, while the latter represents surface area per biomass, reflecting the resource acquisition capacity (Mokany and Ash 2008; Yoshimura et al. 2008). Studies on wheat (Triticum aestivum L., cv. Ayahikari) root shows that differences of special root length and special root surface area result from the allocation of root assimilative matter that is favorable for root elongation growth or root mean diameter variation (Saidi et al. 2010). Plants with higher special root length and special root surface area acquire nutrient easily in relative infertile environment. It is also a representation of plant to enhance root growth and development with lower cost in infertile environment (Eissenstat 1992). The two parameters are also indicated to be affected by nutrient (such as nitrogen and phosphorus) effectiveness in environment (Cedergreen and Madsen 2002; Xie and Yu 2003).

In this study, root morphological parameters of A. retroflexus under wet condition are relatively higher than other treatments, indicating that root of the plant under wet condition has stronger capacity for acquisition and utilization of nutrient and water from soil. The fact is favorable for plant growth steadily. Root mean diameter, root length, root surface area and root volume of the plant under drought condition are significantly lower than that under wet condition, indicating that continuous low water potential has stress influence on root growth of the plant. Correlational researches also indicate that low water potential can inhibit the increase of root mean diameter (Tsakaldimi et al. 2009) and the growth of plant root resulting in lower root length (Zhao et al. 2014). There are two different viewpoints about the root growth and morphology variation under drought condition: one considers that plant can increase allocation to root and enlarge root to shoot ratio after low water potential stress (van der Weele et al. 2000), while another considers that the stress can inhibit root growth and water utilization of aboveground part (Eissenstat 1991). This differentiation may be related to plant biological features and experimental condition (Li et al. 2010). Studies also indicate that low water potential stress can inhibit root growth of old world bluestem (Bothriochloa ischaemum), and water potential in soil is an important factor affecting its root growth (Li et al. 2016). In this study, the results also show that root morphological parameters of A. retroflexus under drought condition are significantly higher than that under flooded condition, which further indicates that the plant has relative strong adaptability to low water potential environment. Flooding stress inhibits root growth of the plant to some extent, and shows negative influence on total biomass accumulation.

However, differed from aforementioned four root morphological parameters, special root length and special root surface area of A. retroflexus under flooded condition are higher than that under drought condition. This may be important representation and
mechanism for the plant adapting to flooded condition, which needs further investigation due to the fact that it is vital for *A. retroflexus* to invade into wetland ecosystem. Furthermore, the plant show significant positive correlation among root biomass, root mean diameter, root length, root surface area and root volume (Table 1). The coordinated variation indicates that root of *A. retroflexus* has strong plasticity to environment. It is related to its ecological amplitude which results from environmental heterogeneity (Sultan 2001; Wu et al. 2008). The strong plasticity of *A. retroflexus* may be one of the important mechanism for it to invade into all kinds of ecosystems successfully.

**Conclusions**

The alien invasive plant *A. retroflexus* is sensitive to flooded condition resulting from high water level. Root growth and development of the plant are inhibited under such condition. However, the plant shows the best growth potential under wet condition caused by moderate water level. Meanwhile, it also shows adaptability to drought condition in low water level. Therefore, growth of *A. retroflexus* can be inhibited by increasing water level to flooded condition. According to water level requirement for wetland ecosystem, increased water level is favorable for rapid construction of plant community in wetland, which can increase stability and resistivity of wetland ecosystem and decrease its invasibility. Meanwhile, the invasiveness of *A. retroflexus* can also be decreased by growth inhibition.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Data availability statement**

The data that support the findings of this study are openly availability in 4TU. ResearchData at [https://doi.org/10.4121/17126780.v1](https://doi.org/10.4121/17126780.v1).

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