

# MORPHOLOGY OF THE INVASIVE AMPHIPHYTE ALTERNANTHERA PHILOXEROIDES UNDER DIFFERENT WATER LEVELS AND NITROGEN CONCENTRATIONS

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Water level fluctuation and inorganic nitrogen enrichment are two serious problems caused by anthropogenic disturbances in aquatic ecosystems. They cause resource fluctuation and thus might influence the invasiveness of alien plants. *Alternanthera philoxeroides* is an amphibious and widespread clonal plant which exhibits significant invasiveness. This experimental study examined the plant's morphological traits under different nitrogen concentrations and water levels. The responses of *A. philoxeroides* to water levels and nitrogen concentrations were similar for both land-like and riverbank-like initial conditions. *A. philoxeroides* showed an escape strategy of shoot elongation when its growth was suppressed by shallow submergence. No toxic symptoms but increased clonal spread was observed at high nitrogen concentrations, suggesting that *A. philoxeroides* not only tolerated but benefitted from nitrogen-polluted water. High nitrogen level mitigated the negative effects of submergence on its leaf survival, thereby enhancing its adaptation to water level fluctuation. Such strong adaptability and clonal spread helps *A. philoxeroides* to grow and invade successfully in shallow eutrophic water.

**Key words:** *Alternanthera philoxeroides*, eutrophication, nitrogen enrichment, plant invasion, submergence, water level.

## INTRODUCTION

Biological invasions have become one of the most serious worldwide threats to global biodiversity and ecosystem integrity as the number of introduced organisms has dramatically increased (Lonsdale, 1999; Funk and Vitousek, 2007). The mechanisms of successful invasion have been investigated in numerous studies, and many hypotheses and theories have been proposed (Lonsdale, 1999; Davis et al., 2000; Blumenthal, 2005; Lockwood et al., 2005). One generally accepted idea is that a plant's invasiveness is enhanced by an increase in the amount of resources (Davis et al., 2000). According to this view of fluctuating resource availability, a disturbance can facilitate the invasion of an alien species into a community by increasing the resources available to the invasive species or by reducing the resource use of the native species

(Hobbs and Huenneke, 1992; Davis et al., 2000; Piola and Johnston, 2008).

In riparian and aquatic ecosystems, water level fluctuation and eutrophication are frequently encountered disturbances. Plants are often submerged due to regular or irregular water level fluctuations, resulting in an oxygen shortage (Voeselek et al., 2003, 2006). Water level fluctuation in river ecosystems can make space and resources available to new species and can inhibit plant growth by submergence (Voeselek et al., 2006; Richardson et al., 2007). Due to the dramatic increase in human activities, eutrophication has become an important problem of water pollution in recent decades (Carpenter et al., 1998). Enrichment of the inorganic nitrogen in water, mainly from agricultural runoff and industrial wastewater effluent, has been found to promote the growth and invasiveness of non-native species (Camargo and Alonso, 2006; Kennedy et al., 2009;

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Wersal and Madsen, 2011). Most often, nitrogen enrichment co-occurs with water level fluctuation, so that aquatic plants encounter the two disturbances together. A variety of co-occurring environmental changes often interactively affect plant performance (Dukes et al., 2011). For instance, high nutrient supply in soil can mitigate the negative effects of high water level on plant growth (Xie et al., 2009).

Whether nitrogen enrichment in the water column will modify the effects of water level fluctuation on plants is unclear. Previous studies have usually focused on the effects of one of the two disturbances on invasive species (Kennedy et al., 2009; Luo et al., 2009, 2011; Wersal and Madsen, 2011). The combined effects of water level variation and nitrogen enrichment on the traits and invasiveness of alien plants require further study.

*Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae) is an invasive perennial weed. It is native to South America and has now invaded many countries including the USA, Australia, New Zealand, India and China (Julien et al., 1995). As a clonal plant, *A. philoxeroides* propagates vegetatively via stolons and roots, and grows into complicated systems which cover a large area and disturb native ecosystems (Sainty et al., 1998; Pan et al., 2007). *A. philoxeroides* is an amphibious weed and can grow vigorously in a wide range of habitats, from dry terrestrial to aquatic (Sainty et al., 1998; Pan et al., 2006). In aquatic systems it often roots on the edge of water bodies and spreads from a terrestrial to an aquatic habitat (Sainty et al., 1998). Many studies have examined the growth, performance and clonal integration of *A. philoxeroides* under various conditions, including resource level (Geng et al., 2006; Liu et al., 2008; Tao et al., 2009; Xu et al., 2010), disturbance (Schooler et al., 2007; Dong et al., 2010, 2012), water depth (Wang et al., 2009) and submergence (Wang et al., 2008; Luo et al., 2011). Morphological and physiological acclimation to frequent fluctuation of water level is a crucial element of the successful establishment of this species in zones of water level fluctuation, such as riparian areas (Luo et al., 2009, 2011). The growth and invasion of *A. philoxeroides* have been reported to be facilitated by high soil nutrients but its responses to nitrogen enrichment in the water column have not been well studied (Geng et al., 2006). In view of the increased pace of environmental change, we need more knowledge of the combined effects of factors such as nitrogen enrichment and frequent water level fluctuation on the invasiveness of alien plants.

In an experiment, we examined the short-term effects of water level and nitrogen concentration on morphological traits of *A. philoxeroides*. Since it is an amphibious plant, we simulated two initial habi-

tats (land and riverbank) to determine whether plants originating from different habitats would differ in their responses to the same changes in water level and nitrogen concentration. The plants were exposed to two increased water levels and two nitrogen concentrations in water. Several morphological traits were measured and statistically analyzed. We wanted to know whether (1) increased water level and nitrogen would affect the growth of *A. philoxeroides* in the short term; (2) addition of nitrogen would modify the effects of water level on *A. philoxeroides*; and (3) would the responses of *A. philoxeroides* to increased water level and nitrogen concentration vary between plants from different initial habitats. The study is novel in investigating the dynamics of an invasive plant under combined environmental changes, with a view toward understanding the performance of *A. philoxeroides* and the mechanism of its invasiveness in eutrophic waters under changing conditions.

## MATERIALS AND METHODS

### PLANT MATERIALS

Plants of *A. philoxeroides* were collected from land near the edge of Nansi Lake in Shandong Province, China in late July 2012, and were propagated vegetatively in a laboratory of North China Electric Power University in Beijing. After about three weeks of cultivation, plants of similar size (~15 cm long) were collected and planted in plastic pots (11 cm diameter, 9 cm high, one plant per pot) filled with washed river sand mixed with 1 g Osmocote (N-P-K 14-14-14, Scotts Company, USA) per pot.

### EXPERIMENTAL DESIGN

The plants were randomly divided into two groups to be acclimated to two simulated habitats, land and riverbank (close to water). For the land habitat group the pots were well drained and watered with tap water containing 1.15 mg l<sup>-1</sup> total N and 0.01 mg l<sup>-1</sup> total P. For the riverbank group the pots were placed in tanks (62 × 40 cm) filled with tap water reaching the surface of the pot substrate. After the plants were acclimatized for ~10 days to their respective initial habitat they were randomly exposed to the combined water level/nitrogen treatments.

For the simulated land habitat group, plants were randomly subjected to one of six combined treatments: three water levels (well-drained; at substrate surface; 25 cm above substrate surface – plants completely submerged) combined with two nitrogen concentrations: 0 mg l<sup>-1</sup> and 10 mg l<sup>-1</sup> NH<sub>4</sub><sup>+</sup>-N from (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, the latter simulating nitrogen pollution much worse than Grade V of the

Chinese national standards for surface water quality (GB 3838–2002). For the simulated riverbank habitat group, plants were randomly assigned to one of four combined treatments: two water levels (at substrate surface, 25 cm above substrate surface) and two nitrogen concentrations (as above). The tanks were randomly assigned to one of the combined treatments, with six replicates per treatment. Temperature during the treatments was 24–30/15–20°C day/night and the maximum photosynthetic photon flux density (PPFD) at leaf level was  $\sim 1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  on a sunny day. Tap water with the respective nitrogen concentration was replenished every week to maintain the water level of each treatment. The plants in the well-drained treatments were watered every two days with the corresponding concentrations of nitrogen solution to keep the substrate moist.

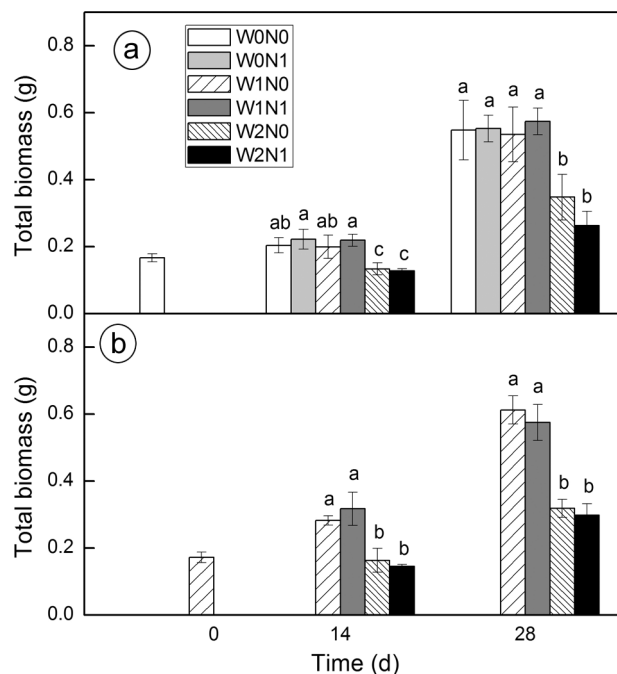
The experiment lasted 38 days from August 21 to September 28, 2012. The combined treatments lasted four weeks (September 1–28).

#### MEASUREMENTS

As the plants were of similar size before the treatments, another six plants were harvested from each initial habitat to determine total biomass. Three plants from each treatment were harvested at the midpoint of the experiment. During the experiment, shoot length was measured once a week. The number of leaves per plant was recorded every two or three days, including the numbers of new leaves and dead leaves. At the end of the experiment they were harvested and divided into leaves, stems and roots. The numbers of internodes and axillary buds and the length of each internode were measured. Then each part of the plants was dried at 80°C for 48 h and weighed. Total biomass was calculated as the sum of leaves, stems and roots. All of the measurements were repeated six times at the end of the experiments, and three times at the midpoint of the experiment. One plant in the treatment of submergence without nitrogen died during the experiment and was excluded from analysis at the end of the experiment.

#### DATA ANALYSIS

Two-way ANOVA was performed to test the effects of water level and nitrogen concentration on traits of *A. philoxeroides*, separately for the two initial habitats. Multiple comparisons employed Duncan's tests. Root biomass was log<sub>10</sub>-transformed before analysis to improve the normality and homogeneity of variance. The figures show only untransformed data. All analyses used SPSS ver. 13.0 (SPSS Inc., Chicago, IL) at  $P=0.05$ . The figures were drawn using OriginPro ver. 8.0 (OriginLab Co., Northampton, MA).



**Fig. 1.** Total biomass of *Alternanthera philoxeroides* plants from the two initial habitats before, at the midpoint, and at the end of the experiment. (a) Land-like habitat, (b) Riverbank-like habitat. All plants from each initial habitat were under the same respective conditions before treatment. W0N0 and W0N1 – plants growing in well-drained (control) substrate without (W0N0) and with (W0N1)  $10 \text{ mg l}^{-1} \text{ NH}_4^+\text{-N}$  in the water; W1N0 and W1N1 – water level at 0 cm above substrate surface without (W1N0) and with (W1N1)  $10 \text{ mg l}^{-1} \text{ NH}_4^+\text{-N}$  in the water; W2N0 and W2N1 – water level at 25 cm above substrate surface without (W2N0) and with (W2N1)  $10 \text{ mg l}^{-1} \text{ NH}_4^+\text{-N}$  in the water. Values are means  $\pm$  SE. Bars bearing different letters differ significantly between treatments at a given time point ( $P < 0.05$ ).

## RESULTS

#### BIOMASS ACCUMULATION

Total biomass of *A. philoxeroides* increased during the experiment. Biomass accumulation increased much more during the second half of the experiment (Fig. 1). Submergence significantly reduced the accumulation of biomass as compared with plants having shoots above the water surface (Fig. 1). This effect appeared at the midpoint and was much more pronounced after one month (Tabs. 1, 2, Fig. 1). Nitrogen concentration did not significantly affect total biomass and there was no significant interaction between water level and nitrogen concentration (Tabs. 1, 2). The response of total biomass to the treatments was similar in plants from the land-like and riverbank-like initial habitats. Leaf, stem and

TABLE 1. Two-way ANOVA for the effects of water level and nitrogen concentration on total biomass of *Alternanthera philoxeroides* at the midpoint of the experiment, and biomass accumulation and morphology at the end of the experiment in plants sourced from the land-like habitat

Parameter	Water level (W)			Nitrogen (N)			W × N		
	SS	df	P	SS	df	P	SS	df	P
At midpoint of experiment									
Total biomass (g)	0.026	2	0.007	0.001	1	0.588	0.001	2	0.837
At end of the experiment									
Total biomass (g)	0.459	2	0.001	0.002	1	0.793	0.023	2	0.617
Leaf biomass (g)	0.057	2	< 0.001	0.001	1	0.419	0.003	2	0.472
Stem biomass (g)	0.140	2	0.002	0.001	1	0.740	0.024	2	0.292
Root biomass (g)	1.927	2	0.001	0.039	1	0.531	0.376	2	0.161
Shoot length (cm)	128.73	2	0.004	0.843	1	0.771	61.317	2	0.059
Number of internodes	3.008	2	0.020	0.053	1	0.694	1.269	2	0.168
Mean length of internodes (cm)	1.720	2	0.099	0.019	1	0.815	0.268	2	0.680
Leaf number	137.531	2	< 0.001	21.004	1	0.064	50.684	2	0.020
Number of axillary buds	13.669	2	0.016	5.888	1	0.051	8.575	2	0.064

TABLE 2. Two-way ANOVA for the effects of water level and nitrogen concentration on total biomass of *Alternanthera philoxeroides* at the midpoint of the experiment, and biomass accumulation and morphology at the end of the experiment, in plants sourced from the riverbank-like habitat

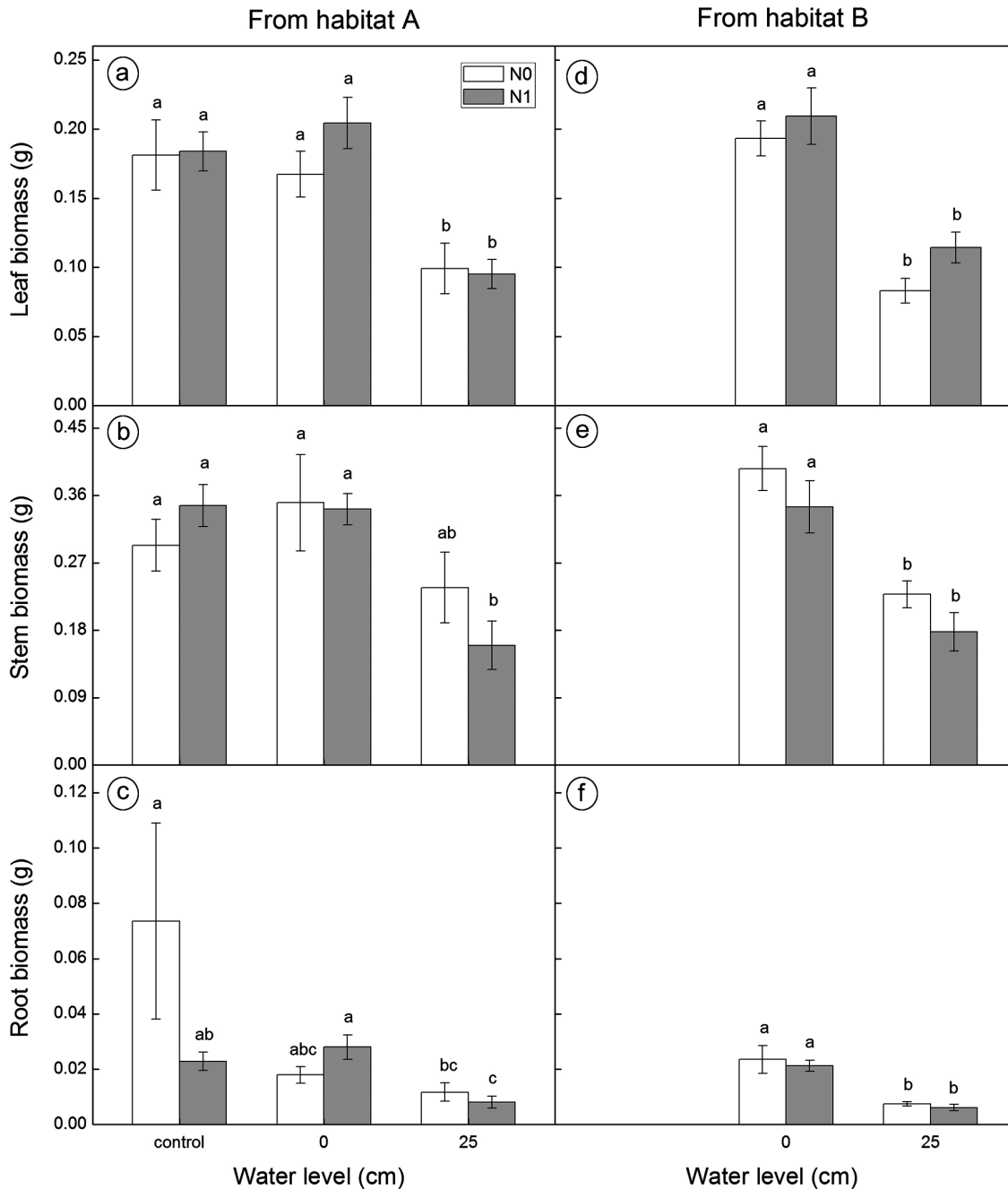
Parameter	Water level (W)			Nitrogen (N)			W × N		
	SS	df	P	SS	df	P	SS	df	P
At midpoint of experiment									
Total biomass (g)	0.064	1	0.002	0.000	1	0.792	0.002	1	0.425
At end of the experiment									
Total biomass (g)	0.477	1	< 0.001	0.004	1	0.568	0.001	1	0.785
Leaf biomass (g)	0.063	1	< 0.001	0.004	1	0.119	0.000	1	0.598
Stem biomass (g)	0.162	1	< 0.001	0.013	1	0.122	8.18E-005	1	0.901
Root biomass (g)	1.690	1	< 0.001	0.034	1	0.376	0.040	1	0.335
Shoot length (cm)	26.670	1	0.170	3.450	1	0.614	1.084	1	0.777
Number of internodes	0.042	1	0.784	0.042	1	0.784	0.042	1	0.784
Mean length of internodes (cm)	0.050	1	0.715	0.152	1	0.526	0.007	1	0.889
Leaf number	251.435	1	< 0.001	44.800	1	0.015	18.514	1	0.102
Number of axillary buds	48.167	1	< 0.001	13.500	1	0.010	6.000	1	0.074

root biomass significantly decreased when the plants were submerged (Tabs. 1, 2, Fig. 2) but was not significantly affected by nitrogen concentration (Tabs. 1, 2). The biomass pattern for each plant part in response to different water levels was also similar in the two initial-habitat groups (Fig 2).

#### SHOOT LENGTH AND INTERNODES

The water depth of the submergence treatment was shallow for the plant, so the duration of complete submergence was short. Almost all shoot apices of

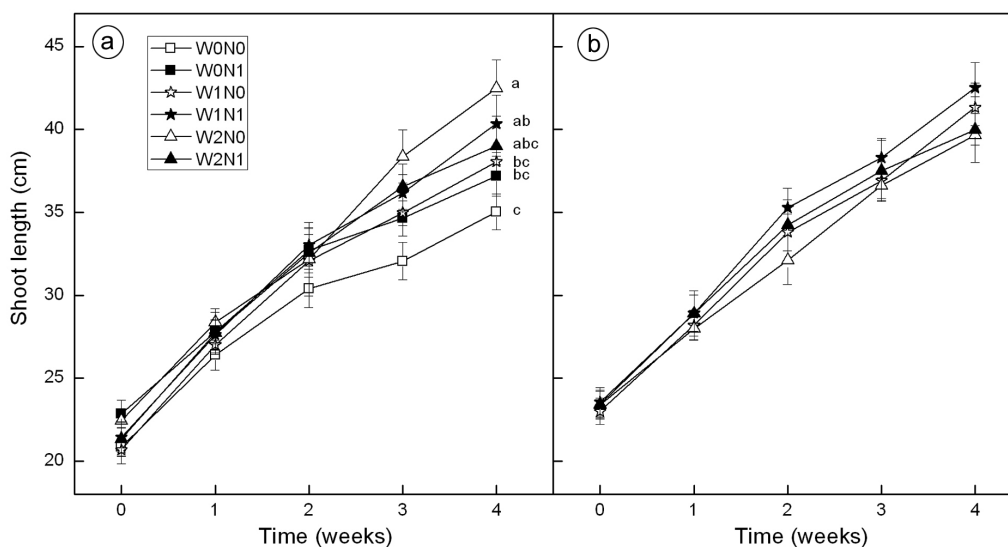
the measured plants emerged above the water one week after the start of the treatments. Shoots elongated continuously during the experiment (Fig. 3). Among the plants from the simulated land habitat, the shoots in the submergence treatment were significantly longer than those in the well-drained treatment at the end of the experiment (Tab. 1, Fig. 3a). Nitrogen concentration did not affect shoot length significantly (Tab. 1). Among the plants from the simulated riverbank habitat, neither water level nor nitrogen concentration affected shoot length significantly (Tab. 2).



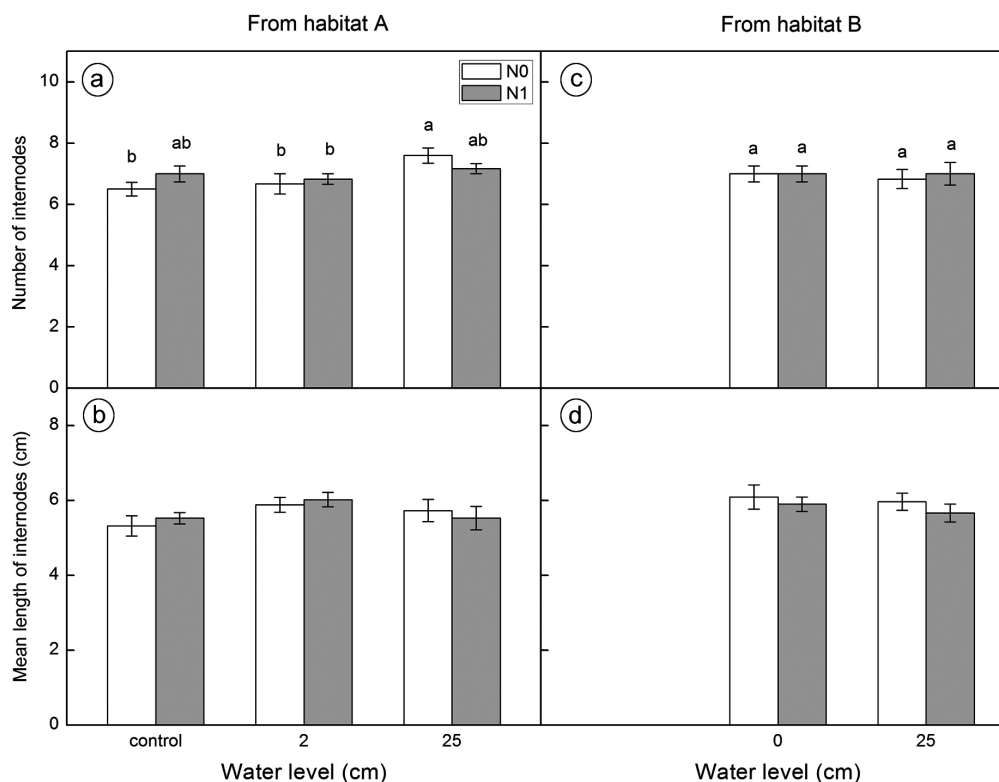
**Fig. 2.** Biomass of *Alternanthera philoxeroides* plant parts under different water levels and nitrogen concentrations. Left panels – plants sourced from the land-like habitat (habitat A), right panels – plants sourced from the riverbank-like habitat (habitat B). Water levels: control (well-drained), at substrate surface (0 cm), 25 cm above substrate surface. Nitrogen conditions: without (N0) and with (N1)  $10 \text{ mg l}^{-1} \text{ NH}_4^+\text{-N}$  in the water. Values are means  $\pm$  SE. Bars bearing different letters differ significantly between treatments ( $P < 0.05$ ).

Complete submergence also greatly increased the number of internodes of the plants from the land-like habitat, (Tab. 1, Fig. 4a); nitrogen concentration did not significantly affect their number of internodes (Tab. 1, Fig. 4a). Neither water level nor nitrogen con-

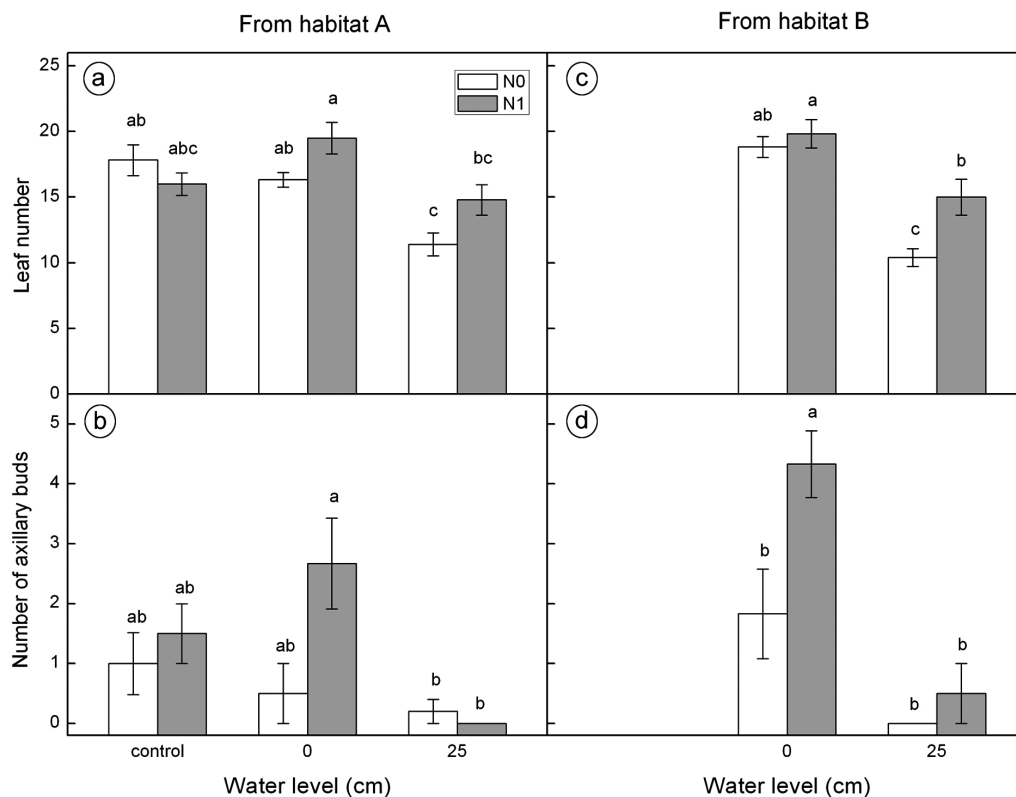
centration affected the number of internodes in the plants from the riverbank-like habitat (Tab. 2, Fig. 4c). The mean length of internodes was not affected by water level or the nitrogen concentration in either habitat group (Tabs. 1, 2, Fig. 4b, d).



**Fig. 3.** Changes in *Alternanthera philoxeroides* shoot length in plants sourced from the land-like habitat (a) and the riverbank-like habitat (b). W0N0 and W0N1 – plants growing in well-drained (control) substrate without (W0N0) and with (W0N1) 10 mg l<sup>-1</sup> NH<sub>4</sub><sup>+</sup>-N in the water; W1N0 and W1N1 – water level at 0 cm above substrate surface without (W1N0) and with (W1N1) 10 mg l<sup>-1</sup> NH<sub>4</sub><sup>+</sup>-N in the water; W2N0 and W2N1 – water level at 25 cm above substrate surface without (W2N0) and with (W2N1) 10 mg l<sup>-1</sup> NH<sub>4</sub><sup>+</sup>-N in the water. Values are means ± SE. Bars bearing different letters differ significantly between treatments (P < 0.05).



**Fig. 4.** Number of internodes and mean length of internodes of *Alternanthera philoxeroides* under different water levels and nitrogen concentrations. Left panels – plants sourced from the land-like habitat (habitat A), right panels – plants sourced from the riverbank-like habitat (habitat B). Water levels: control (well-drained), at substrate surface (0 cm), 25 cm above substrate surface. Nitrogen conditions: without (N0) and with (N1) 10 mg l<sup>-1</sup> NH<sub>4</sub><sup>+</sup>-N in the water. Values are means ± SE. Bars bearing different letters differ significantly between treatments (P < 0.05).



**Fig. 5.** Leaf number and number of axillary buds of *Alternanthera philoxeroides* under different water levels and nitrogen concentrations. Left panels – plants sourced from the land-like habitat (habitat A), right panels – plants sourced from the riverbank-like habitat (habitat B). Water levels: control (well-drained), at substrate surface (0 cm), 25 cm above substrate surface. Nitrogen conditions: without (N0) and with (N1)  $10 \text{ mg l}^{-1} \text{ NH}_4^+ \text{-N}$  in the water. Values are means  $\pm$  SE. Bars bearing different letters differ significantly between treatments ( $P < 0.05$ ).

#### LEAF NUMBER

Among the plants from the land-like habitat, leaf number was significantly affected by the interaction of nitrogen concentration and water level (Tab. 1). In the zero nitrogen treatment, submergence markedly decreased the number of leaves (Fig. 5a), mainly due to the death of many leaves around the midpoint of the experiment (Fig. 6c). Under high nitrogen, submergence did not negatively affect leaf number (Figs. 5a, 6c). New leaves grew out constantly during the experiment, so that the total number of leaves continued to increase in every treatment except for zero nitrogen/submergence (Fig. 6a, b).

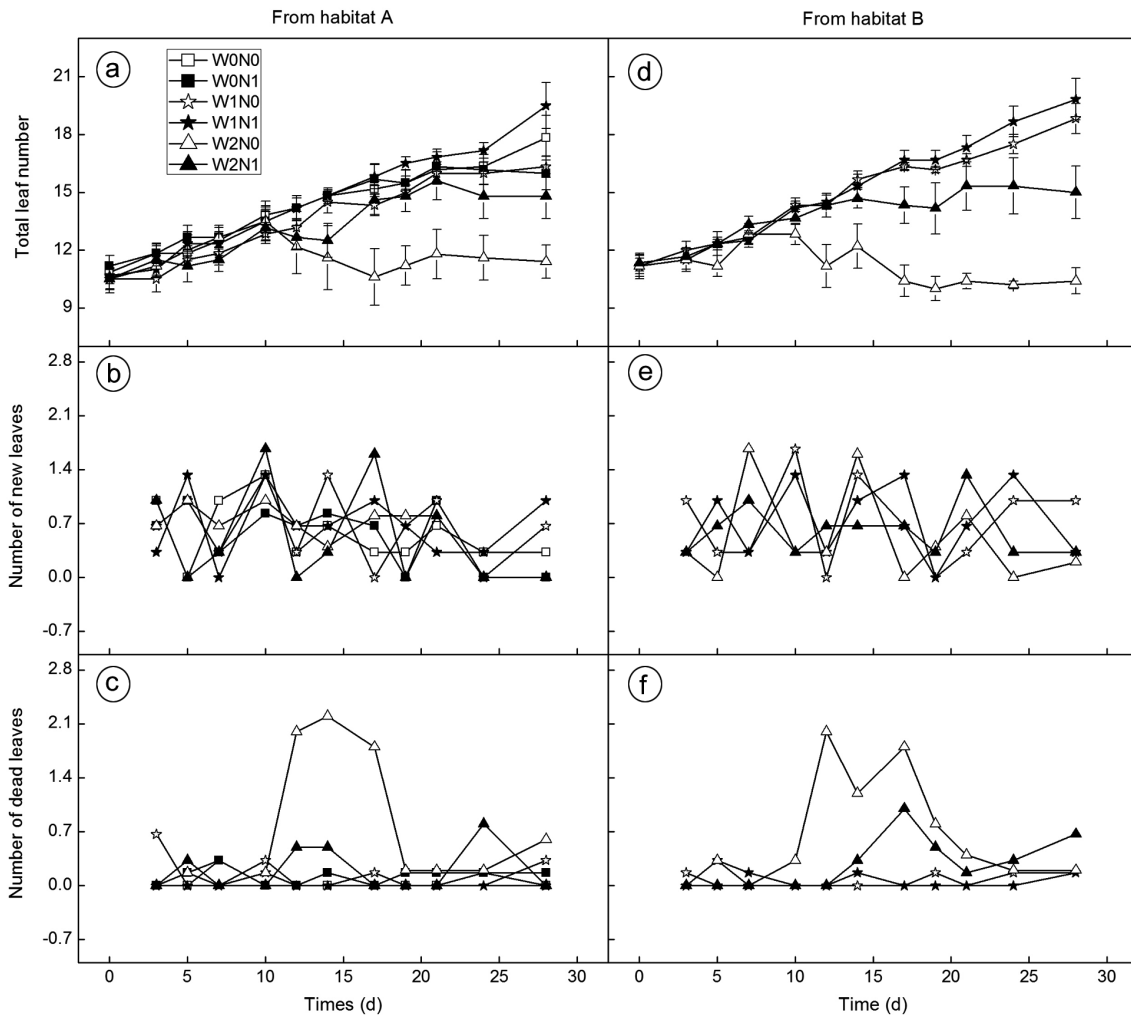
Among the plants from the riverbank-like habitat, submergence also decreased leaf number (Tab. 2, Fig. 5c). Among the submerged plants, high nitrogen increased the number of leaves as compared to the zero nitrogen treatment (Fig. 5c); this effect is attributable to weakening of the effect of submergence on leaf death under high nitrogen (Fig. 6f). The dynamic of leaf number in response to the different treatments was similar in the two initial-habitat groups (Fig. 6a, d).

#### NUMBER OF AXILLARY BUDS

No new ramets or stolons were produced during the experiment but axillary buds grew at some nodes. Submergence inhibited the formation of axillary buds on the plants from the land-like habitat (Tab. 1, Fig. 5b). Their number of axillary buds was highest in the treatment with high nitrogen and the water level at the substrate surface (Fig. 5b). Among the plants from the riverbank-like habitat, axillary bud formation was also significantly suppressed by submergence (Tab. 2, Fig. 5d), and high nitrogen significantly increased the number of axillary buds, especially when the water level was at the substrate surface (Fig. 5d).

#### DISCUSSION

The results suggest that the growth of *A. philoxeroides* was inhibited by shallow submergence even for a short time (Fig. 1). The inhibitory effect of shallow and brief submergence was similar in the plants from the two initial habitats (simulated land, simu-



**Fig. 6.** Total leaf number, new leaves and dead leaves of *Alternanthera philoxeroides* at different water levels and nitrogen concentrations. Left panels – plants sourced from the land-like habitat (habitat A), right panels – plants sourced from the riverbank-like habitat (habitat B). W0N0 and W0N1 – plants growing in well-drained (control) substrate without (W0N0) and with (W0N1)  $10 \text{ mg l}^{-1} \text{ NH}_4^+\text{-N}$  in the water; W1N0 and W1N1 – water level at 0 cm above substrate surface without (W1N0) and with (W1N1)  $10 \text{ mg l}^{-1} \text{ NH}_4^+\text{-N}$  in the water; W2N0 and W2N1 – water level at 25 cm above substrate surface without (W2N0) and with (W2N1)  $10 \text{ mg l}^{-1} \text{ NH}_4^+\text{-N}$  in the water. Values are means  $\pm$ SE in figure a and d, and values are means in others.

lated riverbank). These results are consistent with findings on restraint of growth by flooding or submergence in *A. philoxeroides* (Luo et al., 2009, 2011) and other species (Luo et al., 2009, 2011; Xie et al., 2009). For submerged plants, oxygen shortage is one of the most important factors limiting plant growth. It inhibits aerobic respiration and results in energy deficit or even cell death in some tissues (Blom and Voesenek, 1996; Voesenek et al., 2003). In our study, leaf senescence and death underwater occurred several days after the *A. philoxeroides* plants were submerged. This resulted in lower leaf number and leaf biomass in submerged plants as compared with those grown in the other water level

treatments. Anaerobic respiration exacerbates an insufficiency of carbohydrates, with less ATP yield (Sauter, 2013). The lower biomass accumulation of submerged stems and roots was most likely due to energy and carbohydrate deficits.

Although *A. philoxeroides* growth was inhibited by submergence, the plants could adapt morphologically to submergence and continue growing. As shown in our experiment, shoot elongation is one of the morphological strategies the plant employed to adapt to submergence. Elongation of shoot organs, observed in many aquatic or semi-aquatic species, is considered to be an adaptive response to flooding, enabling the leaves to have contact with the air,



reducing submergence-caused inhibition of respiration and photosynthesis, and thereby restoring growth (Blom and Voesenek, 1996; Voesenek et al., 2003). *A. philoxeroides* can tolerate submergence by its escape strategy of stem elongation in shallow water (Wang et al., 2008; Luo et al., 2009, 2011), and shows great morphological plasticity in different water conditions (Pan et al., 2006; Geng et al., 2006, 2007). This high adaptability allows *A. philoxeroides* to deal with water level fluctuations and exist in diverse aquatic environments.

Submergence-induced shoot elongation is mediated by phytohormone interactions. Ethylene, which accumulates in tissues of submerged plants due to its slow diffusion rate in water, is the key regulator initiating the elongation of leaves, petioles and internodes in submerged conditions (Blom and Voesenek, 1996). Accumulated ethylene along with its interactions with other hormones increases cell wall loosening and extensibility, leading to cell elongation (Voesenek et al., 2003, 2006). Wang et al. (2008) and Luo et al. (2009) found that submerged *A. philoxeroides* elongated the shoot via internode elongation. In our study, however, *A. philoxeroides* shoot elongation relied rather on an increase in the number rather than the length of internodes. The difference in results might be due to differences in water depth between their studies and ours. Wang et al. (2008) and Luo et al. (2009) submerged *A. philoxeroides* in water 1–2 m deep and the plants were kept underwater during submergence. In our study the plants' submergence was shallow, and their shoots were completely submerged only at the beginning of the treatment and could easily emerge during the experiment. Possibly the accumulation of ethylene decreases when the shoots resume contact with the atmosphere, curtailing ethylene-induced elongation (Yu and Yu, 2011). Internode length was not markedly increased in our study entailing shallow and short submergence, but *A. philoxeroides* produced more internodes, which would allow more new leaves to be generated above the water. Leaf recruitment would compensate for the growth loss caused by submergence and would facilitate the plants' survival and growth.

The nitrogen concentration in the water had less of an effect on the growth traits of *A. philoxeroides*, including biomass accumulation and shoot length. Ammonium is an important nitrogen resource for the growth and development of plants but is toxic to plants at high concentrations due to ionic imbalance and acidification (Britto and Kronzucker, 2002). Previous studies have shown that a high ammonium concentration (close to the concentration in our experiment) has negative effects on the survival, growth and reproduction of submerged plants (Cao et al., 2009; Su et al., 2012). In our study, *A. philoxeroide* showed a high degree

of tolerance to the toxicity of high ammonium (high nitrogen) concentrations, as indicated by the lack of effect on growth traits.

High nitrogen reduced leaf death in submerged plants, showing an interaction between nitrogen and water level. The negative effects of high water level on leaf survival were alleviated by the high nitrogen concentration, which would be expected to benefit photosynthesis and consequently plant growth. When plants are exposed to waterlogging or submergence, the structure and function of roots are impaired; membrane integrity and ion transport are compromised, leading to nutrient deficiency and, in turn, senescence and even death (Sauter, 2013). Increased nutrient supply could counterbalance the changes in root morphology, increasing nutrient acquisition and satisfying the requirement for plant physiological functions, compensating for the growth loss induced by flooding (Xie et al., 2009). Our protocol involved nitrogen enrichment of the water column. Nutrient absorption from water would not compensate for growth loss as much as nutrient absorption from soil would, but the high level of nitrogen in the water did reduce submerged leaf mortality. Nitrogen is an essential element of molecules that play key roles in metabolism (e.g., proteins, chlorophylls). High nitrogen could increase the content of crude protein, a material needed for leaf recruitment (Yu and Yu, 2011; Sigua et al., 2012). High nitrogen in the water column may supply it to *A. philoxeroide* through absorption by foliage or adventitious roots (Li et al., 2010; Wersal and Madsen, 2011), alleviating a nutrient deficit and providing more materials for maintenance of leaf function. In this study the negative effects of submergence on leaf survival were mitigated by the high nitrogen level, suggesting that nitrogen enrichment enhances the adaptation of *A. philoxeroides* to water level fluctuation and promotes its invasiveness in highly variable environments.

The high nitrogen concentration also stimulated production of axillary buds when the water level was at the substrate surface. Nitrogen status can regulate the biosynthesis of cytokinin, a critical factor in promoting the outgrowth of axillary buds (Sakakibara, 2006). Axillary buds can develop into new stolons and ramets and finally form extensive networks, which in *A. philoxeroides* is an effective way of reproducing and spreading (Dong et al., 2010, 2012). Clonal plants can increase the intensity of branching under high nutrient supply, raising the production of lateral meristems which grow out and form lateral stolons (de Kroon and Hutchings, 1995). Increased branching intensity helps clonal plants forage more nutrients, propagate and spread. Our finding of increased production of axillary buds under high nitrogen supply suggests that nitrogen-polluted water will boost the clonal growth of

*A. philoxeroides* and its ability to spread from terrestrial to aquatic habitats, expand its area and out-compete native species. This explains, at least in part, the successful invasion of *A. philoxeroides* in shallow eutrophic water.

The plants originating from simulated habitats, land-like and riverbank-like, showed similar effects of increased water level and nitrogen concentration on their growth. It may be that their pretreatment acclimation for 10 days was too short a time for them to acclimate completely to those conditions. This might account for their similar responses to the water level/nitrogen treatments. Alternatively, it is known that the amphibious *A. philoxeroides* can grow in diverse habitats under different water conditions and that it has different anatomical structures enabling it to adapt to terrestrial and aquatic habitats (Tao et al., 2009). When environmental conditions change, its morphological structures can be altered accordingly. The structural or physiological adaptability of *A. philoxeroides* to different habitats might account for the similarity of response to the treatments, as it accounts for its wide distribution in diverse environments. *A. philoxeroides* poses a threat to the integrity and functioning of native ecosystems in many freshwater lakes and rivers. This research has shed some light on the underlying mechanism behind its invasion of nitrogen-polluted water.

## CONCLUSIONS

This research indicates that the growth of *A. philoxeroides* is affected by submergence level and nitrogen concentration.

1. Shallow submergence arising from water level fluctuation suppressed the growth of plants originating from land-like and riverbank-like initial conditions in a short-duration experiment.
2. To adapt to unfavorable submergence conditions, *A. philoxeroides* can regulate its morphological characteristics through elongation of shoots and the production of more internodes. This strong adaptability was determined to be an important element of its survival under diverse water conditions.
3. *A. philoxeroides* can tolerate eutrophication or nitrogen-polluted water. Nitrogen enrichment mitigates the negative effects of submergence on leaf survival, enabling the plant to cope with water level fluctuation and invade environments under changeable water conditions. This work suggests that nitrogen pollution facilitates its invasion by increasing clonal propagation and spatial expansion.
4. *A. philoxeroides* has the ability to adapt to a variety of environmental conditions and can tolerate anthropogenic resource fluctuations. Its

strong adaptability and clonal spread help it to grow and invade successfully in highly disturbed aquatic habitats such as shallow eutrophic water. More attention should be paid to preventing invasions by *A. philoxeroides* in lakes or rivers that might have high nitrogen inputs from agriculture or wastewater.

## AUTHORS' CONTRIBUTIONS

WD designed and performed the experiment, and wrote the manuscript; HZ guided the design of the experiment and commented on the manuscript during its preparation; FZ, LW and SC assisted with the experiment. All authors declare that there are no conflicts of interest.

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