

Differences in Functional traits of invasive (*Wedelia trilobata*) and native (*Wedelia chinensis*) plants under nitrogen enrichment and flooding conditions

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Abstract

Plant invasions are major threat to global change, which can be determined through functional, traits of invasive and native species. Therefore, greenhouse pot experiment was conducted to test whether high water availability, nitrogen enrichment and their interaction promote the growth and functional traits of invasive species (*Wedelia trilobata*, WT), when competing with native species (*Wedelia chinensis*, WC) in monoculture and mixed culture. While, considering the impact of flooding (F) and nitrogen (N) as an individual factor, plant height of WC was non-significant as compared to WT. However, in combination of flooding \times additional nitrogen (F.N, F.2N) physiological parameters of WT were encouragingly higher than those of WC, especially in mixed culture. Variable relative competition intensity at different parameters and higher phenotypic plasticity of WT at different physiological traits make them more dominant than WC at flooding \times additional nitrogen (F.N and F.2N). In conclusion, growth of WT increased under combination of nitrogen \times flooding, because of higher plasticity and better competition intensity that enhanced its competitiveness, playing an important role for the successful invasion of WT in flooding and nitrogen enrichment conditions.

Introduction

Invasive species are a major threat to the ecosystems and are one of the major environmental challenges of global change (Funk et al., 2016). Global environmental changes could create novel environments that directly increase the availability resources for invasive plants. High resources availability facilitate the invasive plant species to grow faster due to their higher phenotypic plasticity (Si et al., 2014) and higher competitive ability (Dostál et al., 2013). Numerous experimental studies have described that global environmental changes enhanced plant invasion. For instance, a recent meta-analysis comparing the growth performance of 74 invasive and 117 native plant species in response to global environmental changes i.e flooding, increase in atmospheric CO₂ concentration, nitrogen enrichment and temperature variations, noted that these changes help invasive species to grow well (Liu et al., 2017). Functional traits play an important role in the success of invasive plant species over native plant species under these global environmental changes (Wan et al., 2018). However, it is very interesting to understand the role of functional traits in enhancing invasive plant species growth under these global environmental changes. Functional traits such as growth characteristics are key indicators of root and shoot development, and leaf functional traits are key indicators of transpiration, evaporation, and photosynthesis (Wang et al., 2016a).

Strong functional traits, especially physiological and growth traits, show the success of invasive species in different environmental conditions. Therefore, to determine the success of invasive species, functional traits and resources are also considered as major factors in the native ecosystems (Jia et al., 2016). The resource ratio hypothesis is one of the explanations for the mechanisms for success of invasive species (Harpole, 2006).

According to the resource ratio hypothesis, plants vary in their nutrient requirements and with the increased availability of a certain nutrient some plants may be benefited and these benefiting plants will inhibit other plants through competitive interactions (Wan et al., 2019). Invasive plant species show better performances under nutrient enrichment and water fluctuations because of higher phenotypic plasticity than native species, and out compete natives species due to interspecific competition (Liu et al., 2018b). Phenotypic plasticity and relative competition intensity are two main features that make invasive plant species to cope with different environmental changes (Liu and van Kleunen, 2017, Van Kleunen et al., 2015), so because of higher phenotypic plasticity and better competition intensity under nutrient enrichment and water fluctuations may create a more suitable environment for invasive species, making them dominant over native species. Relative competition intensity is considered an important factor in determining plant community structure and in promoting invasive potential (Luo et al., 2014).

Mostly competitive ability of invasive and native species depends on the resources of the habitat. Invasive species like to grow in resource-rich habitats. Thus, the competition ability of invasive species may change with the availability of resources. There are several examples where a change in nutrient availability affects the performance of invasive plants. For example, nitrogen (N) enrichment enhanced invasion of *Berberis thunbergii*, *Robinia pseudoacacia*, and competitive ability of *Centaurea stoebe* (He et al., 2012) and addition of potassium made *Taraxacum officinale* successful in grassland, because of higher phenotypic plasticity. Several nutrients have a role in the success of invasive species but N is a major element of global change that disturbs plant community structure, and especially it enhances the abundance of exotic species and decreases the species richness (Duprè et al., 2010). Nitrogen enrichment plays a vital role in the spread of invasive plants. Continued increase in nitrogen may change soil properties that help invasive plants to grow faster in different habitats (Lu et al., 2014). Previous studies have reported that invasive species were more successful than native species in nitrogen-enriched environments because of better competitive ability and these were less successful compared to the native species in the nitrogen-poor habitats (James et al., 2011, Kolb et al., 2002).

Water availability is considered as a key factor responsible for shaping plant communities (Kimball et al., 2014, Rahlao et al., 2010). Water is a vital part of ecosystem facilities, and changes in its availability will modify nutrient cycles resulting in decreased or increased nutrient uptake due to low or high moisture contents in the soil (Ledger et al., 2013, Waraich et al., 2011). The water regime of a habitat can be characterized by the depth, duration, and frequency of flood (Casanova and Brock, 2000). Flooding is the most important factor that plays vital role for the success of invasive species because frequent flooding can produce a more stable environment and shifts in species dominance and species composition (Van Geest et al., 2005). Flooding created more favorable environments, which mostly inhibited the growth of emergent macrophytes (Casanova and Brock, 2000) and preferred submersed aquatic macrophytes, such as evergreen perennial species. Flooding can be observed as a disturbance to the plant community and these disturbances are mostly the main mechanism that facilitates invasion through replacing the native species by the invasive one (Capers et al., 2007). Therefore, understanding the role of nitrogen enrichment and flooding is important to understand the success of invasive species.

Functional traits of invasive plant species play a vital part in its successful invasion in the different environments (Dai et al., 2016b). The response of functional traits of invasive plant species under flooding along with nitrogen enrichment is not well understood. Therefore, we conducted an experiment to examine the functional traits of the invasive species *Wedelia trilobata* and its congener native *Wedelia chinensis* under nitrogen enrichment, flooding, and their interaction, in different plant culture. We hypothesized that flooding along with increased nitrogen concentration, promotes the success of invasive species on native plants due to better physiological and growth responses, which confer them a higher competitive effect, because of higher phenotypic plasticity. In this study, we addressed the following questions: (1) How functional traits of an invasive plant and its native competitor respond to nitrogen enrichment along with flooding? (2) Do flooding and nitrogen enrichment make invasive species more competitive than the native species?

Materials and Methods

This study was conducted from April to July 2019 under greenhouse conditions (had a temperature of $25\pm 5^{\circ}\text{C}$ with 60% relative humidity) to simulate the natural condition. The greenhouse is located at Jiangsu University, Zhenjiang, China (32.20°N , 119.45°E). *Wedelia trilobata* and *Wedelia chinensis* were chosen in this study. *Wedelia trilobata* (W_T) is an annual invasive plant species in China, while *Wedelia chinensis* (W_C) is its congener native species, belonging to the *Asteraceae* family. W_T is a clonal evergreen creeping herb that was also found in the southern region of China in the 1970s on a large scale (Qi et al., 2014). In China, initially, it was introduced as a groundcover species but later it spread rapidly from gardens to roadsides, and then to the agricultural fields, and nowadays, it is found near riverside as well (Song et al., 2010, Talukdar and Talukdar, 2013). W_C is the native congener of W_T , and is mostly used as a medicinal plant. The growth rate of W_C is very slow as compared to W_T (Dai et al., 2016a, Talukdar and Mukherjee, 2008). W_T in China was mostly found in the arid and semi-arid regions. Some of the population of W_T was also found near the Yangzi River in Jiangsu province of China, which probably indicates that water fluctuation and nutrient enrichment made W_T successful in these environments. It prefers growing in nutrient and moisture-rich soils (Dai et al., 2016a). Ramets of W_T and W_C were collected from the same study site of Jiangsu University for the experiment. Ramets of W_T and W_C were growing in the seedling tray with sand as the growth medium. These trays were placed in a greenhouse. The ramets were irrigated with distilled water every day, while nutrition was provided weekly through Hoagland solution. When ramets had two fully expanded leaves, these were transferred to plastic pots (height = 10 cm, diameter = 13 cm) containing sand as a growing-medium. The ramets in the pots were placed in the greenhouse for one week to let them adapt to the greenhouse condition. The two plant species were left growing under two cultures, i.e. mono and mixed cultures. In monoculture, one plant of each species grew; in mixed culture one plant of W_T and W_C grew together. Subsequently, the treatments were implemented as three levels of nitrogen (control = 0.043 g, denoted as CK; additional = 0.130 g, denoted as N and double additional = 0.261 g, denoted as 2N) and two levels of water (normal water = 0.450 L/week and flooding = 0.9 L/week) (Fig. 1). Nitrogen treatments prepared according to (Wan et al., 2018), comprised of equal proportions of KNO_3 and NH_4Cl , and water treatment was made according to (Rahlao et al., 2010). Nitrogen treatments were renewed once a week. The required water amount for water treatments were given three times a week. Two environmental factors were subjected to all pots after transplanting based on factorial design: water (normal or flooding), nitrogen (control, additional, or double additional), and three cultures (two monocultures and one mixed culture), with five replicates. According to this experimental design, there were 90 pots in total (3 nitrogen levels x 2 water levels x 3 cultures x 5 replicates).

Growth and physiological traits measurement

Two months after the treatment, i.e. in the month of July, leaf chlorophyll content (CHI) and leaf nitrogen of both species were measured with portable chlorophyll meter, SPAD; Oakoch OK-Y104, China. Leaf area was measured with ImageJ software every plant with five replicates. Plant height of every plant with five replicates was measured with a measuring scale. The dry weight of above ground (leaf and stem) and below ground (root) biomass of each individual was measured separately, after oven drying to constant weight at 72°C for 48 hours (Parepa et al., 2019).

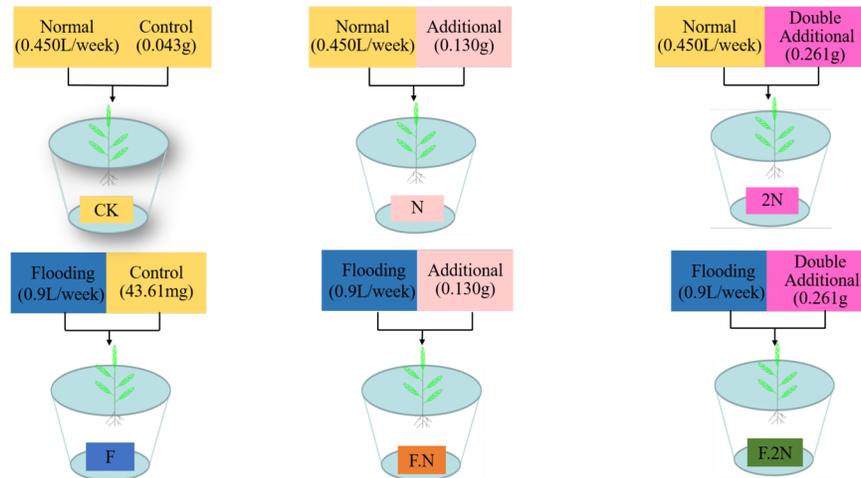


Figure 1. Details of experimental treatments and their combination. Nitrogen treatments were made with equal proportion of KNO_3 and NH_4Cl .

The relative growth rate of total dry weight (RGR_B), relative growth rate of stem length (RGR_{SL}), stem weight ratio (SWR), root weight ratio (RWR) and leaf specific area (SLA) were calculated using the equation shown in Table 1.

Table 1. Equations to measure different growth traits

Traits (Abbreviation)	Equation	Units	Note
Relative growth rate of total dry weight (RGR_B)	$\text{RGR}_B = \frac{(\ln \text{BM}_f - \ln \text{BM}_i)}{t}$	g/day	BM and SL represent total
Relative growth rate of stem length (RGR_{SL})	$\text{RGR}_{SL} = \frac{(\ln \text{Sl}_f - \ln \text{Sl}_i)}{t}$	cm/day	
Stem weight ratio (SWR)	$\text{SWR} = \frac{\text{Total stem weight}}{\text{total weight}}$	g/g	
Root weight ratio (RWR)	$\text{RWR} = \frac{\text{Total root weight}}{\text{total weight}}$	g/g	
Specific leaf area (SLA)	$\text{SLA} = \frac{\text{Leaf area}}{\text{Leaf dry weight}}$	mm^2/mg	

The relative competition intensity (RCI) of different functional traits was calculated by using following equation (Gruntman et al., 2014, He et al., 2012) between W_T and W_C under different nitrogen and water treatments.

$$\text{RCI} = \frac{A_{\text{mix}} - A_{\text{mono}}}{A_{\text{mix}} + A_{\text{mono}}}$$

Where A_{mix} is the trait value of either W_T or W_C in the mixed culture, while A_{mono} is the trait value of either W_T or W_C in the monoculture. RCI values range from -1 to 1. Negative values indicate competition; positive values indicate facilitation; 0 value means neither competitive nor facilitative interactions.

The plasticity index (P_I) of functional traits was calculated by using equation (Funk, 2008, Lamarque et al., 2013) to determine the range of phenotypic plasticity of different functional traits of W_T and W_C under different nitrogen and water treatments within mixed culture.

$$P_I = \frac{\text{Maximum value} - \text{Minimum value}}{\text{Maximum value}}$$

where maximum and minimum value are the maximum and minimum value of one functional trait of W_T or W_C grown in mixed culture under each treatment. The value of P_I ranges from 0 to 1, where one represented the highest P_I .

Statistical analysis

Assumptions of parametric statistics were tested to verify normality and homogeneity of variance using the Shapiro-Wilk normality test and Levene's test before further analysis. Analysis of variance were used to determine the single effects of water, nitrogen and type of culture (categorical variables) and their interactions, on each functional trait ($P \leq 0.05$). Differences in the value of dependent variables among treatment groups were determined with analysis of variance followed by the Tukey test for multiple comparison. To visualize the correlation between traits trends and plant performance Between monoculture of both species and each treatment. We have drawn a heat map of the trait's mechanisms with the help of Pearson Correlation between species under each treatment with reference (Hodgins et al., 2015) and (Shaar-Moshe et al., 2017) to check the correlation between treatments and functional traits. If the pattern for a treatment and plant performance pattern were parallel to each other, then there is positive correlation between change in treatment and plant performance. If the pattern for a treatment and plant performance pattern were opposite to each other then there is negative correlation between change in treatment and plant performance. All analysis was conducted in SPSS:22 and graphs were made in the software Origin Pro9.

Results

Functional Traits

Effect of water (W), nitrogen (N) and culture (C) individually and their interactions ($W \times N \times C$) had affected the functional traits ($P < 0.01$, Table 2). CHI, and SLA were non-significantly affected by the interaction of $W \times N$ and $W \times C$. Plant height and leaf nitrogen was not effective by C. The remaining other functional traits were affected significantly by each of the individual factors and their interaction ($P < 0.01, 0.05$; Table 2). Plant height of W_T and W_C under flooding (F), nitrogen (N) and double additional nitrogen (2N) was non-significant under monoculture but significant under mixed culture. Flooding along with additional and double additional nitrogen (F.N, F.2N) promoted the plant height of W_T compared with the W_C both in monoculture and mixed cultures (Fig. 2a).

Table 2. Functional traits of *Wedelia trilobata* and *Wedelia chinensis* based on ANOVA

Factors	Dry weight	SLA	Plant height	Leaf nitrogen	Chlorophyll content
Water	324.9**	29.90**	38.804**	170.63**	28.239**
Nitrogen	3486.66**	44.36*	118.291**	6.53*	110.400**
Culture	2114.12**	14.09**	2.02 ^{NS}	4.07 ^{NS}	135.467**
Water × nitrogen	297.23**	79.53**	21.33*	2.56 ^{NS}	2.475 ^{NS}
Water × culture	4.147 ^{NS}	3.652 ^{NS}	5.82**	43.46**	3.51 ^{NS}
Nitrogen × culture	665.32**	8.28**	7.07**	45.07*	4.961*
Water × nitrogen × culture	324.98**	3.65**	8.51**	34.12*	11.97**

*significant at $P < 0.05$, **significant at $P < 0.01$

Dry weight (DW) of both the species was significantly affected in all the treatments i.e. W, N, C and their interaction $W \times N \times C$ (Table 2) except only $W \times C$. DW of W_C was low in all the treatments under

monoculture and mixed culture compared to the W_T . The growth rate of W_C was much lower than W_T under the control treatment (CK) (Fig. 2b). W_T had significantly higher growth in all treatments, but higher DW was found in flooding along with additional nitrogen treatments (F.N and F.2N) under mixed culture. These higher DW values indicated that aboveground and belowground biomass of W_T was increased under nutrient-rich environment and outcompeted the competitor (Fig. 2b).

Leaf nitrogen (LN) of both the species varied significantly in all the treatments, i.e. W, N, and their interaction $W \times N \times C$ (Table 2). In the mixed culture at CK, F and N treatments, LN of W_C was higher than W_T , but under flooding and additional nitrogen treatments (F.N and F.2N), leaf nitrogen was higher in W_T than in W_C indicated that nitrogen addition along with water make W_T more dominant competitor than W_C . In the single factor analysis like flooding (F) and nitrogen (N, 2N) W_C had significantly higher leaf nitrogen than W_T , but in combination of nitrogen and flooding (F.N and F.2N) W_T was more successful than W_C (Fig. 2c).

SLA of both the species were significantly affected by all treatment factors i.e. W, N, C and their interaction $W \times N \times C$ (Table 2). W_T had higher SLA than W_C in all the treatments due to higher leaf area and DW of W_T . The SLA of W_T was much higher under mixed culture in flooding along with additional nitrogen treatments (F.N and F.2N); this indicated that resource richness makes invasive species more dominant than native (Fig.2d).

Chlorophyll contents (CHI) were significant in all treatments i.e. W, N, C and their interactions $W \times N \times C$, except $W \times N$ (Table 2). In monoculture under all treatments chlorophyll content of W_T was lower than W_C , but in mixed culture W_T had higher chlorophyll content than W_C (Fig. 2e). Chlorophyll contents of both the species under flooding along with additional nitrogen treatments (F.N and F.2N) were higher than CK that indicated high nitrogen and water made both the species more successful under natural conditions.

Plasticity index

F, N, C and their interaction ($W \times N \times C$) had significant effect on the plasticity index of both the species (Table 3). Dry weight, LN were non-significant under $W \times C$, C and $W \times N$. While F, N, C and their interactions i.e. $W \times N$, $W \times C$, $C \times N$ and $W \times N \times C$ had significant effect on the plasticity index of plant height, DW, SLA, chlorophyll content and leaf nitrogen (Table 3). Out of the five traits, SLA was the most plastic ($F = 118.712$, $P < 0.01$) with a range of 0.13 to 0.325 (Fig. 3g). The plasticity index was higher in all traits of W_T as compared to W_C , except LN and CHI ($P < 0.05$, Fig.3f).

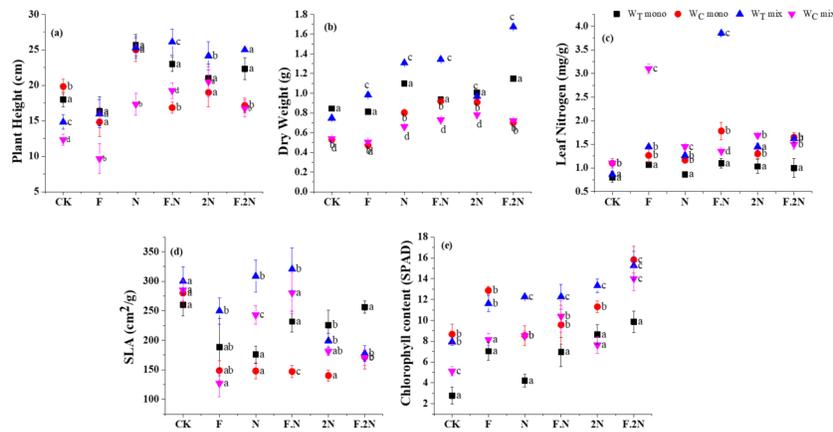


Figure 2. Plant height (a), Dry weight (b), Leaf nitrogen (c), Specific leaf area (d), Chlorophyll content (e) of *Wedelia trilobata* and *Wedelia chinensis* under different treatments, mean \pm SE and different letter represented

significant difference under mono and mixed culture of *Wedelia trilobata* and *Wedelia chinensis*, according to ANOVA and Tukey Test ($P < 0.05$). W_T mono, W_C mono refer to *Wedelia trilobata* and *Wedelia chinensis*, when grown in monoculture; W_T mix and W_C mix refer to *Wedelia trilobata* and *Wedelia chinensis*, when grown in mixed culture.

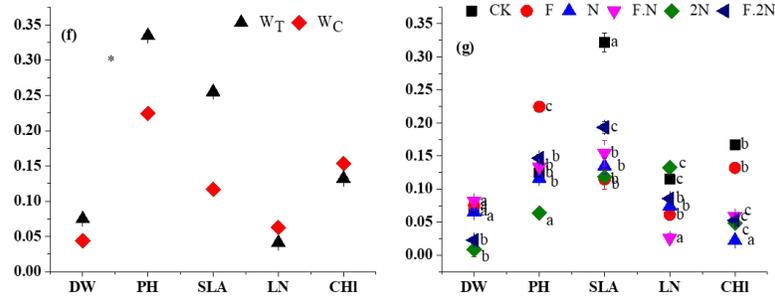


Figure 3. Difference in plasticity indices of physiological traits of *Wedelia trilobata* and *Wedelia chinensis* between plant species and different treatments under mixed culture (f) and (g) representing physiological traits under different treatment; different letters indicate a significant difference ($P < 0.05$) measured ANOVA among groups followed by Tukey Test.

Table 3. ANOVA for the functional traits showing plasticity indices of *Wedelia trilobata* and *Wedelia chinensis*

Factors	SLA	Plant height	Dry weight	Chlorophyll content	Leaf nitrogen
Water	67.19**	147.58**	42.49**	239.56**	228.25**
Nitrogen	273.83**	201.69**	16.98**	174.04**	147.42**
Culture	95.25**	53.88**	44.51**	1130.26**	110.61 ^{NS}
Water × nitrogen	43.84**	192.82**	64.32**	322.92**	100.19**
Water × culture	2.47 ^{NS}	16.76*	5.43*	29.16**	244.38*
Nitrogen × culture	36.784**	33.71**	16.42**	73.07**	3.02 ^{NS}
Water × nitrogen × culture	118.712**	21.94*	10.43**	69.04**	123.18**

*significant at $P < 0.05$, **significant at $P < 0.01$

Relative competition intensity (RCI)

The relative competition intensity (RCI) gave us variable results for traits of both the species, some of which were positive and others were negative (Fig. 4). Plant height of W_T under CK, F and N was affected negatively and showing strong competition, but combination of additional nitrogen and flooding (F.N, F.2N) had a positive effect on plant height, while plant height of W_C in most treatments had RCI below zero, showing strong competition (Fig. 4). LN and CHI of W_C were positive under interaction of nitrogen and flooding (F.N and F.2N) but W_T had negative values under these treatment levels, indicated

that W_T became competitor and W_C became facilitator.

Traits mechanisms

Traits' mechanisms were checked for each species under each treatment and their combinations within monoculture, give us some variable results (Fig. 5). F and N reduced the root weight ratio (RWR) of W_T (-33.91%, -37.73%) and W_C (-83.4%, -90.1%). F increased shoot weight ratio (SWR), relative growth rate of dry weight (RGR_B) and relative growth rate of shoot (RGR_S) of W_T (33.9%, 87.2% and 94.2%) and W_C (83.4%, 29.6%, and 10%). N reduced the RGR_B , RGR_S and RWR of W_C (-59.9%, -18.1%, -90.2%). RGR_B and RGR_S increased in W_T (79.3%, 94.5%). The combination of flooding and additional nitrogen (F.N, F.2N) decreased the RGR_B of W_C (-94.25%, -92.0%), but increased the RGR_B of W_T (42.0%, 33.9%) compared with CK (Fig. 5).

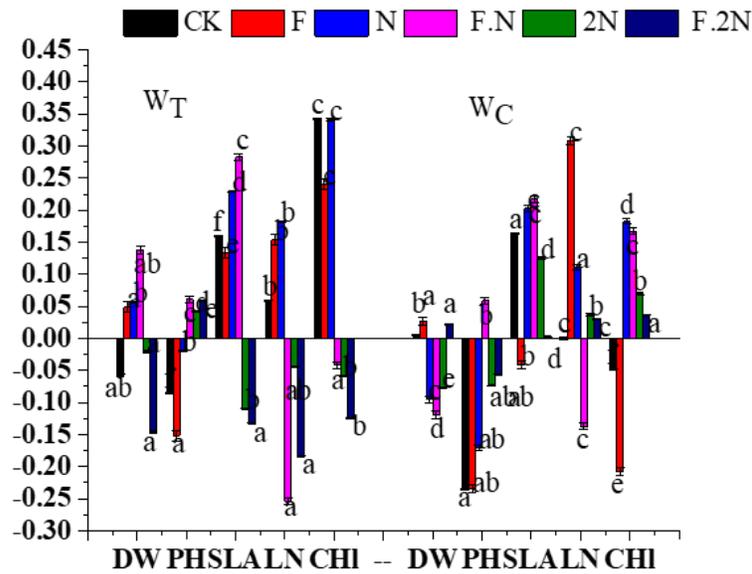


Figure 4. Relative competition intensity of dry weight, plant height, specific leaf area, leaf nitrogen and chlorophyll content of *Wedelia trilobata* (W_T) and *Wedelia chinensis* (W_C) under different nitrogen and water treatments in mixed culture.

Discussion

Functional traits under treatments

The results of this study confirmed the hypothesis that flooding along with additional nitrogen promote the growth of invasive plant species because of higher phenotypic plasticity and better competition intensity. It also indicates that functional traits play an important role for the success of invasive plant species under nitrogen enrichment and flooded habitats. The results showed that W_T was particularly taller than W_C regardless of the water, nitrogen and plant culture methods (monoculture and mixed culture). W_T may be more competitive in resource acquisition due to its tallness, particularly for water and nitrogen, which maybe the most dominant ecological factors that affected plant growth and survival (Drenovsky et al., 2012, Liu et al., 2018a). In addition, SLA of W_T was larger than W_C , supposedly due to larger leaf area and traits response under resource-rich habitats (Matzek, 2012), but some studies pointed out higher growth rate; due to more biomass of leaf rather than leaf structure per unit area (Gallagher et al., 2015, Huang et al., 2016).

However, SLA of W_C was significantly lower than W_T in the treatment under both the cultures, except only CK (Fig. 2d). Hence, SLA may play a role in successful invasion of W_T . While many plant species enhance their growth rate by increasing leaf area, SLA and leaf transpiration rate (LeBel et al., 2013). In this study, higher plant height of W_T serves as a strategy to enhance competition for light; however, this imposes a cost in the form of structural support and water transport (Wang et al., 2017). Therefore, plant species can have higher relative growth rate by increasing plant height and decreasing SLA (Fig. 2a, 2d and Fig. 5), especially in high nitrogen and flooding conditions (F.N and F.2N) (Drenovsky et al., 2012).



Figure 5. Traits difference between different nitrogen and water treatments of *Wedelia trilobata* and *Wedelia chinensis* based on Pearson Correlation under monoculture. For each cell green, orange and red square respectively, indicate Positive, zero and negative percentage difference in the traits value between *Wedelia trilobata* and *Wedelia chinensis* under each treatment. RGR_B, relative growth rate of total dry weight, RGR_{SI}, relative growth rate of stem length, SWR, stem weight ratio, RWR, root weight ratio respectively.

W_T could increase its growth and physiological performance under nitrogen and flooding treatments, particularly in the competition (Fig. 2), because Water and nitrogen are the most important factors for growth and development (Liu et al., 2018c, Tulloss and Cadenasso, 2016). Generally, the role of nitrogen along with water promotes plant growth with increasing plant height, DW, and RGR_B (Čuda et al., 2017, Wang et al., 2016b); that trend was also found in this study (Table 2, Fig. 2 and Fig. 5). DW of W_T was higher under mixed culture because of taller plant height and higher leaf area, that was plant strategy to cope with flooding conditions because higher plant height makes the plant able to get connect with sun light for photosynthesis process (Zhao et al., 2015) and reduce the effect of oxygen deficiency that was created by flooding conditions (Strange et al., 2018). Higher leaf area enhances its photosynthesis and transpiration capacity under flooding and nitrogen enrichment conditions (Wang et al., 2016a, Parepa et al., 2019). Therefore, W_T has higher ability for resource absorption than W_C and also has higher relative growth rate along with reduced resource investment per unit area under combination of nitrogen and flooding (Dalmolin et al., 2012),

Role of relative competitive intensity and plasticity index

Relative competition intensity (RCI) of W_T and W_C indicated improved competition intensity under interspecific competition due to decrease in plant height, leaf nitrogen and increase in SLA and chlorophyll content (Fig.4). Flooding and nitrogen enrichment have great impact on the interspecific competition in the water fluctuation habitat (Zhou et al., 2017). Relative competition intensity is the trade-off among plants between competition and facilitation, which means that under higher resource availability plant shows competition and under stressful condition plant exhibits facilitation (Gratani, 2014). RCI of most functional traits under

additional nitrogen and combination of flooding (2N and F.2N) were negative of W_T that indicated the W_T was more competitive than W_C , especially when both plants grew in mixed culture, because of its survival and better competition ability under flooding and nitrogen enrichment conditions. W_C might be repressed by two factors; one is competition with W_T and the other sensitivity to flooding with higher nutrient availability. Mainly this type of outcome happens under these conditions because every plant species have different tolerance ability under adverse environments (Sun et al., 2019). Here W_T appears to be more dominant due to greater tolerance of flooding and nitrogen enrichment. Furthermore, RCI under combination of flooding and additional nitrogen (F.N and F.2N), LN and CHI of W_T had negative values, but W_C had positive values for these parameters, which indicated that W_T became competitor but W_C behaved like facilitator. Thus, the competition intensity of W_T decreased under combination of flooding and additional nitrogen (F.N and F.2N), but W_C increased (Fig. 4). This can be explained by the flaring of the functional divergence between W_T and W_C under combination of nitrogen and flooding treatments.

Functional traits observed in this study revealed phenotypic plasticity to some extent (Fig. 3). Phenotypic plasticity is the traits mechanism that make plant able to cope with biotic and abiotic environments (Gratani, 2014) and main factor for the success of different plant under different habitats (Legay et al., 2014). According to the results, it was obvious that phenotypic plasticity may play a vital part to adapt to the adverse changes in the environments. However, it was noted that the phenotypic plasticity in functional traits of W_T was higher than W_C . Phenotypic plasticity and relative competitive intensity are closely related to each other because both make invasive plant species able to alter above and below ground functional traits to cope with a wide range of environmental changes (Lamarque et al., 2013). Conflicting to prediction, phenotypic plasticity of LN and CHI in W_T were lower than in W_C . These lower ranges may indicate a fitness cost for plastic physiological traits under complex environments. Leaf construction costs and plant growth rate may be quiet due to lower phenotypic plasticity of LN and CHI (Drenovsky et al., 2012). W_T compensated the negative effects of these adverse environments due to the limited plasticity of functional traits (Quan et al., 2015). Thus, this facilitates invasion and the development of populations in new habitats (Wang et al., 2017). However, plasticity of other indices of W_T was significantly different from W_C (Fig. 3). Although invasive species mostly did not show higher range of plasticity compared with the natives, here W_T showed higher plasticity than W_C because of availability of nitrogen and water. W_T and W_C showed also higher plasticity in plant growth under combination of $W \times N$, which that enhanced their competitiveness (Čuda et al., 2017, Wan et al., 2019). Previous studies also confirmed that invasive and native species could positive respond under competition (Liu et al., 2018a).

Conclusions

Functional traits of W_T and W_C played important role for the success of their growth under nitrogen enrichment and flooding conditions. According to the results, dry weight, and morphological traits of W_T were significantly higher than W_C under combination of flooding and additional nitrogen (F.N and F.2N), due to higher resource acquisition ability of W_T over W_C . LN and CHI of W_T was significantly lower than W_C ; this may confer higher resistance to W_T under the adverse environments. Higher phenotypic plasticity and negative effect of competition of W_T over W_C under combination of flooding and additional nitrogen (F.N and F.2N), make W_T more successful within these complex environments. This study helped us to understanding the role of functional traits for the successful invasion of invasive plant species.

Author Contributions: All authors have read and agree to the published version of the manuscript. Conceptualization, Ahmad Azeem and Qaiser Javed; methodology, Ahmad Azeem; software, Qaiser Javed; validation, Sun Jianfan, Khawar Jabran and Daolin Du; formal analysis, Ahmad Azeem; investigation, Ahmad Azeem; resources, Sun Jianfan.; data curation, Qaiser Javed; writing-original draft preparation, Ahmad Azeem; writing-review and editing, Khawar Jabran; visualization, Qaiser Javed; supervision, Sun Jianfan; project administration, Daolin Du; funding acquisition, Daolin Du.

Data Accessibility Statement

Data will be submitted after acceptance of article according to journal requirement.

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References

- CAPERS, R. S., SELSKY, R., BUGBEE, G. J. & WHITE, J. C. 2007. Aquatic plant community invasibility and scale-dependent patterns in native and invasive species richness. *Ecology*, 88, 3135-3143.
- CASANOVA, M. T. & BROCK, M. A. 2000. How do depth, duration and frequency of flooding influence the establishment of wetland plant communities? *Plant Ecology*, 147, 237-250.
- ČUDA, J., RUMLEROVÁ, Z., BRŮNA, J., SKÁLOVÁ, H. & PYŠEK, P. 2017. Floods affect the abundance of invasive *Impatiens glandulifera* and its spread from river corridors. *Diversity and Distributions*, 23, 342-354.
- DAI, Z.-C., FU, W., QI, S.-S., ZHAI, D.-L., CHEN, S.-C., WAN, L.-Y., HUANG, P. & DU, D.-L. 2016a. Different responses of an invasive clonal plant *Wedelia trilobata* and its native congener to gibberellin: implications for biological invasion. *Journal of chemical ecology*, 42, 85-94.
- DAI, Z.-C., WANG, X.-Y., QI, S.-S., CAI, H.-H., SUN, J.-F., HUANG, P. & DU, D.-L. 2016b. Effects of leaf litter on inter-specific competitive ability of the invasive plant *Wedelia trilobata*. *Ecological research*, 31, 367-374.
- DALMOLIN, Â. C., DALMAGRO, H. J., LOBO, F. D. A., ANTUNES JUNIOR, M. Z., ORTÍZ, C. E. R. & VOURLITIS, G. L. 2012. Effects of flooding and shading on growth and gas exchange of *Vochysia divergens* Pohl (Vochysiaceae) of invasive species in the Brazilian Pantanal. *Brazilian Journal of Plant Physiology*, 24, 75-84.
- DOSTÁL, P., DAWSON, W., VAN KLEUNEN, M., KESER, L. H. & FISCHER, M. 2013. Central European plant species from more productive habitats are more invasive at a global scale. *Global Ecology and Biogeography*, 22, 64-72.
- DRENOVSKY, R. E., KHASANOVA, A. & JAMES, J. J. 2012. Trait convergence and plasticity among native and invasive species in resource-poor environments. *American Journal of Botany*, 99, 629-639.
- DUPRE, C., STEVENS, C. J., RANKE, T., BLEEKER, A., PEPPLER-LISBACH, C., GOWING, D. J., DISE, N. B., DORLAND, E., BOBBINK, R. & DIEKMANN, M. 2010. Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. *Global Change Biology*, 16, 344-357.
- FUNK, J. L. 2008. Differences in plasticity between invasive and native plants from a low resource environment. *Journal of Ecology*, 96, 1162-1173.
- FUNK, J. L., STANDISH, R. J., STOCK, W. D. & VALLADARES, F. 2016. Plant functional traits of dominant native and invasive species in mediterranean-climate ecosystems. *Ecology*, 97, 75-83.
- GALLAGHER, R., RANDALL, R. & LEISHMAN, M. 2015. Trait differences between naturalized and invasive plant species independent of residence time and phylogeny. *Conservation Biology*, 29, 360-369.

- GRATANI, L. 2014. Plant phenotypic plasticity in response to environmental factors. *Advances in botany*, 2014.
- GRUNTMAN, M., PEHL, A. K., JOSHI, S. & TIELBORGER, K. 2014. Competitive dominance of the invasive plant *Impatiens glandulifera*: using competitive effect and response with a vigorous neighbour. *Biological invasions*, 16, 141-151.
- HARPOLE, W. S. 2006. Resource-ratio theory and the control of invasive plants. *Plant and Soil*, 280, 23-27.
- HE, W.-M., MONTESINOS, D., THELEN, G. C. & CALLAWAY, R. M. 2012. Growth and competitive effects of *Centaurea stoebe* populations in response to simulated nitrogen deposition. *PLoS one*, 7, e36257.
- HODGINS, K. A., BOCK, D. G., HAHN, M. A., HEREDIA, S. M., TURNER, K. G. & RIESEBERG, L. H. 2015. Comparative genomics in the Asteraceae reveals little evidence for parallel evolutionary change in invasive taxa. *Molecular ecology*, 24, 2226-2240.
- HUANG, Q. Q., SHEN, Y. D., LI, X. X., LI, S. L. & FAN, Z. W. 2016. Invasive *Eupatorium catarium* and *Ageratum conyzoides* benefit more than does a common native plant from nutrient addition in both competitive and non-competitive environments. *Ecological research*, 31, 145-152.
- JAMES, J., DRENOVSKY, R., MONACO, T. & RINELLA, M. 2011. Managing soil nitrogen to restore annual grass-infested plant communities: effective strategy or incomplete framework? *Ecological Applications*, 21, 490-502.
- JIA, J., DAI, Z., LI, F. & LIU, Y. 2016. How will global environmental changes affect the growth of alien plants? *Frontiers in plant science*, 7, 1623.
- KIMBALL, S., GREMER, J. R., BARRON-GAFFORD, G. A., ANGERT, A. L., HUXMAN, T. E. & VENABLE, D. L. 2014. High water-use efficiency and growth contribute to success of non-native *Erodium cicutarium* in a Sonoran Desert winter annual community. *Conservation physiology*, 2.
- KOLB, A., ALPERT, P., ENTERS, D. & HOLZAPFEL, C. 2002. Patterns of invasion within a grassland community. *Journal of Ecology*, 90, 871-881.
- LAMARQUE, L. J., PORTE, A. J., EYMERIC, C., LASNIER, J.-B., LORTIE, C. J. & DELZON, S. 2013. A test for pre-adapted phenotypic plasticity in the invasive tree *Acer negundo* L. *PLoS one*, 8, e74239.
- LEBEL, P., BRADLEY, R. L. & THIFFAULT, N. 2013. The relative importance of nitrogen vs. moisture stress may drive intraspecific variations in the SLA-RGR relationship: the case of *Picea mariana* seedlings. *American Journal of Plant Sciences*, 4, 1278.
- LEDGER, M. E., BROWN, L. E., EDWARDS, F. K., MILNER, A. M. & WOODWARD, G. 2013. Drought alters the structure and functioning of complex food webs. *Nature Climate Change*, 3, 223.
- LEGAY, N., BAXENDALE, C., GRIGULIS, K., KRAINER, U., KASTL, E., SCHLOTTER, M., BARDGETT, R. D., ARNOLDI, C., BAHN, M. & DUMONT, M. 2014. Contribution of above-and below-ground plant traits to the structure and function of grassland soil microbial communities. *Annals of Botany*, 114, 1011-1021.
- LIU, G., YANG, Y.-B. & ZHU, Z.-H. 2018a. Elevated nitrogen allows the weak invasive plant *Galinsoga quadriradiata* to become more vigorous with respect to inter-specific competition. *Scientific reports*, 8, 3136.
- LIU, Y., LIU, M., XU, X., TIAN, Y., ZHANG, Z. & VAN KLEUNEN, M. 2018b. The effects of changes in water and nitrogen availability on alien plant invasion into a stand of a native grassland species. *Oecologia*, 188, 441-450.
- LIU, Y., ODUOR, A. M., ZHANG, Z., MANEA, A., TOOTH, I. M., LEISHMAN, M. R., XU, X. & VAN KLEUNEN, M. 2017. Do invasive alien plants benefit more from global environmental change than native plants? *Global Change Biology*, 23, 3363-3370.

- LIU, Y. & VAN KLEUNEN, M. 2017. Responses of common and rare aliens and natives to nutrient availability and fluctuations. *Journal of Ecology*, 105, 1111-1122.
- LIU, Y., ZHANG, X. & VAN KLEUNEN, M. 2018c. Increases and fluctuations in nutrient availability do not promote dominance of alien plants in synthetic communities of common natives. *Functional ecology*, 32, 2594-2604.
- LU, X., MAO, Q., GILLIAM, F. S., LUO, Y. & MO, J. 2014. Nitrogen deposition contributes to soil acidification in tropical ecosystems. *Global change biology*, 20,3790-3801.
- LUO, Y., GUO, W., YUAN, Y., LIU, J., DU, N. & WANG, R. 2014. Increased nitrogen deposition alleviated the competitive effects of the introduced invasive plant *Robinia pseudoacacia* on the native tree *Quercus acutissima*. *Plant and soil*, 385, 63-75.
- MATZEK, V. 2012. Trait values, not trait plasticity, best explain invasive species' performance in a changing environment. *PloS one*, 7, e48821.
- PAREPA, M., KAHMEN, A., WERNER, R. A., FISCHER, M. & BOSSDORF, O. 2019. Invasive knotweed has greater nitrogen-use efficiency than native plants: evidence from a 15 N pulse-chasing experiment. *Oecologia*, 191, 389-396.
- QI, S.-S., DAI, Z.-C., MIAO, S.-L., ZHAI, D.-L., SI, C.-C., HUANG, P., WANG, R.-P. & DU, D.-L. 2014. Light limitation and litter of an invasive clonal plant, *Wedelia trilobata*, inhibit its seedling recruitment. *Annals of botany*, 114,425-433.
- QUAN, G., MAO, D., ZHANG, J., XIE, J., XU, H. & AN, M. 2015. Response of invasive *Chromolaena odorata* and two coexisting weeds to contrasting irradiance and nitrogen. *Photosynthetica*, 53, 419-429.
- RAHLAO, S. J., ESLER, K. J., MILTON, S. J. & BARNARD, P. 2010. Nutrient addition and moisture promote the invasiveness of crimson fountaingrass (*Pennisetum setaceum*). *Weed Science*, 58, 154-159.
- SHAAR-MOSHE, L., BLUMWALD, E. & PELEG, Z. 2017. Unique physiological and transcriptional shifts under combinations of salinity, drought, and heat. *Plant physiology*, 174, 421-434.
- SI, C.-C., DAI, Z.-C., LIN, Y., QI, S.-S., HUANG, P., MIAO, S.-L. & DU, D.-L. 2014. Local adaptation and phenotypic plasticity both occurred in *Wedelia trilobata* invasion across a tropical island. *Biological invasions*, 16, 2323-2337.
- SONG, L., CHOW, W. S., SUN, L., LI, C. & PENG, C. 2010. Acclimation of photosystem II to high temperature in two *Wedelia* species from different geographical origins: implications for biological invasions upon global warming. *Journal of experimental botany*, 61, 4087-4096.
- STRANGE, E., HILL, J. & COETZEE, J. 2018. Evidence for a new regime shift between floating and submerged invasive plant dominance in South Africa. *Hydrobiologia*, 817, 349-362.
- SUN, J., JAVED, Q., AZEEM, A., ULLAH, I., SAIFULLAH, M., KAMA, R. & DU, D. 2019. Fluctuated water depth with high nutrient concentrations promote the invasiveness of *Wedelia trilobata* in Wetland. *Ecology and Evolution* .
- TALUKDAR, T. & MUKHERJEE, S. K. 2008. Comparative study of cypselas in three common species of Asteraceae. *Pleione*, 2, 147-149.
- TALUKDAR, T. & TALUKDAR, D. 2013. Response of antioxidative enzymes to arsenic-induced phytotoxicity in leaves of a medicinal daisy, *Wedelia chinensis* Merrill. *Journal of natural science, biology, and medicine*, 4, 383.
- TULLOSS, E. M. & CADENASSO, M. L. 2016. The Effect of Nitrogen Deposition on Plant Performance and Community Structure: Is It Life Stage Specific? *PloS one*, 11, e0156685.

VAN GEEST, G., COOPS, H., ROIJACKERS, R., BUIJSE, A. & SCHEFFER, M. 2005. Succession of aquatic vegetation driven by reduced water-level fluctuations in floodplain lakes. *Journal of Applied Ecology*, 42, 251-260.

VAN KLEUNEN, M., DAWSON, W., ESSL, F., PERGL, J., WINTER, M., WEBER, E., KREFT, H., WEIGELT, P., KARTESZ, J. & NISHINO, M. 2015. Global exchange and accumulation of non-native plants. *Nature*, 525, 100.

WAN, L.-Y., QI, S.-S., ZOU, C. B., DAI, Z.-C., REN, G.-Q., CHEN, Q., ZHU, B. & DU, D.-L. 2019. Elevated nitrogen deposition may advance invasive weed, *Solidago canadensis*, in calcareous soils. *Journal of Plant Ecology*.

WAN, L.-Y., QI, S.-S., ZOU, C. B., DAI, Z.-C., ZHU, B., SONG, Y.-G. & DU, D.-L. 2018. Phosphorus addition reduces the competitive ability of the invasive weed *Solidago canadensis* under high nitrogen conditions. *Flora*, 240, 68-75.

WANG, C., LIU, J., XIAO, H. & DU, D. 2016a. Response of Leaf Functional Traits of *Cerasus yedoensis* (Mats.) Yu Li to Serious Insect Attack. *Polish Journal of Environmental Studies*, 25.

WANG, C., XIAO, H., LIU, J., ZHOU, J. & DU, D. 2016b. Insights into the Effects of Simulated Nitrogen Deposition on Leaf Functional Traits of *Rhus Typhina*. *Polish Journal of Environmental Studies*, 25.

WANG, C., ZHOU, J., LIU, J. & JIANG, K. 2017. Differences in functional traits between invasive and native *Amaranthus* species under different forms of N deposition. *The Science of Nature*, 104, 59.

WARAICH, E. A., AHMAD, R. & ASHRAF, M. 2011. Role of mineral nutrition in alleviation of drought stress in plants. *Australian Journal of Crop Science*, 5, 764.

ZHAO, H., YANG, W., XIA, L., QIAO, Y., XIAO, Y., CHENG, X. & AN, S. 2015. Nitrogen-Enriched Eutrophication Promotes the Invasion of *Spartina alterniflora* in Coastal China. *CLEAN–Soil, Air, Water*, 43, 244-250.

ZHOU, J., CUI, L., PAN, X., LI, W., ZHANG, M. & KANG, X. 2017. Does salt stress affect the interspecific interaction between regionally dominant *Suaeda salsa* and *Scirpus planiculmis*? *PloS one*, 12, e0177497.

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