

Elevated nitrogen deposition and co-evolutionary history shape competition between an invasive plant and its competitors during range expansion

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Abstract

During the range expansion of invasive plants, competitors shared different co-evolutionary history with invasive plants, as well as population differentiation, would have different effects on the response of invaders to global change factors such as increased nitrogen deposition. Disregard the community responses and potential adaptations of invaders during the range expansion might bring misleading answers. To address these challenges, we conducted a greenhouse experiment to explore the synergistic effects between population differentiation during range expansion and competitors on the invasion of *Galinsoga quadriradiata* in response to increased nitrogen deposition. Competitors (new or old that shared short or long co-evolutionary history with the invader, respectively) were set to compete with the invasive central and edge populations under different nitrogen addition treatments. *Galinsoga quadriradiata* from the central population (i.e., with longer residence time since invasion) showed significantly higher total mass, reproduction, interspecific competitiveness when compared to the individuals from the edge population, and the magnitude of response to nitrogen addition treatments was larger in the central population when planted in isolation (single-culture). Nitrogen addition promoted growth and reproductive performance of *G. quadriradiata* in single-culture, in the presence of competitors this effect was weakened. The old competitors acted more effectively than new competitors in inhibiting the invader performance, mainly for the central population. Our results indicate that population differentiation on growth and competitiveness occurred during the range expansion of *G. quadriradiata*, with the central population displaying higher invasiveness than the edge population. The co-evolutionary history between invasive species and its competitors has been suggested to be probably not in favor of invasive plants, especially for central populations. Our results highlight the synergistic and non-additive role of population differentiation and shared co-evolution history between invasive species and its competitors in the range expansion of invaders in the context of global change factors.

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Abstract

During the range expansion of invasive plants, competitors shared different co-evolutionary history with invasive plants, as well as population differentiation, would have different effects on the response of invaders to global change factors such as increased nitrogen deposition. Disregard the community responses and potential adaptations of invaders during the range expansion might bring misleading answers. To address these challenges, we conducted a greenhouse experiment to explore the synergistic effects between population differentiation during range expansion and competitors on the invasion of *Galinsoga quadriradiata* in response to increased nitrogen deposition. Competitors (new or old that shared short or long co-evolutionary history with the invader, respectively) were set to compete with the invasive central and edge populations under different nitrogen addition treatments. *Galinsoga quadriradiata* from the central population (i.e., with longer residence time since invasion) showed significantly higher total mass, reproduction, interspecific competitiveness when compared to the individuals from the edge population, and the magnitude of response to nitrogen addition treatments was larger in the central population when planted in isolation (single-culture). Nitrogen addition promoted growth and reproductive performance of *G. quadriradiata* in single-culture, in the presence of competitors this effect was weakened. The old competitors acted more effectively than new competitors in inhibiting the invader performance, mainly for the central population. Our results indicate that population differentiation on growth and competitiveness occurred during the range expansion of *G. quadriradiata*, with the central population displaying higher invasiveness than the edge population. The co-evolutionary history between invasive species and its competitors has been suggested to be probably not in favor of invasive plants, especially for central populations. Our results highlight the synergistic and non-additive role of population differentiation and shared co-evolution history between invasive species and its competitors in the range expansion of invaders in the context of global change factors.

Keywords: central or edge population, co-evolutionary history, interspecific competition, invasive plant, nitrogen deposition, population differentiation, range expansion

INTRODUCTION

Human activities have dramatically changed the nitrogen cycle in natural ecosystems since the industrial revolution (Liu et al., 2020; Yu et al., 2019; Zhang et al., 2021). This is particularly true for China, as the largest developing country, it is expected to experience a rapid increase in nitrogen deposition: the average flux of total nitrogen deposition for China has been estimated as $20.4 \pm 2.6 \text{ kg ha}^{-1} \text{ yr}^{-1}$ as a result of the industrialization process (Xu et al., 2019; Yu et al., 2019). The increase of nitrogen deposition leads to anthropogenic disturbances that increase available soil nitrogen, and different plants have different responses to this process. Studies have shown that the increase of available soil nitrogen is beneficial to fast-growing plants that can quickly transform nitrogen into new biomass, but not to slow-growing species (Feng et al., 2009; Liu et al., 2017a; Nordin et al., 2005). Many studies have shown that increased nitrogen deposition will promote the colonization, diffusion and other invasive processes of exotic plants, because one of the major characteristics of invasive plants is rapid growth and preemption of resources (Davis et al., 2000; Radford,

2013; Yu et al., 2020; Zheng et al., 2020). Therefore, it is considered that the impact of invasive plants may be further strengthened in the context of future climate change. However, it is still unclear how the increase of nitrogen deposition affects the invasion process of widespread alien plants due to the complexity of interactions during their range expansion.

During their range expansion, invasive plants encounter environmental heterogeneity that might lead to differentiation across populations (Dematteis et al., 2020; Helsen et al., 2020; Zhang et al., 2022). This differentiation may reflect upon species performance and to diverse responses to the increase of nitrogen deposition. Drastic differences across populations usually encountered between a central population where the species initially established in the introduced range and an edge population where the invasive plant has established recently. While the central population shares a longer co-evolutionary history with co-occurring competitors (old competitors), the edge population recently co-occurs with its competitors at the expansion front (new competitors) (Dematteis et al., 2020; Halbritter et al., 2015; Tabassum & Leishman, 2020). The differentiation on growth and competitiveness among invasive populations since time of establishment can significantly affect the competition dynamic with their competitors and determine invaders further expansion (Farooq et al., 2017; Kilkenny & Galloway, 2016). For example, compared with the edge population, the central population of an invasive plant usually needs to deal with the strong competition caused by the denser number of individuals due to the longer time since establishment, so the central population often tends to be more competitive (Phillips et al., 2010; Shine et al., 2011). This differentiation may be driven by evolutionary processes (such as spatial sorting, natural selection, gene flow, mutation, and genetic drift / gene surfing) as well as ecological processes (such as demography, dispersal, expansion speed and shape, and expansion variability), and could be modified by landscape features, trait genetics and biotic interactions (Miller et al., 2020).

The new and old competitors interact differently with the invasive plant along the expansion range (Callaway & Aschehoug, 2000; Sun et al., 2013; Sun & He, 2018), and the outcome of their interactions usually brings idiosyncratic and mixed results. In general, new competitors tend to inhibit the growth of invasive species more significantly than old competitors (Alexander et al., 2015; Sun & He, 2018). Unlike new competitors, the co-evolution between an older competitor and the invader seems lead to a gradual development from competition to coexistence (Huang et al., 2018; Lankau, 2013; Sheppard & Schurr, 2019). Other studies show that invasive species might have an initial advantage over their competitors, but over time, this advantage decreases and competitors may limit the performance of invaders (Oduor, 2013; Saul & Jeschke, 2015; Sheppard & Schurr, 2019). Although the study of pairwise interactions between invasive plants and different competitors is relatively common across the invasion biology literature, there is a lack of understanding on whether old and new competitors have different effects on the response of invasive plants.

Different competitors might have contrasting effects on the response of invasive plants to elevated nitrogen. Many studies have shown that invasive plants use nitrogen more efficiently than native species, with responding more quickly and greatly to the increase of nitrogen deposition (Parepa et al., 2019; Yu et al., 2020). This differential response could lead to a predicted enhanced expansion rate of invasive plants (Liu et al., 2017b; Liu et al., 2019). However, there is no guarantee that invasive plants will succeed in the future, as their success will also depend on the outcome of the competition with their competitors. Some studies have shown that, compared to native species, invasive plants usually maintain a larger magnitude of response to increased nitrogen deposition and increased relative competitive advantage (Eller & Oliveira, 2017; Qin et al., 2018; Valliere et al., 2017). Other studies suggest that the presence of competitors significantly reduces the response magnitude of invasive species to increased nitrogen deposition, and that some native species even gain a higher competitive advantage over invaders as nitrogen availability increases (Eskelinen & Harrison, 2014; Luo et al., 2014; Wang et al., 2017). Thus, the competition outcome between invasive plants and their competitors under elevated nitrogen deposition is still context dependent and needs further clarification.

Galinsoga quadriradiata is an annual invasive forb of Asteraceae, which is native to tropical America (Du et al., 2014; He et al., 2020; Liu et al., 2016b). This invasive plant has strong adaptability and has been widely distributed in most areas of subtropical, temperate and warm temperate zones around the world,

mainly growing in farmland, abandoned land, roadsides, grasslands and other habitats with frequent human disturbance (Kabuce & Priede, 2010; Liu et al., 2016b; Liu et al., 2021b; Zhang et al., 2022). It was first introduced into East China (Jiangxi Province) around the beginning of the last century (earlier than 1943) (Liu et al., 2018; Liu et al., 2016b; Yang et al., 2018). It then gradually spread and established in the Southwest, Central, and North China (Yang et al., 2018). *Galinsoga quadriradiata* arrived at the southern slope of Qinling Mountains, where our study takes place, in Shaanxi Province about 10 years ago (Liu et al., 2016b; Liu et al., 2021b). This invasive plant impacts natural ecosystems and reduce crop production by about 50% when under high invasion (Kabuce & Priede, 2010; Liu et al., 2021a). Whereas its impacts are well understood, the mechanisms of invasion and the predicted expansion under increasing nitrogen deposition, an ongoing scenario in China, are still far from been fully uncovered. In this study, we conducted a common garden experiment to detect the differences in response between central and edge populations of *G. quadriradiata* to their competitors with different co-evolutionary histories in the context of increased soil nitrogen deposition. We hypothesized that: (1) the central population of *G. quadriradiata* would display higher competitive ability and growth when compared to the edge population; (2) nitrogen addition will increase the growth and reproductive performance of both populations, but the central population will have a stronger response to increases in nitrogen deposition than the edge population; (3) the competition outcome in the context of increased nitrogen will depend on the co-evolutionary history between *G. quadriradiata* and its competitors with the new competitors showing a stronger inhibition on the invader's response under high nitrogen, compared to the old competitors.

MATERIALS AND METHODS

Greenhouse experimental design

We chose *Glycine max*, *Zea mays*, and *Glebionis coronaria*, as old competitors which have longer co-evolutionary history with *G. quadriradiata*; while *Medicago sativa*, *Achnatherum splendens*, *Sorghum bicolor*, *Sonchus wightianu*, and *Artemisia capillaris*, as the new competitors. These competitors belong to Poaceae, Fabaceae and Asteraceae respectively. They are common species in the distribution range of *G. quadriradiata*, and they are also the main competitors. Since the invasive plant commonly colonize in crop lands, we chose three common crop species as competitors. The new competitor species are commonly distributed in north and west China, while the old competitors are found in central and east China. We chose the species based on field surveys prior to the beginning of this experiment (Liu et al., 2021a; Liu et al., 2016b; Yang et al., 2018). The seeds of the central population of *G. quadriradiata* and the old competitors were collected from four sampling sites in Lushan, Jiangxi Province in the Fall of 2017, while the seeds of the edge population were collected from three sampling sites (Fig. 1, Table S1) at different elevations in Qinling Mountains, Shaanxi Province in the Fall of 2015. All collected seeds were stored in a 4 °C refrigerator until set to germinate. The seeds of the new competitors were purchased in the market prior to the beginning of the experiment in 2018.

In early May 2018, the seeds of all species were sowed in nursery pots filled with sterile substrate and they were let to germinate in an artificial climate chamber. Four weeks later, the seedlings (about 5 cm high) were transplanted into plastic pots (diameter 16 cm, height 14 cm), and moved into the greenhouse. The pots were filled with sand and soil in a 1:1 proportion by volume. To compare the inter-specific competition intensity, the seedlings were planted in isolation (single-culture) or together with a native competitor (mixed-culture).

In the single-culture treatment, one seedling of the central population or edge population of *G. quadriradiata* was transplanted into a pot. In the mixed-culture treatment, one seedling of *G. quadriradiata* and one seedling of a new or old competitor were planted in the same pot. Seedlings from each population of *G. quadriradiata* were planted with all the other competitors, respectively. After transplanting, all potted plants were subjected to nitrogen treatments in a full factorial design. Each pot was watered with 5 ml deionized water (ambient nitrogen) or 3 g L⁻¹ NH₄NO₃ solution (low nitrogen addition) or 6 g L⁻¹ NH₄NO₃ solution (high nitrogen addition) monthly. The low nitrogen treatment was set to simulate the current nitrogen deposition rate in China (Liu et al., 2013; Zhao et al., 2017) while the high nitrogen addition refers to double the current nitrogen deposition rate. For each combination of treatment, there were 10 replications for the single-culture and 8 replications for the mixed-culture. All pots were randomly arranged in an 80 m² greenhouse to avoid

the influence of herbivores, and watered every two days to keep the soil wet. The experiment had in total 888 pots and 1656 individuals planted.

From July to August 2018, the three uppermost fully expanded young leaves of each of three randomly selected individuals from each treatment were chosen for photosynthetic measurements. Photosynthesis was measured under sunny conditions at a sequence of light levels (1200, 1000, 800, 600, 400, 200, 100, 60, 20, 0 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPF) using a Li-6800 Portable Photosynthesis System with a Red/Blue LED Light Source (Li-Cor, Lincoln, NE, USA). Light curves and maximum net photosynthetic rate (P_{max} , $\mu\text{mol CO}_2 \text{ m}^{-2}$ leaf area s^{-1}) were calculated by a mechanistic model for the photosynthesis–light response based on the photosynthetic electron transport of photosystem II in C_3 and C_4 species (Ye et al., 2013).

In early September 2018, all parts of the plants were harvested. The fresh leaf area was measured immediately after harvest by leaf analysis system WinFOLIA Pro (WinFOLIA™, Régent Instruments Inc., Québec, QC, Canada). The number of capitula per plant was counted before the harvesting as a metric of species performance. The dry weight of the root, leaf, capitula, and stem of each individual was separated and weighted after the samples had been dried to a constant weight in an oven at 60 °C. Elemental analysis for leaf phosphorus concentration (LPC) and leaf nitrogen concentration (LNC) was performed on a continuous flow analyzer (SEAL Auto Analyzer III, Germany). Specific leaf area (SLA) was calculated as the ratio of the leaf area to the leaf dry weight. For each treatment, 0.5 g leaf powder was burned in a muffle furnace at 600 °C for 6 hours to measure an average ash content. The ash content was calculated as ash mass divided by the sample mass. The leaf caloric value was measured by a calorimeter (IKA-C6000, IKA, Germany) with 0.5 g leaf powder from each sample. The SLA, ash content, leaf caloric value, LNC obtained were used to calculate the leaf construction cost per unit of mass (CC_{mass}) following Liu *et al.* (Liu et al., 2016a; Shen et al., 2011). The CC_{mass} was calculated using the ash content, leaf caloric value, SLA, and LNC. Root mass ratio (RMR) was calculated as the ratio of root dry mass and total dry mass.

The relative competition index (RCI), which represents the intensity of interspecific competition between *G. quadriradiata* and competitors, was calculated according to the following equation (Vilà & Weiner, 2004):

$$\text{RCI} = (M_1 - M_2) / M_1 \quad (1)$$

Where, M_1 is the total dry mass of *G. quadriradiata* under single-culture conditions. M_2 is the total dry mass of *G. quadriradiata* in the mixed-culture conditions. The RCI values were generally positive, and the higher RCI value the higher intensity of interspecific competition.

Data analyses

A generalized linear mixed model (GLMM) was performed to evaluate the effects of the nitrogen treatments (NTC), invasive plant population types (PopType), culture types (Culture), and competitor types (CompType, nested in Culture) on the total mass, the number of capitula per plant, RMR, LPC, LNC, or P_{max} . The effects of NTC, PopType, and CompType on the RCI or CC_{mass} were also evaluated by GLMM.

All analyses were conducted using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). The map with the sampling locations was made in ArcGIS version 10.2.2 (Environmental Systems Research Institute, Inc.), and other figures were made by SigmaPlot version 11.0 (Systat Software, Inc., San Jose, CA, USA).

RESULTS

Growth performance of *G. quadriradiata*

The result of the GLMM revealed that NTC (nitrogen addition), PopType (central vs. edge population), Culture (single vs mixed), and CompType (old vs. new competitors; nested in Culture) all had significant effects on plant total mass, and the interactions between CompType and PopType were also significant (Table 1). In general, the total mass of *G. quadriradiata* increased with the nitrogen addition. The central population of *G. quadriradiata* obtained significantly higher total mass than the edge population. The total mass of *G. quadriradiata* in the mixed-culture was significantly lower than that in the single-culture (Fig. 2).

And the old competitors inhibited more the total mass of the central population when compared to the new competitors (Figs 2, S1).

3.2. Intensity of interspecific competition

Both the type of competitors (old vs. new; CompType) and the nitrogen treatments (NTC) had significant effects on the relative competition index (RCI), and the interactions between PopType and NTC were significant (Table 2). In general, the competition was stronger (i.e., higher values of RCI) when *G. quadriradiata* was planted with old competitors then planted with the new competitors (Fig. 3), and the RCI value increased with the nitrogen addition (Fig. 3). When under ambient nitrogen, the interspecific competition was significantly stronger for the edge population when compared to the central population (Fig. S2). There was no statistically significant difference in the interspecific competition between edge and central populations when under low and high nitrogen treatments (Fig. S2).

3.3. Reproduction effort

Culture, NTC, and CompType(Culture) had significant effects on the number of capitula per invasive plant, and the interactions among CompType(Culture) and PopType were also significant (Table 2). Compared to that in the single-culture, *G. quadriradiata* when grown together with competitors had significantly lower number of capitula per plant. This effect was stronger when grown together with old competitors. In general, central populations had significantly higher number of capitula per plant than that of the edge population when grown with new competitors (Figs S3, 4). However, when the invader was mixed with the old competitor, there was no statistical difference between the number of capitula per plant of central vs. edge populations. The number of capitula per plant of *G. quadriradiata* significantly increased with the nitrogen addition. In the ambient nitrogen treatment, the central population obtained lower number of capitula per plant when it was mixed with the old competitors than that mixed with the new competitors (Fig. 4).

3.4. Root mass ratio (RMR)

The result of the GLMM revealed that PopType, Culture, and CompType(Culture) had significant effects on the RMR of *G. quadriradiata*, and the interactions among CompType(Culture) and PopType were also significant (Table 1). The RMR of the central population was significantly lower than that of the edge population when grown alone (Fig. 5). In general, the RMR of *G. quadriradiata* in the mixed-culture was higher than that in the single-culture (Fig. 5, Table 1). The RMR of *G. quadriradiata* was significantly lower when the invader was grown with old than with new competitors. For the central population of *G. quadriradiata*, the RMR was higher when grown with new competitors (Fig. 5). Both new and old competitors had similar effect on the RMR on the edge populations.

3.5. Leaf nutrient (nitrogen and phosphorus) concentration

The nitrogen addition treatments had a significant positive effect on the leaf N content (LNC) of *G. quadriradiata* (Fig. 6a). The interactions among CompType(Culture) and PopType on the LNC were also significant (Table S2). The LNC of the central population was significantly higher than that of the edge population when the invader was grown with old competitors (Fig. 6b).

The GLMM revealed that Culture, NTC, and PopType had significant effects on the leaf P content (LPC) of *G. quadriradiata* (Table S2). In general, the invader LPC was higher in the single-culture than in the mixed-culture; and it was significantly lower under nitrogen addition treatments than when in ambient nitrogen treatment (Fig. 6c). The LPC of the central population was higher than that of the edge population which was further significantly reduced in the presence of the competitors (Fig. 6d).

3.6. Leaf construction cost per unit of mass (CC_{mass})

Competitor and population types (separately) had significant effects on the leaf CC_{mass} , and the interactions among PopType and NTC were also significant (Table 2). The leaf CC_{mass} was significantly lower when *G. quadriradiata* was grown with old competitors than when mixed with new competitors (Fig. 7). In general,

the leaf CC_{mass} of the edge population was significantly lower than that of the central population (Fig. 7), especially under high nitrogen addition treatment (Fig. S4).

DISCUSSION

In this study, we aimed to reveal how population differentiation due time since introduction and shared co-evolutionary history with competitors could affect the invader’s response to increased nitrogen deposition. The results of our experiment suggest that nitrogen addition improves the growth and reproductive performance of the invader *G. quadriradiata*, and enhances its relative competitive advantage on competitors. The central population had both higher growth and reproductive performance when compared to the edge population, and the magnitude of response to nitrogen addition treatments was larger in the central population when in single-culture. In the presence of the competitors, the positive effects of nitrogen addition on invasive plants was diminished. Old competitors with longer shared co-evolutionary history had a stronger competitive inhibition on *G. quadriradiata* performance than the new competitors. These results suggest that the shared co-evolutionary history between competitors and the invader plays an important role in the plant invasion process.

Response to increased nitrogen deposition

The total mass and number of capitula per plant of *G. quadriradiata* were higher under nitrogen addition treatment than in ambient conditions (Figs 1-2). Our results then suggest that increased nitrogen deposition improves the growth and reproduction performance of *G. quadriradiata*, result which align with a number of other invasive plants (Dawson et al., 2012; Parepa et al., 2019; Yu et al., 2020). Invasive species generally tend to be fast-growing plants with high nitrogen use-efficiency (Feng et al., 2009; Liu et al., 2017a). Fast-growing plants often struggle to get enough nitrogen under natural conditions (Bajpai & Inderjit, 2013; Liu et al., 2018). Therefore, under increasing nitrogen deposition, the colonization and expansion of *G. quadriradiata* may be further strengthened, especially in farmlands with frequent artificial fertilization.

While nitrogen addition promoted growth and reproductive performance of *G. quadriradiata* in single-culture, in the presence of competitors this effect was weakened (Figs 1-2). Moreover, the strength of interspecific competition of *G. quadriradiata* increased with nitrogen addition (Fig. 3), suggesting that competitors might also take advantage of the extra soil nitrogen available and compete more intensively with co-occurring invasive species. The competition with species might decrease the positive effect of increased nitrogen deposition on *G. quadriradiata* directly impacting its expansion rate.

Population differentiation

The growth and reproductive performance of the central population of *G. quadriradiata* were higher than the edge population (Figs 2-4), which may mainly be due to the stronger nutrient accumulation capacity (especially in mixed culture) of the central population (Fig. 6). The lower RCI values under ambient nitrogen treatment indicated that the central population was also subjected to less competitive pressure when planted with competitors (Fig. 3). These results suggest a significant population differentiation between the central and edge population of *G. quadriradiata* on growth and competitiveness. Many invasive plants experience population differentiation in the process of range expansion, which helps them to adapt to different environments (Dematteis et al., 2020; Helsen et al., 2020). Generally speaking, the central population of invasive plants may face higher competitive pressure and for longer periods of time (Miller et al., 2020; Phillips et al., 2010; Shine et al., 2011), and subsequently may evolve to be a stronger competitor compared to the edge populations. Our results indicate that, in our study area, the higher competitive ability of the central population of *G. quadriradiata* will potentially help it to better occupy the existing habitats.

When planted alone, the total mass of the central population was significantly higher than that of the edge population only in treatments with nitrogen addition, with no significant differences under ambient nitrogen (Fig. 2). This reveals that the magnitude of response of the central population to elevated nitrogen was larger than that of the edge population, suggesting that populations of invasive plant species with longer time since introduction could have a larger advantage in the context of future increased nitrogen

deposition. Additionally, our results indicate that the central population allocated more nitrogen to growth under increased nitrogen deposition, especially when competing with the old competitors (Fig. 6b). The literature shows that native and invasive populations of invasive plant probably have different nitrogen use strategies, with invasive populations generally showing a quicker return in nitrogen use (Feng et al., 2011). What is still less explored, and we show evidence here, is that different populations of invaders respond uniquely to increases in nitrogen during the range expansion. Therefore, future work should be careful to not overestimate the effects of elevated nitrogen deposition in the range expansion of invasive plants by sampling only the central populations or underestimate them by studying only the edge population.

The effect of competitors with different shared co-evolutionary history

Our results show that the extent of co-evolutionary history between *G. quadriradiata* and its competitors influenced the outcomes of competition in our experimental setup. When *G. quadriradiata* was planted with old competitors, its growth, competitive, and reproductive performances were worse than when growing with the new competitors (Figs 2-4). These results contradict our proposed hypothesis and other studies that have shown better performance of invaders over natives (Alexander et al., 2015; Sun & He, 2018). This may be due to the overlooked adaptations of other species over invasive species with a longer history of co-evolution (Huang et al., 2018; Oduor, 2013; Strauss et al., 2006). It has been suggested that intense competition from invasive plants is a selective factor that eliminates native plant genotypes that cannot tolerate such competition, leading to the accumulation of native plant genotypes that can tolerate intense competition in native plant populations (Leger & Espeland, 2010; Strauss et al., 2006). Genetic variation in traits that are resistant to strong competition from invasive plant species may enable native plants to evolve to adapt to invasive plant species (Oduor, 2013; Strauss et al., 2006). For example, a study on the African savannas suggests that *Parthenium hysterophorus* invasions may have exerted selective pressure on native plants, leading to the differentiation of growth and reproductive traits between invasive and adjacent non-invasive habitats (Oduor, 2022). And it has been found that native plants may evolve a tolerance reducing the negative effects of invasive plants allelopathic compounds (Huang et al., 2018). We speculate that a probable cause to this pattern in our study is due to the lower photosynthetic capacity of *G. quadriradiata* when mixed with the old competitors (Fig. S5).

With respect to the range expansion of *G. quadriradiata*, the old competitors had a stronger competitive inhibition on the central population when compared to the new competitors (Fig. 2). This result was mainly attributed to the lower root allocation of plants from the central population when planted with old competitors than when planted with new competitors (Fig. 5). One potential explanation is that due to intense aboveground competition with the old competitor, invasive plants needed to allocate more biomass aboveground instead of belowground (McCarthy & Enquist, 2007; Puglielli et al., 2021). In contrast, the performance of invasive plants from edge population of *G. quadriradiata* did not significantly differ when growing with new or old competitors (Figs 2-4). The similar performance of the edge population in the face of different competitors and different nitrogen conditions may facilitate the edge population to better adapt to the uncertain environmental factors they may face in the process of range expansion (Alfaro & Marshall, 2019).

Our study explores the competitive relationship between an invasive plant *G. quadriradiata* and eight competitors with different co-evolutionary histories, which represents a large amount of work. However, the conclusion about the influence of the co-evolution history of invasive plants and competitors on their interaction still needs careful extrapolation, because their interaction is also affected by environmental conditions, plant rarity, functional traits and other factors (Oduor, 2013; Zhang & van Kleunen, 2019). And this study only inferred from the phenotypic traits that the co-evolutionary history of competitors and *G. quadriradiata* influenced the relationship between them. However, phenotypic trait expression is influenced by heritable genetic and epigenetic factors (Oduor, 2022). Overall, future studies need to be carried out on co-evolution history of other invasive plants and competitors in more habitats, and multi-generation growth of test plants can be used to test whether trait differences persist over time, and the genetic mechanism should be further explored.

Synergistic effect of nitrogen, population differentiation, and co-evolutionary history

Many abiotic and biotic factors may affect the distribution and spread of invasive plants in the context of disturbance and global climate change (Liu et al., 2017b; Mitchell et al., 2006). These factors often overlap in time and space, and may exert additive, synergistic, or offsetting effects on invasive plants, resulting in complex and unpredictable results (Darling & Cote, 2008; Kersting et al., 2015; NeSmith et al., 2018). For example, in a field experiment in the Californian grassland, the synergistic effect of water and fertilization increased the growth performance of the invaders, which in the presence of surrounding competitors greatly offset the effect (Eskelinen & Harrison, 2014). In our study, we investigated the synergistic effects of nitrogen addition, invasive plant population differentiation, and co-evolutionary history of co-occurring plants on *G. quadriradiata*. All three factors seem to play an important role in the expansion of *G. quadriradiata* from central range to edge range in China. Population differentiation contributed to the stronger invasion potential of the center population, which was further enhanced by increases in nitrogen deposition. However, the invasive species dominance was diminished to some extent in the presence of old competitors. Although the invasive potential of the edge population was relatively low, it was enhanced under increases of nitrogen deposition. Our results echo other studies showing that when studying invasive plants, exploring the interaction of multiple influencing factors and their cumulative effects is pressing (Darling & Cote, 2008; Kersting et al., 2015).

CONCLUSION

In this study, we showed that the nitrogen addition significantly promoted the growth and reproductive performance of *G. quadriradiata*, while central populations had a larger magnitude of response than the edge population when grown in single-culture. However, in the presence of competitors, the response of *G. quadriradiata* to nitrogen addition was reduced. When grown in mixed settings with old competitors that shared a longer co-evolutionary history, *G. quadriradiata* both growth and reproductive performance were worse than when growing with new competitors.

In general, the central population of *G. quadriradiata* had higher population growth, reproductive performance and competitiveness than the edge population. These results suggest that *G. quadriradiata* show trait differentiation among populations in the process of range expansion, which might benefit populations to adapt to different habitat conditions and resident communities.

When considering both the stage of the range expansion and the co-evolutionary history, old competitors more strongly inhibited the central population performance via competition than the new competitors. This effect, however, was not pronounced for the edge population. The difference between the competition strength of new and old competitors on the invasive *G. quadriradiata* confirms that the role of co-evolutionary history between competitors and invaders cannot be ignored when studying the range expansion of invasive plants.

AUTHOR CONTRIBUTIONS

Wen-Gang Zhang: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); software (lead); visualization (equal); writing – original draft (lead); writing – review and editing (equal). **Xing-Jiang Song:** Data curation (equal); investigation (equal); formal analysis (equal); software (supporting); visualization (equal); writing – original draft (supporting); writing – review and editing (equal). **Laís Petri:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Gang Liu:** Conceptualization (equal); funding acquisition (lead); investigation (equal); methodology (equal); writing – original draft (supporting); writing – review and editing (equal). **Xiao-Yan Chen:** Data curation (supporting); investigation (equal); writing – review and editing (equal). **Rui-Ling Liu:** Data curation (supporting); investigation (equal); writing – review and editing (equal). **Fang-Fang Huang:** Writing – original draft (supporting); writing – review and editing (equal). **Jia-Bin Zou:** Investigation (equal); writing – review and editing (equal). **Zhi-Hong Zhu:** Conceptualization (supporting); writing – review and editing (equal).

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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DATA AVAILABILITY STATEMENT

If the paper is accepted for publication, the data will be permanently archived at: <https://www.scidb.cn/en>.

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TABLES

TABLE 1 Effects of various experimental treatments on total mass, number of capitula per plant (NCP), and root mass ratio (RMR) of *Galinsoga quadriradiata* based on GLMM. PopType: edge population and central population. NTC (nitrogen treatments): deionized water, 3 g L⁻¹ NH₄NO₃ solution, and 6 g L⁻¹ NH₄NO₃ solution. Culture: single (invader only) and mixed (invader with competitor). CompType: mixed with new competitors and mixed with old competitors. Bold values indicate that it is statistically significant at $\alpha = 0.05$ level.

Effect	Total mass	Total mass	Total mass	NCP	NCP	NCP	RMR	RMR
	df	F	p-value	df	F	p-value	df	F
Culture	1,637	48.85	<0.0001	1,481	28.85	<0.0001	1,495	7.87
CompType(Culture)	1,637	15.67	<0.0001	1,481	6.98	0.0085	1,495	4.57
NTC	2,637	6.99	0.001	2,481	5.56	0.0041	2,495	2.24
Culture × NTC	2,637	2.09	0.1246	2,481	0.53	0.5864	2,495	0.27
CompType × NTC(Culture)	2,637	0.09	0.917	2,481	0.34	0.7098	2,495	0.84
PopType	1,637	8.01	0.0048	1,481	0.04	0.8372	1,495	6.76
PopType × Culture	1,637	0.09	0.77	1,481	0.47	0.4928	1,495	0.4
CompType × PopType(Culture)	1,637	4.32	0.038	1,481	4.09	0.0436	1,495	4.07
PopType × NTC	2,637	0.97	0.3802	2,481	0.06	0.9462	2,495	0.06
PopType × Culture × NTC	2,637	0.99	0.3714	2,481	0.47	0.6277	2,495	0.01
CompType × PopType × NTC(Culture)	2,637	0.06	0.9399	2,481	0.64	0.5276	2,495	0.55

TABLE 2 The dependence of relative competition index (RCI) and leaf construction cost per unit of mass (CC_{mass}) of *Galinsoga quadriradiata* on the experimental treatments based on GLMM. The invasive plant population types (PopType): edge population and central population. Nitrogen treatments (NTC): deionized water, 3 g L⁻¹ NH₄NO₃ solution, and 6 g L⁻¹ NH₄NO₃ solution. Competitor types (CompType): mixed with

new competitors and mixed with old competitors. The bold value indicates that it is statistically significant at $\alpha = 0.05$ level.

Effect	RCI			CC _{mass}		
	df	F	P	df	F	P
CompType	1,493	13.61	0.0003	1,81	4.52	0.0366
NTC	2,493	3.32	0.0371	2,81	0.8	0.4519
CompType × NTC	2,493	0.27	0.7657	2,81	0.17	0.8477
PopType	1,493	0.75	0.3883	1,81	6.06	0.0159
CompType × PopType	1,493	3.39	0.0662	1,81	0.03	0.8573
PopType × NTC	2,493	3.61	0.0277	2,81	3.44	0.0369
CompType × PopType × NTC	2,493	0.23	0.7933	2,81	0.46	0.6324

FIGURE LEGENDS

FIGURE 1 The location of the central and edge populations of *G. quadriradiata* in China.

FIGURE 2 The total mass of the central and edge populations of *G. quadriradiata* under different treatments. The invasive plant population types are edge (Edge) and central populations (Central). Three levels of nitrogen treatments are deionized water (Ambient N), 3 g L⁻¹ NH₄NO₃ solution (Low N), and 6 g L⁻¹ NH₄NO₃ solution (High N). Culture and competitor types: single-culture with no competitor (None), mixed with new competitors (New competitor), and mixed with old competitors (Old competitor). Different letters indicate significant differences at $p < 0.05$ in each subgraph. Values are means ± SE.

FIGURE 3 Comparison of the relative competition index (RCI) of the central and edge populations of *G. quadriradiata* under nitrogen treatments and different competitor types. The invasive plant population types: edge population (Edge) and central population (Central). Three levels of nitrogen treatments: deionized water (Ambient N), 3 g L⁻¹ NH₄NO₃ solution (Low N), and 6 g L⁻¹ NH₄NO₃ solution (High N). Competitor types: mixed with new competitors (New competitor), and mixed with old competitors (Old competitor). Different letters indicate significant differences at $p < 0.05$. Values are means ± SE.

FIGURE 4 Comparison of the number of capitula per plant (NCP) of the central and edge populations of *G. quadriradiata* under nitrogen treatments, different culture, and different competitor types (nested in Culture). The invasive plant population types: edge population (Edge) and central population (Central). Three levels of nitrogen treatments: deionized water (Ambient N), 3 g L⁻¹ NH₄NO₃ solution (Low N), and 6 g L⁻¹ NH₄NO₃ solution (High N). Culture and competitor types: single-culture with no competitor (None), mixed with new competitors (New competitor), and mixed with old competitors (Old competitor). Different letters indicate significant differences at $p < 0.05$ in each subgraph. Values are means ± SE.

FIGURE 5 Comparison of the root mass ratio (RMR) of the central and edge populations of *G. quadriradiata* under nitrogen treatments, different culture, and different competitor types (nested in Culture). The invasive plant population types: edge population (Edge) and central population (Central). Three levels of nitrogen treatments: deionized water (Ambient N), 3 g L⁻¹ NH₄NO₃ solution (Low N), and 6 g L⁻¹ NH₄NO₃ solution (High N). Culture and competitor types: single-culture with no competitor (None), mixed with new competitors (New competitor), and mixed with old competitors (Old competitor). Different letters indicate significant differences at $p < 0.05$. Values are means ± SE.

FIGURE 6 Comparison of the leaf nitrogen concentration (LNC) and leaf phosphorus concentration (LPC) of *G. quadriradiata* under different treatments. Three levels of nitrogen treatments (NTC): treated with deionized water (Ambient N), 3 g L⁻¹ NH₄NO₃ solution (Low N), or 6 g L⁻¹ NH₄NO₃ solution (High N). The invasive plant population types: edge population and central population. Competitor types: single-culture with no competitor (None), mixed with new competitors (New competitor), and mixed with old competitors (Old competitor). In the bars of figure a and c, the data of no competitors and different competitors summed

together. In the bars of figure b and d, the data of different nitrogen treatments summed together. Different letters indicate significant differences at $p < 0.05$ in each subgraph. Values are means \pm SE.

FIGURE 7 Comparison of the CC_{mass} of the central and edge populations of *G. quadriradiata* under nitrogen treatments and different competitor types. The invasive plant population types: edge population (Edge) and central population (Central). Three levels of nitrogen treatments: deionized water (Ambient N), 3 g L⁻¹ NH₄NO₃ solution (Low N), and 6 g L⁻¹ NH₄NO₃ solution (High N). Competitor types: mixed with new competitors (New competitor), and mixed with old competitors (Old competitor). Different letters indicate significant differences at $p < 0.05$. Values are means \pm SE.

SUPPORTING INFORMATION

TABLE S1 The elevation and coordinates of the seed collection locations of *G. quadriradiata* central and edge populations in China.

TABLE S2 The dependence of maximum net photosynthetic rate (P_{max}), leaf nitrogen concentration (LNC), and leaf phosphorus concentration (LPC) of *Galinsoga quadriradiata* on the experimental treatments based on GLMM. The invasive plant population types (PopType): edge population and central population. Nitrogen treatments (NTC): deionized water, 3 g L⁻¹ NH₄NO₃ solution, and 6 g L⁻¹ NH₄NO₃ solution. Culture type (Culture): single and mixed. Competitor types (CompType): mixed with new competitors and mixed with old competitors. The bold value indicates that it is statistically significant at $\alpha = 0.05$ level.

FIGURE S1 Comparison of the total mass of the central and edge populations of *G. quadriradiata* under different competitor types. The invasive plant population types: edge population and central population. Culture and competitor types: single-culture with no competitor (None), mixed with new competitors (New competitor), and mixed with old competitors (Old competitor). The data of different nitrogen treatments summed together. Different letters indicate significant differences at $p < 0.05$. Values are means \pm SE.

FIGURE S2 Comparison of the relative competition index (RCI) of the central and edge populations of *G. quadriradiata* under nitrogen treatments. The invasive plant population types: edge population and central population. Three levels of nitrogen treatments (NTC): treated with deionized water (Ambient N), 3 g L⁻¹ NH₄NO₃ solution (Low N), or 6 g L⁻¹ NH₄NO₃ solution (High N). The data of no competitors and different competitors summed together. * indicate significant differences at $p < 0.05$. Values are means \pm SE.

FIGURE S3 Comparison of the number of capitula per plant (NCP) of the central and edge populations of *G. quadriradiata* under different competitor types. The invasive plant population types: edge population and central population. Three levels of nitrogen treatments: deionized water (Ambient N), 3 g L⁻¹ NH₄NO₃ solution (Low N), and 6 g L⁻¹ NH₄NO₃ solution (High N). Culture and competitor types: single-culture with no competitor (None), mixed with new competitors (New competitor), and mixed with old competitors (Old competitor). The data of different nitrogen treatments summed together. Different letters indicate significant differences at $p < 0.05$. Values are means \pm SE.

FIGURE S4 Comparison of the Leaf construction cost per unit of mass (CC_{mass}) of the central and edge populations of *G. quadriradiata* under nitrogen treatments. The invasive plant population types: edge population and central population. Three levels of nitrogen treatments (NTC): deionized water (Ambient N), 3 g L⁻¹ NH₄NO₃ solution (Low N), and 6 g L⁻¹ NH₄NO₃ solution (High N). The data of no competitors and different competitors summed together. * indicate significant differences at $p < 0.05$. Values are means \pm SE.

FIGURE S5 Comparison of the maximum net photosynthetic rate (P_{max}) of *G. quadriradiata* under different treatments. Competitor types: mixed with new competitors (New competitor), and mixed with old competitors (Old competitor). Culture type: single-culture (Single) or mixed with competitors (Mixed). The data of different nitrogen treatments summed together. Different letters indicate significant differences at $p < 0.05$ in each subgraph. Values are means \pm SE.









