Elevated CO\textsubscript{2} and Increased N Intensify Competition between Two Invasive Annual Plants in China

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Abstract: As multiple invaders often co-occur, understanding the interactions between different invasive species is important. Previous studies have reported on invasional meltdown and neutral and interference relationships between invasive species. However, interspecific interactions may vary with environmental change owing to the different responses of interacting invaders. To better understand the interaction of notorious invasive alien plants under CO\textsubscript{2} enrichment and N deposition, the growth characteristics of common ragweed (\textit{Ambrosia artemisiifolia}) and redroot pigweed (\textit{Amaranthus retroflexus}) were studied when they were planted in monoculture (4Rag and 4Pig) or mixture (1Rag:3Pig, 2Rag:2Pig, 3Rag:1Pig) under four environmental treatments: elevated CO\textsubscript{2}, increased N, elevated CO\textsubscript{2} + increased N and a control. Increased N positively affected almost all the traits (basal stem diameter, height, shoot biomass, root biomass and total biomass) of common ragweed, except for branch number and root-shoot ratio. But increased N only promoted redroot pigweed’s height and basal stem diameter. interspecific competition promoted basal stem diameter and number of branches but decreased root biomass of common ragweed, and the basal stem diameter was significantly higher in 1Rag:3Pig and 2Rag:2Pig compared to the other two treatments. interspecific competition inhibited almost all the characteristics of redroot pigweed. The interaction between elevated CO\textsubscript{2} and increased N also increased the biomass characteristics (shoot biomass, root biomass and total biomass) of common ragweed. However, elevated CO\textsubscript{2} inhibited the root biomass of redroot pigweed. The results indicated that common ragweed was a superior competitor under conditions of elevated CO\textsubscript{2} and increased N. Moreover, environmental change might strengthen the super-invasive plant common ragweed’s competitive ability.

Keywords: elevated CO\textsubscript{2}; increased N; common ragweed; redroot pigweed; invasional interference; interspecific competition

1. Introduction

Invasive plants often show high adaptability and phenotypic plasticity, which allow them to thrive under altered environmental conditions [1,2]. Numerous case studies have demonstrated that invasive plants benefit from increasing N deposition and elevated CO\textsubscript{2} [3–6]. Elevated CO\textsubscript{2} can facilitate plant invasion by increasing plant photosynthesis, growth rates, efficient resource use, productivity, and seed production [7–12]. N deposition can facilitate plant invasion by increasing N availability, plant growth, and competitive ability [13–16]. These factors often change simultaneously. However, in most studies, these two factors are studied independently.

Understanding the interactive effect of elevated CO\textsubscript{2} and N deposition on invasive alien plants is important. Recent research demonstrated that elevated CO\textsubscript{2} and N deposition synergistically increased the performance (such as biomass and size, survival and reproduction, and photosynthetic rate) of invasive [4,17,18] or naturalized alien plants [6].
than native. Previous studies tested the effect of elevated CO$_2$ and N deposition on alien plants compared with native plants, but few studies have tested the effect among various invasive plants.

As biological invasions increase in frequency, most habitats are invaded by multiple invasive plants, and interactions between invasive species have attracted attention [19–21]. Thus far, three types of interactions have been described: (1) Establishment and impact of one alien invasive species can be facilitated by another invasive species, which is described as invasional meltdown [22]; (2) neutral interactions have also been described [23]; (3) invasion by one species can be negatively impacted by the presence of another invader, termed invasional interference [24–26]. Many authors have noted that a decline in one nonnative species results in a rapid increase in another, which indicates that competition among invasive plants may be common [21,27]. In some cases, these negative relationships may result in an invasive species replacing another invasive species, termed “over-invasion” [20]. For example, the replacement of Spartina anglica by S. alterniflora was found in China [28]. Interactions between invasive plants under elevated CO$_2$ and N deposition remain unclear.

Common ragweed and redroot pigweed are two notorious invasive species both native to North America. They are both included on the list published by the Ministry of Ecology and Environment of the People’s Republic of China [29]. Common ragweed, an annual weed in crop fields, usually forms dense mono-specific stands and produces a considerable amount of pollen [30], and this weed is one of the most problematic aero-allergens [31]. It was introduced into China in the 1930s and, since then, it has spread across twenty provinces [32]. Redroot pigweed occurs in various habitats, including agricultural and ruderal habitats [25,33]. It has a negative impact on ecosystems and native species [34,35] and is regarded as the third most notorious weed in the world [25,36]. Redroot pigweed, introduced into China around 1905, has expanded its distribution in large areas [37]. These two invasive species often co-occur in crops or other habitats [38,39].

In the present study, we tested competitive interactions between these two invasive alien plants in monoculture and mixture under four environmental treatments: elevated CO$_2$, increased N, elevated CO$_2$ and N, and a control. We aimed to answer the following questions: (1) do the growth characteristics of these two invasive alien plants respond to elevated CO$_2$, increased N, and replacement in the same way? (2) which invasive species is more competitive under elevated CO$_2$, increased N deposition, and replacement? (3) how do elevated CO$_2$, N deposition, and replacement affect the reproduction of common ragweed?

2. Materials and Methods

2.1. Plant Materials

We collected seeds of common ragweed and redroot pigweed from Mentougou District in Beijing (100 km from the experiment site) in October 2013. The seeds were treated at low temperatures ($-20 \, ^\circ C$) for two months to break dormancy and then stored at room temperature in paper bags. The seeds of the two species were sown on 7 May 2014, at a depth of 2 cm in two field plots and then watered to field capacity once to stimulate germination. After three weeks, seedlings were transplanted into pots in the OTCs according to the experimental design.

2.2. Experimental Design

The experiment was conducted at the Chinese Research Academy of Environmental Sciences field laboratory, Shunyi District, Beijing, China (116.5875° E, 40.19° N), between 5 June and 8 October 2014.

We tested two CO$_2$ concentrations in our experiment: ambient CO$_2$ (375 ppm) concentrations and elevated CO$_2$ (700 ppm) based on IPCC (Intergovernmental Panel on Climate Change) [40]. Four pairs of open-top chambers (OTCs: 2.2 m in height with an octagonal ground surface area of 6.25 m$^2$) were used. During the experiment, pure CO$_2$ was continuously ventilated into the OTCs of elevated CO$_2$ treatment. Elevated CO$_2$ concentrations were measured at 5-min intervals by monitoring sensors (Qs100). In the elevated CO$_2$
treatment, the achieved level was 695.00 ± 15.67 (mean ± SD) ppm. Ambient CO₂ and elevated CO₂ were randomly assigned to each pair. According to the increase in N deposition rates over the coming decades in China [41,42], two N levels were conducted: ambient N (0 addition) and increased (N 0.8 g/pot).

Four seedlings were planted in each pot under the replacement design: 4Rag:0Pig, 1Rag:3Pig, 2Rag:2Pig, 3Rag:1Pig, and 0Rag:4Pig (where Rag and Pig denote common ragweed and redroot pigweed, respectively). Two N levels, and five replacement levels within each N level, produced 10 pots in each chamber. Thus, 80 pots (32 cm in diameter and 38 cm in depth) were randomly arranged within 8 OTC in the following experimental design: 2 CO₂ levels × 2 N levels × 5 replacement levels × 4 replicates (Figure 1). During the growing season, N was equally divided eight times and uniformly applied in the form of NH₄NO₃ solution, while the control pots were sprayed with the same volume of water.

Figure 1. Experimental design in this study.

To ensure homogeneity of the growing conditions, the pots were filled with a mixture of local soil (80%; collected at a depth of 3–15 cm from a weedy field neighboring the experiment base after excluding topsoil) and vermiculite (20%). Before transplanting, the following soil nutrient contents were measured: organic carbon, 8.4 g·kg⁻¹; total N, 0.73 g·kg⁻¹; ammonium N, 10.77 mg·kg⁻¹; and nitrate N, 6.53 mg·kg⁻¹. One week after transplanting, the elevated CO₂ and N enrichment treatments were initiated. Plants were watered weekly as needed throughout the experiment. In each OTC, pots were rearranged randomly within the chamber every month to minimize location-specific effects.

2.3. Measurements and Calculations

Plant height, basal stem diameter, and branch number per plant of common ragweed and redroot pigweed were measured in October. Shoots of each species in every pot were harvested from above the soil surface and stored in archival paper bags. We turned over the pot, removed the soil of roots with running water, carefully separated the roots of two species in every pot, and then stored them in paper bags. The shoots and roots were oven-dried at 80 °C for 72 h. Shoot biomass and root biomass of each species in each pot were measured, and the total biomass of each species was calculated per pot.

The competitive ability of these two invasive plants was measured by relative yield. RY values > 1 indicate that one species does better when competing against the other species.
than when competing against itself [43]. RY values were calculated using the equation below [43,44].

\[ RY_i = \frac{Y_{ij}}{p_j \times Y_i} \]

where \( Y_{ij} \) is the yield of species \( i \) in the presence of species \( j \), \( p_i \) is the proportion at which species \( i \) is sown, and \( Y_i \) is the yield of species \( i \) in monoculture under the same CO\(_2\) and N treatment as that for \( Y_{ij} \).

To analyze the impact of elevated CO\(_2\), increased N, and replacement on the reproduction of common ragweed, in each pot with common ragweed, seeds were collected by hand in October when the seeds were mature but had not yet begun to drop. The total seed number and the total seed weight were measured as seed yield.

2.4. Statistical Analysis

Split-plot ANOVA was employed to test the effects of CO\(_2\), N, and replacement on the plant performance and relative yield of common ragweed and redroot pigweed, with block as a random factor. When replacement level had a significant effect, significant differences between replacement levels were tested using the Tukey honesty significant difference post-hoc analyses (HSD) \((p < 0.05)\). In all ANOVAs, data were log-transformed to conform to the assumptions of normality and homoscedasticity. All analyses were performed using IBM SPSS Statistics 19 (IBM, 2010, New York, NY, USA).

3. Results

3.1. Growth Characteristics of Two Invasive Alien Plants

Results revealed that height and basal stem diameter of common ragweed were significantly enhanced by increased N in both monoculture and mixtures under both CO\(_2\) concentrations \((p < 0.001)\) (Figure 2a–d; Table 1). At the same time, increased N enhanced \((p < 0.05)\) the height and basal stem diameter \((p < 0.001)\) of redroot pigweed.

Species replacement \((p < 0.001)\) also promoted the basal stem diameter of common ragweed, which was significantly larger in the mixture than in monoculture. The mean stem diameter of ragweed was the largest in the 1Rag:3Pig \((p < 0.05, \text{HSD test})\) (Figure 2a,b), and also significantly larger in the 2Rag:2Pig than in monoculture \((p = 0.014, \text{HSD test})\). Species replacement \((p < 0.001)\) decreased the height and basal stem diameter of redroot pigweed (Figure 2a–d, Table 1). These two characteristics of redroot pigweed in monoculture were all significantly larger than all mixture treatments \((p < 0.001, \text{Figure 2a–d; HSD test})\). Species replacement increased the branch number of common ragweed \((p < 0.001)\), but no branches were observed for redroot pigweed during the experiment.

The impacts of elevated CO\(_2\) on these two invasive plants were not found. A significant interaction of impacts on redroot pigweed was detected in increased N and competition \((p = 0.041)\), elevated CO\(_2\) and species replacement \((p = 0.019)\).
Figure 2. Effects of elevated CO\(_2\) (ambient CO\(_2\), left; elevated CO\(_2\), right), increased N and replacement levels on the growth characteristics of two invasive alien plants. Bars show mean ± SD (4). (a,b). Basal stem diameter. (c,d) Height. (e,f) RY. On the horizontal axis, 1:3 refers to one common ragweed plant and three redroot pigweed plants, 2:2 refers to two common ragweed plants and two redroot pigweed plants, 3:1 refers to three common ragweed plants and one redroot pigweed plant, and 0:4 and 4:0 refer to four redroot pigweed plants or four common ragweed plants, respectively.
Table 1. Results of three-way ANOVA for the growth characters of the alien invasive plants common ragweed and redroot pigweed according to CO$_2$, N and replacement levels: basal stem diameter, height, branch number.

| Species                | Factors                  | Basal Stem Diameter | Height     | Branch Number |
|------------------------|--------------------------|---------------------|------------|---------------|
|                        |                          | F 2.756             | 3.881      | 0.84          |
| common ragweed         | CO$_2$                   | p 0.104             | 0.055      | 0.364         |
|                        |                          | df 1                | 1          | 1             |
|                        |                          | F 32.843            | 17.834     | 0.077         |
|                        | N                        | p <0.001 **         | <0.001 **  | 0.783         |
|                        |                          | df 1                | 1          | 1             |
|                        | replacement              | F 14.961            | 2.446      | 15.668        |
|                        |                          | p <0.001 **         | 0.076      | <0.001 **     |
|                        |                          | df 3                | 3          | 3             |
|                        | CO$_2$ × N               | F 0.751             | 0.062      | 0.161         |
|                        |                          | p 0.334             | 0.755      | 0.101         |
|                        |                          | df 3                | 3          | 3             |
|                        | N × replacement          | F 1.163             | 0.398      | 2.198         |
|                        |                          | p 0.361             | 0.881      | 0.446         |
|                        |                          | df 3                | 3          | 3             |
|                        | CO$_2$ × replacement     | F 0.128             | 0.944      | 1.094         |
|                        |                          | p 0.943             | 0.427      | 0.361         |
|                        |                          | df 3                | 3          | 3             |
|                        | CO$_2$ × N × replacement | F 0.009             | 0.249      | -             |
|                        |                          | p 0.926             | 0.62       | -             |
|                        |                          | df 1                | 1          | 1             |
|                        | N × replacement          | F 2.971             | 0.834      | -             |
|                        |                          | p 0.041 *           | 0.482      | -             |
|                        |                          | df 3                | 3          | 3             |
|                        | CO$_2$ × replacement     | F 0.896             | 3.64       | -             |
|                        |                          | p 0.45              | 0.019 *    | -             |
|                        |                          | df 3                | 3          | 3             |
|                        | CO$_2$ × N × replacement | F 0.289             | 0.712      | -             |
|                        |                          | p 0.833             | 0.55       | -             |
|                        |                          | df 3                | 3          | 3             |

Df represents degrees of freedom, * represents $p < 0.05$, ** represents $p < 0.01$

3.2. Biomass Characteristics of Two Invasive Alien Plants

Shoot biomass, root biomass, and total biomass of common ragweed were all significantly increased by N addition in both monoculture and mixtures under both CO$_2$ concentrations (Figure 3a–d; Table 2). No impacts of N addition were found in biomass characters of redroot pigweed.
Figure 3. Effects of elevated CO\(_2\) (ambient CO\(_2\), left; elevated CO\(_2\), right), N addition and replacement levels on the biomass of two invasive alien plants. Bars show mean ± SD (4). (a,b). Total biomass. (c,d). Root biomass. (e,f). Root–shoot ratio. On the horizontal axis, 1:3 refers to one common ragweed plant and three redroot pigweed plants, 2:2 refers to two common ragweed plants and two redroot pigweed plants, 3:1 refers to three common ragweed plants and one redroot pigweed plant, and 0:4 and 4:0 refer to four redroot pigweed plants or four common ragweed plants, respectively.
Table 2. Results of three-way ANOVA for the biomass characters of the alien invasive plants common ragweed and redroot pigweed according to CO$_2$, N and replacement levels: shoot biomass, root biomass, total biomass, and root–shoot ratio.

| Species      | Factors          | Shoot Biomass | Root Biomass | Total Biomass | Root-Shoot Ratio |
|--------------|------------------|---------------|--------------|---------------|------------------|
|              | $F$              | 2.141         | 0.855        | 2.107         | 0.042            |
|              | $p$              | 0.15          | 0.36         | 0.153         | 0.839            |
|              | df               | 1             | 1            | 1             | 1                |
| common ragweed | $F$              | 140.242       | 73.943       | 142.018       | 2.843            |
|              | $p$              | <0.001 **     | <0.001 **    | <0.001 **     | 0.099            |
|              | df               | 1             | 1            | 1             | 1                |
|              | $F$              | 1.085         | 5.75         | 1.398         | 5.158            |
|              | $p$              | 0.365         | 0.002 **     | 0.255         | 0.004 **         |
|              | df               | 3             | 3            | 3             | 3                |
|              | $F$              | 13.565        | 4.989        | 13.245        | 2.609            |
|              | $p$              | 0.001 **      | 0.030 *      | 0.001 **      | 0.151            |
|              | df               | 1             | 1            | 1             | 1                |
|              | $F$              | 0.399         | 2.804        | 0.651         | 1.275            |
|              | $p$              | 0.754         | 0.05         | 0.587         | 0.294            |
|              | df               | 3             | 3            | 3             | 3                |
|              | $F$              | 0.361         | 0.808        | 0.346         | 0.806            |
|              | $p$              | 0.781         | 0.496        | 0.792         | 0.497            |
|              | df               | 3             | 3            | 3             | 3                |
|              | $F$              | 0.354         | 0.064        | 0.271         | 0.294            |
|              | $p$              | 0.786         | 0.979        | 0.846         | 0.83             |
|              | df               | 3             | 3            | 3             | 3                |

| redroot pigweed | CO$_2 \times$ N | $F$          | 1.92          | 5.08          | 2.63             | 0.037            |
|                 | $p$              | 0.173         | 0.029 *       | 0.112         | 0.849            |
|                 | df               | 1             | 1            | 1             | 1                |
|                 | $F$              | 1.614         | 0.013        | 1.637         | 0.383            |
|                 | $p$              | 0.21          | 0.909        | 0.207         | 0.539            |
|                 | df               | 1             | 1            | 1             | 1                |
|                 | $F$              | 100.968       | 57.074       | 110.475       | 4.281            |
|                 | $p$              | <0.001 **     | <0.001 **    | <0.001 **     | 0.010 **         |
|                 | df               | 3             | 3            | 3             | 3                |
|                 | $F$              | 0.588         | 0.054        | 0.636         | 0.088            |
|                 | $p$              | 0.447         | 0.818        | 0.429         | 0.768            |
|                 | df               | 1             | 1            | 1             | 1                |
|                 | $F$              | 0.512         | 0.182        | 0.472         | 0.184            |
|                 | $p$              | 0.676         | 0.908        | 0.703         | 0.907            |
|                 | df               | 3             | 3            | 3             | 3                |
|                 | $F$              | 0.491         | 0.597        | 0.464         | 1.36             |
|                 | $p$              | 0.69          | 0.62         | 0.709         | 0.267            |
|                 | df               | 3             | 3            | 3             | 3                |
|                 | $F$              | 0.033         | 0.374        | 0.027         | 0.736            |
|                 | $p$              | 0.992         | 0.772        | 0.994         | 0.536            |
|                 | df               | 3             | 3            | 3             | 3                |

Df represents degrees of freedom, * represents $p < 0.05$, ** represents $p < 0.01$

Root biomass and the root-shoot ratio of common ragweed were affected by species replacement. Interspecific and intraspecific competition all impacted root biomass and were higher in monoculture ($p = 0.001$, HSD test) and decreased in 3Rag:1Pig ($p = 0.020$, HSD test) than in 1Rag:3Pig mixture. The root-shoot ratio of common ragweed was significantly lower in the 3Rag:1Pig mixture than in monoculture ($p = 0.011$, HSD test). Shoot biomass, root biomass, and total biomass of redroot pigweed were all significantly suppressed in the mixture compared with monoculture ($p < 0.001$) (Figure 3a–d; Table 2), and these characteristics were all decreased in 3Rag:1Pig than in the other two mixture controls ($p < 0.05$, HSD test). The root-shoot ratio of redroot pigweed was significantly higher in mixtures than in monoculture ($p < 0.05$, HSD test) (Figure 3e, f).
Elevated CO$_2$ inhibited the root biomass ($p = 0.011$) of redroot pigweed in mixtures (Figure 3c, d). An interaction between elevated CO$_2$ and increased N promoted shoot biomass ($p < 0.001$), total biomass ($p < 0.001$), and root biomass ($p = 0.03$) of common ragweed.

3.3. Relative Yields of Two Invasive Alien Species

The relative yields of common ragweed were significantly higher than 1 ($p < 0.001$, $t$-test) irrespective of any treatments; while these values were significantly lower than 1 ($p < 0.001$, $t$-test) for redroot pigweed (Figure 2e,f). The highest relative yield (RY) values of common ragweed reached 4.72, and the mean values of relative yield were larger in 1Rag:3Pig compared to other replacement treatments ($p < 0.001$, HSD test), while these values in 2Rag:2Pig were higher than in 3Rag:1Pig ($p = 0.001$, HSD test). In contrast, the largest relative yield of redroot pigweed was only 0.87, and the mean values of relative yield were higher in 2Rag:2Pig compared to other replacement treatments; however, no significant difference was observed.

3.4. Reproductive Characteristics of Common Ragweed

Increased N enhanced the seed yield ($p < 0.001$; Table 3; Figure 4a,b) and decreased the seed mean weight ($p = 0.001$) of common ragweed (Figure 4c, d), while elevated CO$_2$, competition, and interaction effects showed no significant difference on any of the abovementioned indices (Figure 4; Table 3).

Table 3. Results of three-way ANOVA for the seed characters of the alien invasive plants common ragweed according to CO$_2$, N, and replacement levels: seed yield, seed mean weight.

| Factors            | Seed Number | Seed Total Weight | Seed Mean Weight |
|--------------------|-------------|-------------------|-----------------|
|                    | $F$         | 0.017             | 0.344           | 0.181           |
| CO$_2$             | $p$         | 0.896             | 0.560           | 0.673           |
|                    | $df$        | 1                 | 1               | 1               |
|                    | $F$         | 14.821            | 1.241           | 12.024          |
| N                  | $p$         | $<0.001$ **       | 0.271           | 0.001 **        |
| replacement        | $df$        | 1                 | 1               | 1               |
|                    | $F$         | 2.316             | 2.135           | 0.330           |
| replacement        | $p$         | 0.088             | 0.109           | 0.804           |
|                    | $df$        | 3                 | 3               | 3               |
|                    | $F$         | 1.654             | 0.999           | 0.092           |
| CO$_2$ × N         | $p$         | 0.205             | 0.323           | 0.763           |
|                    | $df$        | 1                 | 1               | 1               |
|                    | $F$         | 0.437             | 0.926           | 0.689           |
| N × replacement    | $p$         | 0.727             | 0.436           | 0.563           |
|                    | $df$        | 3                 | 3               | 3               |
|                    | $F$         | 0.806             | 0.360           | 0.083           |
| CO$_2$ × replacement| $p$         | 0.497             | 0.782           | 0.969           |
|                    | $df$        | 3                 | 3               | 3               |
|                    | $F$         | 1.203             | 0.977           | 1.885           |
| CO$_2$ × N × replacement| $p$     | 0.319             | 0.412           | 0.145           |
|                    | $df$        | 3                 | 3               | 3               |

* represents $p < 0.05$, ** represents $p < 0.01$.
Figure 4. Effects of elevated CO$_2$, N addition and replacement levels on the reproductive characteristics of common ragweed. Bars show mean ± SD (4). (a,b) The number of seeds (c,d) mean weight of seeds. On the horizontal axis, 1:3 refers to one common ragweed plant and three redroot pigweed plants, 2:2 refers to two common ragweed plants and two redroot pigweed plants, 3:1 refers to three common ragweed plants and one redroot pigweed plant, and 4:0 refers to four common ragweed plants.

4. Discussion

In the present study, we found common ragweed and redroot pigweed responded differently to elevated CO$_2$, increased N, and species replacement. We revealed the interspecific competition between common ragweed and redroot pigweed under elevated CO$_2$ and increased N.

Common ragweed showed an apparent competitive advantage over redroot pigweed under mixture treatments, where even one common ragweed plant could strongly inhibit redroot pigweed plants. Common ragweed and redroot pigweed may compete for limited resources. The tall stature of the common ragweed provided a decisive advantage in acquiring light [45]. Common ragweed was higher and more robust than redroot pigweed in mixture treatments (Figures 1 and 2). The height disadvantage of redroot pigweed under competition results in a decrease in its light-capturing capacity [46]. The decline in quality and quantity of light influenced the phenology of pigweed [35,47], so the growth of redroot pigweed was inhibited under competition. We also found the height of redroot pigweed are all positively related to the other characteristics. By comparing the competition between redroot pigweed and common lamb’s quarters (Chenopodium album), it was also found that

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Life 2022, 12, x FOR PEER REVIEW 11 of 16

Figure 4. Effects of elevated CO$_2$, N addition and replacement levels on the reproductive characteristics of common ragweed. Bars show mean ± SD (4). (a,b) The number of seeds (c,d) mean weight of seeds. On the horizontal axis, 1:3 refers to one common ragweed plant and three redroot pigweed plants, 2:2 refers to two common ragweed plants and two redroot pigweed plants, 3:1 refers to three common ragweed plants and one redroot pigweed plant, and 4:0 refers to four common ragweed plants.

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Common ragweed showed an apparent competitive advantage over redroot pigweed under mixture treatments, where even one common ragweed plant could strongly inhibit redroot pigweed plants. Common ragweed and redroot pigweed may compete for limited resources. The tall stature of the common ragweed provided a decisive advantage in acquiring light [45]. Common ragweed was higher and more robust than redroot pigweed in mixture treatments (Figures 1 and 2). The height disadvantage of redroot pigweed under competition results in a decrease in its light-capturing capacity [46]. The decline in quality and quantity of light influenced the phenology of pigweed [35,47], so the growth of redroot pigweed was inhibited under competition. We also found the height of redroot pigweed are all positively related to the other characteristics. By comparing the competition between redroot pigweed and common lamb’s quarters (Chenopodium album), it was also found that
the species which shade the ground with a dense canopy will favorably compete [48]. In our study, we also found that branch numbers of common ragweed increased with competition, but no branches were observed in redroot pigweed. When two alien invasive plants live in the same habitat or require the same scarce resource [49,50], one species limiting the material or space of the other will result in competition.

Growth characteristics (except for branch number and root-shoot ratio) of common ragweed were enhanced by increased N in all treatments. Only the height and basal stem diameter of redroot pigweed increased in the mixtures under increased N (Figures 1 and 2). Our results are consistent with the premise that responsiveness considerably differs between species under N addition [51]. We also found that increased N and elevated CO$_2$ intensified the competitive advantage of common ragweed. For example, biomass characteristics of common ragweed were positively affected by the interaction of increased N and elevated CO$_2$. Lack of an elevated CO$_2$ direct effect on these two invasive alien plants might be explained by indirect effects of CO$_2$ on N limitation, similar to Blumenthal et al. [52]. First, these two invasive alien plants all prefer high levels of nitrogen fertilizer. A previous study showed that the height and dry matter of common ragweed were increased by N addition in both greenhouse and field experiments [53]. Moreover, N addition stimulated the height of redroot pigweed [35] and shoot biomass and root biomass [51]. In the present study, the biomass of redroot pigweed did not increase under N addition in mixtures. Mainly because common ragweed can competitively pre-empt soil nitrogen from redroot pigweed. Secondly, the CO$_2$ response of species might depend on local resource availability in mixed-species competition [4]. Most plants exhibit a positive growth response to elevated CO$_2$ due to increased photosynthesis and/or efficient nutrient use when other factors (e.g., water and nutrients) are not limited [6,54]. Because most invasive plants are sensitive to N availability, the impacts of CO$_2$ on invasive plants varied with N levels [52]. Redroot pigweed increased biomass allocation to the roots to diminish aboveground competitive disadvantage, where plants allocate more biomass to roots to acquire the most limiting resource [55–57]. But elevated CO$_2$ inhibits this increase (Figure 2). A previous study on redroot pigweed revealed lower rates of photosynthesis and stomatal conductivity under elevated CO$_2$ compared to ambient in water stress treatments [58,59]. We speculate that there is an apparent limit of available N for redroot pigweed growth under competition. Third, C3 plants are thought to take more advantage of CO$_2$ enrichment than plants with a C4 [60,61]. In our study, common ragweed was a C3 plant and redroot was a C4 plant. Fourth, allelopathic effects also impacted interspecific competition. Bae et al. found that elevated CO$_2$ may enhance the allelopathic potential of common ragweed [62].

The competition outcome varies with the performance [45] and resource availability of neighboring species [63]. For example, Italian ryegrass has a competitive advantage over common ragweed [64], while redroot pigweed shows higher competitiveness than *Phaseolus vulgaris* [65]. Competitiveness decreased with an increasing density of common ragweed. The highest competitiveness was under 1Rag:3Pig, where the relative yield of common ragweed increased to 350%. Results indicated interspecific and intraspecific generality, as detected in other experiments [4]. It is also possible that release from intraspecific competition allows common ragweed to grow higher and more robust. In contrast, their biomass decreased when redroot pigweed was released from the intraspecific competition (Figures 1 and 2). Intra- or con-specific competition is always stronger than interspecific one [66,67]. Previous studies have shown that elevated CO$_2$ and N deposition might favor performances of invasive plants relative to that of native species [3]. Our results revealed elevated CO$_2$ and increased N prefer the super competitor when two notorious alien invasive plants grew together.

5. Conclusions

The purpose of this study was to understand the differences in growth characteristics of two alien invasive plants under elevated CO$_2$, increased N, and species replacement. The results showed that the biomass of common ragweed was positively enhanced by
increased N and species replacement, but redroot pigweed was negatively inhibited. The relative yield of these two notorious invasive plants revealed a competition interaction. In addition, our results revealed that common ragweed gained a more competitive advantage than redroot pigweed under increased N and elevated CO₂. Our results indicated that common ragweed may be replaced redroot pigweed in heavily invaded regions. The competition or coexistence of redroot pigweed and common ragweed should be discussed under different invasion stages in the future, especially under environmental change. Moreover, understanding the interaction of invasive alien species will help us to manage multispecies invasions in the future.

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References

1. Davidson, A.M.; Jennions, M.; Nicotra, A.B. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecol. Lett. 2011, 14, 419–431. [CrossRef] [PubMed]
2. Liu, Y.; van Kleunen, M. Responses of common and rare aliens and natives to nutrient availability and fluctuations. J. Ecol. 2017, 105, 1111–1122. [CrossRef]
3. Liu, Y.; Oduor, A.M.O.; Zhang, Z.; Manea, A.; Tooth, I.M.; Leishman, M.R.; Xu, X.; van Kleunen, M. Do invasive alien plants benefit more from global environmental change than native plants? Glob. Chang. Biol. 2017, 23, 3363–3370. [CrossRef] [PubMed]
4. Dukes, J.S.; Mooney, H.A. Does global change increase the success of biological invaders? Trends Ecol. Evol. 1999, 14, 135–139. [CrossRef]
5. Mozdzer, T.J.; Langley, J.A.; Mueller, P.; Megonigal, J.P. Deep rooting and global change facilitate spread of invasive grass. Biol. Invasions 2016, 18, 2619–2631. [CrossRef]
6. Sorte, C.J.B.; Ibanez, I.; Blumenthal, D.M.; Molinari, N.A.; Miller, L.P.; Grosholz, E.D.; Diez, J.M.; D’Antonio, C.M.; Olden, J.D.; Jones, S.J.; et al. Poised to prosper? A cross-system comparison of climate change effects on native and non-native species performance. Ecol. Lett. 2013, 16, 261–270. [CrossRef]
7. Dukes, J.S.; Chiaranzo, N.R.; Loarie, S.R.; Field, C.B. Strong response of an invasive plant species (Centaura solstitialis L.) to global environmental changes. Ecol. Appl. 2011, 21, 1887–1894. [CrossRef]
8. Ziska, L.H. Evaluation of the growth response of six invasive species to past, present and future atmospheric carbon dioxide. J. Exp. Bot. 2003, 54, 395–404. [CrossRef]
9. Weltzin, J.F.; Beelte, R.T.; Sanders, N.J. Biological invaders in a greenhouse world: Will elevated CO₂ fuel plant invasions? Front. Ecol. Environ. 2003, 1, 146–153. [CrossRef]
10. Smith, S.D.; Huxman, T.E.; Zitzer, S.F.; Charlet, T.N.; Housman, D.C.; Coleman, J.S.; Fenstermaker, L.K.; Seemann, J.R.; Nowak, R.S. Elevated CO₂ increases productivity and invasive species success in an arid ecosystem. Nature 2000, 408, 79–82. [CrossRef]
11. Stewart, J.; Potvin, C. Effects of elevated CO₂ on an artificial grassland community: Competition, invasion and neighbourhood growth. Funct. Ecol. 1996, 10, 157–166. [CrossRef]
12. Smith, S.D.; Charlet, T.N.; Zitzer, S.F.; Abella, S.R.; Vanier, C.H.; Huxman, T.E. Long-term response of a Mojave Desert winter annual plant community to a whole-ecosystem atmospheric CO₂ manipulation (FACE). Glob. Change Biol. 2014, 20, 879–892. [CrossRef] [PubMed]
13. Wan, L.-Y.; Qi, S.-S.; Zou, C.B.; Dai, Z.-C.; Ren, G.-Q.; Chen, Q.; Zhu, B.; Du, D.-L. Elevated nitrogen deposition may advance invasive weed, Solidago canadensis, in calcareous soils. J. Plant Ecol. 2019, 12, 846–856. [CrossRef]
14. He, W.-M.; Yu, G.-L.; Sun, Z.-K. Nitrogen deposition enhances Bromus tectorum invasion: Biogeographic differences in growth and competitive ability between China and North America. Ecography 2011, 34, 1059–1066. [CrossRef]
15. Zhang, Y.; Ding, W.; Cai, Z.; Valerie, P.; Han, F. Response of methane emission to invasion of Spartina alterniflora and exogenous N deposition in the coastal salt marsh. Atmos. Environ. 2010, 44, 4588–4594. [CrossRef]
16. Limpens, J.; Berendse, F.; Klee, H. N deposition affects N availability in interstitial water, growth of Sphagnum and invasion of vascular plants in bog vegetation. New Phytol. 2003, 157, 339–347. [CrossRef]
17. Lei, Y.B.; Wang, W.B.; Feng, Y.L.; Zheng, Y.L.; Gong, H.D. Synergistic interactions of CO2 enrichment and nitrogen deposition promote growth and ecophysiological advantages of invading Eupatorium adenophorum in Southwest China. Planta 2012, 236, 1205–1213. [CrossRef]
18. Nackley, L.; Hough-Snee, N.; Kim, S.H. Competitive traits of the invasive grass Arundo donax are enhanced by carbon dioxide and nitrogen enrichment. Weed Res. 2017, 57, 67–71. [CrossRef]
19. Shea, K.; Chesson, P. Community ecology theory as a framework for biological invasions. Trends Ecol. Evol. 2002, 17, 170–176. [CrossRef]
20. Russell, J.C.; Sataruddin, N.S.; Heard, A.D. Over-invasion by functionally equivalent invasive species. Ecology 2014, 95, 2268–2276. [CrossRef]
21. Kuebbing, S.E.; Nuñez, M.A. Negative, neutral, and positive interactions among nonnative plants: Patterns, processes, and management implications. Glob. Chang. Biol. 2015, 21, 926–934. [CrossRef] [PubMed]
22. Simberloff, D.; Von Holle, B. Positive Interactions of Nonindigenous Species: Invasional Meltdown? Biol. Invasions 1999, 1, 21–32. [CrossRef]
23. Jackson, M.C. Interactions among multiple invasive animals. Ecology 2015, 96, 2035–2041. [CrossRef] [PubMed]
24. Rauschert, E.S.J.; Shea, K. Competition between similar invasive species: Modeling invasional interference across a landscape. Popul. Ecol. 2017, 59, 79–88. [CrossRef]
25. Yang, Y.; Zhao, W.; Li, Z.; Zhu, S. Molecular Identification of a ‘Candidatus Phytoplasma ziziphi’-related Strain Infecting Amaranth (Amaranthus retroflexus L.) in China. J. Phytopathol. 2011, 159, 635–637. [CrossRef]
26. Rauschert, E.S.J.; Shea, K. Invasional interference due to similar inter- and intraspecific competition between invaders may affect management. Ecol. Appl. 2012, 22, 1413–1420. [CrossRef]
27. Wundrow, E.J.; Carrillo, J.; Gabler, C.A.; Horn, K.C.; Siemann, E. Facilitation and competition among invasive plants: A field experiment with alligatorweed and water hyacinth. PLoS ONE 2012, 7, e48444. [CrossRef]
28. Zhi, Y.; Li, H.; An, S.; Zhao, L.; Zhou, C.; Deng, Z. Inter-specific competition: Spartina alterniflora is replacing Spartina anglica in coastal China. Estuar. Coast. Shelf Sci. 2007, 74, 437–448. [CrossRef]
29. Gong, L.; Li, J.S.; Liu, X.Y.; Zhao, X.J.; Zhao, C.Y. Analysis of invasive alien species in Chinese national nature reserves. Ecol. Sci. 2017, 36, 210–216. [CrossRef]
30. Gentili, R.; Ambrosini, R.; Montagnani, C.; Caronni, S.; Citterio, S. Effect of Soil pH on the Growth, Reproductive Investment and Pollen Allergenicity of Amaranthus retrofexus L. Front. Plant Sci. 2018, 9, 1335. [CrossRef]
31. Ziska, L.H.; Caulfield, F.A. Rising CO2 and pollen production of common ragweed (Ambrosia artemisiifolia), a known allergy-inducing species: Implications for public health. Aust. J. Plant Physiol. 2000, 27, 893–898. [CrossRef]
32. Liu, X.Y.; Li, J.S.; Zhao, C.Y.; Quan, Z.Z.; Zhao, X.J.; Gong, L. Prediction of potential suitable area of Ambrosia artemisiifolia L. in China based on MaxEnt and ArcGIS. J. Plant Protection 2016, 43, 1041–1048. [CrossRef]
33. Valerio, M.; Tomecek, M.B.; Lovelli, S.; Ziska, L.H. Quantifying the effect of drought on carbon dioxide-induced changes in competition between a C3 crop (tomato) and a C4 weed (Amaranthus retrofexus). Weed Res. 2011, 51, 591–600. [CrossRef]
34. Rezaie, F.; Yarnia, M. Allelopathic effects of Chenopodium album, Amaranthus retrofexus and Cynodon dactylon on germination and growth of safflower. J. Food Agric. Environ. 2009, 7, 516–521.
35. Wang, C.; Zhou, J.; Liu, J.; Jiang, K. Differences in functional traits between invasive and native Amaranthus species under different forms of N deposition. Naturwissenschaften 2017, 104, 59. [CrossRef]
36. Ma, J.; Xing, G.; Yang, W.; Ma, L.; Gao, M.; Wang, Y.; Han, Y. Inhibitory effects of leachate from Eupatorium adenophorum on germination and growth of Amaranthus retrofexus and Chenopodium glucocarpum. Acta Ecol. Sin. 2012, 32, 50–56. [CrossRef]
37. Qin, Z.; Zhang, J.E.; Jiang, Y.P.; Wei, H.; Wang, F.G.; Lu, X.N.; Clements, D. Invasion process and potential spread of Amaranthus retrofexus in China. Weed Res. 2018, 58, 57–67. [CrossRef]
38. Marten, G.C.; Andersen, R.N. Forage Nutritive Value and Palatability of 12 Common Annual Weeds1. Crop Sci. 1975, 15, 821–827. [CrossRef]
39. Lei, T.; Cui, G.F.; Sha, H.F.; Li, F.L.; Yan, J.H. Diversity and community distribution characteristics of alien vascular plant species in wetlands of Beijing. J. Beijinig For. Unitis. 2010, 32, 51–57. [CrossRef]
40. IPCC. IPCC Fourth Assessment Report: Climate Change 2007. Available online: https://www.ipcc.ch/assessment-report/ar4/ (accessed on 9 October 2022).
41. Galloway, J.; Townsend, A.; Erisman, J.W.; Bekunda, M.; Cai, Z.; Freney, J.; Martinelli, L.; Seitzinger, S.; Sutton, M. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. Science 2008, 320, 889–892. [CrossRef]
42. Lu, G.; Wang, J.; Sang, W. Effects of nitrogen deposition on invasive and competitive abilities of an alien plant Ambrosia artemisiifolia. J. Northeast Univ. 2012, 40, 60–66. [CrossRef]
43. Williams, A.C.; McCarthy, B.C. A new index of interspecific competition for replacement and additive designs. Ecol. Res. 2001, 16, 29–40. [CrossRef]
44. Weigelt, A.; Jolliffe, P. Indices of plant competition. J. Ecol. 2003, 91, 707–720. [CrossRef]
45. Skalova, H.; Jarosik, V.; Dvorackova, S.; Pysek, P. Effect of Intra- and Interspecific Competition on the Performance of Native and Invasive Species of Impatiens under Varying Levels of Shade and Moisture. PLoS ONE 2013, 8, e62842. [CrossRef]
46. Wang, C.; Cheng, H.; Wei, M.; Wang, S.; Wu, B.; Du, D. Plant height and leaf size: Which one is more important in affecting the successful invasion of Solidago canadensis and Conyza canadensis in urban ecosystems? Urban For. Urban Green. 2021, 59, 127033. [CrossRef]
47. Rajcan, I.; AghaAlikhani, M.; Santantonio, C.J.; Tollenaar, M. Development of redroot pigweed is influenced by light spectral quality and quantity. Crop Sci. 2002, 42, 1930–1936. [CrossRef]
48. Chu, C.-C.; Ludford, P.M.; Ozbun, J.L.; Sweet, R.D. Effects of Temperature and Competition on the Establishment and Growth of Redroot Pigweed and Common Lambsquarters. Crop Sci. 1978, 18, 308–310. [CrossRef]
49. Belote, R.T.; Weltzin, J.F. Interactions between two co-dominant, invasive plants in the understory of a temperate deciduous forest. Biol. Invasions 2006, 8, 1629–1641. [CrossRef]
50. Griffen, B.D.; Guy, T.; Buck, J.C. Inhibition between invasives: A newly introduced predator moderates the impacts of a previously established invasive predator. J. Anim. Ecol. 2008, 77, 32–40. [CrossRef]
51. Blackshaw, R.E.; Brandt, R.N.; Janzen, H.H.; Entz, T.; Grant, C.A.; Derksen, D.A. Differential response of weed species to added nitrogen. Weed Sci. 2003, 51, 532–539. [CrossRef]
52. Blumenthal, D.M.; Kray, J.A.; Ortmans, W.; Ziska, L.H.; Pendall, E. Cheatgrass is favored by warming but not CO\textsubscript{2} enrichment in a semi-arid grassland. Glob. Chang. Biol. 2016, 22, 3026–3038. [CrossRef] [PubMed]
53. Leskovšek, R.; Datta, A.; Knezevic, S.Z.; SimonČič, A. Common ragweed (Ambrosia artemisiifolia) dry matter allocation and partitioning under different nitrogen and density levels. Weed Biol. Manag. 2012, 12, 98–108. [CrossRef]
54. Kimball, B.A.; Kobayashi, K.; Bindi, M. Responses of agricultural crops to free-air CO\textsubscript{2} enrichment. In Advances in Agronomy; Sparks, D.L., Ed.; Elsevier Science: Amsterdam, The Netherlands, 2002; Volume 77, pp. 293–368.
55. Keser, L.H.; Visser, E.J.; Dawson, W.; Song, Y.B.; Yu, F.H.; Fischer, M.; Dong, M.; van Kleunen, M. Herbaceous plant species invading natural areas tend to have stronger adaptive root foraging than other naturalized species. Front. Plant Sci. 2015, 6, 273. [CrossRef] [PubMed]
56. Keser, L.H.; Visser, E.J.; Dawson, W.; Song, Y.B.; Yu, F.H.; Fischer, M.; Dong, M.; van Kleunen, M. Invasive clonal plant species have a greater root-foraging plasticity than non-invasive ones. Oecologia 2014, 174, 1055–1064. [CrossRef] [PubMed]
57. Poorter, H.; Nagel, O. The role of biomass allocation in the growth response of plants to different levels of light, CO\textsubscript{2}, nutrients and water: A quantitative review. Aust. J. Plant Physiol. 2000, 27, 595–607. [CrossRef]
58. Ward, J.K.; Tissue, D.T.; Thomas, R.B.; Strain, B.R. Comparative responses of model C\textsubscript{3} and C\textsubscript{4} plants to drought in low and elevated CO\textsubscript{2}. Glob. Change Biol. 1999, 5, 857–867. [CrossRef]
59. Weller, S.; Florentine, S.; Javaid, M.M.; Welgama, A.; Chauhan, B.S.; Turville, C. Amaranthus retroflexus L. (Redroot Pigweed): Effects of Elevated CO\textsubscript{2} and Soil Moisture on Growth and Biomass and the Effect of Radiant Heat on Seed Germination. Agron. Basel 2021, 11, 728. [CrossRef]
60. Peacey, R.W.; Ehleringer, J. Comparative ecophysiology of C\textsubscript{3} and C\textsubscript{4} plants. Plant Cell Environ. 1984, 7, 1–13. [CrossRef]
61. Poorter, H. Interspecific Variation in the growth-response of plants to an elevated ambient CO\textsubscript{2} concentration. Vegetatio 1993, 104, 77–97. [CrossRef]
62. Bae, J.; Byun, C.; Gyong, A.Y.; Choi, J.H.; Lee, D.; Kang, H. Effect of elevated atmospheric carbon dioxide on the allelopathic potential of common ragweed. J. Ecol. Environ. 2019, 43, 212–218. [CrossRef]
63. Lonsdale, W.M. Global patterns of plant invasions and the concept of invasibility. Ecology 1999, 80, 1522–1536. [CrossRef]
64. Leskovšek, R.; Elter, K.; Batič, F.; Simončič, A. The influence of nitrogen, water and competition on the vegetative and reproductive growth of common ragweed (Ambrosia artemisiifolia L.). Plant Ecol. 2012, 213, 763–781. [CrossRef]
65. Amini, R.; Alizadeh, H.; Yousefi, A. Interference between red kidneybean (Phaseolus vulgaris L.) cultivars and redroot pigweed (Amaranthus retroflexus L.). Eur. J. Agron. 2014, 60, 13–21. [CrossRef]
66. Golivets, M.; Wallin, K.F. Neighbour tolerance, not suppression, provides competitive advantage to non-native plants. Ecol. Lett. 2015, 18, 714–725. [CrossRef]
67. HilleRisLambers, J.; Harpole, W.S.; Tilman, D.; Knops, J.; Reich, P.B. Mechanisms responsible for the positive diversity-productivity relationship in Minnesota grasslands. Ecol. Lett. 2004, 7, 661–668. [CrossRef]