Effects of Drought Stress on Annual Herbaceous Plants under Different Mixed Growth Conditions in Desert Oasis Transition Zone of the Hexi Corridor

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Abstract: Annual herbaceous plants are frequently layered under the artificial sand-fixing forest within the desert oasis transition zone of the Hexi Corridor. The effect of drought stress on annual herbaceous plants is of great significance to the restoration of artificial vegetation as well as the stability of the ecosystem in the desert oasis transition zone. Setaria viridis, Chloris virgata, Halogeton arachnoideus, and Bassia dasyphylla are the typical annual herbaceous plants that occur naturally in the Caragana korshinskii forest and were used as the research subject in this study. Concentration gradient tests were conducted under different mixed growth conditions: 0 (blank control group), 5, 10, and 15 C. korshinskii seeds, and different drought stress conditions: 0%, 2%, 5%, 10%, and 15%, in order to explore the interactive effects of drought stress on annual herbaceous plants. The results demonstrated that the germination percentage and germination rate of annual herbaceous plants was significantly affected by the number of C. korshinskii seeds (p < 0.05), whereby the germination effect was optimal when no C. korshinskii seeds were present. Furthermore, we found that the germination percentage and germination rate of the annual Gramineae was higher than that of the Chenopods. In the growth stage, the biomass and root-shoot ratio of the chenopods were significantly affected by the number of C. korshinskii seeds and drought stress (p < 0.05). We found that the biomass of annual herbaceous plants was the highest at 2% drought stress, and the root-shoot ratio displayed a positive correlation with an increase in drought stress. Notably, the survival rate of annual herbaceous plants was higher when grown in combination with five C. korshinskii seeds, thus indicating a positive interaction; in contrast, the survival rate decreased significantly when they were grown in combination with more than five C. korshinskii seeds, indicating a negative interaction. We observed a decreasing trend in root activity and chlorophyll content when annual herbaceous plants were grown in combination with an increasing number of C. korshinskii seeds and drought stress. The reduced root activity and decline in photosynthetic ability resulted in the inhibition of seedling growth. Furthermore, we found that the root activity and chlorophyll content of the Gramineae was ~1.3–2.0 times higher than that of the Chenopods, which may be the reason behind the lower survival rate of the chenopods.

Keywords: arid desert area; annual herbaceous plants; drought stress; mixed growth; physiological

1. Introduction

Annual herbaceous plants are frequently layered under artificial sand-fixing forests, as they play an essential role in the construction and stabilization of artificial vegetation communities in the transition zone [1]. They invade and settle in large numbers as pioneer species after the establishment of artificial sand-fixing vegetation in the desert oasis.
transition zone, and the biomass increases along with the number of years of plantation, thereby leading to the formation of a mixed community in artificial sand-fixing vegetation systems. These mixed communities are of great ecological importance for the maintenance of stability in the ecosystem, owing to their efficient use of limited water resources and resistance to high temperature and drought stress [2].

Previous studies have focused on the growth, reproduction, and physiology of individuals and populations of artificial sand-fixing vegetation as well as the community responses to biotic and abiotic stresses in arid desert areas. For example, in terms of biotic stressors, Friedman et al. [3] observed a low emergence and high mortality in seedlings of the same species when grown in areas surrounding adult plants in the desert. However, when the adults were removed, the biomass and survival of seedlings increased. Zhang [4] conducted a study on the spatial distribution pattern of the desert plant, *Reaumuria soongorica*, and indicated that these species show an aggregated distribution that is subject to spatial heterogeneity with respect to seed distribution patterns and moisture conditions at larger scales, and the intense water competition among plant individuals creates a spatial pattern of uniform distribution of *Reaumuria soongorica* on smaller scales; Went [5] statistically demonstrated complex interspecific relationships between annuals and shrubs in the Mojave and Sonora Deserts. In addition to the effects of biotic stresses, the effects of abiotic stress factors (moisture conditions, light, temperature, sand burial, etc.) have also been studied extensively by a large number of scholars. Zhou et al. [6] demonstrated that *Nitraria sphaeroarpa* is able to access deep soil water and groundwater as the main source of hydration in habitats with low rates of precipitation, by studying the response of *Nitraria sphaeroarpa* to precipitation in two desert habitats. Han et al. [7] argued that a decrease in the light saturation point (LSP) is the main reason for the change in light demand in four types of sandy shrubs that are found in the northwest of China’s sandy region under arid conditions. Yan et al. [8] demonstrated that the seed germination percentage of *Caragana korshinskii* was at a maximum of 15 °C and gradually decreased as the temperature continued to rise. Wang et al. [9] found that 2 cm is an optimal burial depth for the germination and growth of *Haloxylon ammodendron*, which contributed to the regeneration of the *H. ammodendron* population. However, there are relatively few studies on the interrelationships between dominant species and annual herbaceous plants in plantation forests, specifically with respect to the effects of different stress conditions on the plant relationships in mixed communities.

The Hexi Corridor is a typical desert oasis area in northwest China, with a variable and arid climate, as well as sparse natural vegetation. There are many natural and artificial oases of different shapes and sizes in the vast desert, which are mainly irrigated by water from the Qilian Mountains. Due to the surrounding deserts (Tengger Desert, Badangilin Desert, and Kumutag Desert), severe desertification has occurred at the edge of some of the oases. To prevent further degradation of the oasis areas, artificial sand-fixing vegetation has been widely established with dominant species such as *C. korshinskii* and *H. ammodendron*, as these plants are generally drought tolerant, resistant to wind, and fix sand effectively. Numerous studies have found that herbaceous annuals are able to successively invade and proliferate amongst the artificial sand fixation vegetation, especially a few years after planting. In addition, herbaceous annuals such as *Setaria viridis*, *Chloris virgata*, *Halogoton arachnoideus*, and *Bassia dasyphylla* eventually became the dominant plant taxa in these artificial sand-fixing communities [10]. Aeolian sand activity is affected by dune microtopography, and plant seeds, fine aeolian sand particles, and plant defoliation all gather in the dune lowland, making the lowlands become independent units formed naturally. Dune slacks provide a better living environment for certain plants, owing to less soil moisture, nutrients, and wind and sand activity; however, this results in the concentration of a large number of plants in a limited space, thereby increasing the competition between plant species. Perennial shrubs severely deplete soil water in the deeper regions (50–500 cm), thus causing the deeper soils to become arid layers [11]. This results in the formation of physical or biological crusts on the sand surface and leads to competition between herbaceous
annuals and shrubs for shallow water, which is intensified during the seedling growth stage. The study of the interrelationship between annual plants and dominant plant species in the seedling stage, which are planted on the dune slacks under an arid environment, is important in order to reveal the interspecific association between artificial sand-fixing vegetation communities and the vegetation succession pattern. Here, we simulated the interaction between dominant plants and annual herbaceous plants under arid conditions. We observed the changes in the germination, growth, and physiology of annual herbaceous plants when grown in combination with various numbers of Calligonum korshinskii seeds under drought-stressed conditions. We believe that the results of our study can provide a scientific basis for the regeneration of desert plant populations and the restoration and reconstruction of sandy soil vegetation.

2. Materials and Methods

2.1. Overview of the Study Area

The study area is located in Linze County in the central part of Gansu’s Hexi Corridor (100°07′ E, 39°21′ N; altitude: 1367 m), in the middle reaches of the Heihe River basin, and located at the edge of the oasis in the Batangilin Desert (Figure 1). The area is characterized by a temperate continental desert climate with scarce and concentrated precipitation. The average annual precipitation is 117.1 mm and is mainly concentrated from June to September. The evaporation rate is high (annual evaporation = 2337.6 mm), with an annual average temperature of 7.6 °C. The maximum air temperature is 39.1 °C, and the minimum temperature is −27 °C. The annual sunshine hours in the area are 3045 h, and the frost-free period is 105 d per year. The average wind speed is 3.2 m·s⁻¹, where the main wind direction is northwest and is mainly concentrated from March to May. The soil is predominantly gray-brown desert soil (zonal soil) and wind-sand soil. The soil texture in the study area was coarse, with particles of ~0.05–0.25 mm, which accounted for 80–90% of the total. The desert oasis transition zone was mainly planted with artificial sand-fixing vegetation, including Haloxylon ammodendron and Calligonum korshinskii as the dominant species. The naturally occurring vegetation included Calligonum mongolicum, Nitraria sphaerocarpa, C. virgata, Halogeton arachnoideus, Baccharis dasyphylla, and other annual herbaceous plants.

![Figure 1. Location of the study area.](image-url)
2.2. Experimental Design

Test seeds were collected in September 2020 from an area near the Linze Inland River Basin Integrated Research Station of the Chinese Academy of Sciences in Linze County, central Hexi Corridor, Gansu Province. Mature and intact seeds were collected from different mother plants. The collected seeds were cleaned, left to dry naturally, and then placed in plastic pots in the laboratory. Germination tests were conducted in the laboratory using germination bags 35 cm in length and 25 cm in width. A total of 40 randomly selected full-seeded annuals (Gramineae: 10 seeds each of S. viridis and C. virgata; Chenopods: 10 seeds each of H. arachnoideus and B. dasyphylla) were mixed with 0, 5, 10, and 15 C. korshinskii seeds in order to simulate different mixing conditions, which was performed by spreading them evenly in the bag. Approximately 20 mL of water was added in order to keep the filter paper in the sprouting bag well moistened. The greenhouse temperature was controlled and set at 23 °C during the day and 14 °C at night. Germination was considered to have started when the first sprouts began to appear above the soil surface. After the start of germination, the number of sprouted seeds was recorded daily. After emergence, precipitation of 5 mm was simulated, which was based on the local precipitation rate from June to September (mainly small precipitation events of ~5 mm) [12]. Polyethylene glycol (PEG) is a strong hydrophilic organic substance that is widely used in drought tolerance studies. PEG-6000 solution was used to simulate drought stress in this study, as it is simple and easy to use [13,14]. Drought stress was initiated when three to four true leaves appeared on the sprouted seeds [15]. PEG-6000 solutions, prepared by mixing a mass of PEG-6000 with distilled water, with different osmotic potentials were prepared in a concentration gradient of 0%, 2%, 5%, 10%, and 15%, and recorded as PEG0%, PEG2%, PEG5%, PEG10%, and PEG15% respectively. The osmotic potentials for each concentration were calculated using the equation described by Michel et al. [16] and were as follows: 0, −0.002, −0.06, −0.17, −0.32 MPa, respectively. The filter paper was kept moist during the study and seedling survival was recorded every 2 d. The number of seedlings was counted until the end of the study. Each treatment was replicated five times and the trial period was 30 d. After the tests were complete, the samples were stored in a low-temperature refrigerator at 4 °C and the indices were calculated using the formula below.

\[
\Psi_s = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T
\]

\(\Psi_s\) is the osmotic potential of the solution (bar), 1 bar = 0.1 MPa; \(C\) is the PEG-6000 solution concentration (g·kg\(^{-1}\)); \(T\) is the solution temperature (20 °C).

2.3. Measurement Methods for Indicators

Growth index determination: The direct measurement method was used to measure the length of the stem and roots, with a straightedge accuracy of 0.01 cm. Stem length was measured from above the roots, while the main root was measured to obtain the root length. The total biomass was measured by fresh weight and weighed on a 1/1000 balance. Germination percentage was defined as the percentage of seeds that germinated within the total number of seeds used in the test. Survival rate was defined as the percentage of seedlings that survived until the end of the study within the maximum number of sprouts. The relevant indicators are calculated using the following formula.

\[
\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Number of seeds for testing}} \times 100\% \quad (1)
\]

\[
\text{Germination rate} = \Sigma (100G_i/nt_i) \quad (2)
\]

\[
\text{Root – shoot ratio} = \frac{\text{root biomass}}{\text{aboveground biomass}} \quad (3)
\]

\[
\text{Survival rate} = \frac{\text{final number of germinating seeds}}{\text{maximum number of germinating seeds}} \times 100\% \quad (4)
\]
In Formula (2): \( n \) is the number of seeds used per treatment, \( G_i \) is the number of germinating seeds in \( t_i \) (\( t_i = 0, 1, 2, 3, \ldots, \infty \) days); the higher the germination rate, the faster the seeds germinate [17].

Determination of physiological parameters: root vitality was determined using the TTC (2,3,5-Triphenyltetrazolium chloride) method. TTC solution and phosphate buffer were added to ~0.1 g of fresh leaf sample and then kept in a dark, artificial climate chamber at 37 °C for 3 h. Subsequently, 2 mL of 1 mol·L\(^{-1}\) sulfuric acid solution was added to the mixture in order to terminate the reaction. Thereafter, water-absorbing paper was used to remove all surface water and the resulting pulp was ground. The absorbance was then measured at 485 nm and root vitality \([\text{mg} \cdot (\text{g} \cdot \text{h})^{-1}]\) was calculated. Next, the chlorophyll content was determined using the 80% acetone method. Briefly, 0.1 g of fresh leaf sample was selected and macerated well. Chlorophyll was extracted by immersing the macerated leaf material in 80% acetone solution at room temperature of 20 °C, in a dark environment for 24 h. The absorbance of the extracts was measured at 663 nm, 646 nm, and 470 nm, and the chlorophyll content \((\text{mg} \cdot \text{g}^{-1})\) was calculated using the Lambert-Beer law [18].

2.4. Data Processing

The experimental data were analyzed using the SPSS 21.0 program. Duncan’s multiple range test, the LSD test, and One-Way and Two-way ANOVA were used to compare the differences in seed germination, survival, growth, and physiology under different drought stress treatments. The confidence interval was 0.95. The figures in the text were completed using Origin 2019 software.

3. Results and Analysis

3.1. Characteristics of Plant Germination under Different Mixed Conditions

We observed a significant change in the germination percentage and the germination rate of the annual herbaceous plants as the number of \( C. korshinskii \) seeds in the mixed plant community increased. The germination percentage of the four annual herbaceous species decreased as the number of \( C. korshinskii \) seeds increased from ~0–15 grains. The germination percentage of \( S. viridis \) decreased from 51.6% to 29.6%; \( C. virgata \) from 59.6% to 40.0%; \( H. arachnoideus \) from 21.2% to 13.2%; \( B. dasyphylla \) from 22.6% to 7.6% (Figure 2a). A decreasing trend was observed in the germination rate of \( S. viridis \) and \( C. virgata \) as the number of \( C. korshinskii \) seeds increased. The germination rate of \( S. viridis \) decreased from 34.4 to 26.8, while the germination rate of \( C. virgata \) decreased from 70.0 to 50.9, thus indicating only slight changes to the germination rate in the Chenopods \((p > 0.05)\). In contrast, the germination rate of \( H. arachnoideus \) (saltbush) increased slightly from 8.0 to 9.0, while the germination rate of \( B. dasyphylla \) increased slightly from 16 to 19 (Figure 2b), and the highest germination percentage and germination rate was observed in the \( C. virgata \) species.

3.2. Effects of Drought Stress on the Growth of Annual Plants under Different Mixed Conditions

3.2.1. Amount of Biomass

Table 1 shows us that a significant change in the biomass of the annual herbaceous plants resulted from changes in the number of \( C. korshinskii \) seeds and drought stress \((p < 0.01)\). Only slight changes to biomass were observed in the Gramineae; however, the Chenopods were significantly affected by the number of \( C. korshinskii \) seeds and drought stress \((p > 0.05)\). The biomass of annual herbaceous plants displayed an initial increase, followed by a decreasing trend as the severity of drought stress and \( C. korshinskii \) plant density increased. The highest biomass was observed in the group exposed to 0–2% drought stress; however, this began to decrease as the drought stress increased from ~2–15%, with the lowest biomass observed in the group exposed to 15% drought stress. The biomass was recorded for each species at different \( C. korshinskii \) planting densities as follows (0, 5, 10, and 15 seeds): \( S. viridis \), 1.30, 1.20, 0.93, and 0.72 g, respectively (Figure 3a); \( C. virgata \), 1.30, 1.30, 0.85, and 0.65 g, respectively (Figure 3b); \( H. arachnoideus \), 0.75, 10.75, 0.50, and 0.15 g, respectively (Figure 3c); \( B. dasyphylla \),
of 0.60, 0.50, 0.35, and 0.15 g, respectively. The highest value of biomass of *B. dasyphylla* under the influence of 15 *C. korshinskii* seeds was located at 0% drought stress (0.15 g) (Figure 3d).

![Figure 2. Effects of different seed density of Caragana korshinskii on germination of annual herbaceous plants](image)

**Figure 2.** Effects of different seed density of *Caragana korshinskii* on germination of annual herbaceous plants (a) germination percentage; (b) germination rate (Different letters represent significant differences in the number of seeds of the same plant, *Caragana korshinskii*).

**Table 1.** Two-way ANOVA on the effects of seed number of *Caragana korshinskii* and drought stress on each index of annual herbaceous plants.

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Plant Species</th>
<th><em>C. korshinskii</em> Number</th>
<th>Drought Stress</th>
<th><em>C. korshinskii</em> Number × Drought Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Biomass</td>
<td><em>S. viridis</em></td>
<td>3</td>
<td>13.236</td>
<td>&lt;0.01</td>
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<td></td>
<td><em>C. virgata</em></td>
<td>7</td>
<td>2.999</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td><em>H. arachnoideus</em></td>
<td>29.644</td>
<td>&lt;0.01</td>
<td>49.406</td>
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<tr>
<td></td>
<td><em>B. dasyphylla</em></td>
<td>24.561</td>
<td>&lt;0.01</td>
<td>32.890</td>
</tr>
<tr>
<td></td>
<td><em>S. korshinskii</em></td>
<td>37.877</td>
<td>&lt;0.01</td>
<td>39.560</td>
</tr>
<tr>
<td></td>
<td><em>C. virgata</em></td>
<td>133.251</td>
<td>&lt;0.01</td>
<td>223.270</td>
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<tr>
<td></td>
<td><em>H. arachnoideus</em></td>
<td>123.698</td>
<td>&lt;0.01</td>
<td>65.628</td>
</tr>
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<td><em>B. dasyphylla</em></td>
<td>0.407</td>
<td>0.749</td>
<td>15.470</td>
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<tr>
<td></td>
<td><em>S. korshinskii</em></td>
<td>0.509</td>
<td>0.678</td>
<td>12.853</td>
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<tr>
<td></td>
<td><em>C. virgata</em></td>
<td>13.812</td>
<td>&lt;0.01</td>
<td>8.412</td>
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<td><em>S. korshinskii</em></td>
<td>6.817</td>
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<td><em>B. dasyphylla</em></td>
<td>4.889</td>
<td>0.01</td>
<td>9.432</td>
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<td><em>C. virgata</em></td>
<td>29.707</td>
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<td>21.619</td>
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<td><em>S. korshinskii</em></td>
<td>16.844</td>
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<td>15.991</td>
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<td></td>
<td><em>H. arachnoideus</em></td>
<td>6.153</td>
<td>&lt;0.01</td>
<td>8.607</td>
</tr>
<tr>
<td></td>
<td><em>B. dasyphylla</em></td>
<td>57.240</td>
<td>&lt;0.01</td>
<td>15.386</td>
</tr>
<tr>
<td></td>
<td><em>C. virgata</em></td>
<td>27.544</td>
<td>&lt;0.01</td>
<td>13.279</td>
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<tr>
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<td>0.509</td>
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<td>27.544</td>
<td>&lt;0.01</td>
<td>13.279</td>
</tr>
</tbody>
</table>
3.2.2. Survival Rate

The survival rate of annual herbaceous plants was significantly affected by drought stress and the number of \textit{C. korshinskii} seeds ($p < 0.01$). Furthermore, drought stress significantly affected the number of \textit{C. korshinskii} seeds ($p < 0.05$). The survival rate of annual herbaceous plants demonstrated a decreasing trend with the increase of drought stress and \textit{C. korshinskii} plant density. The highest survival rate was observed at 0\% drought stress. The survival rate of different species at different \textit{C. korshinskii} seed numbers are as follows (0, 5, 10, and 15 seeds): \textit{S. viridis} displayed survival rates of 95.3\%, 100.0\%, 96.0\%, and 87.7\%, respectively (Figure 4a); \textit{C. virgata} displayed survival rates of 34.7\%, 100.0\%, 50.7\%, and 45.0\%, respectively (Figure 4b); \textit{H. arachnoideus} displayed survival rates of 83.7\%, 100.0\%, 100.0\%, and 55.3\%, respectively (Figure 4c); \textit{B. dasyphylla} displayed survival rates of 50.3\%, 86.0\%, 66.3\%, and 32.8\%, respectively (Figure 4d). Among them, the survival rate of Chenopods seedlings was 0\% when they were highly affected by drought stress and \textit{C. korshinskii} seed germination.

3.3. Effects of Drought Stress on the Root-Shoot Ratio of Annual Plants under Different Mixed Conditions

The results demonstrated that the root-to-crown ratio of annual herbaceous plants was significantly affected by drought stress ($p < 0.01$). Furthermore, the number of \textit{C. korshinskii} seeds combined with drought stress significantly affected the Chenopods sprouts ($p < 0.05$); however, it did not significantly affect the sprouts belonging to the Graminaceae family. We observed that with an increase in drought stress and \textit{C. korshinskii} planting density, the root-to-crown ratio of annual herbaceous plants tended to increase, with the highest ratio observed in the 15\% drought stress group. The root-to-crown ratios of the plant species in different mixed planting groups are as follows (0, 5, 10, and 15 \textit{C. korshinskii} seeds): \textit{S. viridis} had root-to-crown ratios of 0.70, 0.82, 0.79, and 0.80 respectively (Figure 5a); the root-to-crown ratios of \textit{C. virgata} were 0.76, 0.78, 0.60, and 0.75, respectively (Figure 5b); the root-to-crown ratios of \textit{H. arachnoideus} were 0.80 and 0.75, respectively (Figure 5c) and that of \textit{B. dasyphylla} was 0.76 (Figure 5d). Overall, the average root-shoot ratio of Chenopods seedlings was 0.81 when grown in drought-stressed conditions combined with \textit{C. korshinskii} seedlings.
3.4. Plant Physiology under Different Drought Stress Intensities

3.4.1. Root Vitality

We observed that drought stress and *C. korshinskii* planting density had a highly significant effect on the root vitality of annual herbaceous plants (*p* < 0.01). Our results indicated that *B. dasyphylla* was the most affected by drought stress and *C. korshinskii* planting density (*p* < 0.05), while the remaining three species were not significantly affected (*p* > 0.05). We found that the root vitality of annual herbaceous plants displayed a decreasing trend due...
to the increase in drought stress and \textit{C. korshinskii} seed numbers. In all of the annuals, the highest root vitality was observed at 0\% drought stress. The following root vitality values were recorded in each mixed planting group (0, 5, 10, and 15 \textit{C. korshinskii} seeds): the root vitality values for \textit{S. viridis} were 8.0 mg·(g·h)$^{-1}$, 7.7 mg·(g·h)$^{-1}$, 6.3 mg·(g·h)$^{-1}$, and 5.7 mg·(g·h)$^{-1}$, respectively (Figure 6a); those of \textit{C. virgata} were 7.8 mg·(g·h)$^{-1}$, 7.7 mg·(g·h)$^{-1}$, 6.0 mg·(g·h)$^{-1}$, and 5.6 mg·(g·h)$^{-1}$, respectively (Figure 6b); those of \textit{H. arachnoideus} were 4.2 mg·(g·h)$^{-1}$, 4.3 mg·(g·h)$^{-1}$, 3.7 mg·(g·h)$^{-1}$, and 3.0 mg·(g·h)$^{-1}$, respectively (Figure 6c); those of \textit{B. dasyphylla} were 4.3 mg·(g·h)$^{-1}$, 3.5 mg·(g·h)$^{-1}$, 2.4 mg·(g·h)$^{-1}$, and 2.0 mg·(g·h)$^{-1}$, respectively (Figure 6d). Overall, Chenopods seedlings suffered root mortality due to severe drought stress and \textit{C. korshinskii} seed germination.

3.4.2. Chlorophyll Content

Our results indicated that drought stress and \textit{C. korshinskii} planting density significantly affect the chlorophyll content in annual herbaceous plants ($p < 0.01$). \textit{B. dasyphylla} was the most affected by drought stress and \textit{C. korshinskii} planting density ($p < 0.05$), while the remaining three species were not significantly affected ($p > 0.05$). We observed a decrease in the chlorophyll content of annual herbaceous plants with the increase in drought stress and \textit{C. korshinskii} seed numbers. The highest chlorophyll content was found in the group exposed to 0\% drought stress. The following chlorophyll contents were recorded for each species in the different mixed planting groups (0, 5, 10, and 15, and \textit{C. korshinskii} seeds): the chlorophyll contents for \textit{S. viridis} were 0.80 mg·g$^{-1}$, 0.77 mg·g$^{-1}$, 0.58 mg·g$^{-1}$, and 0.51 mg·g$^{-1}$, respectively (Figure 7a); those for \textit{C. virgata} were 0.76 mg·g$^{-1}$, 0.74 mg·g$^{-1}$, 0.60 mg·g$^{-1}$, and 0.53 mg·g$^{-1}$, respectively (Figure 7b); those for \textit{H. arachnoideus} were 0.53 mg·g$^{-1}$, 0.50 mg·g$^{-1}$, 0.33 mg·g$^{-1}$, and 0.26 mg·g$^{-1}$, respectively (Figure 7c); and those for \textit{B. dasyphylla} were 0.55 mg·g$^{-1}$, 0.45 mg·g$^{-1}$, 0.31 mg·g$^{-1}$, and 0.20 mg·g$^{-1}$, respectively (Figure 7d). Among them, Chenopods seedlings died when they were more affected by drought stress and \textit{C. korshinskii} seed germination.
Figure 7. Effects of drought stress and seed number of Caragana korshinskii on chlorophyll content of annual herbaceous plants (a) Setaria viridis; (b) Halogeton arachnoideus; (c) Bassia dasyphylla; (d) Chloris virgata; N: seedling death.

4. Discussion

4.1. Effect of Different Mixing Conditions on the Germination of Annual Herbaceous Plants

Seed germination is a vital part of the annual plant life cycle and plays an essential role in the reproduction and settlement of plant species [19,20]. The results of this study demonstrated that under mixed conditions, the germination percentage and germination rate of annual herbaceous plants tended to decrease as the number of C. korshinskii seeds increased. C. korshinskii is a dominant shrub species used for sand fixation, and the water required for germination is much higher due to its larger seeds. Furthermore, an increased plant density is known to increase the competition for limited resources in an area, which results in a reduction of the germination percentage and germination rate in annual herbaceous plants. We found that a higher degree of clustering resulted in less competition. This is similar to the conclusions of Li [21], who studied the effects of weed clustering levels on crop competition. Furthermore, we found that annual herbaceous grasses displayed a better germination rate than did the Chenopods seedlings when planted in combination with C. korshinskii seeds, suggesting that Graminoids respond more rapidly to competition for resources during germination, and are able to facilitate the occupation of resources as seedlings [22]. The delayed germination of Chenopods seeds contributes to the seed bank and helps to reduce the risk of withering and drying due to sudden changes in environmental conditions, thus effectively reducing the loss of seedlings and creating an effective risk-sharing strategy [23,24]. We believe this to be an important mechanism for the adaptation of annual herbaceous plants to desert environments.

4.2. Effects of Different Mixed Planting and Drought Stress on the Growth and Survival of Annual Herbaceous Plants

Drought is the main environmental factor affecting plant seedling establishment and ecosystem stability in desert areas [25]. The results of this study indicated that the biomass of annual herbaceous plants initially increased and then began to decrease with an increase in drought stress, suggesting that mild drought stress (2%) is beneficial to the growth of annual herbaceous plants; however, growth is inhibited above a certain range (2%) and as the stress level increases, the inhibition becomes more pronounced [26]. These results are similar to those of Sang et al. [27], who studied the effects of drought stress...
on the growth of *Mchella liliflora* DC. seedlings. In addition, we observed less variation in the biomass of annual herbaceous plants at *C. korshinskii* seed numbers 0–5; however, a greater decline in biomass was observed in the presence of 10 or more *C. korshinskii* seeds. These results indicate that when *C. korshinskii* is present in small numbers, annual herbaceous plants will participate in a mutualistic relationship with the sand-fixing shrub. However, as the resource requirement for growth increases, along with a decrease in space, *C. korshinskii*’s claim on limited resources intensifies and the growth of annual herbaceous plants is inhibited.

Studies have demonstrated that populations that are spatially aggregated tend to be regulated by density constraints [28], and mortality is one way by which plants respond to population density [29]. In this study, we found that the combination of mixed planting and drought stress significantly affected the survival of annual herbaceous plants (*p* < 0.05). The survival rate of annual herbaceous plants decreased when drought stress and *C. korshinskii* seed numbers increased. A possible reason for this is the shallow roots of annual plants—during the early stages of seedling emergence, as they must effectively use the moisture of the rapidly drying surface sand layer in order to survive in artificial sand-fixation areas. If the plant density and total water demand are low, the individual roots can reach the wet sand layer before the surface sand layer dries; thus, plants can use the wet sand layer moisture to sustain their survival, and their mortality rate will be lower.

In contrast, the greater the population density and the demand for water, the faster the water in the surface sand layer will be used, resulting in a more rapid moisture decline; this will result in the root systems of many plants failing to reach the wet sand layer before the surface sand layer dries, thus leading to a higher individual mortality rate [30]. We also observed a parabolic trend in the survival rate of annual herbaceous plants when grown in combination with *C. korshinskii* under drought-stressed conditions. In the absence of *C. korshinskii* seeds, the survival rate of annual herbaceous plants was low; however, when the number of *C. korshinskii* seeds increased to five, the survival rate increased. This suggests that a certain degree of mixed community is likely to promote water and nutrient uptake by both plants compared to monocultures [31], due to the presence of a mutualistic relationship, which is characterized by positive interactions and promotion of development and growth. In contrast, when biotic stress exceeds a certain threshold, negative interactions and interspecific competition may occur. In this study, five *C. korshinskii* seeds could be considered as the turning point for positive and negative interactions, which is consistent with the asymmetric type of mutualism (biased symbiosis) among the different mutualism models explored by Lin et al. [32], who demonstrated that mixing of plant species under mild to moderate stress can increase plant survival, due to the improved stress gradient hypothesis (SGH) [33].

It has been shown that drought stress has a beneficial effect on the primary root growth of desert plants [34]. In the present study, we found that the root-to-crown ratio of annual herbaceous plants tended to increase with an increase in drought stress. This finding is consistent with the results obtained by Liu et al. [35], who studied the effects of drought stress on biomass allocation in *Glycyrrhiza uralensis* Fisch. This is because shallow-rooted annuals in desert environments are strongly responsive to water, and biomass allocation during drought-stressed conditions flows more to the roots in order to enable the plant to adapt to the arid environment. In this case, stem and leaf biomass becomes relatively low, thereby reducing water transpiration to maintain optimal growth [36], which is also consistent with the functional balance theory proposed by Brouwer [37]. Similar conclusions were reported by Zeng et al. [38] through drought stress studies on *C. korshinskii*, *Hedysarum scoparium*, and *Artemisia sphaerocephala*. Therefore, researchers have proposed that annual herbaceous seedlings deploy a more favorable strategy when resources are limited, that is, increasing the root-to-crown ratio to adapt to the environment. Furthermore, two-way ANOVA showed that the biomass and the root-to-crown ratio of Chenopods were significantly (*p* < 0.05) influenced by the combination of mixed planting and drought stresses. Both *C. korshinskii* and Chenopods are taproots, and the growth of
Chenopods is more susceptible to water predation by *C. korshinskii* due to overlapping ecological niches, demonstrating a negative interaction. This may be the reason for the lower biomass and root-to-crown ratio of Chenopods compared to Gramineae and even the high mortality rates of seedlings.

### 4.3. Effects of Different Mixes and Drought Stress on the Physiology of Annual Herbaceous Plants

Studies have shown that root vigor significantly affects water uptake, and the stronger the vigor, the more efficient the water uptake [39]. In this study, we found that the root vigor of annual herbaceous plants was the strongest under normal moisture conditions and gradually decreased with an increase in drought stress. When plants are subjected to drought stress, the root-to-crown ratio increases to ensure that the root system absorbs more water from the soil; however, as drought intensifies, plant root vigor is unable to meet the demands of seedling growth, ultimately leading to a decrease in the survival of the plants.

Chlorophyll content is a physiological indicator of plant sensitivity to drought stress. Researchers have found that drought stress causes a linear decrease in chlorophyll content by inhibiting chlorophyll synthesis and accelerating its breakdown [40]. In this study, the chlorophyll content of annual herbaceous plants decreased with an increase in drought stress and the number of *C. korshinskii* seeds present. It has been shown that chlorophyll content is reduced under moderate and severe drought-stressed conditions, causing severe effects on plant growth and metabolism [41]. In our trials, the root vigor and chlorophyll content of Gramineae were 1.3–2.0 times higher than those of Chenopods, which may be the reason for the lower survival rate of Chenopods compared to Gramineae. Two-way ANOVA indicated that root vigor and chlorophyll content were not significantly affected by mixed plantings and drought stress, with the exception of *B. dasyphylla*, which was significantly affected. This indicates that the interaction between rooting and drought on mixing and drought was not significant, and the interaction between the two may be a cumulative effect over time.

### 5. Conclusions

This study showed that the germination percentage and germination rate of annual herbaceous plants varied significantly under mixed growth conditions. The high germination percentage of Gramineae indicated that these plants prioritize essential resources, while the delayed germination of Chenopods reduces its risk of withering and drying due to sudden changes in environmental conditions. The interaction effect of mixed planting and drought stress significantly affected the growth and survival of annual Chenopod seedlings. The moderate density of interplant mixing increased plant survival, while high density beyond the threshold resulted in a decreased survival rate. Under drought-stressed conditions, annual herbaceous plants can increase root biomass input, but the root vigor and chlorophyll content decrease with the increase of drought stress, which eventually affects photosynthesis and the effective growth of seedlings. Therefore, among the annual herbaceous plants, annual Gramineae plants are more vigorous, while annual Chenopods plants with taproots are susceptible to water depletion by artificial *C. korshinskii*, which are also taproots; due to this, we suggest establishing mixed plants with different types of root during plantation construction and ecological restoration.

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