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Effects of Water and Nitrogen on Eco-physiological Characteristics of Two Dominant Grassland Species on the Semiarid Loess Plateau

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Abstract:

To investigate the eco-physiologcial traits in response to water and nitrogen supplying conditions of native species is of importance for understanding their eco-adaptation to the local environment, and also finding rational agronomy measures for using them in artificial grassland construction. In this study, using *Bothriochloa ischaemum* (C4 grass) and *Lespedeza davurica* (C3 legume) as research materials, a pot experiment was conducted to compare the leaf photosynthetic traits, leaf fluorescence parameter characteristics and leaf relative water content of the two dominant native species under three different soil moisture conditions (80%FC, 60%FC and 40%FC) and two nitrogen supplying (0 g N kg^{-1} and 0.025 g N kg^{-1}) conditions when sown individually or in mixture. Results showed that as soil water content decreased from 80% FC (field capacity) to 40% FC, leaf net photosynthetic rate (*Pn*), transpiration rate (*Tr*), intercellular CO_2 concentration (*Ci*), and stomatal conductance (*Gs*) of monocultured plants showed an increasing trend, while the photosynthetic parameters of mixculture plants generally exhibited a decreasing trend. The *Pn* values of *L. davurica* were significantly higher than those of *B. ishchaemum* ($P<0.05$), and the maximum value was 12.50 mmol m^{-2} s^{-1}. As soil water content decreased, the initial fluorescence values (*Fo*), maximum potential quantum yield of PSII (*Fv/Fo*), and maximum photochemical efficiency (*Fv/Fm*) values under no nitrogen treatment showed no significant change ($P>0.05$). Overall, the data indicated that when *B. ischaemum* and *L. davurica* were mixcultured, there existed a significant mutualistic effect under low soil water situation, indicating mixculture of *B. ischaemum* and *L. davurica* was beneficial for coping with drought in the semiarid region.

Keywords: Photosynthetic rate, transpiration rate, leaf relative water content, water stress, nitrogen, *Bothriochloa ischaemum*, *Lespedeza davurica*

1. Introduction

Climate change has induced great impact globally on terrestrial ecosystem that cannot be ignored, such as the long-term trend of increasing air average temperature and CO_2 concentration (Malhi et al., 2020). Rainfall pattern also has changed, and which greatly alter the growth process of plants, as well as bring atmospheric nitrogen compounds into the earth surface through precipitation and dry deposition. Changes in water conditions would alter the eco-adaptation of local plant species to the environment and affect their growth and distribution. Increased nitrogen deposition can alter soil nitrogen availability, and which would affect the uptake and utilization of nitrogen by plants, causing changes in the community composition and natural distribution patterns of species (Rivero-Villar et al., 2021), and all these will affect the stability and succession trend of terrestrial ecosystems.

The response of plant communities in response to climate change mainly depends on the response of dominant species. Dominant species are the components that determine the formation, structure and function of the community (Avolio et al., 2019). In the context of altered rainfall patterns and increased nitrogen deposition in grasslands, the main ecological process would be changed, such as plant photosynthesis, biomass production accumulation, and species composition and diversity (Carly et al., 2022). At the same time, the distribution pattern of dominant plant species will be also altered, and their spatial aggregation would change under climate change conditions (Bai et al., 2021). Therefore, studying the adaptation to water and nutrient conditions is beneficial for clarifying the mechanisms for the distribution patterns of dominant species, and also the rational planting methods during artificial grassland construction (Zhang et al., 2022).

Grassland construction is an important part of vegetation rehabilitation measures in terrestrial ecosystems. Nowadays, the main problems facing grassland construction in China include soil desiccation caused by irrational use of plant species during vegetation rehabilitation due to limited water resources (Shao et al., 2015), negative environmental effects caused by excessive revegetation, separation of production and ecological functions of artificial grassland, and lack of grassland models with special characteristics (Li et al., 2016). At this stage, long-term observation and analysis of eco-physiological characteristics such as water-use and requirement and eco-adaptability to drought or water stress is important, which can provide a basis for species choosing and grassland construction (Shao et al., 2015). Therefore, the selection of appropriate species and the appropriate planting method are important to be considered.

Adaptation assessments of plant species in dry regions from drought-resistant and eco-physiological perspectives are commonly employed. For instance, Li et al. (2019) indicated that stress-related physiological indices showed that peanuts exhibited higher drought tolerance than rice under drought stress conditions. There are lots of index indicating plant adaptation ability to drought, and physiological parameters are frequently used. Xu et al. (2020) evaluated the integrated drought resistance of 12 herbaceous species by studying changes in photosynthetic rates under soil progressive drought conditions. Ding et al. (2017) investigated the effects of drought stress on photosynthesis, water relations, and physiological and biochemical aspects of five xerophytic plants in the northwest arid region. Wang et al. (2018) compared and determined the responses of three soybean varieties to different levels of soil water contents through analysis of chlorophyll fluorescence. Therefore, leaf photosynthetic rate, transpiration rate, and relative water content of plant leaves were mainly selected for drought-adaptation assessment in this paper.

Studies have been conducted to evaluate the drought tolerance of typical native herbaceous. For example, in order to explore the differences in seedling drought resistance of Old World bluestems (*Bothriochloa ischaemum*) in 10 different wild populations, Zhong et al. (2018) measured the changes in plant physiological indexes and enzyme activities, and finally analyzed several indexes by the subordinate function to obtain the order of strength of seedling drought resistance of *B. ischaemum* using the potting method with different water gradients. Ma et al. (2012) studied the drought resistance of five species of *Lespedeza* by using PEG treatment to artificially simulate soil water stress and used principal component analysis and the subordinate function method to comprehensively evaluate their drought resistance.

Sowing methods may also affect the drought resistance performance of diffident species. For example, Ding et al. (2014) set up seven combination ratios and two moisture level treatments according to the plant ratio of *B. ischaemum* and *Lespedeza davurica* through a controlled trial in pots, and results showed that the mixture ratio could increase the PSII activity of the grass species under both normal water supply and moisture stress. Li (2019) used *B. ischaemum* and *L. davurica* as materials and set up nine combination ratios according to the row ratios of the two species, and used a randomized block experimental design to compare their biomass production, nutritional value, and soil nutrient contents, and comprehensively evaluated and ranked their performance in terms of production and nutritional value.

Bothriochloa ischaemum and *L. davurica* are two typical native grassland species in the semiarid region on the

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Loess Plateau of China. *B. ischaemum* is a perennial C_4 herbaceous plant of the Gramineae family, which is the dominant species of grassland community in the semi-arid regions, featuring superb regeneration ability, high drought resistance and trampling resistance, remarkable regional adaptability, and is a high-quality natural forage species (Liu et al., 2017). *Lespedeza davurica*, a C_3 perennial leguminous subshrub species, is one of the dominant species in the semiarid natural grassland communities in northwest China and is an excellent natural species due to high quality and adaptability (Duan et al., 2022). Since the distribution of the above two plants overlap, and thus both may have a reciprocal effect on their mixture growth (Wang et al., 2019). Therefore, the two species were taken as research subjects in this study, and their photosynthetic physiological characteristics and leaf relative water content were compared under three different soil water and two nitrogen fertilization conditions to simulate water stress and nitrogen deposition in both monoculture and mix culture.

2. Materials and Methods

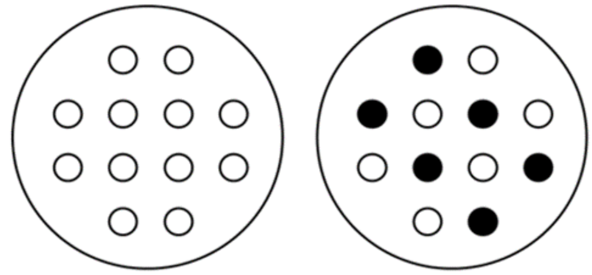
2.1 Plant and soil materials

The experimental plant materials were *B. ischaemum* and *L. davurica*, seeds of which were collected from natural grassland in Ansai District, Yan'an City, Shaanxi Province in 2022. The germination rates of *B. ischaemum* and *L. davurica* were determined before the experiment began, which were 43% and 80% respectively. The soil used was 0-20cm yellow loamy soil from farmland in Ansai, with a field capacity (FC) of 20% and a wilting point (WP) of 4%. The soil nutrient contents were as follows: organic matter content 3.8 g kg^{-1}, total nitrogen content 0.028 mg kg^{-1}, total phosphorus content 0.071 mg kg^{-1}, total potassium content 2.01 mg kg^{-1}, and pH 8.57.

2.2 Experimental design

The seeds were sown on April 5, 2023, and thinned in early June, with 12 plant individuals retained per pot. The planting patterns were monoculture of *B. ischaemum*, monoculture of *L. davurica*, and mixculture of *B. ischaemum* and *L. davurica* at a 1:1 ratio. Each has six replications.

The planting pattern and distribution in the pot was shown as in Figure 1.



12:0 (monoculture of each species) 6:6 (mixculture of the two species)

Fig. 1 Schematic diagram of plantation (black and white colors represent *B. ischaemum* and *L. davurica*, respectively)

Soil water contents were set at three levels, *i.e*. high water regime (80%±5% FC), medium water regime (60%±5% FC), and low water regime (40%±5% FC), simulating sufficient, normal, and drought stress conditions in the region, respectively. Soil water content was controlled and adjusted by the gravimetric method at 18:00 daily. Two nitrogen (N) treatments, *i.e*. N0 (0 g N kg^{-1}) and N1 (0.025 g N kg^{-1}) were applied, with urea (H_2NCONH_2) as the N fertilizer. The N1 level was equivalent to an annual N application of 83 kg N ha^{-1} in the region. In total, there were three planting methods, three soil water regimes, two nitrogen treatments, and six repetitions, which resulted in 3 (planting methods) × 3 (water levels) × 2 (nitrogen treatments) × 6 (repetitions) = 108 pots. The experiment was conducted in the outdoor rainfall shed of the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau. The pots used were plastic buckets with the size of 30 cm deep and 20 cm in diameter. Water was added through a PVC pipe that was 30 cm long and 2 cm in diameter, attached to the inner side of the bucket.

2.3 Measurements

Leaf photosynthetic gas exchange parameters, chlorophyll fluorescence characteristics and relative water content (RWC) were measured at the flowering stage (August 5-10, 2023) as referenced to the growth period of *B. ischaemum*.

2.3.1 Photosynthetic gas exchange parameters

The measurements were also taken on newly fully expanded leaves from the upper part of the same plant from 9:00 am to 11:30 am, following the determination of chlorophyll fluorescence parameters using the Licor-6800 photosynthesis system (LICOR, NE, USA). The measurement conditions were as follows: an artificial light source provided by the leaf chamber with an incident light intensity of 1200 μmol m^{-2} s^{-1}, CO_2 concentration was set at 400 μmol mol^{-1}, flow rate was set at 0.5 L min^{-1}, relative humidity was maintained at 55-70%, and temperature was set to match the outdoor temperature. The parameters determined include net photosynthetic rate (*Pn*, μmol m^{-2} s^{-1}), transpiration rate (*Tr*, mmol m^{-2} s^{-1}), intercellular CO_2 concentration (*Ci*, μmol mol^{-1}), and stomatal conductance (*Gs*, mol m^{-2} s^{-1}). The leaf instantaneous water use efficiency (WUEi, μmol $mmol^{-1}$) was calculated as: WUEi = *Pn* / *Tr*.

2.3.2 Leaf chlorophyll fluorescence characteristics

The measurements were conducted using the Continuous Light Induction Protocol (CLIP) with the Handy-PEA Plant Efficiency Analyser (Hansatech Instruments Ltd., UK). For each treatment, newly fully expanded leaves of each species were randomly selected. The leaves were dark-adapted for 30 min using a leaf clip before the measurements. The main characteristics parameters obtained included initial chlorophyll fluorescence (*Fo*), maximum potential quantum yield of PSII (*Fv/Fo*), and maximum photochemical efficiency of PSII (*Fv/Fm*). Each measurement was replicated three times.

2.3.3 Relative water content (RWC)

10 newly fully expanded upper leaves from the plants of each species under different treatment were sampled between 6:00 am and 8:00 am. The leaves were immediately put in an ice box and then taken back to the lab. The fresh weight (W_1) was obtained using an analytical balance (0.0001 g), and then the leaves were immersed completely in deionized water for 24 hours. The turgid weight (W_2) was determined after wiping off the surface water. Then, the leaves were oven-dried at 65℃ to a constant dry weight (W_3). The leaf relative water content was calculated as: RWC = (W_1 - W_3) / (W_2 - W_3) × 100% (Barrs and Weatherley, 1962).

All data are presented as mean ± standard deviation (n=3). The significant differences between the two species and under different soil water and nitrogen treatments were tested using Tukey's one-way ANOVA ($p<0.05$). The effects of soil water regime, nitrogen treatment, planting method, and their interactions on leaf RWC, gas exchange traits, and chlorophyll fluorescence parameters of the two species were tested using three-way ANOVA ($p<0.05$).

3. Results

3.1 Photosynthetic gas exchange characteristics

3.1.1 Net photosynthetic rate (Pn)

According to Fig. 2, in monocultured *B. ishaemum*, when soil water content decreased, there was no significant influence on *Pn* under N0 condition, while under N1 condition there was a significant increasing trend, and the *Pn* values under N1 and both 60%FC and 40%FC were significantly higher than that under N0 ($P<0.05$). In monocultured *L. davurica*, when soil water content decreased, *Pn* values increased first and then decreased under both N0 and N1 conditions; *Pn* value was significantly higher in 60%FC than in other two soil water supplying regimes ($P<0.05$), and the value under N0 was significantly higher than that under N1 treatment ($P<0.05$). In the mixculture, with the decrease in soil water content, the *Pn* value of *B. ischaemum* under 40%FC was significantly lower than those of 60%FC and 80%FC under N0 condition ($P<0.05$) but showed a significant decreasing trend under N1 condition ($P<0.05$). However, *L. davurica* showed a downward trend under N1 condition, and *Pn* value in 80%FC was significantly higher than the other two soil water regimes ($P<0.05$), while under N0 condition, *Pn* value in 60%FC was significantly higher than that in 80%FC.

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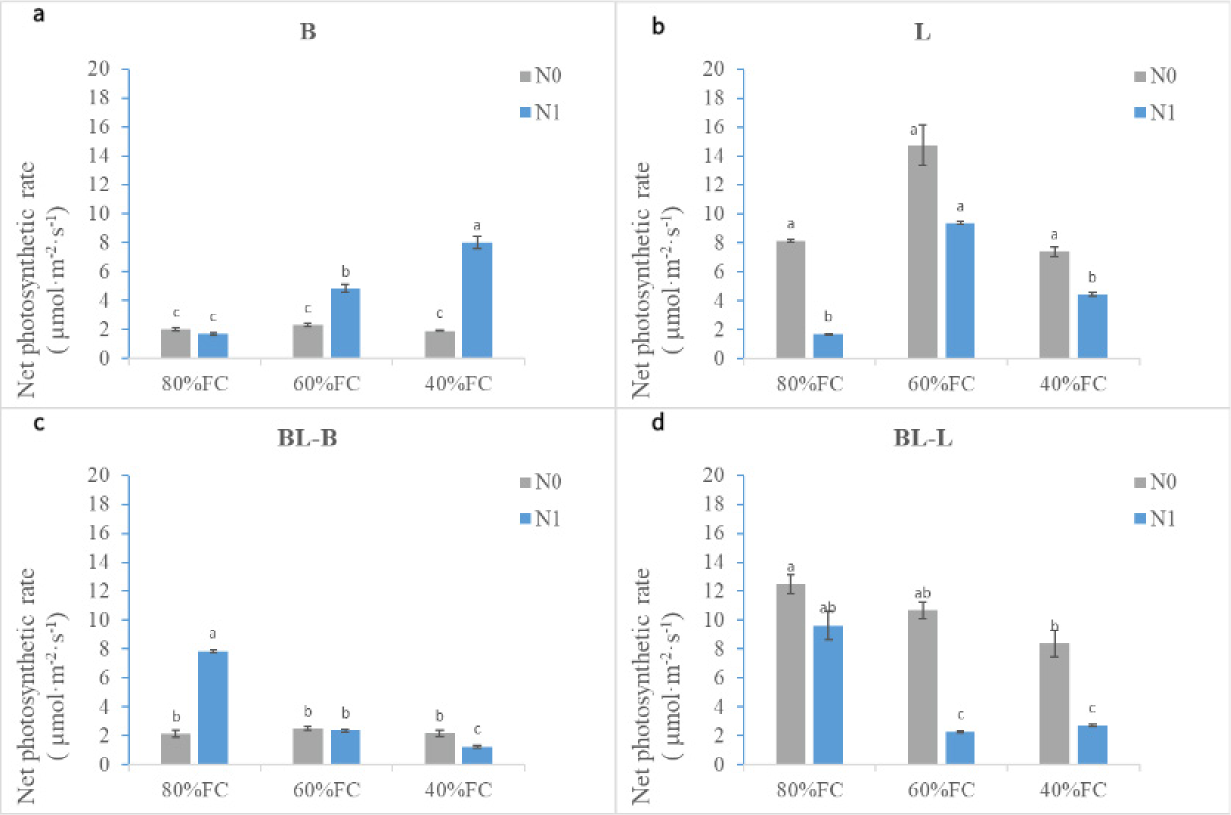

Fig. 2 Net photosynthetic rate (*Pn*) of the two species under each treatment

Note: B means *B. ischaemum* monoculture, L means *L. davurica* monoculture , BL-B means *B. ischaemum* in mixculture, BL-L means *L. davuric* in mixculture. Different small letters above columns indicate significant differences between all soil water and nitrogen application treatments (P<0.05). Same as below.

3.1.2 Transpiration rate (Tr)

According to Fig. 3, in the monocultured *B. ischaemum*, when soil water content decreased, *Tr* under N0 had no significant change (P>0.05), while *Tr* under N1 had a significant increase trend among each soil water regime (P<0.05), and *Tr* value reached the highest under 40%FC. In the monocultured *L. davurica*, *Tr* under both N0 and N1 treatments increased first and then decreased when soil water content decreased, and Tr reached the significantly maximum value in 60%FC (P<0.05). In the two species mixculture, when soil water content decreased, the *Tr* value of *B. ischaemum* showed no significant change trend under N0 condition (P<0.05) but showed a significant decline trend under N1 condition (P<0.05). The *Tr* value of *L. davurica* increased first and then decreased under N0 condition, and the *Tr* value in 60% FC was significantly higher than that in 80%FC. Under the condition of N1, the *Tr* value of *L. davurica* showed a downward trend that *Tr* value in 80% FC was significantly higher than both that in 60% and 40% FC (P<0.05). In both monoculture and mixculture, the *Tr* value of *L. davurica* was significantly higher than *Tr* value of B under N0 condition, and reached the maximum value when FC is 60% (P<0.05).

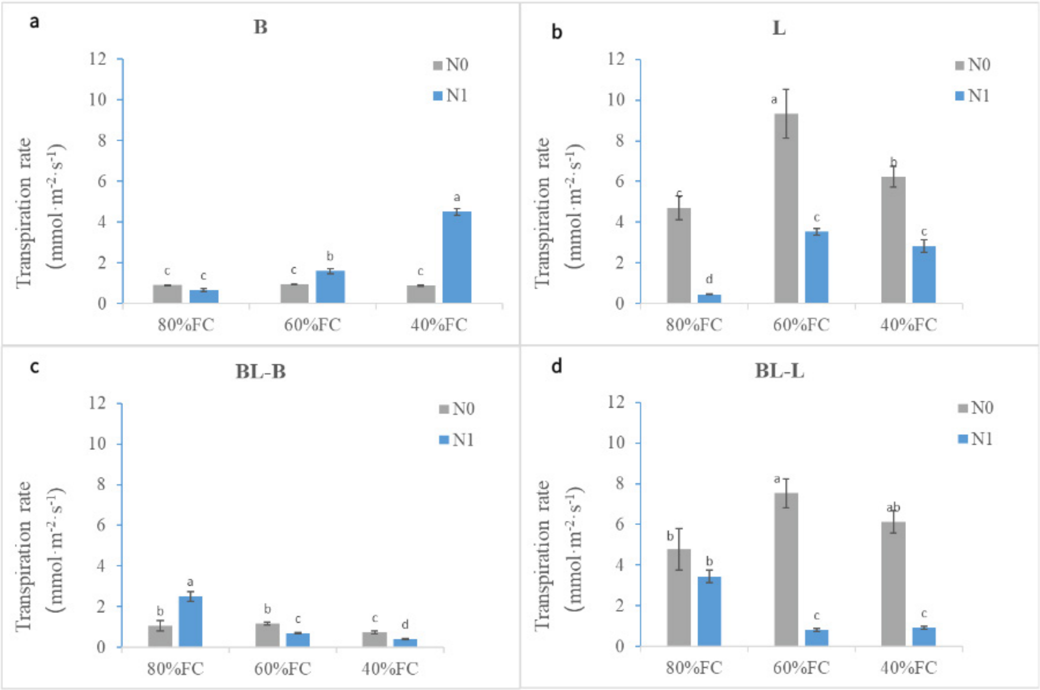

Fig. 3 Transpiration rate (*Tr*) of the two species under each treatment

3.1.3 Intercellular CO_2 concentration (Ci)

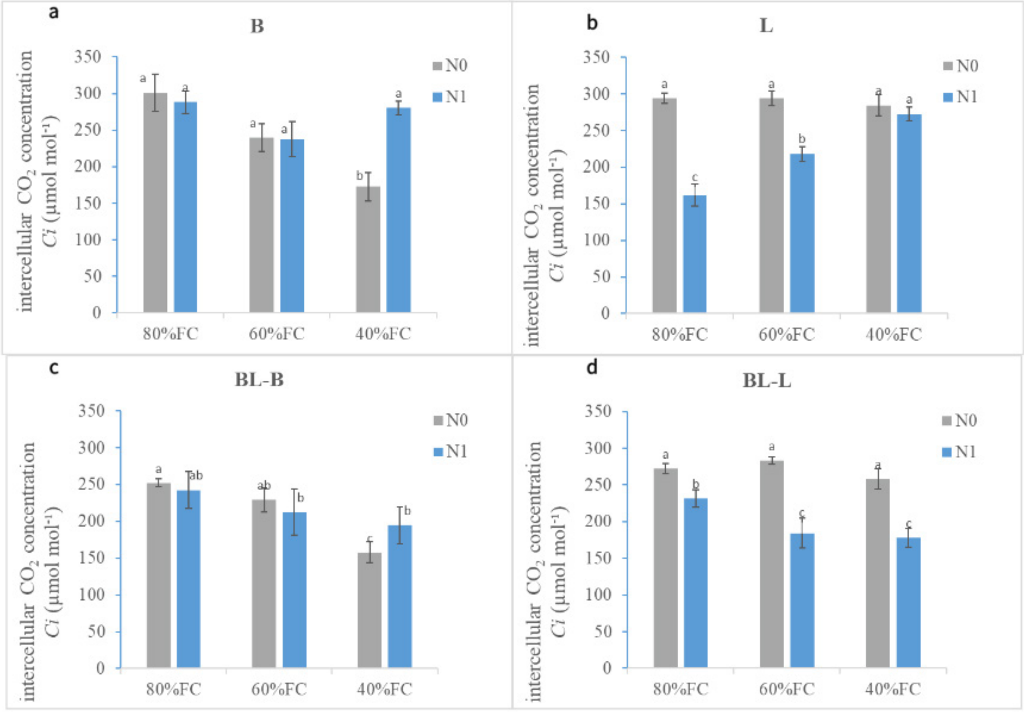

Fig. 4 Intercellular CO_2 concentration (*Ci*) of the two species under each treatment

According to Fig.4, in the monocultured *B. ischaemum*, with the decrease in soil water content, *Ci* showed a decreasing trend under N0 condition, and was significantly smaller at 40%FC ($P<0.05$). Under N1 condition, there was no significant change between different levels of FC ($P>0.05$). In the monocultured *L.davurica*, the decrease

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of FC had almost no effect on the *Ci* value under N0 condition (*P*>0.05), and the *Ci* value significantly increased under N1 condition (*P*<0.05), reaching the maximum value at 40%FC. In the mixculture group, when FC decreased, the *Ci* value of *B. ischaemum* showed a decreasing trend under both N0 and N1 conditions (*P*>0.05). The *Ci* value of *L. davurica* was not significantly affected by the decrease of FC under N0 condition (*P*>0.05), and the *Ci* value of *L. davurica* decreased with the decrease of FC under N1 condition, and the *Ci* value in 80%FC was significantly higher than that in 60%FC and 40% FC.

3.1.4 Stomatal conductance (Gs)

According to Fig.5, in the group of monocultured *B. ischaemum* with the decrease of soil water content, the variation range of *Gs* under N0 condition between 60% and 40% FC was not significant (*P*>0.05), and the *Gs* value in 80%FC was significantly high (*P*<0.05), while *Gs* under N1 condition was significantly increased (*P*<0.05), reaching the highest value in 40%FC. In the monocultured *L. davurica*, with the decrease of FC, *Gs* showed a trend of first increasing and then decreasing under both N0 and N1 conditions, and the *Gs* value of group N0 was significantly higher than that of group N1 (*P*<0.05).

In the mixcultured group, the change in soil water content had no significant effect on the *Gs* value of B under N0 condition, while under N1 condition, the *Gs* value was significantly highest (*P*<0.05). The *Gs* value of mixcultured *L. davurica*in under 40%FC was significantly lower than those of 60% FC and 80% FC under N0 condition (*P*<0.05), and under N1 condition, the *Gs* value was significantly higher at 80%FC (*P*<0.05).

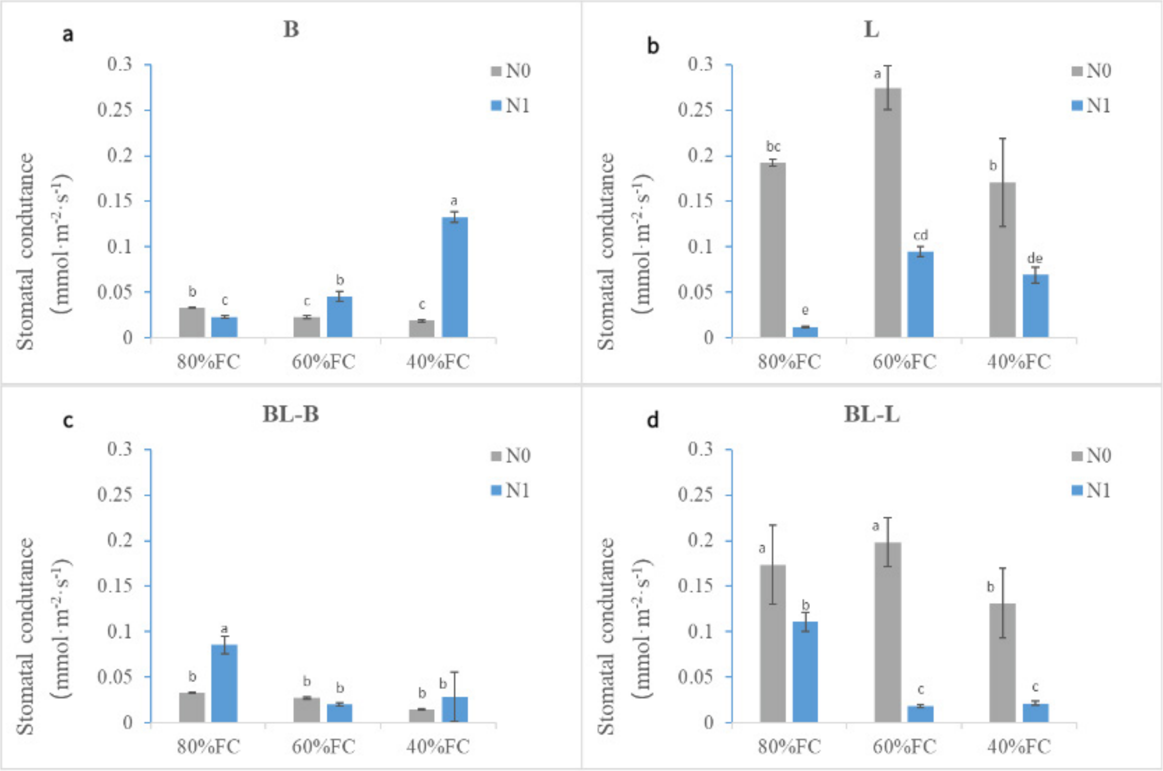

Fig. 5 Stomatal conductance (*Gs*) of the two species under each treatment

3.1.5 Water use efficiency (WUEi)

According to Fig.6, in the monocultured *B. ischaemum*, with the decline of soil water content, *WUEi* showed a trend of first increasing and then decreasing under both N0 and N1, and there was no significant change in N0 condition. In the monocultured *L. davurica*, under the condition of N0, *WUEi* value of 80%FC was significantly high (*P*<0.05), while under the condition of N1, *WUEi* of *L. davurica* significantly decreased with the decrease of FC (*P*<0.05). In the mixculture, *WUEi* level of *B. ischaemum* was significantly higher at 40%FC under N0 condition than the other two soil water levels (*P*<0.05), and was not significantly affected by soil water content changes under N1 condition (*P*>0.05).

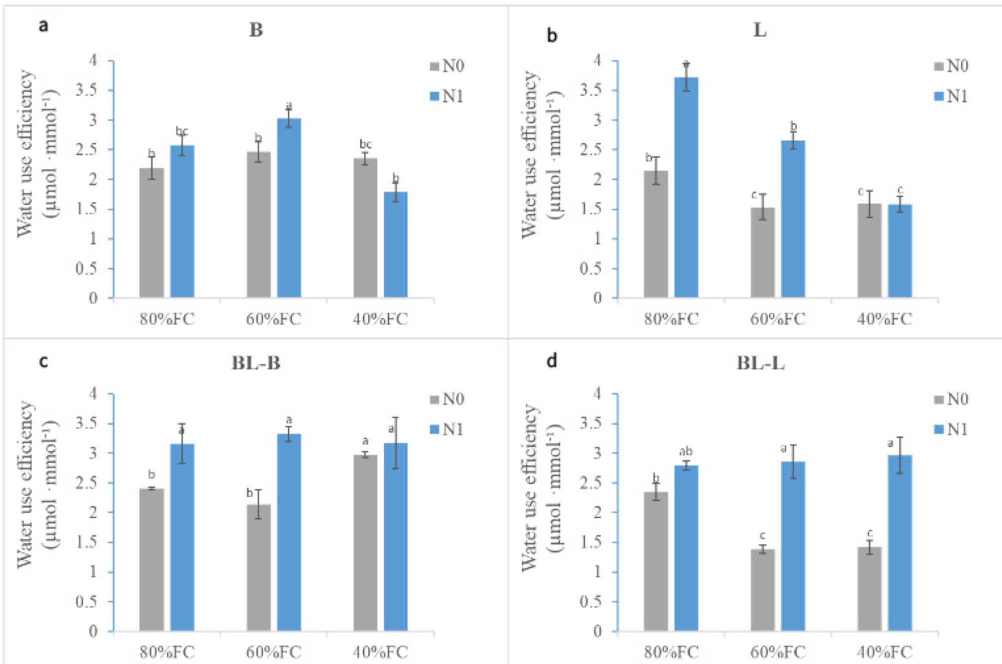

Fig.6 Leaf instantaneous water use efficiency (*WUEi*) of the two species under each treatment

3.2 Chlorophyll fluorescence parameters

3.2.1 Leaf initial fluorescence (Fo)

At 80% FC, the *Fo* values of monocultured *L. davurica* were significantly higher than that of monocultured *B. ischaemum*. Compared to 80% FC, when soil moisture dropped to 60%, mixcultured *B. ischaemum* had the largest increase under N1 conditions, followed by monocultured *B. ischaemum* under N1 conditions. At 40%FC, *Fo* values for monocultured *L. davurica* and mixcultured *L. davurica* were the highest, but there were no significant differences between them (P>0.05).

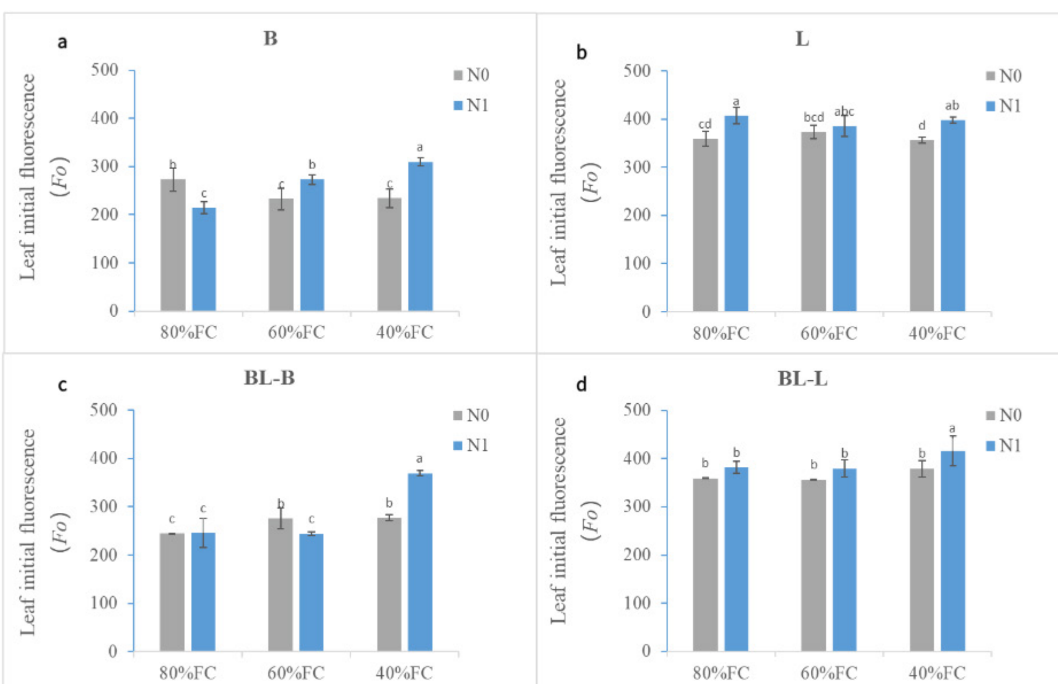

Fig. 7 Leaf initial fluorescence (*Fo*) values of the two species under each treatment

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3.2.2 Maximum potential quantum yield of PSII (Fv/Fo)

Under N0 condition, monocultured *L. davurica* and mixcultured *L. davurica* had the highest *Fv/Fo* values at 80%FC. As soil water content decreased to 60%FC, there was a significant decrease in mixcultured *B. ischaemum* (P<0.05), while there were no significant changes in other groups (P>0.05). As soil water content decreased from 60%FC to 40%FC, there was a significant decrease in monocultured *B. ischaemum* (P<0.05), while there were no significant changes in other groups (P>0.05). As soil water content decreased from 80% FC to 40% FC under N1 condition, there were no significant changes in both monocultured *L. davurica* and *B. ischaemum* (P>0.05), while there were significant changes in both mixcultured *L. davurica* and *B. ischaemum* (P<0.05).

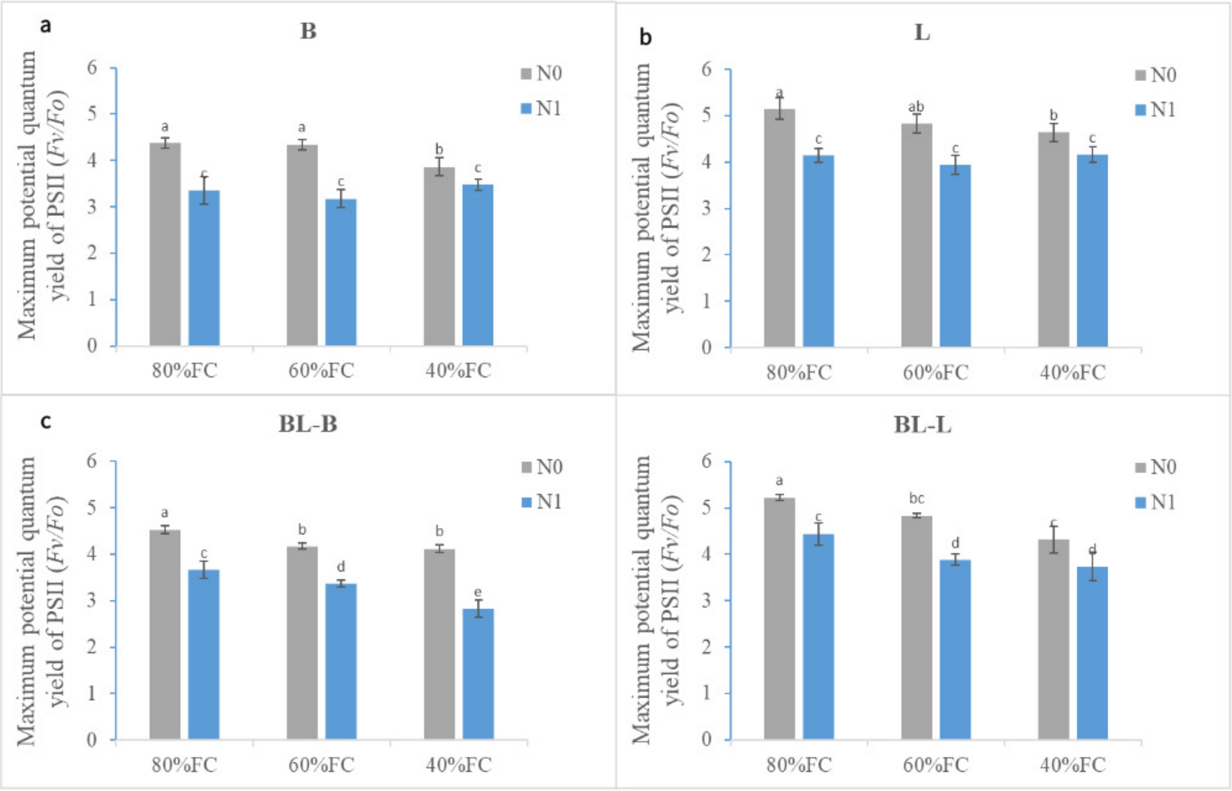

Fig. 8 Maximum potential quantum yield of PSII (*Fv/Fo*) of the two species under each treatment

3.2.3 Maximum Photochemical Efficiency (Fv/Fm)

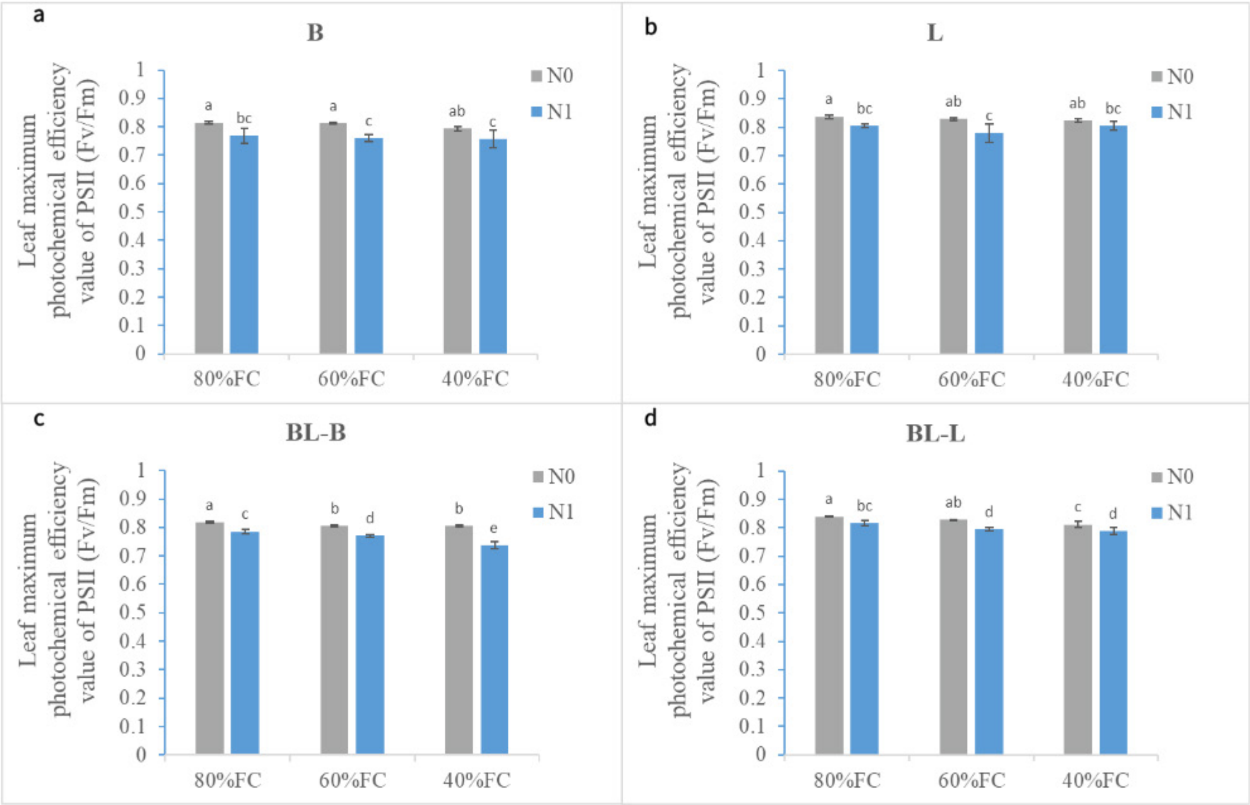

Fig. 9 Leaf maximum photochemical efficiency values of PSII (*Fv/Fm*) of the two species under each treatment

At 80% FC under N0 condition, there were no significant differences in *Fv/Fm* values among the groups. As soil water content decreased to 60%FC under N0 conditons, there was a significant decrease in mixcultured *B. ischaemum* ($P<0.05$), while there were no significant changes in other groups ($P>0.05$). As soil water content decreased from 60%FC to 40%FC under N0 conditons, there was a significant decrease in mixcultured *L. davurica* ($P<0.05$), while there were no significant changes in other groups ($P>0.05$).

Under N1 condition, as the soil water content decreased, both monocultured *B. ischaemum* and *L. davurica* showed no significant changes ($P>0.05$), while mixcultured *B. ischaemum* showed a significant decreasing trend ($P<0.05$).

3.3 Leaf relative water content (RWC)

At 80% FC, the RWC values of mixcultured *L. davurica* under both N0 and N1 conditions were the lowest, both significantly lower than those of the other groups, with no significant differences among the RWC values of the other groups. When the soil moisture dropped to 60%, the RWC value of mixcultured *L. davurica* under the N0 condition decreased to the lowest value, showing a trend of first rising and then falling, while the RWC values of the other groups did not exhibit significant rises or falls.

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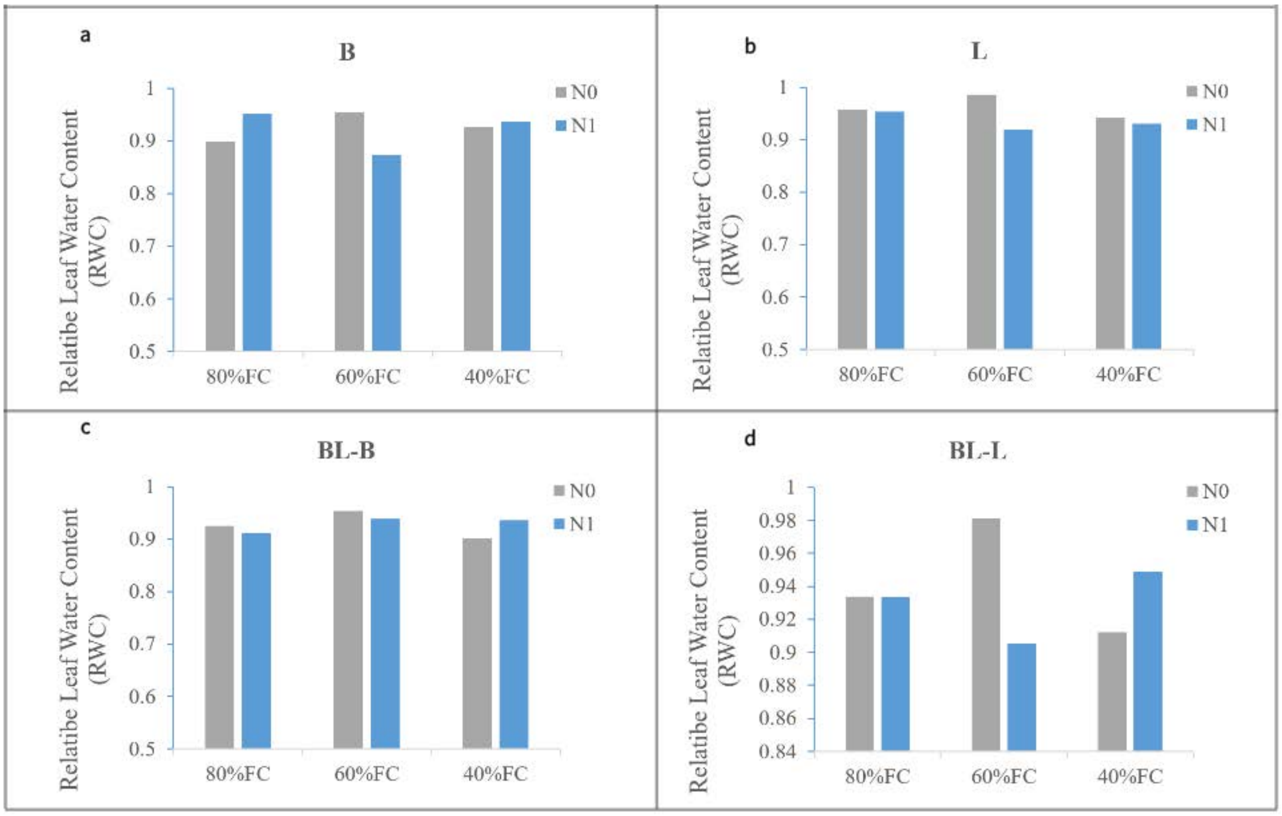

Fig. 10 Leaf relative water content (RWC) of the two species under each treatment

4. Discussion

Water is the most crucial factor influencing the photosynthetic physiological characteristics of plants in arid and semiarid regions. Water stress affects a series of photosynthetic activities, including stomatal opening and closing, chloroplast activity, electron transport, and photo-phosphorylation (Silva and Arrabaca, 2008). Chlorophyll fluorescence parameters are often used to evaluate the effects of environmental stress on the structure and function of plant leaf photosynthetic organs. The initial fluorescence (*Fo*) value, which is the fluorescence yield when the PSII reaction center is fully open, indicates the degree of damage to the PSII reaction center, with higher *Fo* values indicating more severe damage. The maximum potential quantum yield of PSII (*Fv*/*Fo*) reflects the activity of the PSII reaction center, while the high or low maximum photochemical efficiency (*Fv*/*Fm*) is closely related to the rate of plant photosynthesis.

In this study, when soil water content decreased from 80%FC to 60%FC under N0 condition, the *Pn* values of monocultured and mixcultured *B. ischaemum* showed no significant changes (P>0.05), while the *Pn* values of monocultured and mixcultured *L. davurica* showed significant change (P<0.05). There was no significant change in *Fv/Fm values* (P>0.05) among all treatments, indicating that the decrease in *Pn* might be due to the decrease in *Gs*, resulting in insufficient CO_2 supply within the cells, *i.e*. stomatal limitation. When soil water content dropped from 60% to 40% under N0 condition, the *Pn* values of the monocultured *L. davurica* and mixcultured *B. ischaemum* showed significant declines (P<0.05), while the *Fv/Fm* values did not change significantly (P>0.05) , suggesting that the decrease in *Pn* at this stage was still caused by stomatal limitation. During the decline in soil water content, the *Pn* and *Gs* values of *L. davurica* were the highest, particularly under the N0 condition, where they were greater than those of other treated plants under the same conditions, indicating that *L. davurica* exhibits active photosynthetic physiological activities compared to *B. ischaemum* under soil water content limitation and without nitrogen deposition.

Under 40%FC, the *Pn* and *Gs* values of *L. davurica* under N0 condition were significantly higher than under N1 treatment in both monoculture and mixculture (P<0.05). The *Pn* and *Gs* values of monocultured *B. ischaemum* under N0 condition was significantly lower than that under N1 condition (P<0.05), and the *Pn* value of mixcultured *B. ischaemum* under N0 condition was significantly higher than that under N1 condition (P<0.05), indicating that the grass species was sensitive to nitrogen. The *WUEi* value of mixcultured *L. davurica* under N1 condition was significantly higher than that under N0 condition, while for monocultured and mixcultured *B. ischaemum* and monocultured *L. davurica*, the *WUEi* values under N1 condition compared to N0 condition did not show significant deviation (P>0.05). At 40% FC, the photosynthetic physiological efficiency of mixcultured *L. davurica* and *B. ischaemum* was significantly lower under nitrogen treatment than that without nitrogen treatment, indicating that nitrogen deposition may reduce the physiological efficiency of the two species.

In drought environments, plants may enhance leaf water use efficiency (*WUEi*) by adjusting the relationship between carbon assimilation and water consumption. In this study, both *Pn* and *Tr* decreased, suggesting that plants can improve water use efficiency by reducing transpiration rates, which is one of the primary drought resistance strategies (Lawlor and Cornic, 2002). At 40% FC, the *WEUi* of plants in the mixture was higher than that of monoculture, except for *L. davurica* in the mixture at N0, where the *WUEi* was significantly lower than that of its monoculture. There were no significant differences in *WUEi* values for other plants at 40% FC under both N conditions, indicating that under nutrient-deficient conditions, *L. davurica* was in a relatively disadvantageous position in competition with *B. ischaemum* when mixcultured, with weaker nutrient competition ability, resulting in lower water use efficiency; while under nutrient-sufficient conditions, *B. ischaemum* could obtain more nutrients, so it has higher vitality and higher water use efficiency. The RWC values of *L. davurica* during mixcultured were also significantly lower than that of other plants, possibly because, during mixculture, *L. davurica* and *B. ischaemum* competed for resources, resulting in limited water and nutrient conditions that were insufficient to maintain leaf water contents.

5. Conclusion

In summary, *L. davurica* in monoculture was more significantly affected by changes in soil water content and nitrogen application compared to *B. ischaemum*, while in the mixculture of the two species, they exhibited significantly higher drought resistance than in monoculture. In the mixculture, both exhibited significantly higher adaptability comparing to their counterparts in monoculture under the same soil water and nitrogen supplying conditions, which was due to the reciprocal effect between grass and legume in their mixture, and which may be due to the enhanced nitrogen fixation and use efficiency, resulting to improve drought-resistance ability for both *B. ischaemum* and *L. davurica* under soil water stress condition. Therefore, the two species mixculture was recommend for artificial grassland construction in the region because of generally low soil water and low soil nutrient situation in the natural conditions.

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