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ORIGINAL ARTICLE

**Bing-Cheng Xu · Wei-Zhou Xu · Zhi-Juan Gao
Jing Wang · Jin Huang**

Biomass production, relative competitive ability and water use efficiency of two dominant species in semiarid Loess Plateau under different water supply and fertilization treatments

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Abstract Water stress and nutrient deficiency are considered to be the main environmental factors limiting plant growth and species interaction in semiarid regions. However, less is known about the interactive effects of soil water, nitrogen and phosphorus on native species growth and relative competitive ability. A replacement series design method was used with 12 mixed plants of *Bothriochloa ischaemum* and *Lespedeza davurica* grown in a pot experiment under three water regimes and four fertility treatments. Intercropping systems were assessed on the basis of indices such as biomass production and allocation, relative competitive ability, aggressivity, relative yield total and water use efficiency (WUE). Water stress decreased significantly the total biomass production for each species, either in monoculture or in mixtures. N, P, or NP application can significantly improve biomass production of the two species in their mixtures. There was no obvious change trend in root/shoot ratio of *B. ischaemum* or *L. davurica* in different mixture proportions. Relative yield total (RYT) values ranged from 0.98 to 1.39. Aggressivity values of *B. ischaemum* to *L. davurica* were positive in all water regimes and fertilizations, implying that *B. ischaemum* was the dominant species. Relative competition intensity values of *B. ischaemum* (i.e., RCI_B) were less than zero, while greater than zero for *L. davurica* (i.e., RCI_L), indicating that the effects of intraspecific competition with *L. davurica* were stronger for *B. ischaemum*, and the opposite for *L. davurica*. WUE increased gradually as the proportion of *B. ischaemum* increased in mixtures, and a 10:2 *B. ischaemum*:*L. davurica* mixture proportion had significantly higher WUE. Results suggest that it is advantageous to grow the two species together to maximize biomass production and the recommended mixture ratio was 10:2 of *B. ischaemum* to *L. davurica* because it gave higher RYT and significantly higher WUE under conditions of water deficit.

Keywords *Bothriochloa ischaemum* · *Lespedeza davurica* · Relative yield total · Aggressivity · Relative competition intensity · Fertilization · Water stress

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B.-C. Xu (✉) · W.-Z. Xu · Z.-J. Gao · J. Wang · J. Huang
State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, China
E-mail: Bcxu@ms.iswc.ac.cn
Tel.: +86-29-87016571
Fax: +86-29-87012210

B.-C. Xu · J. Huang
Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, Shaanxi 712100, China

Introduction

Plant competition has been recognized as an important factor determining species distribution, species composition, community structure, function and dynamics in natural vegetation (Vilà and Sardans 1999; Naeem and Wright 2003). Plant competition includes all limiting effects that plants may have on each other indirectly or directly, and the balance of positive and negative interactions may change, depending on plant life history and resource availability (Callaway and Walker 1997; Armas and Pugnaire 2005; Michalet 2007). Understanding species interactive mechanisms under different resource supply conditions is important in explaining species coexistence in natural environments, and in constructing artificial groups for vegetation rehabilitation and agricultural development with complementarity or facilitation in resource use and yield (Vilà and Sardans 1999; Zhang and Li 2003). Results showed that competition would be less important with soil water deficiency or under infertile conditions because plant growth would be limited by these factors rather than by competition (Wedin and Tilman 1993; Callaway and Walker 1997; Holmgren et al. 1997). Conversely, other results have suggested no change in competition intensity with varying water availability (Belcher et al. 1995; Wetzel and Van der Valk 1998).

Recently, there has been mounting interest in diversified agricultural production systems to obtain the improved plant protection, increased productivity and profitability that can be offered by intercropping system such as higher yields, greater land-use efficiency and improvement of soil fertility (Ghosh 2004; Li et al. 2007; Liu et al. 2013). For example, legume–cereal intercropping is practiced widely especially in arid and semiarid regions. Intercropping is based on the manipulation of plant interactions to maximize their growth and productivity, as intercropping components might utilize resources more efficiently (Hauggaard-Nielsen et al. 2009). Investigation of species responses under intercropping systems is not only important for understanding species interactive mechanisms, but also useful for constructing agricultural systems with higher resource-use efficiency (Zhang and Li 2003; Ghosh 2004; Li et al. 2007).

Water is the primary limiting factor for plant growth, vegetation distribution and agricultural development in the semiarid Loess Plateau of China. Besides water deficit, the soil environment is harsh due to serious soil erosion, causing considerable losses of N, P and other soil nutrients in the region (Shan and Chen 1993). *Bothriochloa ischaemum* and *Lespedeza davurica* are two co-dominant species in the natural grassland communities in the area. *B. ischaemum* is a C_4 perennial grass species, and *L. davurica* is a C_3 perennial leguminous subshrub. These two species are of great importance in reducing soil and water loss and maintaining distinctive natural sceneries in the area. Besides acting as an excellent natural pasture species, many agronomic attributes make them ideal forage species due to their high adaptability and quality (Xu et al. 1997). Understanding how their growth characteristics respond to competition under different water and nutrient (e.g., nitrogen and phosphorus) availability will advance our knowledge of their co-existence mechanisms, their potential role in structuring natural plant communities, and their use in creating artificial grasslands. In the semiarid Loess Plateau, artificial grassland construction is currently limited by problems such as limited grass species and use of simple structures dominated by single species with low eco-adaptation. Addressing these problems would involve strengthening research in this area, and breeding of native and wild grass species with greater adaptability to local environments (Shan and Xu 2009). Thus, in the present study, we used a replacement series design method to examine the effects of water supplement and fertilization on the growth and competitive abilities of these two species in a pot experiment. We assumed the existence of intercropping advantages of the two species in mixtures, and that these would be affected by water regime, fertilization treatment and mixture ratio. In this paper, we aimed to address three questions: (1) is the biomass production of the two species in mixtures higher than in their respective monoculture? (2) Is the ability of each of the two species to respond competitively to altered soil water and nutrient availability due to species-specific physiological responsiveness? (3) What is the optimal mixture ratio of the two species considering water supply and fertilization? We examined the biomass production and allocation, and relative competitive ability of each species in their mixtures, and compared relative yield total (RYT) and water use efficiency (WUE) of the two species mixtures under three soil water regimes and four fertilization treatments.

Materials and methods

Plant materials

The two species used were *Bothriochloa ischaemum* L. Keng and *Lespedeza davurica* (Laxm.) Schindl. Seeds were obtained from the experimental fields at Ansai Research Station (ARS) of the Chinese Academy of Sciences (CAS) (36°51′30″N, 109°19′23″E, altitude 1,068–1,309 m a.s.l.) in 2009, which is located at the center of the semiarid hilly-gully region on the Loess Plateau. Seed germination rates were above 90 % when germinated on moist filter paper in Petri dishes at 25 °C before the experiment started.

Growth conditions

The experiment was conducted under a rainout shelter in State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Yangling, Shaanxi Province, China (34°12′N, 108°7′E, altitude 530 m a.s.l.). The mean annual temperature is 13.0 °C, with a maximum mean monthly temperature of 26.7 °C in July and a minimum temperature of −1 to −2 °C in January. Mean annual precipitation is 650 mm. The soil for the study was collected from the upper 20 cm of an arable field in ARS. The soil was sandy loam with 55 % porosity, and its bulk density was 1.2 g cm^{-3}. Soil gravimetric water content at field capacity (FC) and wilting point were 20.0 and 4.0 %, respectively. Soil organic matter content, total N, total P and total K contents were 0.27, 0.017, 0.063 and 1.97 %, respectively, and the soil available N, P and K contents were 11.21, 6.55 and 94.85 mg kg^{-1}, respectively.

Plants were grown in cylindrical plastic pots sized Ø20 cm (inner diameter) × 30 cm (height). Each pot contained 9.0 kg dry soil. The experiment had four fertilizer treatments which were CK (control), N (0.025 g per kg dry soil), P (0.1 g per kg dry soil) and NP (0.025 g N + 0.1 g P per kg dry soil). N and P were applied as CON_2H_4 and KH_2PO_4, and the amounts were 0.48 and 3.95 g per pot, respectively. All the fertilizers were mixed with soil uniformly during barreling. One plastic pipe (Ø 2.0 cm) was inserted adjacent to pot inner wall to supply water at about 10 cm distance from the bottom.

Species combination

A replacement series design was conducted with a density of 12 plants in each pot (Connolly et al. 2001). Seven planting mixture ratios (12:0, 10:2, 8:4, 6:6, 4:8, 2:10 and 0:12) were used.

Water treatments

Seeds were sown on 31 March 2010. During the period of seedling establishment, soil water content (SWC) was maintained above 80 % FC. Water treatments started on 21 June 2010 when the seedling biomass of each species was about 0.2 g per seedling biomass dry weight (DW). Just before the water treatments initiated, 40 g perlite was placed on the soil surface of each pot (about 2.0 cm) to reduce the soil compaction caused by watering and evaporation. At that time, *B. ischaemum* was at the five-leaf stage, and *L. davurica* had no new branching. From then, the pots were watered regularly after weighing to maintain three watering regimes: 80 ± 5 % FC (HW), 60 ± 5 % FC (MW) and 40 ± 5 % FC (LW) until the experiment ended.

Biomass sampling

Plant biomass was sampled before and after imposing the water treatments on 21 June and 19 October 2010, respectively. The first sampling was use to calibrate water regime. At each sampling, 12 seedlings in each pot were harvested, and shoots and roots were collected after removing roots from soil and washing carefully. Dry weight (DW) was determined after drying at 75 °C for 48 h in an air-dried oven. Root/shoot (R/S) ratio was calculated as the ratio of root to shoot dry biomass.

Competition indices

Several indicators for species interaction have been used in intercropping research (Connolly et al. 2001; Ghosh 2004). Two indices for studying the intensity of competition [e.g., relative competition intensity (RCI) and aggressivity (A)] and one index for analyzing competition effects [e.g., relative yield total (RYT)] were employed in this study (Weigelt and Jolliffe 2003). All were calculated from the biomass dry weight data and their various proportions within the replacement series.

Relative competition intensity

Relative competition intensity (RCI) is used to compare the competitive ability of different plants, measuring competitive changes within a given combination. It can be calculated according to biomass production or numbers of plants under different mixtures (Facelli et al. 1999; Sammul et al. 2000). In this study, RCI was calculated as:

$$RCI_{ab} = \frac{(Y_{aa} \times Z_{ab} - Y_{ab})}{Y_{aa} \times Z_{ab}}, \quad (1)$$

where Y_{aa} (or Y_{bb}) the biomass production of species *a* (or *b*) in monoculture, Y_{ab} (or Y_{ba}) is the biomass production for species *a* (or *b*) in mixture with species *b* (or *a*). Z_{ab} (or Zba) are sown proportions of crop "a" and "b" in the mixture. Letters "*a*" and "*b*" represent *B. ischaemum* and *L. davurica*, respectively throughout this paper. If RCI = 0, the interspecific competition equals the intraspecific competition, and if RCI is positive then interspecific competition is higher, while a negative value for RCI means that intraspecific competition is higher (Wang et al. 2012).

Aggressivity

Aggressivity (A) is another index that represents how much the relative yield increase in crop 'a' is greater than that of crop 'b' in a mixture. It measures the interspecific competition in intercropping by relating the yield changes of the two component plants to their respective monoculture (Ghosh 2004). In this paper, the aggressivity concept was used to evaluate the difference between the extent to which intercropped species 'a' and 'b' varied from their respective monoculture:

$$A_{ab} = \frac{Y_{ab}}{Y_{aa} \times Z_{ab}} - \frac{Y_{ba}}{Y_{bb} \times Z_{ba}}, \quad (2)$$

where Y_{ab}, Y_{ba}, Y_{aa}, and Y_{bb} are as defined in Eq (1). A_{ab} is the difference between the relative change in yield of *B. ischaemum* in mixture and the corresponding value for *L. davurica*. If $A_{ab} = 0$, both species are equally competitive, and if A_{ab} is positive then *B. ischaemum* is the dominant species, while a negative value for A_{ab} means that *B. ischaemum* is the dominated species.

Relative yield total

RYT gives an accurate assessment of the greater biological efficiency of the intercropping. RYT values = 1.0 means that the two species have equal demands for the same limiting resources. RYT values > 1.0 indicate the mixtures are advantageous for biomass production compared to monocultures. RYT values < 1.0 indicates mutual antagonism. RYT was calculated as:

$$\mathrm{RYT} = (\mathrm{RY}_{ab} + \mathrm{RY}_{ba}) = \left\{ \left(\frac{Y_{ab}}{Y_{aa}} \right) + \left(\frac{Y_{ba}}{Y_{bb}} \right) \right\}, \quad (3)$$

in which RY_{ab} is the relative yield for species *a* in mixture with species *b*, and RY_{ba} is the relative yield for species *b* in mixture with species *a*.

Water use efficiency

Daily evapo-transpiration was obtained by weighing the pots at 1800 hours, and then watering to the desired level through the plastic pipes. Three identical pots filled with soil but without plants were used to estimate soil evaporation. Soil evaporation was subtracted from total water consumption. Thus, WUE was same as transpiration efficiency (TE, g DW kg^{-1} H_2O^{-1}), and defined as the amount of total biomass produced per unit volume of water transpired by plants during the water treatment.

Statistical analysis

Our treatments were a factorial combination of two species, seven mixture ratios, four fertilizations and three water regimes, arranged into a completely randomized design with three replications. Values from all sampling pots within each treatment were averaged, and the values are expressed as mean ± SE (standard error) of the three replicates. Data were prepared using the Microsoft office Excel 2003 for windows. One-way ANOVA with Tukey method was used to detect possible differences among the three replications. Tukey's HSD test was used to partition the main effects of species, mixture ratios, water regime, fertilization and their interactions.

Three-way ANOVA was used to evaluate effects of water regime, fertilization and mixture ratio on RYT and WUE of the two species combinations, and aggressivity values of *Bothriochloa ischaemum* and RCI of the species in their mixtures. Aggressivity and relative competition intensity values were analyzed by species-specific ANOVAs. In these analyses, aggressivity values of *Bothriochloa ischaemum* to *Lespedeza davurica* calculated from total, shoot and root biomass was modeled as the response variable, while water regime (HW, MW and LW), fertilization (CK, N, P and NP), mixture ratio (except monoculture) and the interaction (water regime: fertilization: mixture ratio) were used as categorical explanatory variables. Also, RCI values of each species calculated from total biomass was modeled as the response variable, while water regime (HW, MW and LW), fertilization (CK, N, P and NP), mixture ratio (except monoculture) and the interaction (water regime: fertilization treatment: mixture ratio) were used as categorical explanatory variables.

Four-way ANOVA was used to evaluate effects of species, water regime, fertilization and mixture ratio on the biomass (shoot, root and total) production and root/shoot (R/S) ratio of each species. In these analyses, biomass production and partition ratio were modeled as the response variable, while species, water regime (HW, MW and LW), fertilization (CK, N, P and NP), mixture ratio (except monoculture) and the interaction (water regime: fertilization treatment: mixture ratio) were used as categorical explanatory variables.

Differences were considered significant at $P < 0.05$ throughout this paper. Statistical analyses were performed using SPSS 17.0. All the figures were drawn using SmartDraw 7.0 (http://www.smartdraw.com/).

Results

Biomass production and allocation

Irrespective of fertilization, water stress significantly decreased the total biomass production for each species, either in monoculture or in their mixtures (Fig. 1). Under each water regime, N, P, or NP treatments, especially N treatment, can significantly improve biomass production of the two species in their different mixture proportions (Fig. 1). Under sufficient water supply (HW) or moderate water stress (MW) regimes, N and NP treatments had similar biomass production, and both were higher than P treatment. Under a serious water stress regime (LW), NP treatment had much higher biomass than N and P treatments. *L. davurica* had the highest biomass when grown alone, and its biomass production decreased gradually as its proportion decreased in the mixture, while for *B. ischaemum*, the highest biomass production was obtained when grown at 10:2 *B. ischaemum*:*L. davurica* mixture proportion, under most water regimes or fertilizations. Replacement series diagrams based on total biomass production illustrated that the curves of *B. ischaemum* were concave, while the curves of *L. davurica* were convex (Fig. 1). The lines intersected to the left of the *B. ischaemum*:*L. davurica* 6:6 mixture proportions, i.e., the proportion of *B. ischaemum* was lower in the mixture. The effect of each individual factor (species, water regime, fertilization and mixture ratio), and all their interactions on total, shoot or root biomass were statistically significant (see Table 5 in the Supplementary Material).

There was no obvious change trend in root/shoot ratio of *B. ischaemum* or *L. davurica* in their mixture proportions (Figs. 2, 3). On average, both species had significant higher mean root/shoot ratio values under the LW regime. Compared with CK, N and NP treatments decreased the root/shoot ratios of *B. ischaemum*, while they increased under P treatment (Fig. 2). For *L. davurica*, fertilization decreased the root/shoot ratios, irrespective of water regime, and the trend of reduction extent was NP > P > N (Fig. 4). The effect of each individual factor (species, water, fertilization and mixture ratio), and all their interactions except spe-

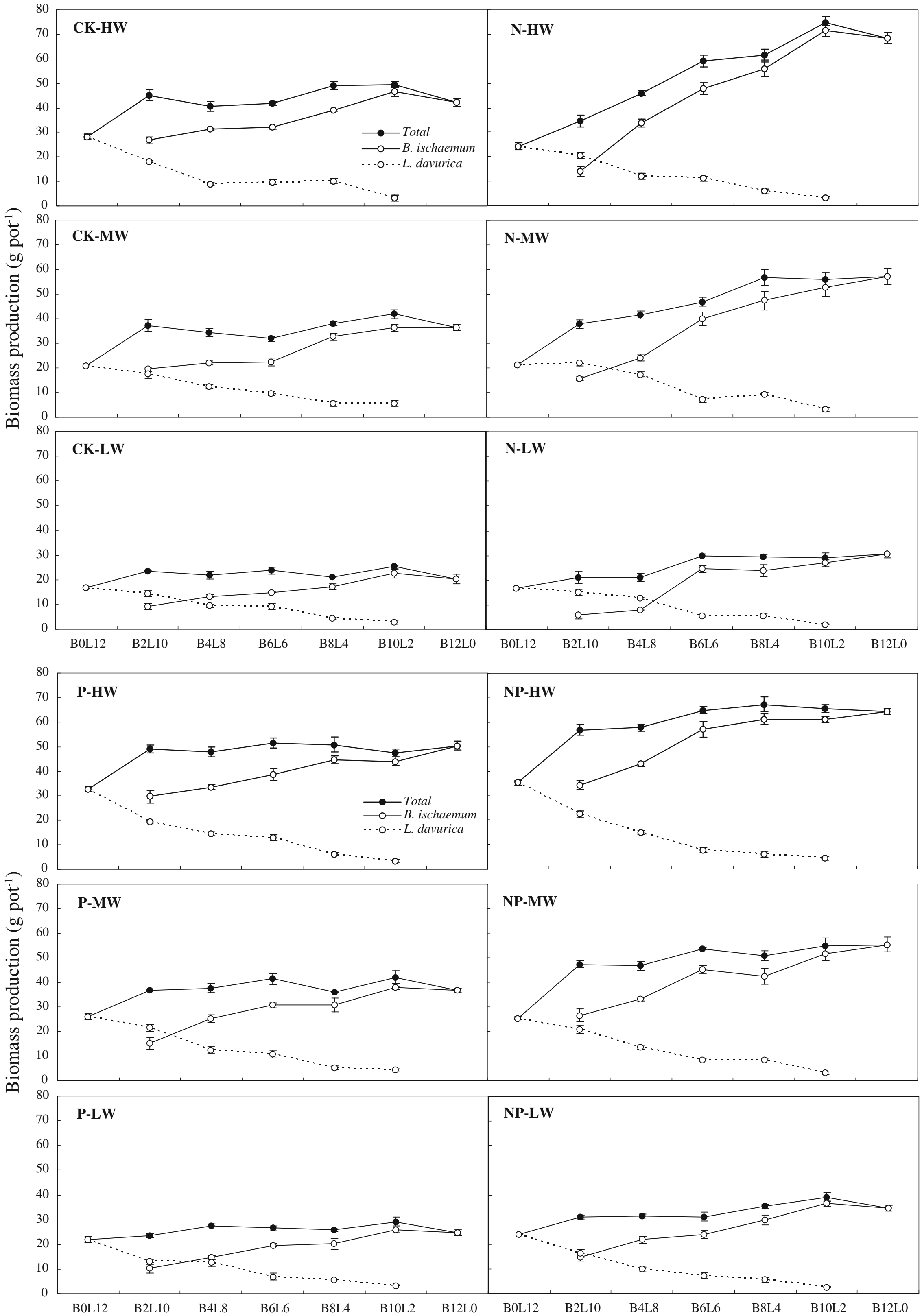

Fig. 1 Total biomass production of the two species (B, *Bothriochloa ischaemum*; L, *Lespedeza davurica*) in the replacement series under three water regimes [*HW* sufficient water supply, 80 ± 5 % FC (field capacity); *MW* moderate water stress, 60 ± 5 % FC; *LW* severe water stress, 40 ± 5 % FC) and four fertilizations (CK, N, P, NP). *Error bars* Standard error

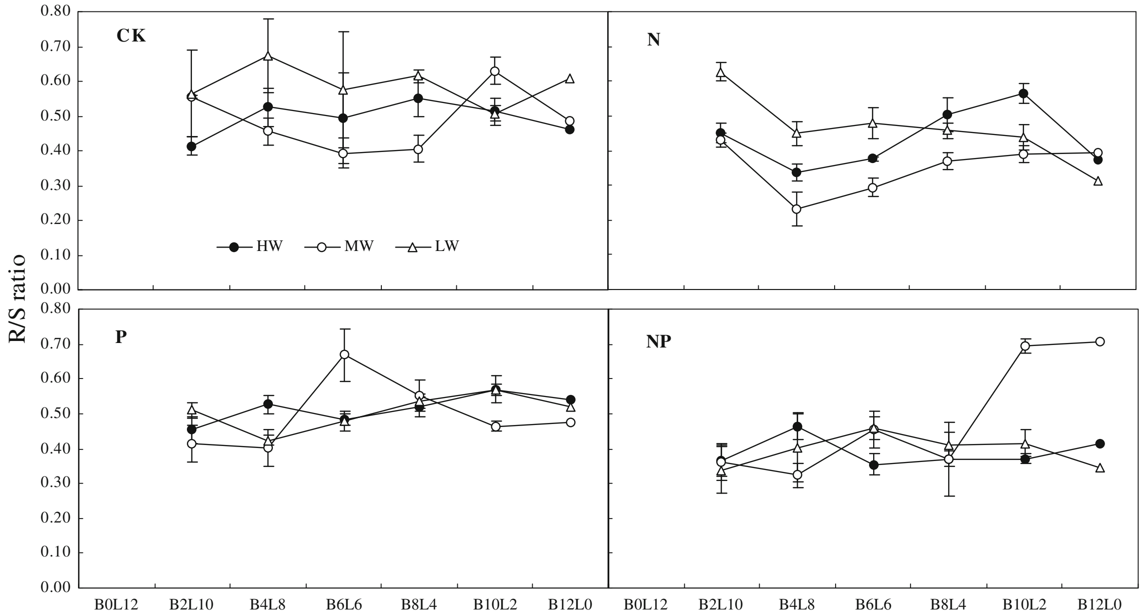

Fig. 2 Root/shoot (*R*/*S*) ratio of *B. ischaemum* in the replacement series under different water and fertilization regimes. See Fig. 1 for treatments. *Error bars* Standard error

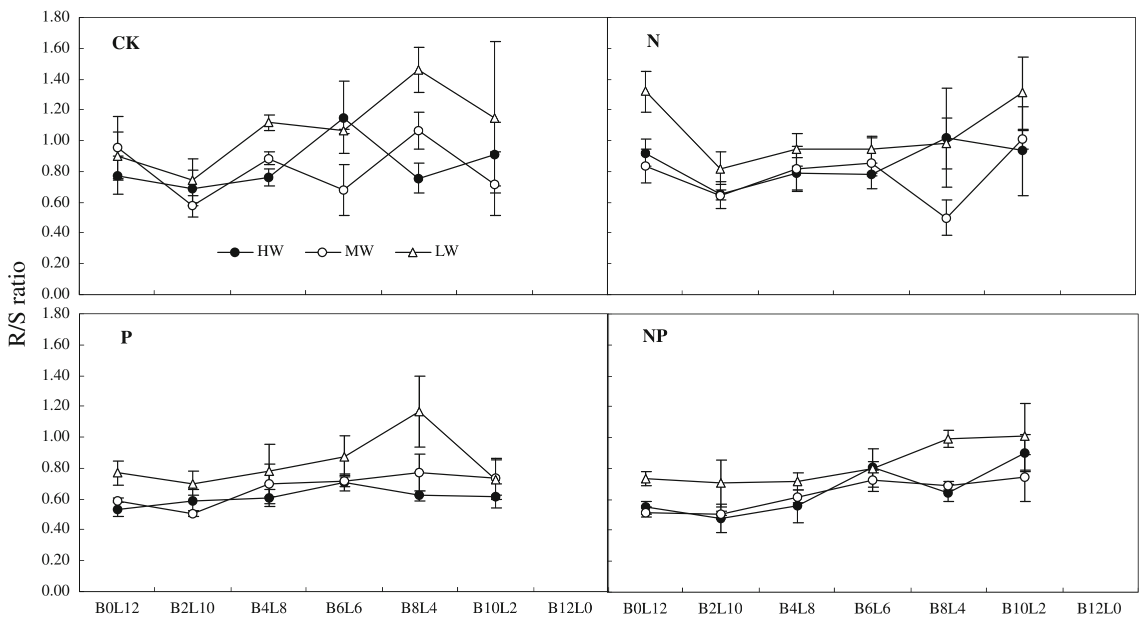

Fig. 3 Root/shoot (*R*/*S*) ratio of *L. davurica* in the replacement series under different water and fertilization regimes. See Fig. 1 for treatments. *Error bars* Standard error

cies × water regime × fertilizer on root/shoot ratio were statistically significant (see Table 5 in the Supplementary Material).

Relative yield total

RYT values for total biomass production in this study ranged from 0.98 to 1.39, and there were no obvious changing trend as the mixture proportion changed under different fertilizations or water regimes (Table 1). Except in three cases (4:8 and 10:2 *B. ischaemum*:*L. davurica* mixture proportion under HW with N and P application, respectively, and 6:6 *B. ischaemum*:*L. davurica* proportion under LW with NP), RYT values were all greater than 1.0 (Table 1). RYT > 1.0 indicates that there was a yield advantage of their mixture in terms of biomass produc-

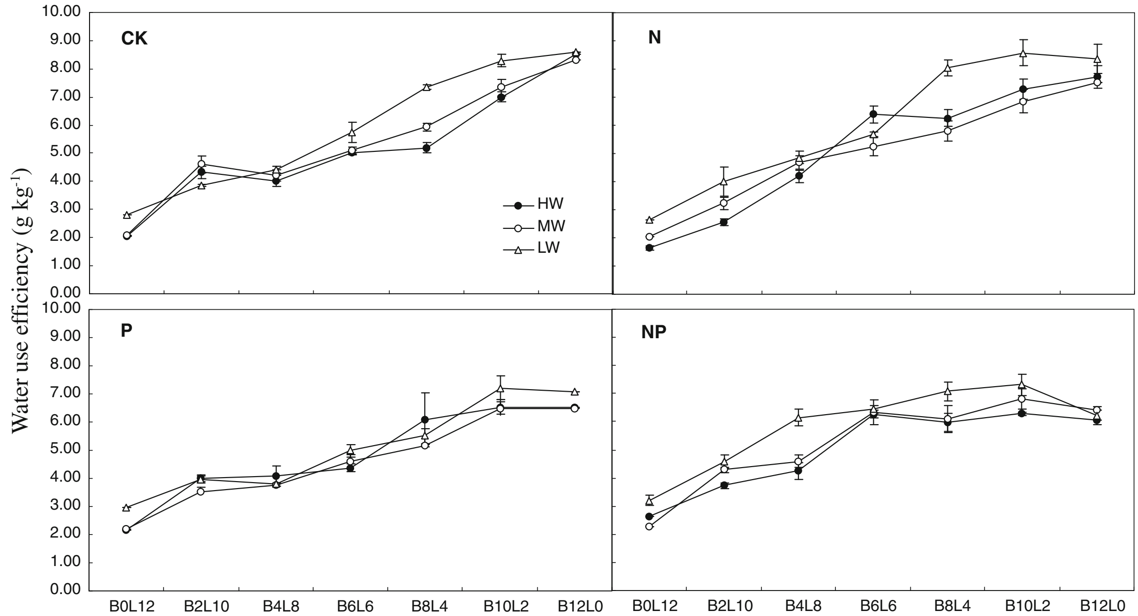

Fig. 4 Water use efficiency (WUE) based on total biomass production and water transpired from *B. ischaemum* and *L. davurica* in the replacement series under different water and fertilization regimes. See Fig. 1 for treatments. *Error bars* Standard error

Table 1 Relative yield total (RYT) values for total biomass of *Bothriochloa ischaemum* and *Lespedeza davurica* under each water and fertilizer treatment within the replacement series

Water treatment	Planting scheme (B/L)[a]	Fertilizer treatment			
		CK	N	P	NP
HW	2/10	1.29 ± 0.05	1.06 ± 0.08	1.18 ± 0.03	1.17 ± 0.06
	4/8	1.07 ± 0.09	0.99 ± 0.02	1.11 ± 0.06	1.09 ± 0.01
	6/6	1.11 ± 0.06	1.16 ± 0.01	1.16 ± 0.07	1.11 ± 0.03
	8/4	1.29 ± 0.09	1.06 ± 0.02	1.07 ± 0.07	1.12 ± 0.06
	10/2	1.22 ± 0.08	1.18 ± 0.07	0.98 ± 0.06	1.07 ± 0.04
MW	2/10	1.39 ± 0.08	1.31 ± 0.07	1.24 ± 0.03	1.30 ± 0.03
	4/8	1.20 ± 0.08	1.24 ± 0.07	1.17 ± 0.06	1.14 ± 0.05
	6/6	1.07 ± 0.03	1.03 ± 0.01	1.25 ± 0.05	1.15 ± 0.05
	8/4	1.16 ± 0.03	1.27 ± 0.09	1.04 ± 0.02	1.10 ± 0.08
	10/2	1.27 ± 0.04	1.07 ± 0.04	1.20 ± 0.08	1.06 ± 0.05
LW	2/10	1.31 ± 0.03	1.11 ± 0.09	1.02 ± 0.07	1.11 ± 0.04
	4/8	1.22 ± 0.06	1.04 ± 0.05	1.18 ± 0.06	1.04 ± 0.04
	6/6	1.27 ± 0.11	1.14 ± 0.05	1.11 ± 0.07	0.99 ± 0.04
	8/4	1.10 ± 0.06	1.12 ± 0.05	1.07 ± 0.04	1.09 ± 0.02
	10/2	1.28 ± 0.11	1.00 ± 0.09	1.20 ± 0.02	1.16 ± 0.04

HW sufficient water supply, 80 ± 5 % FC (field capacity); *MW* moderate water stress, 60 ± 5 % FC; *LW* severe water stress, 40 ± 5 % FC. Values are mean ± SE, $n = 3$

[a] B/L represents the density of *B. ischaemum* as the first species versus *L. davurica* in each pot

tion. Combined with the results shown in Fig. 1, it can be deduced that *B. ischaemum* component yields in mixtures were higher than expected on the basis of their proportion, whereas *L. davurica* component yields in mixtures were lower than expected. The effect of water, fertilization, mixture ratio and all their interactions on RYT values calculated from total, shoot or root biomass were statistically significant, except the effect of water on RYT values calculated from root biomass of the two species in their mixtures (see Table 6 in Supplementary Material).

Aggressivity

In nearly all mixtures, the A values of *B. ischaemum* to *L. davurica* were positive, which implied that *B. ischaemum* was the dominant species (Table 2). Except under N treatment, in which the A values of *B. ischaemum* to *L.*

davurica showed no obvious trend as the proportion of *B. ischaemum* increased in the mixtures. Under CK, P and NP treatments, the A values of *B. ischaemum* to *L. davurica* decreased as the proportion of *B. ischaemum* increased in the mixtures, irrespective of water regime. In each water regime, there were no obvious changing trends of A values of *B. ischaemum* to *L. davurica* calculated from total biomass, shoot biomass or root biomass (Table 2). Water regime, fertilization, mixture ratio and all their interactions on RYT values calculated from total, shoot or root biomass were statistically significant (see Table 6 in the Supplementary Material).

Relative competition intensity

In nearly all mixture ratios and treatments, the RCI values of *B. ischaemum* (i.e., RCI_B) were less than zero, indicating that the effects of intraspecific competition with *L. davurica* were stronger than those of interspecific competition, while for *L. davurica*, RCI_L values were greater than zero, suggesting that interspecific competition was stronger, especially under P and NP treatments (Table 3). Under CK, P and NP treatments, RCI_B values increased as the proportion of *B. ischaemum* increased in the mixture, and the absolute value in each proportion decreased as water stress increased, while there was no obvious changing trend in each water regime under N treatment (Table 3). For *L. davurica*, the RCI_L values did not show any obvious trend under each water and fertilization treatment. The effect of water regime, fertilizer, mixture ratios and all their interactions on RCI_B values calculated from total, shoot or root biomass were statistically significant (Table 4). Except for the interaction of water regime × fertilizer on RCI_L value based on total biomass, water regime, fertilizer, mixture ratio and all their interactions on RCI_L values calculated from total, shoot or root biomass were also statistically significant (Table 4).

Water use efficiency

Water use efficiency (WUE) increased gradually as the proportion of *B. ischaemum* increased while that of *L. davurica* decreased in the mixture (Fig. 4). *L. davurica* monoculture had significant lower WUE across all mixture proportions, irrespective of water or fertilization. On average, LW treatment had significantly higher mean WUE than HW and MW treatments, and there were no significant difference between the later two. Under CK treatment, *B. ischaemum* monoculture had significant higher WUE in each water regime, while under fertilization, 10:2 *B. ischaemum*:*L. davurica* mixture proportion had significantly higher WUE, especially under LW regime (Fig. 4). Water regime, fertilizer, mixture ratio and all their interactions statistically significantly affected WUE values of the two species mixtures (see Table 7 in the Supplementary Material).

Table 2 Aggressivity (A) values based on total, shoot and root biomass of *B. ischaemum* to *L. davurica* under each water and fertilizer treatment within the replacement series

Water treatment	Planting scheme (B/L)	CK Total	CK Shoot	CK Root	N Total	N Shoot	N Root	P Total	P Shoot	P Root	NP Total	NP Shoot	NP Root
HW	2/10	3.06 ± 0.31	3.05 ± 0.39	2.73 ± 0.55	0.24 ± 0.08	0.07 ± 0.02	0.70 ± 0.23	2.81 ± 0.14	3.05 ± 0.16	2.37 ± 0.17	2.44 ± 0.09	2.66 ± 0.09	1.88 ± 0.09
	4/8	1.77 ± 0.12	1.67 ± 0.06	1.98 ± 0.33	0.75 ± 0.15	0.62 ± 0.02	0.72 ± 0.21	1.40 ± 0.16	1.45 ± 0.18	1.39 ± 0.04	1.37 ± 0.07	1.30 ± 0.02	1.53 ± 0.19
	6/6	0.82 ± 0.11	0.90 ± 0.09	0.73 ± 0.32	0.49 ± 0.05	0.41 ± 0.05	0.58 ± 0.13	0.74 ± 0.05	0.88 ± 0.07	0.48 ± 0.01	1.33 ± 0.08	1.47 ± 0.05	1.03 ± 0.17
	8/4	0.37 ± 0.02	0.21 ± 0.28	0.36 ± 0.07	0.52 ± 0.10	0.41 ± 0.13	0.86 ± 0.18	0.77 ± 0.09	0.82 ± 0.10	0.68 ± 0.06	0.91 ± 0.15	0.98 ± 0.09	0.92 ± 0.16
	10/2	0.84 ± 0.12	0.65 ± 0.30	0.49 ± 0.10	0.53 ± 0.04	0.43 ± 0.07	0.89 ± 0.10	0.36 ± 0.05	0.44 ± 0.11	0.50 ± 0.13	0.50 ± 0.02	0.58 ± 0.10	0.29 ± 0.06
MW	2/10	2.19 ± 0.19	1.80 ± 0.39	2.72 ± 0.12	0.48 ± 0.08	0.26 ± 0.08	0.79 ± 0.19	1.60 ± 0.16	1.62 ± 0.20	1.56 ± 0.09	2.01 ± 0.24	2.45 ± 0.08	0.98 ± 0.17
	4/8	0.93 ± 0.07	0.94 ± 0.13	0.87 ± 0.22	0.14 ± 0.01	0.17 ± 0.05	−0.36 ± 0.15	1.46 ± 0.08	1.57 ± 0.13	1.34 ± 0.12	0.99 ± 0.17	1.55 ± 0.09	0.32 ± 0.16
	6/6	0.38 ± 0.05	0.53 ± 0.10	0.41 ± .18	0.74 ± 0.14	0.86 ± 0.16	0.52 ± 0.06	0.83 ± 0.19	0.69 ± 0.12	1.34 ± 0.06	0.96 ± 0.09	1.33 ± 0.10	0.41 ± 0.14
	8/4	0.47 ± 0.02	0.70 ± 0.16	0.44 ± 0.10	−0.05 ± 0.04	−0.34 ± 0.04	0.31 ± 0.12	0.66 ± 0.09	0.65 ± 0.18	0.69 ± 0.09	0.16 ± 0.06	0.55 ± 0.04	−0.44 ± 0.14
	10/2	−0.43 ± 0.10	−0.77 ± 0.14	−0.14 ± 0.05	0.21 ± 0.10	0.19 ± 0.03	0.38 ± 0.09	0.35 ± 0.01	0.42 ± 0.03	0.26 ± 0.04	0.53 ± 0.08	0.57 ± 0.05	0.42 ± 0.12
LW	2/10	1.90 ± 0.32	1.35 ± 0.14	1.65 ± 0.36	0.16 ± 0.07	−0.31 ± 0.21	0.52 ± 0.05	1.76 ± 0.13	1.75 ± 0.19	1.43 ± 0.04	1.77 ± 0.22	1.64 ± 0.21	1.63 ± 0.22
	4/8	1.14 ± 0.25	1.16 ± 0.30	1.15 ± 0.36	−0.46 ± 0.05	−0.58 ± 0.11	−0.34 ± 0.14	1.09 ± 0.10	1.18 ± 0.22	0.89 ± 0.10	1.18 ± 0.06	1.20 ± 0.25	1.54 ± 0.08
	6/6	0.46 ± 0.05	0.51 ± 0.19	0.24 ± 0.06	0.94 ± 0.10	0.78 ± 0.12	1.09 ± 0.22	0.98 ± 0.10	1.02 ± 0.18	0.92 ± 0.11	0.78 ± 0.14	0.69 ± 0.16	1.27 ± 0.13
	8/4	0.58 ± 0.09	0.85 ± 0.04	0.37 ± 0.04	0.22 ± 0.03	0.07 ± 0.03	0.28 ± 0.07	0.46 ± 0.06	0.59 ± 0.03	0.40 ± 0.05	0.69 ± 0.07	0.66 ± 0.14	0.67 ± 0.19
	10/2	0.31 ± 0.06	0.16 ± 0.03	0.31 ± 0.03	0.29 ± 0.04	0.40 ± 0.12	0.23 ± 0.04	0.43 ± 0.10	0.31 ± 0.10	0.57 ± 0.15	0.63 ± 0.08	0.65 ± 0.12	0.85 ± 0.08

Mean ± SE, $n = 3$

Table 3 Relative competition intensity (RCI) of *B. ischaemum* (RCI_B) and *L. davurica* (RCI_L) calculated from the total biomass of each species under each water and fertilizer treatment within the replacement series

Water treatments	Planting scheme (B/L)	CK		N		P		NP	
		RCI_B	RCI_L	RCI_B	RCI_L	RCI_B	RCI_L	RCI_B	RCI_L
HW	2/10	−2.84 ± 0.31	0.22 ± 0.01	−0.23 ± 0.16	−0.02 ± 0.06	−2.52 ± 0.11	0.29 ± 0.04	−2.20 ± 0.10	0.23 ± 0.06
	4/8	−1.25 ± 0.17	0.52 ± 0.05	−0.49 ± 0.11	0.26 ± 0.05	−1.00 ± 0.22	0.34 ± 0.06	−1.00 ± 0.05	0.37 ± 0.01
	6/6	−0.52 ± 0.07	0.30 ± 0.09	−0.40 ± 0.02	0.09 ± 0.03	−0.53 ± 0.10	0.21 ± 0.05	−0.77 ± 0.06	0.56 ± 0.04
	8/4	−0.39 ± 0.06	−0.09 ± 0.15	−0.22 ± 0.06	0.27 ± 0.09	−0.33 ± 0.09	0.44 ± 0.05	−0.43 ± 0.09	0.48 ± 0.10
	10/2	−0.32 ± 0.05	0.31 ± 0.32	−0.25 ± 0.08	0.21 ± 0.13	−0.05 ± 0.08	0.38 ± 0.08	−0.14 ± 0.05	0.26 ± 0.15
MW	2/10	−2.21 ± 0.09	−0.02 ± 0.11	−0.66 ± 0.17	−0.24 ± 0.07	−1.50 ± 0.21	0.01 ± 0.06	−1.89 ± 0.26	0.02 ± 0.08
	4/8	−0.82 ± 0.11	0.11 ± 0.07	−0.27 ± 0.10	−0.22 ± 0.06	−1.05 ± 0.19	0.28 ± 0.07	−0.80 ± 0.16	0.19 ± 0.03
	6/6	−0.23 ± 0.07	0.09 ± 0.07	−0.40 ± 0.06	0.34 ± 0.08	−0.66 ± 0.10	0.16 ± 0.12	−0.63 ± 0.09	0.33 ± 0.04
	8/4	−0.35 ± 0.08	0.22 ± 0.12	−0.25 ± 0.12	−0.32 ± 0.06	−0.25 ± 0.02	0.40 ± 0.08	−0.16 ± 0.10	0.01 ± 0.05
	10/2	−0.20 ± 0.05	−0.62 ± 0.33	−0.11 ± 0.03	0.09 ± 0.23	−0.23 ± 0.11	0.00 ± 0.17	−0.13 ± 0.12	0.27 ± 0.16
LW	2/10	−1.72 ± 0.44	−0.03 ± 0.09	−0.19 ± 0.20	−0.10 ± 0.07	−1.48 ± 0.15	0.27 ± 0.06	−1.58 ± 0.14	0.19 ± 0.08
	4/8	−0.98 ± 0.22	0.17 ± 0.06	0.20 ± 0.17	−0.16 ± 0.07	−0.81 ± 0.29	0.14 ± 0.06	−0.89 ± 0.18	0.39 ± 0.06
	6/6	−0.47 ± 0.09	−0.07 ± 0.15	−0.61 ± 0.08	0.33 ± 0.07	−0.58 ± 0.02	0.36 ± 0.15	−0.38 ± 0.05	0.40 ± 0.10
	8/4	−0.27 ± 0.12	0.24 ± 0.08	−0.17 ± 0.07	−0.02 ± 0.10	−0.22 ± 0.06	0.24 ± 0.02	−0.30 ± 0.04	0.32 ± 0.13
	10/2	−0.34 ± 0.17	0.01 ± 0.24	−0.06 ± 0.13	0.27 ± 0.06	−0.26 ± 0.04	0.11 ± 0.08	−0.27 ± 0.04	0.36 ± 0.07

Mean ± SE, $n = 3$

Table 4 Summary (*F* and *P* values) of analysis of variance for the effects of water, fertilization and mixture ratio on RCI of *B. ischaemum* (RCI_B) and *L. davurica* (RCI_L) in mixtures, respectively

Source	*df*	RCI_B						RCI_L					
		Total		Shoot		Root		Total		Shoot		Root	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
WR	2	27.378	< 0.001*	30.987	< 0.001*	26.675	< 0.001*	62.568	< 0.001*	42.684	< 0.001*	26.863	< 0.001*
FT	3	98.408	< 0.001*	139.482	< 0.001*	49.043	< 0.001*	48.605	< 0.001*	57.826	< 0.001*	5.591	0.001*
MR	4	325.855	< 0.001*	461.432	< 0.001*	159.321	< 0.001*	14.381	< 0.001*	17.609	< 0.001*	4.688	0.002*
WR × FT	6	2.672	0.018*	9.368	< 0.001*	10.665	< 0.001*	1.788	0.107	3.776	0.002*	4.077	0.001*
WR × MR	8	9.623	< 0.001*	12.921	< 0.001*	4.389	< 0.001*	4.227	< 0.001*	2.712	0.009*	3.375	0.002*
FT × MR	12	34.716	< 0.001*	49.010	< 0.001*	18.007	< 0.001*	9.402	< 0.001*	8.435	< 0.001*	3.703	< 0.001*
WR × FT × MR	24	4.320	< 0.001*	5.418	< 0.001*	4.143	< 0.001*	8.173	< 0.001*	6.783	< 0.001*	3.460	< 0.001*

WR Water regime, *FT* fertilization treatment, *MR* mixture ratio
* Probabilities considered statistically significant

Discussion

Herbaceous-legume intercropping is a widely used agricultural system with significant advantage in yield and resource utilization, and is considered as an important practice in sustainable agricultural development in resource-limited environments (Ghosh 2004; Li et al. 2007). Improvements in intercropping yields may be achieved through an understanding of yield advantages due to species interactions such as species competition or facilitation mechanisms in their mixtures (Semere and Froud-Williams 2001). Experimental studies of plant interference have usually focused on the effect of one biotic or abiotic factor on the growth of one or several competing populations of plants (Connolly et al. 2001). While in reality, plants may face different kinds of environmental stress as well as species interaction that will affect their growth and performance in the community. The purpose of this work was to examine the effects of both water and fertilization on a replacement series of *B. ischaemum* and *L. davurica*. The replacement series design method has been used routinely to characterize the interactions in plant interference studies, especially cereal-legume intercropping. This design has been criticized because it apparently cannot adequately differentiate between interspecific and intraspecific competition (Connolly et al. 2001). However, the method is useful in evaluating relative growth in monoculture, interference effects between two species (or plant types) at a single total density, and assessing interference interactions between species in mixtures (Gealy et al. 2005).

Biomass production is the most important index in evaluating species competition (Gibson 1999). In this study, biomass production of the two species was highly significantly affected by both fertilization and irrigation, which confirms that herbaceous production is strongly co-limited by water and nutrients in the semiarid Loess

Plateau (Shan and Chen 1993). The highest biomass production (grams plant^{-1}) for *B. ischaemum* was obtained when intercropped with *L. davurica*, while the highest biomass production (g plant^{-1}) for *L. davurica* was obtained when grown alone (Fig. 1). These suggested that *B. ischaemum* grew better with intraspecific rather than with interspecific competition, while *L. davurica* grew better with interspecific competition. Fleming et al. (1988) reported that the more aggressive species in the mixture increased in dry weight with increased proportions compared to the less aggressive species. Aminpanah and Javadi (2011) also reported that the more aggressive rice Deylamani had higher aboveground biomass under intercropping with rice barnyard grass, while the less competitive rice Hahsemi had higher aboveground biomass under monoculture. Under intercropping, equal competition between species would be represented by lines with constant slopes across all ratios, resulting in an intersection point of the two curves at a ratio of 6:6. As shown in Fig. 1, the two curves did not intersect at the 6:6 ratio under all water and fertilization regimes, indicating the frequent occurrence of interspecific competition based on the response of total biomass. The figure shows that *B. ischaemum* and *L. davurica* lines intersect to the left of the 6:6 mixture ratios, indicating that *B. ischaemum* is more competitive than *L. davurica* (Aminpanah and Javadi 2011).

Relative yield total represents the sum of the proportional changes in yield that occurred in the mixtures and measures the degree to which the two-species mixture make demands on the same resources. RYT is one of the indicators most commonly used in analyzing species interaction and intercropping productivity (Connolly et al. 2001). The RYT values of mixtures were generally greater than 1.0, regardless of water regime and fertilization, indicating partial resource complementarity between *B. ischaemum* and *L. davurica* in their mixtures (Table 1). The RYT values of these mixtures were 0.98–1.39, which means that a maximum of 39 % more land area or other resources would be required by a monoculture system to equal the biomass yield of intercropping system (Gao et al. 2009; Lithourgidis et al. 2011). Therefore, the *B. ischaemum* and *L. davurica* intercropping system may improve land-use efficiency considerably. Most studies of legume/non-legume intercrops have given RYT values of greater than 1.0 (Semere and Froud-Williams 2001; Lithourgidis et al. 2011), which have been attributed to differences in root and shoot characteristics, and the use of different soil sources (Banik et al. 2000). Results have shown that water, fertilizer, mixture ratio and all their interactions had significant effects on RYT, showing that the degree of complementarity was affected by water resources and the two species combination ratio (Table 6 of Supplementary Material). The results of A values confirmed that *B. ischaemum* was the dominant species (A values positive) in *B. ischaemum* and *L. davurica* mixtures (Table 2). Generally, the aggressivity of *B. ischaemum* to *L. davurica* decreased as water stress increased, indicating a decrease in its ability to make use of the water supply. Semere and Froud-Williams (2001) found that water stress decreased the relative competitive ability of maize to pea in a two-level water regime pot experiment.

Relative competition intensity is generally considered to provide the most ecologically valid measure for comparing the effect of competition (Weigelt and Jolliffe 2003). In our experiment, competition between individuals of the same species seems to be more intense for *L. davurica* (Table 3), which can partially explain its discrete distribution in nature. The high intraspecific and low interspecific competitive ability might explain the nearly single-species community of *B. ischaemum* in the area (Xu et al. 1997). Besides interspecific competition, interspecific facilitation also exists in the legume–cereal association. Legumes may transfer fixed N to intercropped cereals during their joint growing period and this N is an important resource for the cereals (Zhang and Li 2003). Some early studies indicated that the non-legume was much more competitive than the legume for soil N, forcing the legume to rely mainly on N_2 fixation for its N nutrition (Corre-Hellou et al. 2007; Hauggaard-Nielsen et al. 2009). Also, the dominance of cereals over legumes in intercrops is often attributed to the fact that their roots access a larger volume of soil. Thus, the greater root access to soil resources and a higher N demand are probably the main reasons for the much higher competitive ability of *B. ischaemum* for soil N (Corre-Hellou et al. 2007). When the nitrogen availability was improved, the competitive dynamics for nitrogen may change directly or it may create a situation where another resource (e.g., phosphorus) becomes more limiting (LeJeune and Seastedt 2001). In the present study, except under N treatment, the A values of *B. ischaemum* decreased as its proportion increased, while RCI values increased as its proportion increased in the mixtures, irrespective of water regime (Tables 2, 3), which confirmed the conclusion that intraspecific competition between individuals of *B. ischaemum* was higher than interspecific competition, and also indicated that the competition between *B. ischaemum* and *L. davurica* for soil phosphorus became intense when soil N availability increased.

Water use efficiency—here actually transpiration WUE—is defined as the amount of total biomass produced per unit volume of water transpired by mixtures during water treatment. In intercropping systems, WUE waiss influenced by plant density and crop proportion (Morris and Garrity 1993). Generally, WUE increased as the proportion of *B. ischaemum* increased in the mixture, and was affected by water and fertilization (Fig. 4). Thus, to improve WUE in the *B. ischaemum* and *L. davurica* intercropping system, their planting proportion should be adjusted appropriately. It is very difficult to separate water use by two crops during the co-growth period (Morris and Garrity 1993; Gao et al. 2009). This study did not attempt to investigate water use by *B. ischaemum* and *L. davurica* separately in the intercropping system. The sap flow and carbon isotope discrimination technique, which can measure directly the transpiration rate of each plant, may be a helpful tool to study water absorption by each crop in an intercropping system (Davies et al. 2010; He et al. 2012).

Studies have shown a general reduction in growth associated with smaller pot sizes (Townend and Dickinson 1995; Whitfield et al. 1996; Ray and Sinclair 1998). Ray and Sinclair (1998) pointed out that, for both maize and soybean, and in both well-watered control and water-deficit regime, there was a significant reduction in shoot dry weight and total transpiration with decreasing pot size. Compared with the results of our former experiment, which used smaller pots (Xu et al. 2011a, b), average WUE was higher in the present study, and large differences existed as the proportion of *B. ischaemum* increased in the mixtures (Fig. 4). The reason for this was that biomass production increased as pot size increased, while pot size did not influence the response of transpiration to soil water content (Ray and Sinclair 1998). In addition, the C_4 photosynthetic pathway followed by *B. ischaemum* offers higher water and nitrogen use efficiencies, compared to the less efficient C_3 photosynthetic pathway, and *L. davurica* favors higher competitive ability for *B. ischaemum* under limited resource conditions.

Precipitation regimes are predicted to become more variable, with more extreme rainfall events punctuated by longer intervening dry periods, and water-limited ecosystems are likely to be highly responsive to altered precipitation regimes (Schwinning and Ehleringer 2001). Globally, both precipitation patterns and nitrogen deposition rates show directional changes over time. It is uncertain how the dominant species respond to concurrent changes in water and nutrient availability in semiarid regions. Soil N and water content are coupled tightly to the growth of plants in semiarid region on the Loess Plateau of China. Our results provided increasing evidence that the positive response of *B. ischaemum* to water and nitrogen may lead to an increase in the biomass of this species, and perhaps ultimately the number of individuals in relatively wet seasons, years or regions compared with *L. davurica*, while decreasing its competitive ability in the semiarid Loess Plateau.

Recently, the rational use of native dominant species for vegetation construction and rehabilitation has gained increasing attention in the semiarid Loess Plateau of China (Jiao et al. 2007; Shan and Xu 2009; Wang et al. 2011). Our research suggests the existence of intercropping advantages and resource use complementarity when growing these species together, either under adequate or deficit water conditions, and with or without fertilization. Therefore, the two-species intercropping system may be helpful to ensure forage production and improve WUE, and is worth putting into practice in the semiarid Loess Plateau. By the way, the study also implies that fertilizer, especially nitrogen, could be applied to the natural grassland community to increase the growth of two dominant species, in order to accelerate vegetation establishment, natural successional process and mitigate desertification in the region.

Conclusions

The results indicated that mixtures of *B. ischaemum* and *L. davurica* had a biomass production advantage for exploiting the available environmental resources compared with their respective monocultures. Total biomass yield in *B. ischaemum* and *L. davurica* intercropping rose by a maximum of 39 %, compared to their yields in monoculture. Competition indices indicated that *B. ischaemum* was the dominant species in the mixture. Average WUE in the intercropping system was less than that of *B. ischaemum* in monoculture, but greater than that of *L. davurica* in monoculture. Among all the mixtures, the *B. ischaemum* to *L. davurica* 10:2 mixture was found to use water most efficiently under a water-deficit regime. Both *B. ischaemum* and *L. davurica* are perennial species, and other factors such as morphology, physiology and the different requirements for nutrients will affect performance in their mixtures. Thus, further research is required to study those factors, especially in the field with long-term intervals.

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