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ORIGINAL ARTICLE

Morphological changes in roots of *Bothriochloa ischaemum* intercropped with *Lespedeza davurica* following phosphorus application and water stress

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Abstract
Root morphological characteristics are important parameters for the evaluation of plant adaptation to stress environment. In this study, a pot experiment was conducted to investigate changes in the root morphology of *Bothriochloa ischaemum* intercropped with *Lespedeza davurica* under three soil water regimes and two phosphorus (P) fertilizer treatments. Results showed that root biomass (RB) per *B. ischaemum* plant decreased as its proportion increased in the mixtures. There were no significant differences in root:shoot ratio (RSR) among the mixture ratios, and P application did have consistent effects on the RSR. *Bothriochloa ischaemum* tended to have smaller root surface area (RSA), root average diameter (RAD), specific root length (SRL), and specific root area (SRA) under water stress conditions. There was a negative linear relationship between RB and RSA under each water and P treatment. Negative and positive linear relationships were found between RB and TRL (total root length), TRL and RSA, respectively, except under severe water stress without P application. P application decreased the RAD and increased the SRA and SRL of *B. ischaemum* under water stress. All these suggest two apparent response mechanisms for *B. ischaemum* under water stress and P application: an increase in length of small diameter roots and decrease in root weight density.

Keywords: *Root average diameter, root surface area, specific root area, specific root length, total root length*

Introduction

Plant growth and productivity are limited by water and phosphorus (P) availability in most terrestrial ecosystems, especially in arid and semi-arid regions. Plants exhibit an array of physiological responses to water and P availability, including morphological responses of the root system (Raghothama 1999; Ho et al. 2004). Root trait responses that influence the root system's spatial exploitation of the soil are particularly important for the acquisition of P, which is relatively immobile in most soils, especially in heavily weathered soils (Ma et al. 2001; Li et al. 2007; Delgado et al. 2012). The identification of adaptive strategies, including the associated physiological and ecological trade-offs, is essential in understanding plant adaptation to water and P limited ecosystems. Plants develop various strategies for enhancing P acquisition from P-deficient soils, including increase in root surface area (RSA), specific root length (SRL), root:shoot ratio (RSR), and root exudation of carboxylates, as well as decrease in proton release (Hinsinger et al. 2003; Li et al. 2007; Tang et al. 2009).

Root data have become more important when evaluating the environmental impacts of agriculture, especially in arid and semi-arid regions (Campbell & de Jong 2001). Many studies on root distributions in pure and mixed stands have been carried out for crops, shrubs, trees, and some grasses, but root growth and morphological adaptations for enhanced P-acquisition have received less attention (Lynch & Brown 2008). Various morphological parameters, such as the branching pattern, root length density, specific root area (SRA), and SRL that are influenced by genetic variability and environmental conditions, have been

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used as potential indicators of soil nutrient status (Ostonen et al. 2007). The use of such traits by plants for P acquisition efficiency would be most evident in competitive environments, including those experienced by wild plants, and plants in agroecosystems or high-density monocultures (Li et al. 2007; Lynch & Brown 2008). Minimizing resource competition between plants while maximizing the use of available resources is central to improve yield and overall productivity in multi-species cropping systems (Li et al. 2007; Zamora et al. 2007). Theoretical modeling showed that interplant competition can be important in determining an optimal balance of plastic and non-plastic root phenotypes under conditions of P-stress and combined P and water stresses (Lynch & Brown 2008). In contrast to the aboveground competition for a single resource (i.e., light), belowground competition among plants is for multiple resources and can be more intense and can reduce plant performance more than aboveground competition (Casper & Jackson 1997; Zhang et al. 2012). Thus, an analysis based on the appearance of aboveground plant parts alone is not sufficient to clarify the essence of species competition in plant mixtures or natural communities.

Bothriochloa ischaemum and *Lespedeza davurica* are two co-dominant species in natural grassland communities in the semi-arid Loess Plateau region of China. *Bothriochloa ischaemum* is a C_4 perennial grass species while *L. davurica* is a C_3 perennial leguminous subshrub, and both are distributed mainly in the temperate zones of the world. Besides their potential as excellent natural pasture species, many agronomic attributes make these two wild species ideal for breeding as forage species due to their high eco-adaptability and quality. We found that the two species grown together in pots would maximize biomass production due to their complementary effects (Xu et al. 2011a,b). To the best of our knowledge, no study on the root morphology of *B. ischaemum* has been reported, especially when grown in mixtures with *L. davurica* under both P and water deficit conditions. Root morphological characteristics play an important role in belowground resource acquisition for plants, affecting plant competitive ability in community or mixtures. Therefore, this experiment was carried out to clarify the root morphological characteristics of *B. ischaemum* in the mixtures, and to investigate potential mechanisms associated with the changes in root morphology in response to resources availability under controlled conditions with or without added P under three water regimes.

Materials and methods

Plant materials

The species used were *Bothriochloa ischaemum* L. Keng and *Lespedeza davurica* (Laxm.) Schindl. Seeds were obtained in the autumn of 2008 from the experimental fields at the Ansai Research Station (ARS) of the Chinese Academy of Sciences (36°51′30″N, 109°19′23″E, altitude 1068–1309 m a.s.l.), located at the center of the semi-arid 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.

Growth conditions

The experiment was conducted under a rainout shelter in 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. Mean annual precipitation is 650 mm. The sandy loam, loessial soil used was collected from the upper 20 cm of an arable field in ARS. In the pot experiment, soil was packed to a bulk density of 1.2 g cm^{-3}, a porosity of 55% and soil gravimetric moisture contents at field capacity (FC) and wilting point of 20.0% and 4.0%, respectively. The soil organic matter content was 0.33%, and total N, P, and K contents were 0.019%, 0.061%, and 2.01%, respectively; available N, P, and K contents were 27.72, 3.99, and 83.40 mg kg^{-1}, respectively.

Seeds of each species were sown in 3.67-l plastic pots (17.6 cm × 19 cm × 14.7 cm; height × upper inner diameter × lower inner diameter). Each pot contained 3.0 kg of dry soil. A plastic pipe was inserted into the soil adjacent to pot inner wall to supply water. During seedling establishment period, the soil moisture content was maintained above 80% FC.

Species combination design

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

Water and P treatments

The experiment began on 28 March 2009. Three soil water regimes were applied on 30 June when the seedling biomass of each species was about 0.2 g per seedling (dry weight). Just before the water regimes were imposed, the soil surface of each pot was covered with 40 g of perlite (2.0 cm deep) to reduce evaporation. At that time, *B. ischaemum* was at the five-leaf stage, and *L. davurica* had no new branching. Subsequently, pots were watered daily to replace water losses determined by weighing, in order to maintain the three water regimes: 80 ± 5% FC (HW, sufficient water supply), 60 ± 5% FC

(MW, moderate water stress), and 40 ± 5% FC (LW, severe water stress). Two P treatments were applied during pot packing: one being the application of P (0.1 g P_2O_5 per kg dry soil) and the other had no P added, i.e., P was at the initial level found in the soil taken from the field.

Experimental design

The experiment used a factorial design of seven mixture ratios, two P treatments and three water regimes, arranged in a completely randomized design beneath the rainout shelter. Treatments were in four replications.

Shoot and root samplings

At the end of the growth period (5 October), three randomly selected pots within each treatment were selected for destructive sampling to obtain root morphology estimations prior to biomass measurements. At each sampling time, the 12 seedlings in each selected pot were harvested, and the shoots and roots were separated after carefully removing the roots from the soil and washing. The root system was separated from the soil using a gentle water jet over a sieve that collected any detached root fragments. Since the root systems were large, only about 30% of each root system was used for the morphological measurements. The root system subsample was dyed by immersing it in 0.5% methylene blue solution for 5 min to improve the contrast of the root image, gently dried with absorbent paper, and placed between two transparent plastic sheets to keep it in a fixed position. A scanned (BENQ color scanner 5560) image was analyzed (DT-Scan, Delta T-Devices) to determine the total root length (TRL), RSA, and root average diameter (RAD; Tsakaldimi et al. 2005). The shoot and whole root system dry biomass were obtained after drying at 75°C for 48 h in an oven. The RSR was calculated as the ratio of root biomass (RB) to shoot biomass. For each individual plant, the SRL ($cm\,g^{-1}$) was determined from the TRL divided by the RB (Trubat et al. 2006; Cortina et al. 2008), and the specific root area (SRA) was the total surface area divided by the total RB (Hill et al. 2006).

Statistical analysis

Data were processed using Microsoft Office Excel 2003. An analysis of variance (Procedures in SPSS 17.0) was used to partition the main effects of mixture ratios, P treatments, water levels, and their interactions. Treatment means were compared using Tukey's HSD (honestly significant difference) test at the 0.05 probability level.

Results

RB and RSR

For each water regime, the RB of individual *B. ischaemum* plant generally increased with P application, and the relative increase was notably greater when its proportion in the mixtures with *L. davurica* was lower (Table I). Water regime, P level, mixture ratio, and their interactions all significantly affected the RB of *B. ischaemum* (Table II). The RSR without P application ranged between 0.44 and 0.59, and there were no significant differences among mixture ratios under each water regime (Table I). When P was applied, there were significant increases in the RSR of *B. ischaemum* in only five mixtures, i.e., the ratios of *B. ischaemum* to *L. davurica* were 2:10 and 4:8 under HW, and 2:10, 6:6, and 10:2 under LW (Table I). There were no obvious changes in RSR of *B. ischaemum* in the different mixtures receiving P across the three water levels (Table I). The effect of each individual factor (P, water regime, mixture ratio) on RSR was significant as was the interaction of water × P (Table II).

Root surface area

Water stress significantly decreased the RSA of *B. ischaemum*, and RSA also decreased as the proportion of *B. ischaemum* increased in the mixtures under both P treatments (Figure 1). Without P application, MW and LW reduced the RSA of *B. ischaemum* across mixtures by 28.0% and 66.1% and in monocultures by 26.6% and 59.7%, as comparing with those under HW regime. Compared with MW, LW decreased the RSA by 52.9% and 45.1% in the mixtures and monoculture, respectively (Figure 1). When P was applied, MW and LW reduced the RSA of *B. ischaemum* by 19.9% and 57.4% across mixtures, and by 11.9% and 50.0% in monocultures, comparing with those under HW regime. Compared with MW, LW decreased the RSA by 46.6% and 43.2% in the mixtures and monoculture, respectively (Figure 1). The effects of P, water regime, mixture ratio, and the interactions of water × ratio and P × ratio on RSA were significant (Table III). Significant linear correlations were observed between RSA and RB (Table IV).

Total root length

TRL per plant followed a similar trend as RSA and decreased as the proportion of *B. ischaemum* increased in the mixtures for each water regime (Figure 2). Generally, water stress decreased the TRL, but the differences between any two water regimes decreased as the proportion of *B. ischaemum* increased (Figure 2). Water regime, P level, mixture

Table I. RB (g plant^{-1}) and RSR per plant of *Bothriochloa ischaemum* (B) intercropped with *Lespedeza davurica* (D) under three water regimes and two levels of P.

Water treatments	Mixture ratio (B/D)	RB		RSR	
		−P	+P	−P	+P
HW	2/10	0.90 ± 0.03 a(b)	1.24 ± 0.14 a(a)	0.44 ± 0.07a(b)	0.48 ± 0.05abc(a)
	4/8	0.67 ± 0.02 bc(b)	0.82 ± 0.07 b(a)	0.44 ± 0.01 a(b)	0.46 ± 0.01 abc(a)
	6/6	0.67 ± 0.04 b(b)	0.74 ± 0.04 b(a)	0.50 ± 0.06 a(a)	0.52 ± 0.02 ab(a)
	8/4	0.59 ± 0.05 bcd(a)	0.58 ± 0.05 c(a)	0.49 ± 0.05 a(a)	0.46 ± 0.01 bc(a)
	10/2	0.46 ± 0.01 defg(a)	0.50 ± 0.04 cde(a)	0.50 ± 0.04 a(a)	0.49 ± 0.04 abc(a)
	12/0	0.40 ± 0.08 fghi(a)	0.45 ± 0.02 cdef(a)	0.46 ± 0.07 a(a)	0.46 ± 0.09 abc(a)
MW	2/10	0.54 ± 0.14 cdef(b)	0.84 ± 0.14 b(a)	0.45 ± 0.14 a(a)	0.48 ± 0.08 abc(a)
	4/8	0.58 ± 0.03 bcd(b)	0.78 ± 0.02 b(a)	0.58 ± 0.03 a(a)	0.61 ± 0.02 a(a)
	6/6	0.54 ± 0.03 bcde(a)	0.52 ± 0.03 cd(a)	0.59 ± 0.04 a(a)	0.55 ± 0.05 ab(a)
	8/4	0.44 ± 0.06 efgh(a)	0.44 ± 0.04 cdefg(a)	0.52 ± 0.05 a(a)	0.48 ± 0.06 abc(a)
	10/2	0.39 ± 0.03 ghij(a)	0.41 ± 0.03 defg(a)	0.56 ± 0.06 a(a)	0.56 ± 0.06 ab(a)
	12/0	0.36 ± 0.06 ghij(a)	0.35 ± 0.03 efgh(a)	0.58 ± 0.03 a(a)	0.49 ± 0.06abc(a)
LW	2/10	0.33 ± 0.06 hijk(b)	0.46 ± 0.03 cdef(a)	0.55 ± 0.11 a(a)	0.35 ± 0.04 c(b)
	4/8	0.32 ± 0.03 hijk(a)	0.37 ± 0.08 defgh(a)	0.58 ± 0.10 a(a)	0.47 ± 0.09 abc(a)
	6/6	0.30 ± 0.02 ijk(a)	0.32 ± 0.01 fgh(a)	0.61 ± 0.03 a(a)	0.54 ± 0.02 ab(b)
	8/4	0.26 ± 0.03 jk(a)	0.23 ± 0.04 h(a)	0.48 ± 0.08 a(a)	0.43 ± 0.08 bc(a)
	10/2	0.23 ± 0.01 k(a)	0.24 ± 0.01 h(a)	0.52 ± 0.04 a(a)	0.42 ± 0.04 bc(b)
	12/0	0.22 ± 0.02 k(b)	0.28 ± 0.01 gh(a)	0.59 ± 0.09 a(a)	0.51 ± 0.02 ab(a)

Notes: The replacement series B/L represents the numbers of *B. ischaemum* and *L. davurica* plants in each pot (HW: sufficient water supply, 80 ± 5% FC; MW: moderate water stress, 60 ± 5% FC; LW: severe water stress, 40 ± 5% FC). + P or − P represents with or without P addition. Values followed by different letters are significantly different within a column ($P < 0.05$) or within a row for different letters in parentheses. Data indicate means ± SE ($n = 3$).

ratio, and their interactions all had significant effects on TRL except the interaction of water × P × mixture ratio (Table III). Significant linear correlations were observed between TRL and RB, TRL and RSA except under LW without P application. There were significant linear correlations between TRL and RSA when not considering water regimes (Table IV).

Root average diameter

There were no obvious trends in the RADs of *B. ischaemum* in the various mixtures whether in water or P treatments. However, RAD values were significantly higher in the monoculture without P application under both HW (0.71 mm) and MW (0.63 mm) than under LW (0.42 mm) (Figure 3). In mixtures without P application, the mean RAD values were 0.41 for HW, 0.42 for MW, and 0.41 mm for LW, but there were no significant differences among them. With P application, the mean RAD values were 0.41 for HW, 0.40 for MW, and 0.37 mm for LW in the mixtures, while in the monocultures they were 0.39, 0.37, and 0.32 mm, respectively (Figure 3). Considering the mean RAD of all the mixtures, including the monoculture, the P application had little effect under HW and MW, but both were significantly higher than that under LW. For each mixture ratio, there were no obvious differences between P treatments, but in the monoculture, P application was associated with significantly lower RADs for each water regime (Figure 3). Water

Table II. Analysis of variance of effects of mixture ratio, water and P treatments on RB (g plant^{-1}), and RSR per plant of *Bothriochloa ischaemum* intercropped with *Lespedeza davurica*.

Effect	df	RB		RSR	
		F value	*P* value	*F* value	*P* value
Phosphorus level (PL)	1	68.449	< **0.001**	9.925	**0.002**
Water regime (WR)	2	539.521	< **0.001**	9.267	< **0.001**
Mixture ratio (MR)	5	157.530	< **0.001**	5.730	< **0.001**
WR × PL	2	4.157	**0.018**	8.076	**0.001**
MR × PL	5	20.328	< **0.001**	0.211	0.957
MR × WR	10	22.215	< **0.001**	1.826	0.064
MR × WR × PL	10	2.036	**0.036**	1.018	0.433

Note: Probabilities considered statistically significant ($P < 0.05$) are indicated in bold.

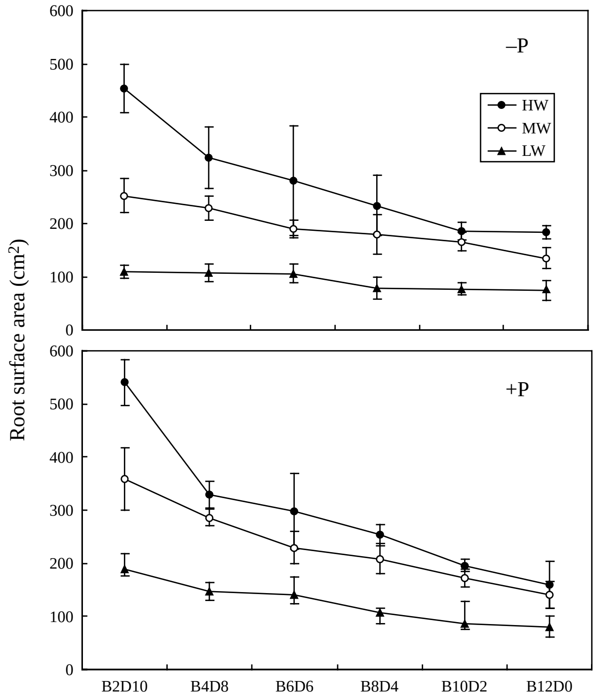

Figure 1. RSA of *Bothriochloa ischaemum* in a replacement series with *Lespedeza davurica* grown under three water regimes (HW: sufficient water supply, 80 ± 5% FC; MW: moderate water stress, 60 ± 5% FC; LW: severe water stress, 40 ± 5% FC) without (− P) or with (+ P) addition of P. BnDn in abscissa represents planting ratio of B: *B. ischaemum* and D: *L. davurica*. Mean ± standard error, $n = 3$.

regime, P level, mixture ratio, and all their interactions, except for water × P, significantly affected the RAD values of *B. ischaemum* (Table III).

SRA and SRL

Without P application, the mean SRA of all mixture ratios differed significantly across water regimes in the order: HW > MW > LW. However, with P application, the MW regime had the highest mean SRA ($P < 0.05$), but there was no significant difference between HW and LW (Figure 4). Plants under HW without P application had significantly higher SRA, whereas those treated with P under MW and LW had significantly higher SRA. Mean SRL followed similar trends as SRA (Figure 5). Water and the interaction of water × P significantly affected SRA and SRL (Table III).

Discussion

Drought and soil infertility, especially P deficiency, often co-occur and are primary constraints to plant production in ecological and agricultural communities in most arid and semi-arid terrestrial ecosystems (Raghothama 1999; Ho et al. 2004). Although numerous studies have focused on the interactions of

Table III. Analysis of variance of effects of mixture ratio, water and P treatments on RSA, RAD, TRL, SRA, and SRL of *Bothriochloa ischaemum* intercropped with *Lespedeza davurica*.

Effect	df	RSA (cm^2)		RAD (mm)		RTL (cm)		SRA ($cm^2\,g^{-1}$)		SRL ($m\,g^{-1}$)	
		F value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value
Phosphorous level (PL)	1	20.053	**<0.001**	37.305	**<0.001**	29.532	**<0.001**	0.495	0.484	1.947	0.167
Water level (WL)	2	223.454	**<0.001**	6.597	**0.002**	240.640	**<0.001**	7.432	**0.001**	5.045	**0.009**
Mixture ratio (MR)	5	65.863	**<0.001**	7.586	**<0.001**	73.662	**<0.001**	1.800	0.124	1.214	0.312
WL × PL	2	0.816	0.446	0.219	0.804	3.383	**0.039**	3.742	**0.028**	3.971	**0.023**
MR × PL	5	<3.738	**0.005**	11.380	**<0.001**	2.531	**0.036**	1.969	0.094	0.561	0.729
MR × WL	10	10.334	**<0.001**	3.672	**0.001**	10.469	**<0.001**	0.743	0.682	0.688	0.733
MR × WL × PL	10	0.151	0.999	2.012	**0.044**	0.250	0.989	0.548	0.850	0.536	0.859

Note: Probabilities considered statistically significant ($P < 0.05$) are indicated in bold.

Table IV. Linear regressions among RSA (cm^2), RB ($g\,plant^{-1}$), and TRL (cm) under the three water and two P application treatments.

Water regime	P(±)	*Y*	*X*	Slope	Intercept	R^2	*P*
HW	−	RSA	RB	−71.80	548.01	0.65	<0.001
	+	RSA	RB	−106.03	742.25	0.78	<0.001
MW	−	RSA	RB	−33.38	293.64	0.80	<0.001
	+	RSA	RB	−73.00	472.53	0.67	<0.001
LW	−	RSA	RB	−20.31	128.16	0.44	<0.01
	+	RSA	RB	−37.55	199.87	0.47	<0.01
HW	−	TRL	RB	−176.47	1371.2	0.48	<0.01
	+	TRL	RB	−244.34	1762.2	0.73	<0.001
MW	−	TRL	RB	−111.87	855.61	0.78	<0.001
	+	TRL	RB	−187.45	1256.9	0.75	<0.001
LW	+	TRL	RB	−91.02	523.37	0.45	<0.01
HW	−	RSA	TRL	0.37	15.62	0.83	<0.001
	+	RSA	TRL	0.39	10.25	0.85	<0.001
MW	−	RSA	TRL	0.22	78.33	0.52	<0.001
	+	RSA	TRL	0.37	−1.31	0.78	<0.001
LW	+	RSA	TRL	0.29	24.94	0.53	<0.001
HW + MW + LW	−	RSA	TRL	0.36	13.25	0.87	<0.001
	+	RSA	TRL	0.39	−5.9	0.87	<0.001

Notes: HW: sufficient water supply, 80 ± 5% FC; MW: moderate water stress, 60 ± 5% FC; LW: severe water stress, 40 ± 5% FC). + P or − P represents with or without P addition.

water and P availability in relation to plant growth, studies describing specific root traits that enable a plant to acquire these resources have not been well documented (Ho et al. 2005). A common response of plants to P deficiency is to increase RSR (Lynch & Brown 2008). Increased relative allocation to root is obviously beneficial for P acquisition, but may slow overall plant growth because of the increased

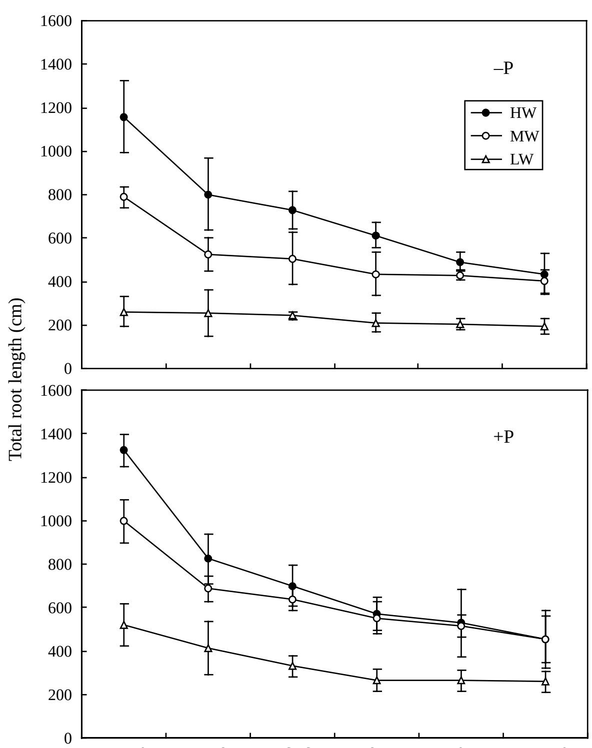

Figure 2. TRL of *Bothriochloa ischaemum* in a replacement series with *Lespedeza davurica* grown under three water regimes (HW: sufficient water supply, 80 ± 5% FC; MW: moderate water stress, 60 ± 5% FC; LW: severe water stress, 40 ± 5% FC) without (− P) or with (+ P) addition of P. BnDn in abscissa represents planting ratio of B: *B. ischaemum* and D: *L. davurica*. Mean ± standard error, *n* = 3.

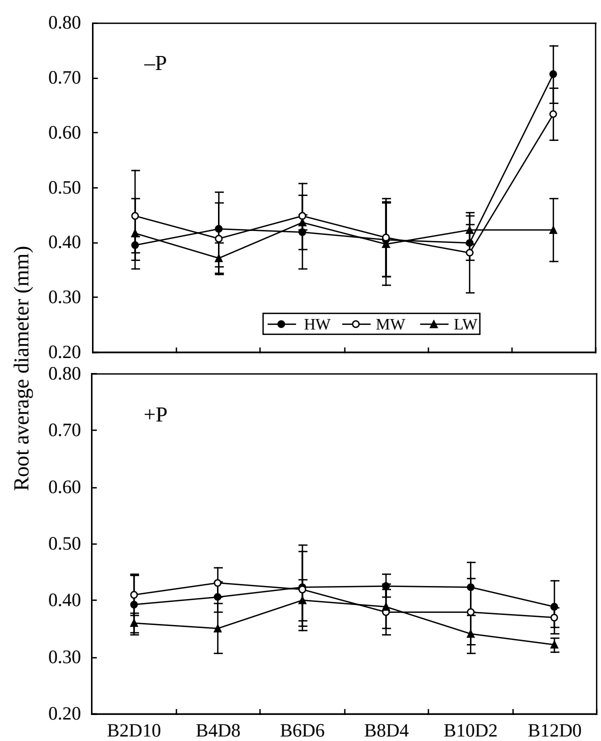

Figure 3. RAD of *Bothriochloa ischaemum* in a replacement series with *Lespedeza davurica* grown under three water regimes (HW: sufficient water supply, 80 ± 5% FC; MW: moderate water stress, 60 ± 5% FC; LW: severe water stress, 40 ± 5% FC) without (− P) or with (+ P) addition of P. BnDn in abscissa represents planting ratio of B: *B. ischaemum* and D: *L. davurica*. Mean ± standard error, *n* = 3.

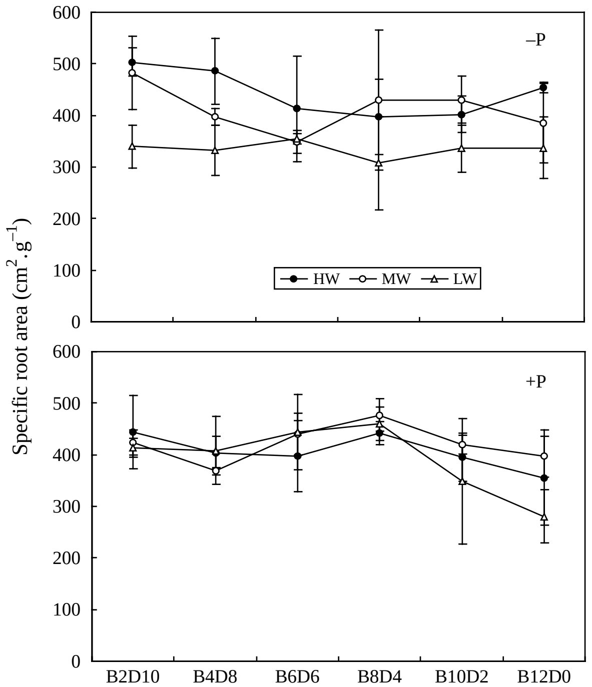

Figure 4. SRA of *Bothriochloa ischaemum* in a replacement series with *Lespedeza davurica* grown under three water regimes (HW: sufficient water supply, 80 ± 5% FC; MW: moderate water stress, 60 ± 5% FC; LW: severe water stress, 40 ± 5% FC) without (−P) or with (+P) addition of P. BnDn in abscissa represents planting ratio of B: *B. ischaemum* and D: *L. davurica*. Mean ± standard error, $n = 3$.

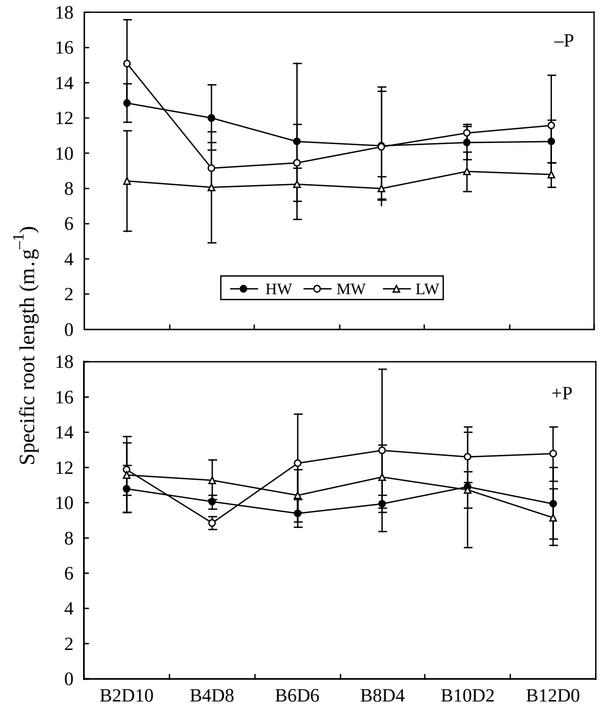

Figure 5. SRL of *Bothriochloa ischaemum* in a replacement series with *Lespedeza davurica* grown under three water regimes (HW: sufficient water supply, 80 ± 5% FC; MW: moderate water stress, 60 ± 5% FC; LW: severe water stress, 40 ± 5% FC) without (−P) or with (+P) addition of P. BnDn in abscissa represents planting ratio of B: *B. ischaemum* and D: *L. davurica*. Mean ± standard error, $n = 3$.

respiratory burden on root tissue (Nielsen et al. 2001; Fan et al. 2003). Depending on the plant species and growth conditions, more than 50% of the daily carbon is consumed by root respiration (Nielsen et al. 2001; Fan et al. 2003). In this experiment, P application significantly improved the RSR in only three mixture ratios (i.e., 2:10, 6:6, and 10:2) for plants under serious water stress (LW) (Tables I and II), implying that species competition may play an important role in biomass allocation (van Auken & Bush 2010).

The RAD has been considered to be an important root property to evaluate plant adaptability (Gahoonia & Nielsen 2004). The root diameter is effectively related to soil volume contact when a given amount of photosynthate is invested. Decreasing root diameter is one important mechanism for plant adaptation to water deficit environment, and this is significant because it increases the surface-to-volume ratio, thus facilitating water uptake and root elongation (van der Weele et al. 2000). Root systems under water stress have been observed to produce thin roots in most plant species (van der Weele et al. 2000). In this study, only serious water stress (i.e., LW) significantly decreased RAD, and P application also significantly decreased RAD across water level (Table III; Figure 3). Most species decreased root diameter in response to P deficiencies (Hill et al. 2006), but there are others, e.g., *Arabidopsis thaliana*, whose root diameters were larger under low P conditions (Ma et al. 2001). In this study, the contradictory results can be mainly attributed to the significant effect of species competition for limited water and P (Table III). Plant root systems have an inherent capability to adjust to prevailing environmental conditions through their morphological and physiological plasticities (Zamora et al. 2007). Possibly, *B. ischaemum* also has high heritability of root diameter, since in this experiment, although water stress had significant effects, the RAD values of *B. ischaemum* only showed small fluctuations in all the mixture ratios (Figure 3). Under each water regime, P application significantly increased RSA (Figures 1 and 3), which corresponded to Leuschner et al. (2004) who pointed out that plants can increase RSA for a given amount of carbon investment by producing thinner roots that have a larger RSA with P application.

SRL is another indicator of the absorptive surface produced per unit root volume, and has been used in root morphological variations in relation to different nutrient levels, water content, and soil types (Tsakaldimi et al. 2005; Zobel et al. 2006; Ostonen et al. 2007). The variation in SRL between plant species and genotypes is well documented, because it is believed to characterize the economic aspects of root systems (Fan et al. 2003; Gahoonia & Nielsen 2004; Xie et al. 2006). Increasing SRL is one possible intense strategy by which roots can acquire more nutrients by increasing the volume of soil exploited per unit biomass invested in the roots (Ostonen et al. 2007). Longer, finer roots would be more efficient in nutrient acquisition and can form larger root systems for a lower carbon cost than shorter, thicker roots to adapt to infertile or competitive environments (Nicotra et al. 2002; Trubat et al. 2006; Xie et al. 2006). Therefore, higher SRL values indicate thinner roots and higher exploitation efficiency under intensive competition conditions (Eissenstat 1992). However, this "traditional" interpretation does not appear to be consistently applicable in practice. For example, the interpretation appears to explain increases in SRL reported by Trubat et al. (2006) for some Mediterranean woody species in response to water limitation, but appears to fail to explain low SRL values reported by Nicotra et al. (2002). Furthermore, the SRL of fine roots has been shown to increase, decrease, or stay constant in response to nutrient limitation (Ostonen et al. 2007). For our results, the interpretation for increased SRL could apply to the plants that did not receive additional P, while the contradictory behavior was observed when P was applied (Figure 5). The contradictory response has been found in native perennial grasses that have evolved in both a nutrient and water-limited environment (Hill et al. 2006; Ostonen et al. 2007). In these cases, the contradictory response was attributed to the benefits of higher root mass density. Furthermore, lower SRL may lead to greater longevity of the root system, which is beneficial to the plant in terms of conserving the carbon budget.

The SRA is considered to be the best index for characterizing the overall root structure. The more the growing conditions differ from the optimal, the greater SRA becomes. Disregarding differences in the density of plant tissues, SRA characterizes the ratio of root area to volume and is inversely proportional to the mean diameter of the roots (Lohmus et al. 1989). Both SRA and SRL values were significantly lower for *B. ischaemum* grown under water stress (MW, LW) regimes with P application (Figures 4 and 5). Higher SRA and SRL values indicate that the carbon costs of root system construction were lower. Species with high SRA and SRL are more likely to be smaller plants (e.g., annual species) with high growth rates and low green shoot biomasses (Roumet et al. 2006). Root system morphology and fine root distribution are fundamental factors in determining the magnitude of interspecific belowground competition in mixed species systems (George et al. 1996). There will be higher root hair densities and longer root hairs in plants grown in P-limited conditions (Bates & Lynch 2000; Ma et al. 2001). Changes in root SRL that lead to increased SRA were important for most plant species adapting to low P conditions (Hill et al. 2006). In this study, significantly higher SRL and SRA values of *B. ischaemum* were only observed under well-watered conditions (HW) without P application, while the SRL and SRA values were significantly higher under moderate (MW) and severe (LW) water deficits when P was added (Figure 5). The relatively larger increases in SRL of plants growing under LW (Figure 5) with P application were a consequence of the plant adapting to conditions by decreasing RAD (Figure 3) and increasing RB (Table I).

Data from our previous studies showed strong competitions between *B. ischaemum* and *L. davurica*, and *B. ischaemum* was the dominant species in their mixtures, there was an apparent complementarity between them under different soil water regimes with or without P application (Xu et al. 2011a,b). Although mixture ratio, water regime, and P affected RB yield of *B. ischaemum*, in this study, regardless of the mixture ratio and water regime, there was a consistent response whereby the RAD decreased and RB, TRL, and RSA all increased. LW not only decreased the RB, TRL, RSA, and RAD but also the SRA and SRL, thus the root tissue density increased, which implied resource conservation and increased root longevity strategy to harsh environments (Useche & Shipley 2010). All these suggest that there were two apparent response mechanisms for *B. ischaemum* when P was added: (1) an increase in the length of fine roots (to increase the surface area for absorption) and (2) a decrease in root weight density (to increase metabolic activity) (Table IV) (Zobel et al. 2006).

Animal husbandry is one of the traditional industries in semi-arid regions of the Loess Plateau of China (Shan & Chen 1993), but there is a lack of fine species with high adaptability and good quality for establishing artificial pastures or meadows. To find an appropriate mixture ratio is important for sustainable biomass production and rational use of the two co-dominant species in the area. Our results suggest that, in the semi-arid Loess Plateau of China, which has degraded loess soil with low P availability and limited rainfall, the improvement of root morphological traits by the application of a proper amount of P fertilizer might offset the adverse effects of water deficit and increase its abilities in competing and acquiring for limited resources.

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