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ROOT GROWTH AND MORPHOLOGY OF SWITCHGRASS (*PANICUM VIRGATUM* L.) AND BUSH CLOVER (*LESPEDEZA DAVURICA* S.) IN MIXED PLANTATION UNDER VARYING SOIL WATER AND PHOSPHORUS SUPPLY CONDITIONS

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Abstract

Water and phosphorus (P) are two major factors affecting plant growth on the Loess Plateau of China. To clarify root response of introduced species to native species in mixture under varying water and P supplies would be favorable for assessing their interactions. This study investigated the effects of soil water, P and mixture ratio on root growth and morphology of switchgrass (*Panicum virgatum* L.) (an introduced C_4 perennial herbaceous grass) and bushclover (*Lespedeza davurica* S.) (a C_3 perennial leguminous subshrub) in mixtures in a pot experiment. Two soil water regimes [75 ± 5% field capacity, FC (high water, HW) and 35 ± 5% FC (low water, LW)], three P treatments (addition of 0, 0.05 and 0.1 g of P_2O_5 per kg dry soil) and 5 mixture ratios of the two species (12:0, 8:4, 6:6, 4:8, 0:12) were complemented. Results showed that switchgrass tended to decrease root average diameter (RAD) and increase specific root length (SRL) under LW comparing with HW, while bushclover showed the opposite trends. P application significantly ($p<0.05$) deceased the RAD of switchgrass under LW, while had no effect on SRL, resulting in a thinner root system with higher root tissue density. Regardless of soil water regimes, SRL of bushclover decreased significantly after P application. Root biomass and total root length of switchgrass were increased with the decrease of mixture ratio, while both parameters decreased in bushclover. In mixture with switchgrass, bushclover decreased SRL and proportion of fine root length (0-0.5 mm diameter class). The present study implied that switchgrass would be superior when mixed with bushclover, and P application might increase the abilities of switchgrass in competing and acquiring for limited resources.

Key words: Mixture ratio, Root competition, Root biomass, Root diameter class, Specific root length.

Introduction

As a common farming practice, intercropping growing two or more plant species or genotypes together for a time, may reduce environmental stress and optimize the utilization of limited resources (Li *et al*., 2001; Brooker *et al*., 2015; Li *et al*., 2016). In many low-input/resource-limited environments, intercropping is important because it is favorable for stabilizing yield with decreased inputs (Brooker *et al*., 2015; Bargaz *et al*., 2016). Legume and non-legume mixtures confer more efficient exploitation of limited resources for higher yield production through stimulation of nodulation, root distribution and associated-biochemical changes, and thus widely practiced in resource-limited environments (Li *et al*., 2001, 2016; Xu *et al*., 2011; Bargaz *et al*., 2016). Although mixed plantation shows many advantages, the competition between co-existing species is critical for this superiority persistence in production practice (Ren *et al*., 2010; Ren *et al*., 2016). Competition inequality between shoots (*i.e.* light) (Ren *et al*., 2010; Poorter & Ryser, 2015) and roots (*i.e.* water and nutrients) (Bargaz *et al*., 2016; Ren *et al*., 2016) may lead to the changes in community structure, and the dominated species may disappear eventually (Xu *et al*., 2008). In humid regions, shoot competition for light may be the main determinant of mixture productivity (Friday & Fownes, 2002; Ren *et al*., 2010), while in arid and semiarid regions, root competition would be the key factor limiting the productivity of mixtures (Ren *et al*., 2010; Bargaz *et al*., 2016).

Phosphorus (P) is one key nutrient among the crucial abiotic constraints for plant growth (Suriyagoda *et al*., 2010; Fan *et al*., 2015). Plants growing in low P environment typically have a greater specific root length (SRL), thinner root diameter as well as more fine roots and root hairs (Suriyagoda *et al*., 2010; Zou *et al*., 2019). Under low P conditions, faster root growth has been reported correlated with higher P uptake, and such phenomenon is more obvious in cereals than legumes (Li *et al*., 2006; Bargaz *et al*., 2017). Plants sensitive to P improvement also tend to grow faster in mixed plantation, therefore occupy soil volume rapidly and take the dominant position in the competition (Xia *et al*., 2013). Moreover, P availability is closely related to soil moisture because diffusion is the main mechanism for its transfer from soil to roots (Fan *et al*., 2015). An understanding of root growth in mixtures in response to both soil water and P supply is important than that of either individual alone (Suriyagoda *et al*., 2010; Fan *et al*., 2015).

On the Loess Plateau of China, severe soil erosion due to leaching, surface runoff and wind erosion, results in a larger amount of P output than input, and thus soil P content is low (Oelmann *et al*., 2007; Liu *et al*., 2013;

Zhou *et al*., 2017). Besides soil erosion, due to long-term irrational exploration and over-grazing, P has become a key limiting factor for plant growth and biomass productivity in this area (Li *et al*., 2015; Zhou *et al*., 2017). Enhancing vegetation cover is an effective and economical way to lessen P losses by decreasing soil erosion (Liu *et al*., 2013; Zhou *et al*., 2017). Study indicated that an appropriate (cheap and effective) amount of P fertilization could alleviate soil P deficiency, maintain biodiversity and improve pasture production, even during low rainfall years (Zhou *et al*., 2017). Water deficiency is the primary constraint on plant distribution and growth in semiarid region on the Loess Plateau (Turner *et al*., 2011; Feng *et al*., 2021). The comparatively low rainfall amount in such areas (long-term annual rainfall is 510 mm) and uneven temporal distribution restrict not only the grassland production but also the availability of P fertilizer (Turner *et al*., 2011; Fan *et al*., 2015). There always exist the problems of lack of high-quality gramineous species, and cropping structure is also simple in artificial grasslands (Xu *et al*., 2011). Switchgrass (*Panicum virgatum* L.) (a perennial C_4 grass) is often used as pasture and high-quality biomass energy crop (Ma *et al*., 2011). It showed a great eco-adaptability and soil and water conservation ability in the region since it was introduced from North America in 1990s (Cooney *et al*., 2017; Gao *et al*., 2017). Our previous studies indicated that switchgrass gradually took the dominant positions when intercropped with two cultivated legume species [*i.e*. milkvetch (*Astragalus adsurgens* Pall.) and sainfoin (*Onobrychis viciaefolia* Scop.)], while the two legume species disappeared in the fifth year after sowing under 2:1 row spacing (Xu *et al*., 2008). The root growth competition compared with shoot of these two species may be the main reason for the instability of community and the disappearance of vulnerable species (Ren *et al*., 2010; Bargaz *et al*., 2016). However, few studies focus on switchgrass and wild native legume species intercropping.

Comparing with cultivated species, wild native species are more adaptable to the specific environment (Guan *et al*., 2013). As a co-dominant species, bushclover (*Lespedeza davurica* S.) (a perennial C_3 leguminous subshrub) occupies diverse positions in natural grassland communities in the region. Compared with the alfalfa (*Medicago sativa* L) and milkvetch, bushclover is more drought-resistant and the yield was more stable after establishment in semiarid region (Guan *et al*., 2013). There has complementary effects to grow bushclover with a native gramineous species (*i.e*. Old World bluestems) together, because higher biomass production and water use efficiency were detected in their mixtures comparing with their respective sole cropping (Xu *et al*., 2011; Wang *et al*., 2018). Thus, a pot experiment was conducted using switchgrass and bushclover in mixtures under two water regimes with three P fertilization treatments, focusing on their root growth and morphological traits in the mixtures. The aims were to: (1) investigate root growth of these two species in response to mixture plantation, (2) clarify their root morphological characteristics in the mixtures, and (3) characterize the root growth response to mixtures under different water and P conditions. We plan to further assess the ecological invasive risk of switchgrass, and to provide an experimental basis for growing them to construct artificial grass-legume meadowland in the region.

Materials and Methods

Plant materials: Seeds of switchgrass and bushclover were harvested in October 2014 at the Ansai Research Station (ARS) of the Chinese Academy of Sciences (36°51′60″N, 109°19′23″E, 1068 to 1309 m a.s.l.) located in the semiarid region on the Loess Plateau. Switchgrass seeds were collected from the experimental fields established in 1999. The cultivar was ‘Alamo’ sourced from America. Bushclover were harvested from natural grasslands. All seeds were stored after air-drying in a natural state, and their germination rates were above 90% at 25ºC in the culture chamber.

Growth conditions: The experiment was conducted in the Institute of Soil and Water Conservation located in Yangling, Shaanxi Province, China (34°12′N, 108°7′E, 530 m a.s.l.). The average annual temperature is 13.0ºC, and the mean monthly temperature ranged from -1.2ºC in January to 26.7ºC in July. Pots utilized were 30 cm × 20 cm PVC pipes (depth × diameter) with a plastic pipe (2 cm in diameter) adjacent to the inside of the wall for watering from the bottom. Each pot was filled with 9 kg dry loess soil. The soil utilized was sandy loam, and got from the upper 20 cm layer of a tillable land in ARS. The soil field capacity (FC) was 20% and pH value was 8.3. The soil organic matter content was 0.26%, and available P and available N were 6.67 and 2.80 mg kg^{-1} dry soil, respectively. The soil total P and total N were 0.61 g kg^{-1} and 0.97 g kg^{-1} dry soil, respectively.

Plant density, mixture ratio, water regime and P treatment were referred from previous studies (Xu *et al*., 2012, 2015). Seeds were sown by a replacement series design on 17 April 2016, and the density was 12 plants per pot. Five mixture ratios (12:0, 8:4, 6:6, 4:8, 0:12) , two soil water regimes [high water: 75 ± 5% FC (field capacity) and low water: 35 ± 5% FC] and three P treatments (no extra P application as control, 0.05 and 0.1g P_2O_5 per kg dry soil) were conducted with five replications of each mixture ratio × water regime × P treatment combination. P as calcium superphosphate were applied to the soil during potting. All pots were put under a rainout shelter, and were adequately watered (80% FC) until water regimes were imposed on July 20 2016, when switchgrass was at the tilling stage. Before soil water regimes were imposed, each pot was covered by a 2.0 cm layer of perlite to decrease soil evaporation. All pots were weighed to control soil water content at 18:00 every day, and required water was supplied from the inner pipe of the barrel.

Root sampling and measurement: Plants were harvested on 28 October 2016. After aboveground was harvested, the roots with soil in each pot were put on a sieve (60 meshes), and carefully washed by a gentle water jet (Xu *et al*., 2011). Roots of each species were carefully separated in water. Because the root systems were large, two individual plants of each species in each pot were used for morphological measurements. Root samples were then scanned using a desktop scanner at 300 dpi (Epson Perfection V800, Long Beach, CA, USA). The scanned images were analyzed (WinRHIZO, 2009b, Regent Instrument Inc., Canada) to obtain total root length (TRL, m), root surface area (RSA, m^2), root average diameter (RAD, mm), and root length in different root diameter class. Fine roots are the most efficient part to absorb soil water and nutrients, accounting for a great proportion of TRL and RSA in herbaceous and woody species (Mou *et al*., 2013; Li *et al*., 2014). Those of herbaceous species tend to be composed of terminal two root orders, and can be differentiate from coarse roots by a great change in diameter class (Comas *et al*., 2013). Depending on root length distribution in diameter class, 0.5 mm is a jump in diameter of switchgrass and bushclover in this study, and root length in 0-0.5 mm diameter class of both species was account for approximately 80% of TRL (Table 1). Thus, roots in 0-0.5 mm diameter class were fine roots of both species, and we divided root diameter class by 0.5 mm. Proportion of root length (%) in each 0.5 mm diameter class was calculated by root length in corresponding diameter class divided by TRL. Selected samples were then oven-dried at 80ºC for 48 h to attain the root biomass (RB, g). Specific root length (SRL, $m\ g^{-1}$) and specific root area (SRA, $cm^{-2}\ g^{-1}$) were calculated by TRL and RSA divided by RB, respectively (Xu *et al*., 2015).

Table 1. Proportion of root length in different root diameter classes of switchgrass (S) and bushclover (B) at different mixture ratios under each water and P treatment.

WR	PT	MR	Diameter class of switchgrass (mm)			Diameter class of bushclover (mm)			
			0-0.5	0.5-1.0	>1.0	0-0.5	0.5-1.0	1.0-1.5	>1.5
HW	P_0	S12B0	0.83±0.01	0.16±0.01	0.01±0.00				
		S8B4	0.83±0.01	0.15±0.01	0.02±0.00	0.80±0.01	0.13±0.01	0.03±0.00	0.04±0.01
		S6B6	0.84±0.01	0.15±0.01	0.02±0.00	0.80±0.01	0.13±0.00	0.04±0.00	0.03±0.00
		S4B8	0.83±0.01	0.15±0.01	0.02±0.01	0.82±0.01	0.12±0.01	0.03±0.00	0.03±0.00
		S0B12				0.85±0.02	0.10±0.02	0.03±0.00	0.02±0.00
	$P_{0.05}$	S12B0	0.81 ± 0.01	0.17 ± 0.01	0.02 ± 0.01				
		S8B4	0.81 ± 0.01	0.16 ± 0.01	0.02 ± 0.01	0.78 ± 0.01	0.13 ± 0.00	0.05 ± 0.00	0.03 ± 0.00
		S6B6	0.81 ± 0.02	0.15 ± 0.02	0.04 ± 0.01	0.82 ± 0.01	0.11 ± 0.01	0.04 ± 0.00	0.02 ± 0.00
		S4B8	0.82 ± 0.01	0.16 ± 0.01	0.02 ± 0.01	0.83 ± 0.01	0.12 ± 0.01	0.03 ± 0.00	0.02 ± 0.00
		S0B12				0.87 ± 0.01	0.08 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
	$P_{0.1}$	S12B0	0.83 ± 0.01	0.15 ± 0.01	0.02 ± 0.00				
		S8B4	0.82 ± 0.01	0.15 ± 0.01	0.03 ± 0.00	0.80 ± 0.02	0.11 ± 0.01	0.05 ± 0.02	0.04 ± 0.01
		S6B6	0.83 ± 0.01	0.14 ± 0.01	0.03 ± 0.00	0.81 ± 0.01	0.14 ± 0.01	0.04 ± 0.01	0.02 ± 0.00
		S4B8	0.82 ± 0.01	0.14 ± 0.01	0.03 ± 0.01	0.81 ± 0.01	0.12 ± 0.01	0.05 ± 0.01	0.03 ± 0.00
		S0B12				0.84 ± 0.01	0.11 ± 0.01	0.03 ± 0.00	0.02 ± 0.00
LW	P_0	S12B0	0.83 ± 0.01	0.16 ± 0.01	0.01 ± 0.00				
		S8B4	0.84 ± 0.01	0.15 ± 0.01	0.01 ± 0.00	0.81 ± 0.01	0.11 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
		S6B6	0.84 ± 0.02	0.15 ± 0.01	0.01 ± 0.00	0.81 ± 0.02	0.12 ± 0.02	0.04 ± 0.01	0.03 ± 0.00
		S4B8	0.84 ± 0.01	0.15 ± 0.01	0.01 ± 0.00	0.82 ± 0.01	0.11 ± 0.01	0.03 ± 0.00	0.03 ± 0.00
		S0B12				0.87 ± 0.02	0.09 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
	$P_{0.05}$	S12B0	0.85 ± 0.01	0.14 ± 0.01	0.01 ± 0.00				
		S8B4	0.84 ± 0.01	0.15 ± 0.01	0.01 ± 0.00	0.79 ± 0.02	0.14 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
		S6B6	0.84 ± 0.01	0.15 ± 0.01	0.01 ± 0.00	0.82 ± 0.02	0.12 ± 0.02	0.03 ± 0.00	0.03 ± 0.00
		S4B8	0.83 ± 0.01	0.16 ± 0.01	0.01 ± 0.00	0.80 ± 0.01	0.13 ± 0.01	0.04 ± 0.01	0.03 ± 0.00
		S0B12				0.86 ± 0.01	0.10 ± 0.01	0.02 ± 0.01	0.03 ± 0.00
	$P_{0.1}$	S12B0	0.84 ± 0.01	0.15 ± 0.01	0.01 ± 0.00				
		S8B4	0.83 ± 0.01	0.16 ± 0.01	0.01 ± 0.00	0.76 ± 0.01	0.14 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
		S6B6	0.84 ± 0.00	0.15 ± 0.01	0.01 ± 0.00	0.78 ± 0.01	0.13 ± 0.02	0.04 ± 0.00	0.04 ± 0.00
		S4B8	0.85 ± 0.02	0.14 ± 0.02	0.01 ± 0.00	0.79 ± 0.02	0.12 ± 0.02	0.05 ± 0.01	0.04 ± 0.00
		S0B12				0.81 ± 0.02	0.12 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
WR			** (0.008)	n.s.	** (0.003)	n.s.	n.s.	n.s.	** (0.003)
PT			n.s.	n.s.	* (0.004)	** (0.007)	n.s.	** (0.006)	* (0.004)
MR			n.s.	n.s.	n.s.	** (0.008)	** (0.013)	** (0.007)	** (0.005)
WR × PT			n.s.	n.s.	* (0.006)	n.s.	n.s.	n.s.	* (0.006)
WR × MR			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PT × MR			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
WR × PT × MR			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

HW: 75 ± 5% FC, LW: 35 ± 5% FC, WR: water regime, PT: P treatment, MR: mixture ratio. * and ** represent significance at p = 0.05 and 0.01 levels, respectively. n.s. means not significant. The numbers in parentheses are the least significant difference (LSD) values at p = 0.05 level

Statistical analysis

The experiment was a three-factorial (water regime, P treatment and mixture ratio) randomized complete block design. Root biomass and root morphology of switchgrass and bushclover were analyzed by three-way analysis of variance (ANOVA) using GenStat 19.0 (VSN International Ltd., England). Means of each treatment among two water regimes, three P treatments and five mixture ratios were performed by least significant difference (LSD) test at 5% level. Data were presented as the mean value of single plant for both species under each treatment. The relationships among RB, TRL and RSA were determined by linear regression analysis for each species at each soil water regime with different P application conditions.

Results

Root biomass (RB): Irrespective of mixture ratio or P treatment, RB of switchgrass under HW was significantly higher than those under LW (28.0-183.5% increase in average) (p<0.05) (Fig. 1). P application significantly increased RB of switchgrass at each mixture ratio under HW (19.8-54.8% in average), and also under LW (13.6% in maximum). The soil water × P interaction significantly affected RB of switchgrass, and also that of soil water × mixture ratio, while P × mixture ratio and soil water × P × mixture ratio had no interactive effects on RB. RB of switchgrass showed an increased trend as its ratio decreased in mixtures irrespective of P treatment under both water regimes. Comparing with monoculture, RB of switchgrass in mixtures averagely increased 64.9% under HW and 24.5% under LW.

RB of bushclover under HW was also significantly higher than those under LW (13.5-51.1% in average), irrespective of mixture ratio or P treatment (p<0.05) (Fig. 1). Interactions of soil water × mixture ratio and soil water × P × mixture ratio significantly affected RB, while P × mixture ratio had no effect on RB of bushclover. Under HW, RB of bushclover in mixtures averagely decreased by 10.7%, 18.2% and 31.3% in P_0, $P_{0.05}$ and $P_{0.1}$ treatment, respectively. Under LW, generally no significant differences were detected for RB of bushclover in the mixtures, except 6:6 (i.e. S6B6, means the plant numbers of switchgrass: bushclover in the pot) mixture ratio in P_0 treatment and 4:8 ratio in $P_{0.1}$ treatment, the former was lower while the latter was higher than monoculture.

Total root length (TRL): TRL of switchgrass under HW were significantly higher than those under LW at each P treatment and mixture ratio (27.9-63.6% in average) (Fig. 2). Under HW, TRL of switchgrass with or without P application showed an increased trend (10.9-105.5% more in average) as its mixture ratio decreased in mixtures. Similar trends were found in P_0 treatment under LW comparing with monoculture, TRL of switchgrass averagely increased 40.3% in mixtures. In $P_{0.05}$ treatment, TRL of switchgrass significantly increased about 48.9% in mixtures comparing with switchgrass monoculture, while in $P_{0.1}$ treatment, only TRL of switchgrass at a ratio of 4:8 significantly increased by 25.7%. Except the integrated interaction of three factors, each individual factor and their pairwise interactions significantly affected TRL of switchgrass.

TRL of bushclover under HW were significantly greater than those under LW, irrespective of mixture ratio or P treatment (Fig. 2). P application significantly increased TRL of monoculture under HW while decreased under LW. Under HW, TRL of bushclover showed an increased trend as its mixture ratio increased in mixtures irrespective of P treatment. Comparing with monoculture, TRL of bushclover in mixtures decreased about 17.8-41.9% without P application, 6.7-53.5% in $P_{0.05}$ and 38.4-67.7% in $P_{0.1}$. Under LW, TRL of bushclover in mixtures significantly decreased comparing with monoculture. Similar trends were found in $P_{0.05}$ treatment, while in $P_{0.1}$ treatment, TRL of bushclover at 4:8 ratio was the highest among mixture ratios. Each individual factor and their interactions significantly affected TRL of bushclover except the P × mixture ratio interaction.

Root surface area (RSA): RSA of switchgrass under HW were significantly greater than those under LW, irrespective of mixture ratio or P treatment (Fig. 3). P application significantly increased RSA of switchgrass at each mixture ratio under HW and slightly increased the value under LW. Under HW, RSA of switchgrass showed an increased trend as its ratio decreased in mixtures in each P treatment, averagely increased by 23.2-115.6%, comparing with monoculture. Similar trends were found under LW, and comparing with monoculture, RSA of switchgrass in mixtures significantly increased about 12.0-51.8% in P_0 treatment and 43.1-54.7% in $P_{0.05}$, while in $P_{0.1}$ treatment only at 4:8 ratio it increased by 23.2% (p<0.05). Each individual factor and their interactions significantly affected RSA of switchgrass.

RSA of bushclover under HW were significantly greater than those under LW at each P treatment and mixture ratio (Fig. 3). P application significantly increased RSA of monoculture under HW while decreased under LW. Under HW, comparing with monoculture, the averaged RSA of bushclover in mixtures significantly decreased about 8.6-35.1% without P application and 28.5-60.2% in $P_{0.1}$ treatment. In $P_{0.5}$ treatment, RSA of bushclover at 8:4 and 6:6 ratios significantly decreased by 45.9% and 39.6% respectively, and no significant difference was detected between bushclover monoculture and 4:8 ratio. Under LW, RSA of bushclover in mixtures were significantly decreased comparing with monoculture in P_0 treatment. Similar trends were detected in $P_{0.05}$ treatment, while in $P_{0.1}$ treatment, RSA at 4:8 ratio was the highest among mixture ratios. Each factor and their interactions significantly affected RSA of bushclover except P × mixture ratio interaction.

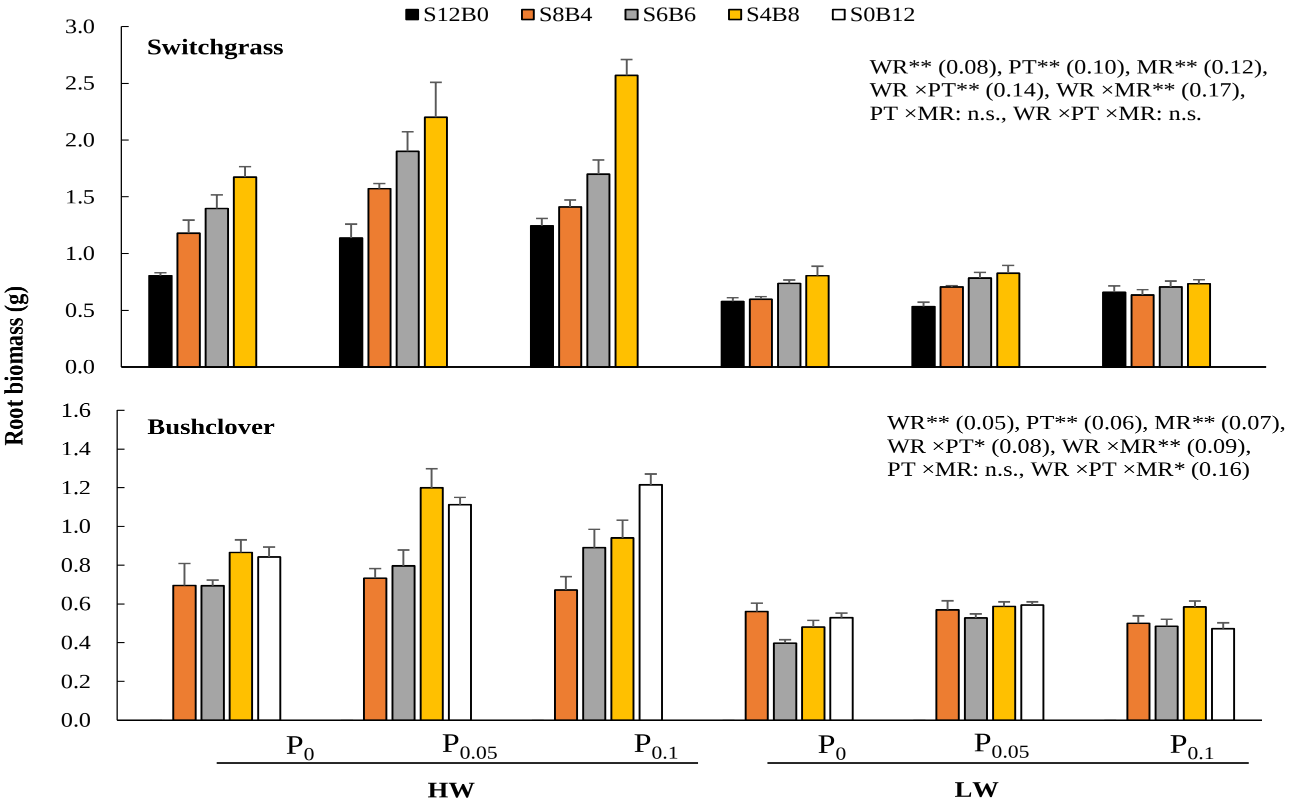

Fig. 1. Root biomass (RB) of switchgrass (S) and bushclover (B) per plant at different mixture ratios under two water and three P treatments. HW: 75 ± 5% FC, LW: 35 ± 5% FC, WR: water regime, PT: P treatment, MR: mixture ratio. * and ** represent significance at $p = 0.05$ and 0.01 levels, respectively. n.s. means not significant. The numbers in parentheses are the least significant difference (LSD) values at $p = 0.05$ level.

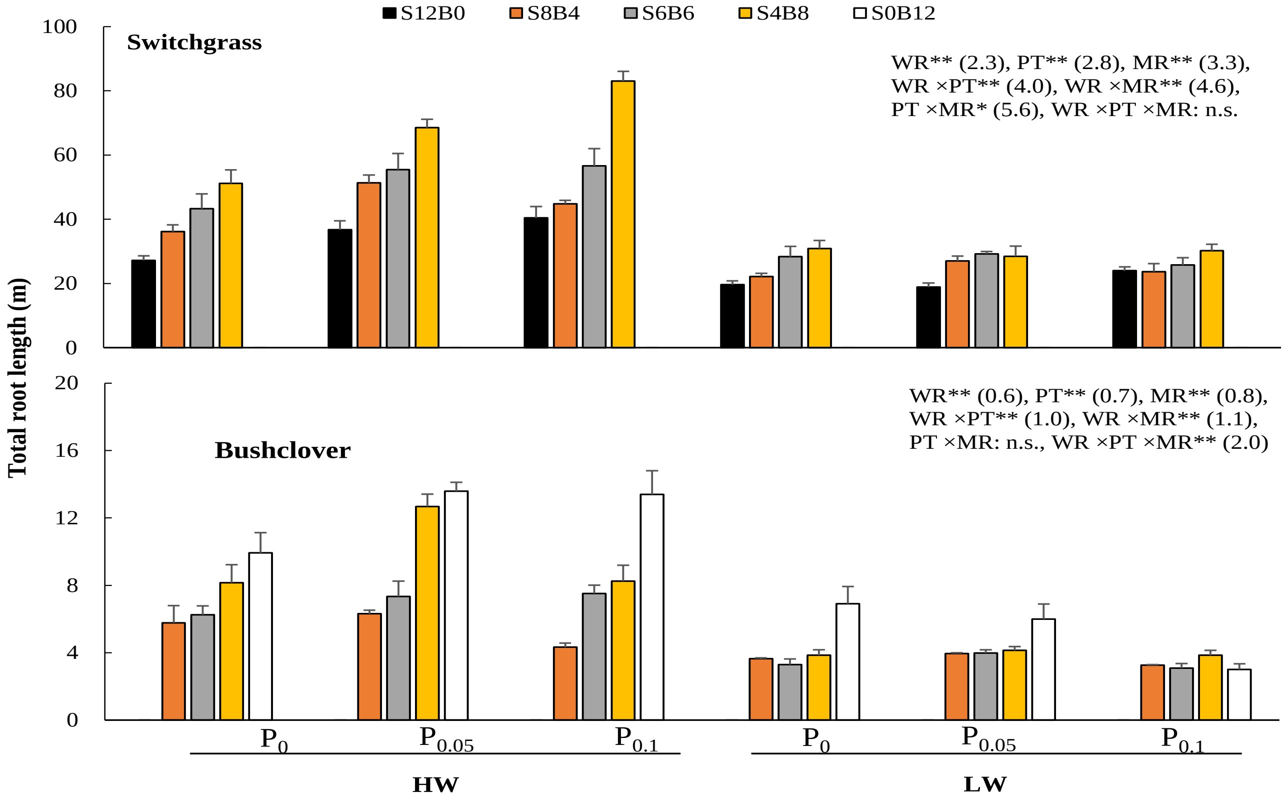

Fig. 2. Total root length (TRL) of switchgrass (S) and bushclover (B) per plant at different mixture ratios under two water and three P treatments. HW: 75 ± 5% FC, LW: 35 ± 5% FC, WR: water regime, PT: P treatment, MR: mixture ratio. * and ** represent significance at $p = 0.05$ and 0.01 levels, respectively. n.s. means not significant. The numbers in parentheses are the least significant difference (LSD) values at $p = 0.05$ level.

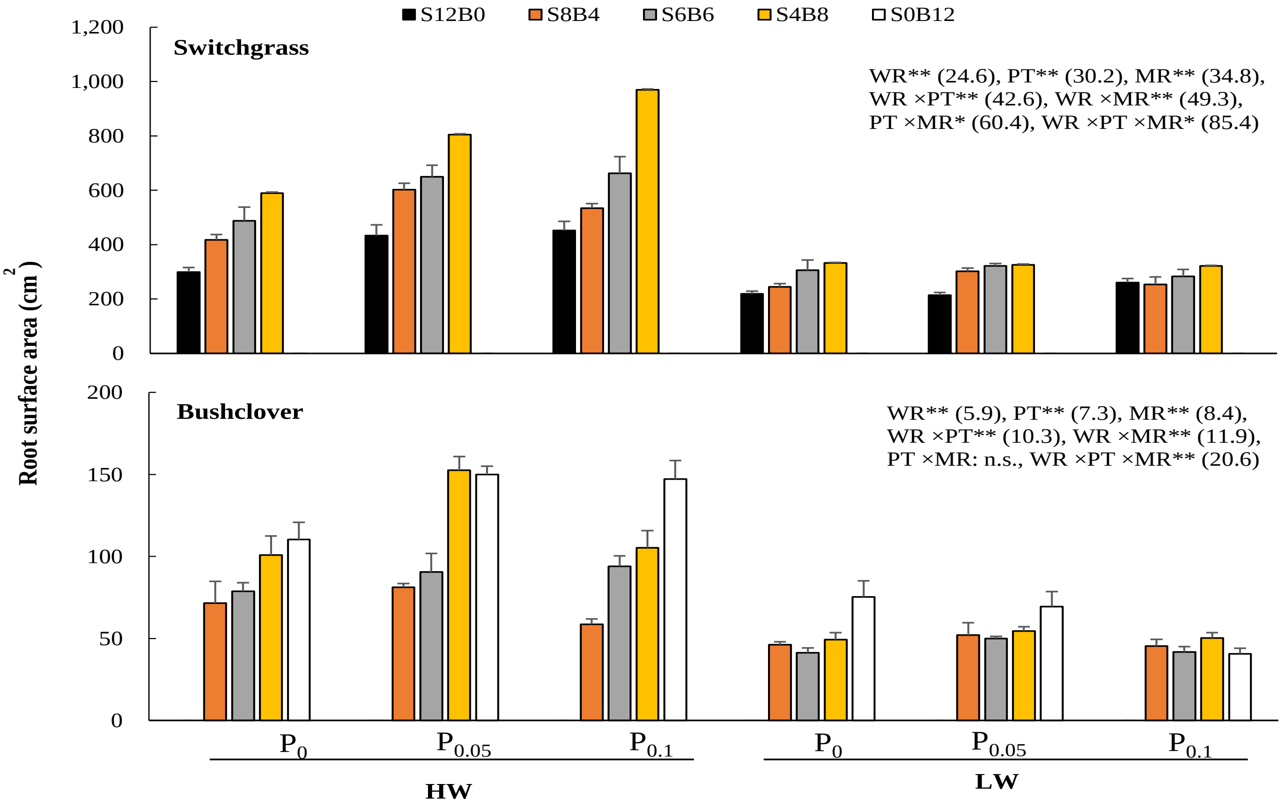

Fig. 3. Root surface area (RSA) of switchgrass (S) and bushclover (B) per plant at different mixture ratios under two water and three P treatments. HW: 75 ± 5% FC, LW: 35 ± 5% FC, WR: water regime, PT: P treatment, MR: mixture ratio. * and ** represent significance at $p = 0.05$ and 0.01 levels, respectively. n.s. means not significant. The numbers in parentheses are the least significant difference (LSD) values at $p = 0.05$ level.

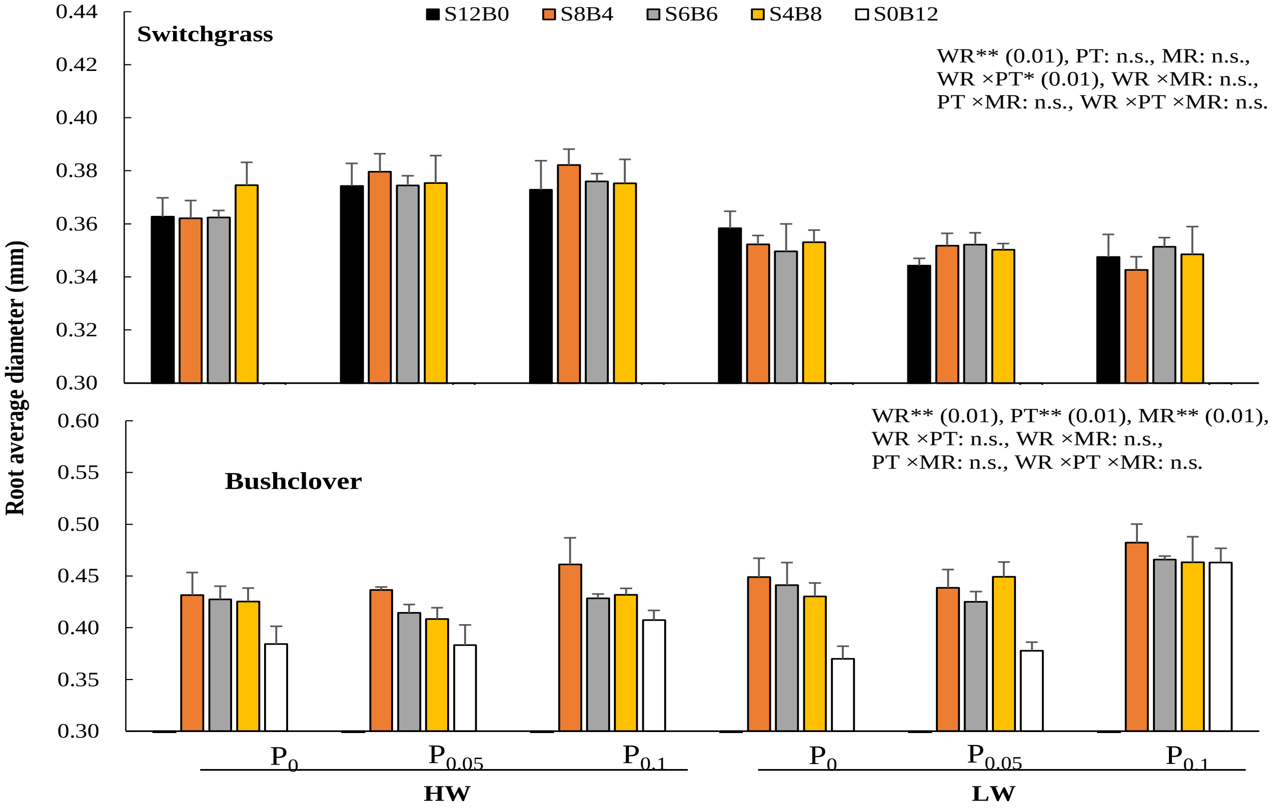

Fig. 4. Root average diameter (RAD) of switchgrass (S) and bushclover (B) at different mixture ratios under two water and three P treatments. HW: 75 ± 5% FC, LW: 35 ± 5% FC, WR: water regime, PT: P treatment, MR: mixture ratio. * and ** represent significance at $p = 0.05$ and 0.01 levels, respectively. n.s. means not significant. The numbers in parentheses are the least significant difference (LSD) values at $p = 0.05$ level.

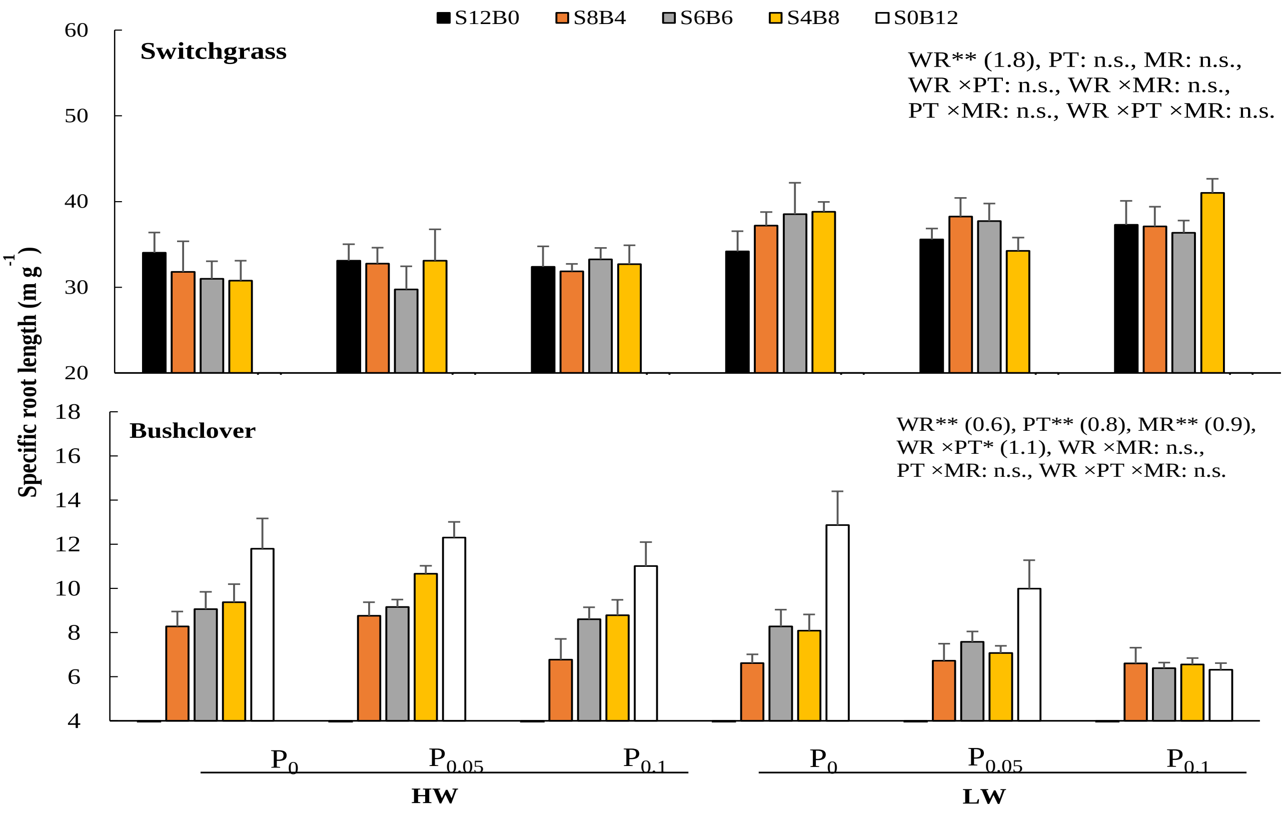

Fig. 5. Specific root length (SRL) of switchgrass (S) and bushclover (B) at different mixture ratios under two water and three P treatments. HW: 75 ± 5% FC, LW: 35 ± 5% FC, WR: water regime, PT: P treatment, MR: mixture ratio. * and ** represent significance at $p = 0.05$ and 0.01 levels, respectively. n.s. means not significant. The numbers in parentheses are the least significant difference (LSD) values at $p = 0.05$ level.

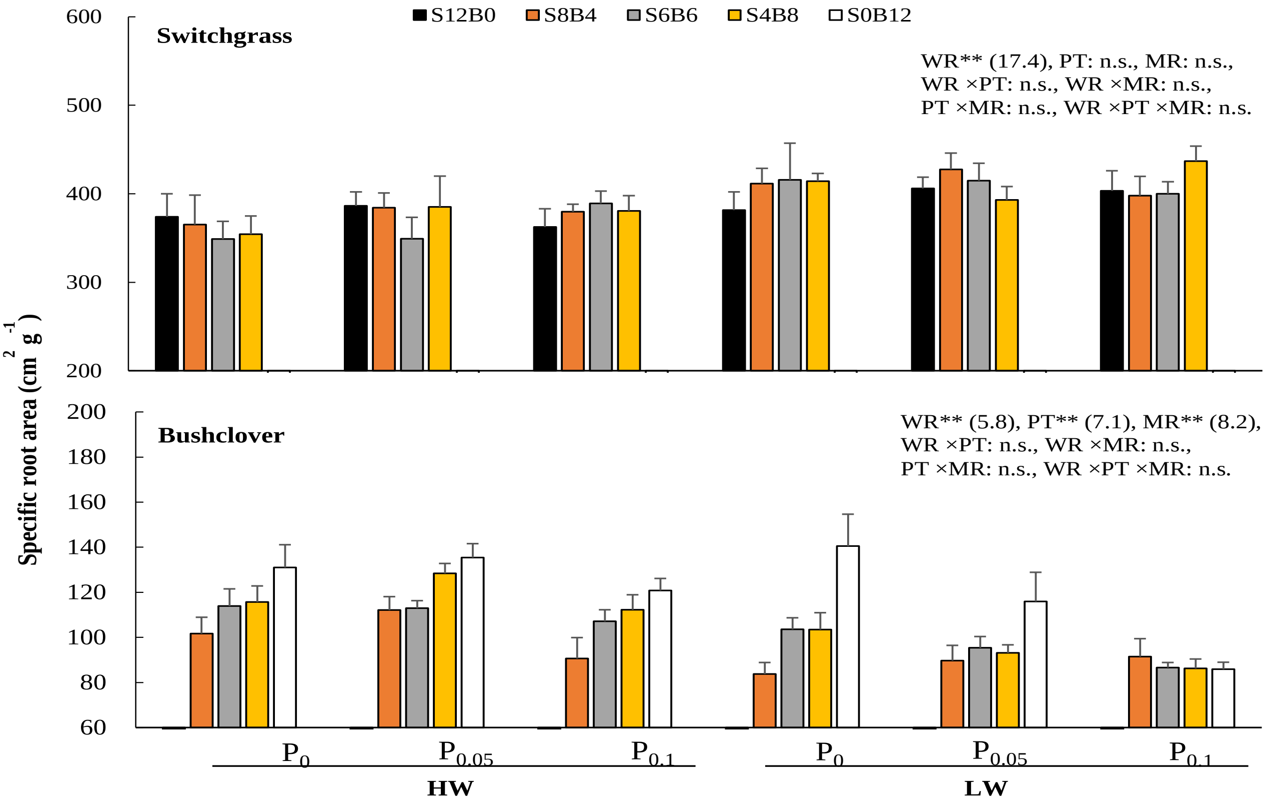

Fig. 6. Specific root area (SRA) of switchgrass (S) and bushclover (B) at different mixture ratios under two water and three P treatments. HW: 75 ± 5% FC, LW: 35 ± 5% FC, WR: water regime, PT: P treatment, MR: mixture ratio. * and ** represent significance at $p = 0.05$ and 0.01 levels, respectively. n.s. means not significant. The numbers in parentheses are the least significant difference (LSD) values at $p = 0.05$ level.

Root diameter length: Root length in 0-0.5 mm diameter class accounted for 81-85% (Table 1). Water regime significantly affected root length proportions in 0-0.05 and >1.0 mm diameter classes of switchgrass. Under HW, root length proportions in 0-0.5 mm diameter class generally significantly lower compared to LW at each mixture ratio and P treatment, while those in >1.0 mm diameter class showed the opposite. P application had no effect on the root length proportion in 0-0.5 mm diameter under both soil water regimes, but significantly increased the proportions of root length in > 0.10 mm diameter under HW, and had no effect under LW. Mixture ratio and its interaction with water and P treatment had no significantly effect on proportion of root length in each diameter class.

About 77-87% of total root length of bushclover was in 0-0.5 mm diameter class (Table 1). Water regime only significantly affected root length proportions in >1.5 mm diameter class. In $P_{0.05}$ and $P_{0.1}$ treatments, root length proportions in >1.5 mm diameter class under HW were significantly lower than those of LW. P application significantly decreased root length proportion in 0-0.5 mm diameter class, and increased those in >1.5 mm diameter class under both water regimes. Mixture ratio significantly affected root length proportions in each diameter class. Overall, root length proportions in 0-0.5 mm diameter class at monoculture were significantly higher than those in mixtures, while other diameter classes showed the opposite.

Root average diameter (RAD): Mixture ratio and its interaction with water or P treatment had no effect on RAD, while water regime and its interaction with P significantly affected RAD of switchgrass (Fig. 4). The averaged RAD of switchgrass for all mixture ratios and P treatments under HW was significantly larger than that under LW. P application significantly increased the averaged RAD of switchgrass for all mixture ratios under HW, and decreased those under LW.

Each individual factor significantly affected RAD of bushclover (Fig. 4). RAD values of bushclover under HW were averagely lower than those under LW. $P_{0.1}$ treatment significantly increased RAD under both soil water regimes, while no difference was detected between $P_{0.05}$ and P_0 treatments. Under HW, RAD of bushclover with or without P application showed a decreasing tendency as its mixture ratio increased. Under LW, RAD were significantly increased in mixtures comparing with bushclover monoculture in both P_0 and $P_{0.05}$ treatments, but no significant difference was detected among mixture ratios in $P_{0.1}$ treatment.

Specific root length (SRL) and specific root area (SRA): Only water regimes significantly affected SRL and SRA of switchgrass (Figs. 5 and 6). SRL and SRA of switchgrass under HW were averagely higher than those under LW. SRL and SRA values of switchgrass were 30-34 m g^{-1} and 348 -397 cm^2 g^{-1} under HW, while 34-42 m g^{-1} and 381-438 cm^2 g^{-1} under LW, respectively.

Each individual factor (P, water, mixture ratio) significantly affected SRL and SRA of bushclover (Figs. 5 and 6). SRL and SRA values of bushclover under HW were averagely significantly higher than those under LW. Under HW, SRL and SRA of bushclover in $P_{0.1}$ treatment significantly decreased comparing with P_0 treatment, and similar trends were found in both $P_{0.05}$ and $P_{0.1}$ treatments under LW. SRL and SRA of bushclover showed an increased trend as its mixture ratios increased in mixtures.

Discussion

During plant growth process, the demand and utilization of nutrients of plants are in sync with soil water conditions, and competition intensity between species or plants are closely related to soil water and nutrient availability (Bush & van Auken, 2010; Ren *et al*., 2010). In soil water deficit environments, species competition may be weaker, because the reduction in plant growth would decrease shoot shading and root intermingle degree (Adiku *et al*., 2001). In this study, RB of both switchgrass and bushclover decreased significantly under low soil water regime (Fig. 1), indicating that soil water was the key factor limiting their root growth (Xu *et al*., 2012, 2015; Wang *et al*., 2018). Under HW regime, P application was beneficial for root growth of both switchgrass and bushclover, while under LW such effects were not significant, implying that the effects of P on root growth were related to soil water conditions (Fig. 1) (Fan *et al*., 2015; Ren *et al*., 2016).

Total root length (TRL) and root surface area (RSA) reflect the absorption capacity of plant roots (Li *et al*., 2014; Feng *et al*., 2021). Under both water regimes, switchgrass increased TRL and RSA when mixed with bushclover, while bushclover showed the opposite, implying mixed plantation was beneficial for the absorption capacity of switchgrass rather than bushclover (Figs. 2 and 3) (Xu *et al*., 2012, 2015; Bargaz *et al*., 2016). Some 80% of the total roots were fine roots in both plants, indicating fine roots contributed the majority to TRL and RSA (Table 1). Fine roots are more efficient for soil water and nutrients absorption (Li *et al*., 2014), and are the most sensitive part of plant to changes with soil environment (Mou *et al*., 2013; Li *et al*., 2014; Wang *et al*., 2018), which may be increased, decreased, or constant in response to non-self-neighbors (Cardinael *et al*., 2015; Shu *et al*., 2018). Mixture ratio had no significant effect on root length distribution in different diameter classes of switchgrass (Table 1), implying an overall root growth of switchgrass in mixtures (Shu *et al*., 2018). Root length proportions of fine roots were decreased in bushclover when mixed with switchgrass, suggesting that TRL and RSA of bushclover were decreased in their mixtures mainly because of the reduction of fine roots growth (Figs. 2-4, Table 1) (Li *et al*., 2014). The reduction of fine roots growth would weaken its ability to absorb soil resources, while the increase in the coarse roots allocation is benefit for root deepening (Cardinael *et al*., 2015).

Root average diameter (RAD) is considered as an important root traits in evaluating plant adaptability (Xu *et al*., 2012; Padilla *et al*., 2013). Root diameter shows an effective correlation with soil contact area at a certain amount of photosynthate (Padilla *et al*., 2013; Poorter & Ryser, 2015). Under water deficit environment, decreasing or increasing in root diameter reflect different adaptation strategies (Zhou *et al*., 2018). Decreasing root diameter is among the mechanisms for water absorption and root elongation by increasing the surface-to-volume ratio (Ostonen *et al*., 2007; Comas *et al*., 2013). Increasing root diameter is a strategy to conserve water and reduce the consumption of photosynthate by root metabolism (Kadam *et al*., 2015). The change of RAD was caused by the overall change of coarse and fine roots diameters or the change of root distribution in different diameter class (Poorter & Ryser, 2015). Switchgrass and bushclover showed different responses of RAD to low soil water. Reduced RAD in switchgrass under low water regime is mainly due to fine roots growth, which is beneficial for soil resource absorption, whilst bushclover increased the growth of coarse roots (Fig. 4, Table 1). Under P deficiency, most species decreased their root diameters, but this is not always the case (Zobel *et al*., 2006; Pang *et al*., 2010). In this study, P application increased RAD in bushclover through decreasing the fine root proportion under both soil water regimes compared to low P supply (Fig. 4). Switchgrass tended to decrease root diameter in response to soil P improvement, which would facilitate its ability in soil resource uptake (Fig. 4, Table 1) (Comas *et al*., 2013).

Specific root length (SRL) and specific root area (SRA) are indicators of the absorptive surface produced per unit root biomass (Zobel *et al*., 2006; Pang *et al*., 2010). Greater SRL and SRA imply a lower photosynthate cost for root construction and a more efficient soil exploitation (Zobel *et al*., 2006; Wang *et al*., 2018). On the other hand, lower SRL and SRA are beneficial to conserve the carbon budget for its greater longevity and lower root respiration (Zobel *et al*., 2006; Xu *et al*, 2015). The increase in SRL and SRA in switchgrass and decrease in bushclover in response to low water regime suggested that switchgrass developed a water acquisition strategy through thinner roots, while bushclover had a water conservation strategy via producing thicker roots (Figs. 4-6, Table 1) (Padilla *et al*., 2013; Kadam *et al*., 2015). It is generally expected that in low-P environment, plants tend to have more fine roots although there are exceptions (Fan *et al*., 2015; Xu *et al*., 2015). In low-P conditions, SRL of fine roots may increase, decrease, or stay constant (Zobel *et al*., 2006). The present study showed that under low water regime, switchgrass had thinner root system and higher root tissue density after P application (Figs. 4-6, Table 2) (Ostonen *et al*., 2007; Poorter & Ryser, 2015), which might increase the root absorption capacity and root life span (Ostonen *et al*., 2007).

Table 2. Linear regressions among root biomass (RB, g), total root length (TRL, cm), and root surface area (RSA, cm^2) of switchgrass (S) and bushclover (B) under two water regimes and three P treatments.

WR	PT	Y	X	Switchgrass				Bushclover			
				Slope	Intercept	R^2	*p*	Slope	Intercept	R^2	*p*
HW	P_0	TRL	RB	24.61	8.37	0.68	<0.001	11.15	-1.10	0.51	<0.001
	$P_{0.05}$	TRL	RB	18.19	22.02	0.57	<0.001	12.45	-1.98	0.79	<0.001
	$P_{0.1}$	TRL	RB	30.65	3.14	0.88	<0.001	12.20	-2.97	0.68	<0.001
LW	P_0	TRL	RB	38.40	0.80	0.68	<0.001	-	-	-	0.053
	$P_{0.05}$	TRL	RB	33.78	1.84	0.78	<0.001	14.84	-3.93	0.42	<0.01
	$P_{0.1}$	TRL	RB	34.99	1.98	0.54	<0.001	6.76	-0.15	0.62	<0.001
HW	P_0	RSA	TRL	11.18	7.38	0.99	<0.001	9.92	15.72	0.94	<0.001
	$P_{0.05}$	RSA	TRL	11.10	34.49	0.95	<0.001	10.36	15.16	0.89	<0.001
	$P_{0.1}$	RSA	TRL	11.61	2.21	0.98	<0.001	10.72	13.72	0.98	<0.001
LW	P_0	RSA	TRL	10.98	-1.46	0.98	<0.001	9.34	11.70	0.98	<0.001
	$P_{0.05}$	RSA	TRL	10.43	20.98	0.97	<0.001	9.77	12.39	0.98	<0.001
	$P_{0.1}$	RSA	TRL	10.61	5.41	0.97	<0.001	11.02	8.17	0.93	<0.001

HW: 75 ± 5% FC, LW: 35 ± 5% FC, WR: water regime, PT: P treatment; MR: mixture ratio

Conclusions

Under low soil water condition, switchgrass developed a water acquisition strategy through thinner roots, while bushclover had a water conservation strategy by producing thicker roots. P application significantly deceased RAD in switchgrass under soil water deficit condition, indicating a thinner root system with higher root tissue density was formed. Bushclover increased SRL to adapt low P conditions regardless of water regime. Comparing with monoculture, the RB of switchgrass in mixtures increased under both water regimes, while those of bushclover showed the opposite trends under high soil water regime. Under all water and P treatments, mixture ratio had no effect on RAD, SRL and root length distribution in different diameter classes of switchgrass, indicating an overall growth change of fine and coarse roots in their mixtures. Decreased TRL and RSA in bushclover when mixed with switchgrass implied that switchgrass would be superior to bushcover in their communities, and P application might increase the abilities of switchgrass to acquire and compete for limited resources.

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