



**Full Length Article**

## Reasonable Coupling of Water and Phosphorus Improves Hay Yield, and Water and Phosphorus Use Efficiency of Alfalfa

Qianbing Zhang<sup>\*†</sup>, Junying Liu<sup>†</sup>, Shengyi Li, Xuanshuai Liu, Weihua Lu, Xuzhe Wang and Chunhui Ma<sup>\*</sup>

The College of Animal Science & Technology, Shihezi University, Shihezi, 832003, China

<sup>\*</sup>For correspondence: qbz102@163.com; chunhuima@126.com

<sup>†</sup>Contributed equally to this work and are co-first authors

Received 13 December 2019; Accepted 14 January 2020; Published 02 April 2020

### Abstract

Alfalfa (*Medicago sativa* L.) is an important forage legume grown in arid areas, but shortages of water resources and low utilization of fertilizers have restricted its growth. In a 2-year field experiment in Xinjiang, China, alfalfa was grown using different amounts of irrigation, *i.e.*, 3750, 4500, and 5250 m<sup>3</sup> ha<sup>-1</sup>, under varying phosphorus (P) levels (0, 50, 100, and 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>). The results showed that the soil total phosphorus (TP) content increased gradually with increasing P application, and the TP content for the 150 kg ha<sup>-1</sup> treatment was significantly higher than that of 50 and 0 kg ha<sup>-1</sup> treatments under same irrigation conditions ( $P < 0.05$ ). Nonetheless, the hay yield, water use efficiency (WUE), and agronomic efficiency of P fertilizer (AEPF) of alfalfa and soil available P contents increased with increasing P level and then decreased; the 100 kg ha<sup>-1</sup> treatment reached the maximum values, and these values were significantly higher than those of the 0 kg ha<sup>-1</sup> treatment. Under the same P application conditions, with increasing irrigation amount, the hay yield of alfalfa increased to a maximum under the 4500 m<sup>3</sup> ha<sup>-1</sup> treatment and then decreased, and that of the 4500 m<sup>3</sup> ha<sup>-1</sup> treatment was significantly larger than those of the 3750 m<sup>3</sup> ha<sup>-1</sup> and 5250 m<sup>3</sup> ha<sup>-1</sup> treatments ( $P < 0.05$ ). The soil TP and available phosphorus (AP) contents gradually decreased with increasing soil depth under the same irrigation amount and P application. In conclusion, P application at 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> under irrigation rate of 4500 m<sup>3</sup> ha<sup>-1</sup> seemed a viable technique to improve hay yield and agronomic efficiency of P fertilizer of alfalfa. © 2020 Friends Science Publishers

**Keywords:** Alfalfa; Drip irrigation; Phosphorus; Hay yield; Water use efficiency

### Introduction

Alfalfa (*Medicago sativa* L.) is an important forage crop due to its relatively high yield, palatability, digestibility and excellent nutritional value. Alfalfa is the most efficient forage legume (Zhang *et al.* 2016) and plays an important role in promoting the development of animal husbandry in China (Geisseler *et al.* 2010). Additionally, alfalfa is a kind of water-loving plant, and irrigation significantly affects the yield and quality of alfalfa (Ismail and Almarshadi 2013; Altinok *et al.* 2015; Rahmana *et al.* 2016). However, recent research has shown that irrigation water has little effect on alfalfa plant height and stem diameter but plays an important role in increasing the branch number, thus indirectly affecting the hay yield (Saeed and El-Nadi 1997). Under water stress, the growth rates of mature alfalfa leaves and stems decreased significantly, and the hay yield decreased (Fiasconaro *et al.* 2012). The number and length of the stem nodes of alfalfa plants increased and the photosynthetic rate of the leaves became stronger under the full irrigation rate (Zhang *et al.* 2016). Therefore, an

appropriate irrigation quota is conducive to improve the hay yield of alfalfa (Fosu-Mensah and Mensah 2016).

Like water, phosphorus (P) is an indispensable nutrient element in plants and one of the main limiting factors of crop yield (Rehim *et al.* 2016; Ijaz *et al.* 2018). After the application of P fertilizer, the grass yield per unit area and plant regeneration speed of alfalfa was affected by P fertilizer (Song *et al.* 2018). The results show that irrigation rate can not only regulate the fertilizer effect but also affect the absorption of water by herbage (Singh 1999). Additionally, the increase in alfalfa hay yield and nutrients is due to the positive coordination of water and fertilizer application (Berg *et al.* 2018). Therefore, reasonable coupling of water and fertilizer is of great significance to alfalfa yield.

The oasis area of Xinjiang is one of the main areas of alfalfa cultivation in China. The soil in this area is mainly grey desert soil. Because of the lack of water and poor soil fertility in this area, alfalfa cultivation is difficult. In particular, the success of plantings in the planting year has a great influence on the hay yield and nutrient quality of

alfalfa in the later periods of alfalfa growth. Hence, a rational combination of water and fertilizer is the key to a high yield of alfalfa in the same year. In recent years, research on alfalfa has mainly focused on autumn dormancy characteristics, growth period, mowing methods, and grass yield (Gu *et al.* 2018), while research on alfalfa production performance in the year of establishment is relatively limited. Therefore, the objectives of this study were to establish an optimal fertilization model, to clarify the effect of water and P coupling on the nutrition quality and the P use efficiency (PUE) of alfalfa under drip irrigation and to determine the relationship between PUE and the hay yield of alfalfa.

## Materials and Methods

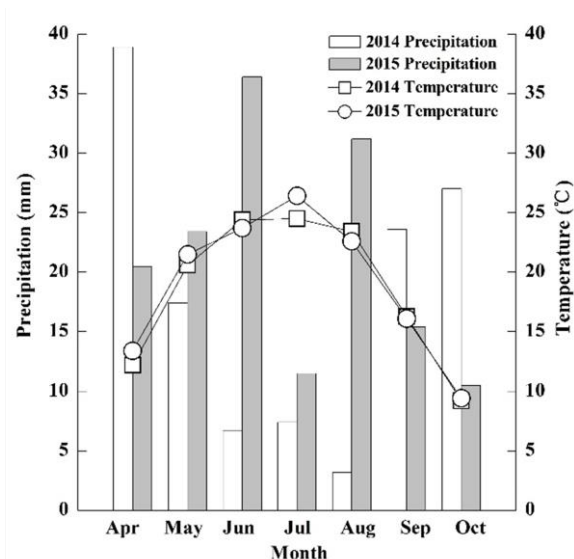
### Experimental site description

Field experiments were conducted in 2014 at the agricultural College Test Station of Shihezi, Xinjiang, China (44°26'N, 85°95'E) and the experimental field of Shihezi Tianye Group Agricultural Demonstration Park (44°31'N, 85°52'E) in 2015. The physical and chemical properties of the 0–20 cm plough layer soil are shown in Table 1. The physical and chemical properties of the soil are basically the same in the two test sites, so their effects on the subsequent water test results of this paper are negligible. The soil type was grey desert soil.

### Experimental design

The alfalfa crop was grown using different irrigation amounts, *i.e.*, 3750, 4500, and 5250 m<sup>3</sup>·ha<sup>-1</sup>, under varying P levels (0, 50, 100, and 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) with three replications. The experiment was designed following a randomized complete block design with factorial arrangement. The crop was irrigated six times each year, on April 26, June 10, June 26, July 12, August 13 and August 28, 2014 and on May 2, June 19, July 1, July 16, August 14, and August 29, 2015. The average precipitation and temperature in each month during the test period are shown in Fig. 1.

In this experiment, WL354HQ alfalfa seeds taken from Beijing Zhengdao Ecology Technology Co., Ltd. were sown on April 19, 2014 and on April 16, 2015, using the drill method with a sowing depth of 1.5~2.0 cm, row spacing of 20 cm, and seed rate of 18.0 kg ha<sup>-1</sup> in plots with an area of 5.0 m × 8.0 m. A walkway 1.0 m wide between the plots was left to prevent water and P infiltration between the plots. The drip irrigation belt was shallowly buried in the 8~10 cm surface soil at a distance of 60 cm. In addition to the water and fertilizer factors, other management methods were carried out according to the local high-yield field of alfalfa under the conditions of drip irrigation.



**Fig. 1:** Average temperature and precipitation in growth stages during 2014-2015 (from Shihezi Meteorological Bureau, Xinjiang)

### Soil sample collection

Soil samples of 0–20 cm, 20–40 cm and 40–60 cm were taken from soil drills in each plot by the "S" sampling method in October of each year. Five soil samples from the same soil layer were mixed to make composite soil samples. After removing impurities such as alfalfa roots and stones, the soils were brought back to the laboratory and dried to constant weight in an oven at 65°C. The soil samples were ground, and the fine soil was sifted through a 100 mesh sieve for reserve.

### Measurement index and method

**Hay yield determination:** In the initial flowering stage (5–10% plants in bloom), alfalfa plants with uniform growth were selected using the "S" sampling method in the experimental plot. Using a 1 m × 1 m sample, scissors were used to cut the alfalfa plants in the sampling plot (stubble height 5 cm) to weigh the plants, and the fresh grass yield of plants was recorded. This process was repeated three times, and the hay yield (kg ha<sup>-1</sup>) of alfalfa was calculated (Formula 1). The concrete formula is as follows:

$$Y = FY \times (1 - M (\%)) \quad (1)$$

Y represents the alfalfa hay yield, FY represents the alfalfa fresh grass yield and M represents the alfalfa moisture contents

### Determination total phosphorus and available phosphorus in soil

The total phosphorus (TP) content of soil was determined using the sulfuric acid perchlorate digestion method, and the

available phosphorus (AP) content was determined using  $\text{NaHCO}_3$  leaching molybdenum antimony KangFa determination (Lu 2000).

### Calculation of water use efficiency (WUE) and agronomic efficiency of phosphorus fertilizer (AEPF) of alfalfa

The WUE of alfalfa was defined as the ratio of alfalfa hay yield ( $Y$ ,  $\text{kg ha}^{-1}$ ) to transpiration ET (mm), that is,

$$\text{WUE} = Y/ET \quad (2)$$

The ET was calculated by the following formula (Li *et al.* 2018):

$$\text{ET} = P + I + \Delta\omega \quad (3)$$

$P$ ,  $I$  and  $\Delta\omega$  represent mowing twice during rainfall, irrigation quantity and 0–2 m of difference in the soil water content, respectively.

The AEPF of alfalfa was calculated using the following equation:

$$\text{AEPF} = (Y_f - Y_0) / F_i \quad (4)$$

AEPF represents the agronomic efficiency of P fertilizer,  $Y_f$  represents the alfalfa hay yield in the fertilized area,  $Y_0$  represents the alfalfa hay yield in the non-fertilized area, and  $F_i$  represents the amount of P fertilizer used.

### Data processing and analysis

Microsoft Excel 2010 was used for data processing, and DPS 7.05 (Data Processing System, China) was used for data processing and analysis. The data were analysed by two-way ANOVA to determine the significance of individual factors and their interactions. The LSD test was used to compare the differences among means, and Origin 8.0 software (OriginLab OriginPro, U.S.A.) was used for drawing.

## Results

### Hay yield of alfalfa

The hay yield of alfalfa first increased and then decreased with increasing P application and reached the highest value under the  $100 \text{ kg} \cdot \text{ha}^{-1}$  treatment (Table 2). Under the same P application conditions, the hay yield of the  $4500 \text{ m}^3 \text{ ha}^{-1}$  treatment with increasing irrigation amount was significantly greater than those of the  $3750 \text{ m}^3 \text{ ha}^{-1}$  and  $5250 \text{ m}^3 \text{ ha}^{-1}$  treatments ( $P < 0.05$ ). There were no significant differences between the treatments except  $3750 \text{ m}^3 \text{ ha}^{-1}$  and  $5250 \text{ m}^3 \text{ ha}^{-1}$ , which were cut for the first time in 2014 ( $P < 0.05$ ). The hay yield of alfalfa in the second cut was higher than that in the first cut under the same water P treatment (except the  $5250 \text{ m}^3 \text{ ha}^{-1}$  condition in 2014).

### WUE and AEPF of alfalfa

The WUE and AEPF of alfalfa increased first and then decreased with increasing P application (Table 3), and the WUE of alfalfa under P application was significantly higher than that without P application ( $P < 0.05$ ). Except for  $0 \text{ kg ha}^{-1}$  and  $100 \text{ kg ha}^{-1}$  in 2014, there were no significant differences in the water use efficiencies of alfalfa under  $50 \text{ kg ha}^{-1}$  in 2015, and there was a significant difference in the water use efficiency under different irrigation amounts and the same P application ( $P < 0.05$ ). Appropriate irrigation rates and P levels of at  $4500 \text{ m}^3 \text{ ha}^{-1}$  and  $100 \text{ kg ha}^{-1}$ , respectively, significantly improved the WUE and AEPF of alfalfa. The WUEs of the first and second cut alfalfa were  $1.66 \text{ kg mm}^{-1} \text{ ha}^{-1}$  and  $1.94 \text{ kg mm}^{-1} \text{ ha}^{-1}$ , respectively, in 2014, and it was  $1.59 \text{ kg mm}^{-1} \text{ ha}^{-1}$  and  $2.36 \text{ kg mm}^{-1} \text{ ha}^{-1}$ , respectively, in 2015.

### Soil TP contents

The TP contents in the soil with P application were significantly higher than that without P application under the same irrigation conditions in the same soil layer (Table 4). Except for the 0–20 cm soil layer, the TP content from the  $100 \text{ kg ha}^{-1}$  treatment was significantly greater than those of the  $0 \text{ kg ha}^{-1}$  and  $50 \text{ kg ha}^{-1}$  treatments under  $4500 \text{ m}^3 \text{ ha}^{-1}$  conditions ( $P < 0.05$ ), and that of the  $150 \text{ kg ha}^{-1}$  treatment reached the maximum value. Except in the 20–40 cm soil layer, the TP content from the  $4500 \text{ m}^3 \text{ ha}^{-1}$  treatment was significantly higher than those of the  $3750 \text{ m}^3 \text{ ha}^{-1}$  and  $5250 \text{ m}^3 \text{ ha}^{-1}$  treatments under  $50 \text{ kg ha}^{-1}$  ( $P < 0.05$ ), and the total P contents of the other treatments increased with increasing irrigation. The content of total P in the 0–20 cm soil layer was the highest, and that at 60 cm was the lowest under the same irrigation and P application conditions.

### Soil AP content

The AP contents in the soil with P application were significantly higher than that without P application under the same irrigation conditions in the same soil layer (Table 5). The content of available P in the soil first increased and then decreased with increasing P application. The contents of available P in the soil reached maximum values for  $3750 \text{ m}^3 \text{ ha}^{-1}$  and  $4500 \text{ m}^3 \text{ ha}^{-1}$  under the  $100 \text{ kg ha}^{-1}$  treatment, and those of the  $100 \text{ kg ha}^{-1}$  treatment were significantly higher than those of the  $0 \text{ kg ha}^{-1}$  treatment ( $P < 0.05$ ). The content of soil available P reached a maximum value of  $5250 \text{ m}^3 \text{ ha}^{-1}$  under the  $150 \text{ kg ha}^{-1}$  treatment, and those of the  $100 \text{ kg ha}^{-1}$  and  $150 \text{ kg ha}^{-1}$  treatments were significantly higher than those of the  $0 \text{ kg ha}^{-1}$  treatment ( $P < 0.05$ ). The content of soil available P varied with increasing irrigation amount under the same conditions. In the same soil layer, for  $0 \text{ kg ha}^{-1}$ , the content of available P in the  $4500 \text{ m}^3 \text{ ha}^{-1}$  treatment was the highest; in other conditions, and that of the  $5250 \text{ m}^3 \text{ ha}^{-1}$  treatment was significantly higher

**Table 1:** Basic physical and chemical properties of the test soils

Site	Bulk density (g cm <sup>-3</sup> )	Alkaline-N (mg kg <sup>-1</sup> )	Organic matter (g kg <sup>-1</sup> )	Available P (mg kg <sup>-1</sup> )	Available K (mg kg <sup>-1</sup> )
A	1.48	70.2	24.3	22.1	186.3
B	1.56	60.8	25.5	25.5	330.2

A: Agricultural College Test Station, Shihezi, Xinjiang, China; B: Agricultural Demonstration Park of the Tianye Group Agricultural Research Institute, Shihezi, Xinjiang, China

**Table 2:** Effect of phosphorus application on hay yield of alfalfa under different irrigation treatments

Treatments	Hay yield at first cut (kg ha <sup>-1</sup> )					
	2014			2015		
	W <sub>1</sub>	W <sub>2</sub>	W <sub>3</sub>	W <sub>1</sub>	W <sub>2</sub>	W <sub>3</sub>
P <sub>0</sub>	3064.82 ± 2.83Cd	3330.72 ± 18.38Bd	3984.76 ± 14.14Ad	3539.51 ± 12.23Cd	3708.38 ± 18.66Bd	4146.93 ± 8.76Ad
P <sub>1</sub>	3372.64 ± 7.07Cc	4286.02 ± 31.11Bb	4348.93 ± 7.07Ab	3737.43 ± 0.35Cc	3966.99 ± 47.65Bc	4268.96 ± 2.51Ac
P <sub>2</sub>	4034.02 ± 1.41Ca	4667.46 ± 7.07Ba	5280.97 ± 0.28Aa	4141.21 ± 1.49Ca	4759.4 ± 58.29Aa	4601.22 ± 4.94Ba
P <sub>3</sub>	3600.09 ± 18.38Bb	4079.28 ± 4.24Ac	4066.59 ± 0.57Ac	3808.93 ± 9.08Cb	4639.07 ± 9.6Ab	4380.95 ± 22.97Bb
	Hay yield at second cut (kg ha <sup>-1</sup> )					
P <sub>0</sub>	3610.73 ± 1.13Cd	4802.77 ± 0.79Ad	3641.07 ± 1.39Bd	3844.01 ± 5.87Cd	5008.38 ± 11.59Bd	5446.93 ± 4.52Ad
P <sub>1</sub>	3773.02 ± 2.83Bc	5622.03 ± 1.1Ab	3695.2 ± 0.54Cc	4057.43 ± 13.79Cc	5266.99 ± 8.92Bc	5568.96 ± 2.51Ac
P <sub>2</sub>	4461.93 ± 0.42Ca	5696.8 ± 0.51Aa	5466.32 ± 0.99Ba	4441.21 ± 12.66Ca	6059.4 ± 1.72Aa	5901.22 ± 14.84Ba
P <sub>3</sub>	4300.37 ± 1.56Bb	4922.24 ± 0.48Ac	3963.7 ± 1.34Cb	4108.93 ± 11.91Cb	5939.07 ± 9.6Ab	5680.95 ± 8.82Bb

Different capital letters indicate significant differences at the 0.05 level within the same column means while different small letters indicate significant differences at the 0.05 level within the same row means

P<sub>0</sub>, P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> represent 0 kg ha<sup>-1</sup>, 50 kg ha<sup>-1</sup>, 100 kg ha<sup>-1</sup> and 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> respectively and W<sub>1</sub>, W<sub>2</sub>, and W<sub>3</sub> represent 3750 m<sup>3</sup> ha<sup>-1</sup>, 4500 m<sup>3</sup> ha<sup>-1</sup> and 5250 m<sup>3</sup> ha<sup>-1</sup>, respectively

**Table 3:** Effect of phosphorus application on WUE (kg·mm<sup>-1</sup>·ha<sup>-1</sup>) and AEPF (kg kg<sup>-1</sup>) of alfalfa under different irrigation treatments

Treatments	2014				2015			
	First cut WUE	Second cut WUE	First cut AEPF	Second cut AEPF	First cut WUE	Second cut WUE	First cut AEPF	Second cut AEPF
W <sub>1</sub> P <sub>0</sub>	1.26 ± 0.0012Ad	1.41 ± 0.0004Bd	—	—	1.35 ± 0.0047Ad	1.76 ± 0.0027Cd	—	—
W <sub>1</sub> P <sub>1</sub>	1.38 ± 0.0029Bb	1.47 ± 0.0011Bc	12.25 ± 0.05Cb	6.45 ± 0.13Bc	1.42 ± 0.0001Ac	1.85 ± 0.0063Cc	7.92 ± 0.5Bb	8.54 ± 0.32Bb
W <sub>1</sub> P <sub>2</sub>	1.65 ± 0.0006Aa	1.74 ± 0.0002Ba	19.89 ± 0.45Ba	17.67 ± 0.57Ba	1.58 ± 0.0006Aa	2.03 ± 0.0058Ba	12.03 ± 0.21Ba	11.94 ± 0.37Ba
W <sub>1</sub> P <sub>3</sub>	1.47 ± 0.0075Ac	1.68 ± 0.0006Ab	6.84 ± 0.09Bc	9.25 ± 0.06Ab	1.45 ± 0.0035Bb	1.88 ± 0.0054Cb	3.59 ± 0.28Bc	3.53 ± 0.24Bc
W <sub>2</sub> P <sub>0</sub>	1.18 ± 0.0065Bc	1.64 ± 0.0003Ad	—	—	1.24 ± 0.0062Bd	1.95 ± 0.0045Ad	—	—
W <sub>2</sub> P <sub>1</sub>	1.52 ± 0.0110Aab	1.92 ± 0.0004Ab	37.2 ± 0.99Aa	32.33 ± 0.40Aa	1.32 ± 0.0159Bc	2.05 ± 0.0035Ac	10.34 ± 1.16Ac	10.34 ± 0.82Ac
W <sub>2</sub> P <sub>2</sub>	1.66 ± 0.0025Aa	1.94 ± 0.0002Aa	25.73 ± 0.57Ab	17.65 ± 0.304Bb	1.59 ± 0.0194Aa	2.36 ± 0.0007Aa	21.02 ± 1.54Aa	21.02 ± 0.27Aa
W <sub>2</sub> P <sub>3</sub>	1.45 ± 0.0015Bb	1.68 ± 0.0002Ac	10.19 ± 0.06Ac	1.80 ± 0.20Cc	1.55 ± 0.0032Ab	2.32 ± 0.0037Ab	12.41 ± 0.38Ab	12.41 ± 0.28Ab
W <sub>3</sub> P <sub>0</sub>	1.25 ± 0.0044Ad	1.10 ± 0.0004Cd	—	—	1.23 ± 0.0026Bd	1.85 ± 0.0015Bd	—	—
W <sub>3</sub> P <sub>1</sub>	1.36 ± 0.0022Cc	1.12 ± 0.0002Cc	14.01 ± 0.54Bb	2.41 ± 0.49Cc	1.27 ± 0.0007Cc	1.89 ± 0.0009Bc	4.88 ± 0.25Cb	4.88 ± 0.08Cb
W <sub>3</sub> P <sub>2</sub>	1.65 ± 0.0001Aa	1.65 ± 0.0003Ca	25.99 ± 0.17Aa	36.35 ± 0.32Aa	1.36 ± 0.0015Ba	2.01 ± 0.0050Ca	9.09 ± 0.08Ca	9.09 ± 0.21Ca
W <sub>3</sub> P <sub>3</sub>	1.27 ± 0.0002Cb	1.2 ± 0.0004Bb	1.15 ± 0.08Cc	4.40 ± 0.10Bb	1.30 ± 0.0068Cb	1.93 ± 0.0030Bb	3.12 ± 0.19Bc	3.12 ± 0.06Bc

Different capital letters indicate significant differences at the 0.05 level within the same column means while different small letters indicate significant differences at the 0.05 level within the same row means

P<sub>0</sub>, P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> represent 0 kg ha<sup>-1</sup>, 50 kg ha<sup>-1</sup>, 100 kg ha<sup>-1</sup> and 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> respectively and W<sub>1</sub>, W<sub>2</sub>, and W<sub>3</sub> represent 3750 m<sup>3</sup> ha<sup>-1</sup>, 4500 m<sup>3</sup> ha<sup>-1</sup> and 5250 m<sup>3</sup> ha<sup>-1</sup>, respectively

**Table 4:** Effect of phosphorus application on soil TP contents (g kg<sup>-1</sup>) under different irrigation treatments

Treatments	2014			2015		
	0-20 cm	20-40 cm	40-60 cm	0-20 cm	20-40 cm	40-60 cm
W <sub>1</sub> P <sub>0</sub>	0.360 ± 0.068Ab	0.206 ± 0.001Bd	0.115 ± 0.001Ad	0.222 ± 0.006Bb	0.176 ± 0.031Bc	0.146 ± 0.016ABb
W <sub>1</sub> P <sub>1</sub>	0.426 ± 0.064Ab	0.278 ± 0.004Cc	0.167 ± 0.005Bc	0.287 ± 0.026Bb	0.252 ± 0.008Ab	0.223 ± 0.026Ba
W <sub>1</sub> P <sub>2</sub>	0.445 ± 0.066Bb	0.335 ± 0.003Bb	0.225 ± 0.005Cb	0.333 ± 0.021Ab	0.242 ± 0.032Bb	0.215 ± 0.046Ba
W <sub>1</sub> P <sub>3</sub>	0.447 ± 0.081Aa	0.351 ± 0.009Aa	0.237 ± 0.003Ca	0.370 ± 0.074Aa	0.319 ± 0.020Aa	0.232 ± 0.016Ba
W <sub>2</sub> P <sub>0</sub>	0.370 ± 0.064Ab	0.213 ± 0.001Bd	0.124 ± 0.001Ad	0.246 ± 0.010ABb	0.192 ± 0.013ABb	0.164 ± 0.011Bc
W <sub>2</sub> P <sub>1</sub>	0.464 ± 0.004Aab	0.429 ± 0.008Aa	0.169 ± 0.010Bc	0.303 ± 0.033ABab	0.257 ± 0.017Aab	0.202 ± 0.004Ab
W <sub>2</sub> P <sub>2</sub>	0.530 ± 0.086Aa	0.337 ± 0.001Bc	0.243 ± 0.003Bb	0.323 ± 0.062Aab	0.300 ± 0.024Aab	0.248 ± 0.004Aa
W <sub>2</sub> P <sub>3</sub>	0.541 ± 0.011ABa	0.406 ± 0.006Ab	0.283 ± 0.004Ba	0.367 ± 0.038Ab	0.330 ± 0.040Ab	0.254 ± 0.001ABa
W <sub>3</sub> P <sub>0</sub>	0.429 ± 0.008Ab	0.256 ± 0.001Ac	0.103 ± 0.001Bd	0.256 ± 0.013Ab	0.212 ± 0.008Ac	0.197 ± 0.012Ab
W <sub>3</sub> P <sub>1</sub>	0.560 ± 0.073Aa	0.406 ± 0.001Bb	0.197 ± 0.009Ac	0.327 ± 0.004Aa	0.273 ± 0.018Ab	0.245 ± 0.036Aab
W <sub>3</sub> P <sub>2</sub>	0.604 ± 0.003Aa	0.415 ± 0.001Ab	0.335 ± 0.003Aa	0.345 ± 0.019Aa	0.292 ± 0.028Ab	0.257 ± 0.024Aa
W <sub>3</sub> P <sub>3</sub>	0.638 ± 0.011Aa	0.519 ± 0.008Aa	0.304 ± 0.008Ab	0.353 ± 0.032Aa	0.337 ± 0.032Aa	0.260 ± 0.037Aa

Different capital letters indicate significant differences at the 0.05 level within the same column means while different small letters indicate significant differences at the 0.05 level within the same row means

P<sub>0</sub>, P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> represent 0 kg ha<sup>-1</sup>, 50 kg ha<sup>-1</sup>, 100 kg ha<sup>-1</sup> and 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> respectively and W<sub>1</sub>, W<sub>2</sub>, and W<sub>3</sub> represent 3750 m<sup>3</sup> ha<sup>-1</sup>, 4500 m<sup>3</sup> ha<sup>-1</sup> and 5250 m<sup>3</sup> ha<sup>-1</sup>, respectively

than that of the 3750 m<sup>3</sup> ha<sup>-1</sup> treatment ( $P < 0.05$ ). The content of soil available P decreased with increasing soil depth under the same irrigation and P application conditions.

### Relationship between TP and AP contents in soil

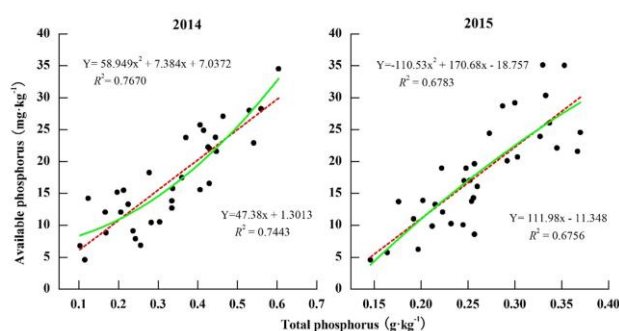
To clarify the relationship between the soil TP and AP contents, the soil TP and AP contents were fitted and the

**Table 5:** Effect of phosphorus application on soil AP contents (mg kg<sup>-1</sup>) under different irrigation treatments

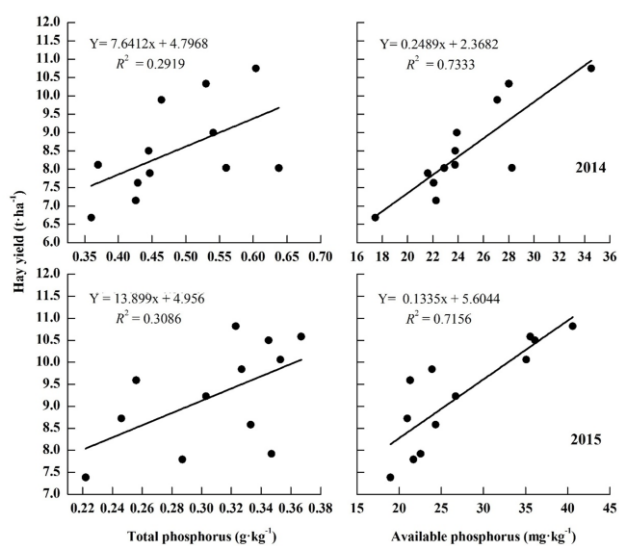
Treatments	2014			2015		
	0-20 cm	20-40 cm	40-60 cm	0-20 cm	20-40 cm	40-60 cm
W <sub>1</sub> P <sub>0</sub>	17.45 ± 0.51Cc	12.05 ± 0.02Bc	4.60 ± 0.07Cd	18.97 ± 0.33Ac	13.70 ± 1.10Ac	4.56 ± 0.89Bc
W <sub>1</sub> P <sub>1</sub>	22.25 ± 0.15Bb	18.24 ± 1.82Ba	12.06 ± 0.43Bb	28.70 ± 1.40Ab	17.04 ± 1.87Cb	12.07 ± 0.44Cab
W <sub>1</sub> P <sub>2</sub>	23.78 ± 0.14Ca	13.82 ± 0.32Cb	13.27 ± 0.06Aa	30.35 ± 1.94Aa	23.33 ± 1.60Ba	13.24 ± 1.56Ba
W <sub>1</sub> P <sub>3</sub>	21.59 ± 0.64ABb	7.68 ± 0.06Cd	9.12 ± 5.74Bc	24.55 ± 0.54Ba	17.18 ± 0.60Bb	10.26 ± 1.88Bb
W <sub>2</sub> P <sub>0</sub>	23.75 ± 0.17Ab	15.49 ± 0.52Aa	14.2 ± 0.64Aa	16.97 ± 1.23Ac	11.07 ± 1.43ABd	5.71 ± 0.39Ac
W <sub>2</sub> P <sub>1</sub>	27.09 ± 0.15Aa	16.55 ± 0.01Ca	8.84 ± 0.78Cc	20.70 ± 1.24Bb	19.63 ± 1.72Bc	13.88 ± 0.68Bb
W <sub>2</sub> P <sub>2</sub>	28.02 ± 0.65Ba	15.77 ± 0.68Aa	7.88 ± 1.08Bc	40.59 ± 1.41Bb	29.18 ± 2.81Ab	18.94 ± 1.02Aa
W <sub>2</sub> P <sub>3</sub>	22.91 ± 1.19Ab	15.57 ± 0.17Aa	10.43 ± 0.34Ab	21.57 ± 0.57Aa	35.13 ± 2.33Aa	13.77 ± 1.60Ab
W <sub>3</sub> P <sub>0</sub>	22.06 ± 0.68Bc	6.84 ± 1.05Cc	6.78 ± 0.70Bd	14.31 ± 1.68Cc	9.87 ± 1.92Cd	6.22 ± 0.29Ac
W <sub>3</sub> P <sub>1</sub>	28.25 ± 0.29Ab	25.73 ± 0.25Aa	15.19 ± 0.30Aa	23.91 ± 1.53Bab	24.4 ± 1.48Aa	10.06 ± 1.18Aa
W <sub>3</sub> P <sub>2</sub>	34.53 ± 1.11Aa	24.87 ± 1.11Ba	12.67 ± 0.36Ab	22.11 ± 1.54Cb	20.11 ± 1.57Cb	8.56 ± 1.03Cb
W <sub>3</sub> P <sub>3</sub>	20.91 ± 0.11Bc	13.02 ± 0.64Bb	10.53 ± 0.19Ac	35.08 ± 0.97Aa	26.06 ± 0.57Cc	16.07 ± 0.59Cc

Different capital letters indicate significant differences at the 0.05 level within the same column means while different small letters indicate significant differences at the 0.05 level within the same row means

P<sub>0</sub>, P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> represent 0 kg ha<sup>-1</sup>, 50 kg ha<sup>-1</sup>, 100 kg ha<sup>-1</sup> and 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> respectively and W<sub>1</sub>, W<sub>2</sub>, and W<sub>3</sub> represent 3750 m<sup>3</sup> ha<sup>-1</sup>, 4500 m<sup>3</sup> ha<sup>-1</sup> and 5250 m<sup>3</sup> ha<sup>-1</sup>, respectively



**Fig. 2:** Relationships between total phosphorus and available phosphorus in soil



**Fig. 3:** Relationships between soil TP and AP and hay yield of alfalfa

results show that (Fig. 2) quadratic equations fit the soil TP and AP contents better than linear equations, but the determination coefficient ( $R^2$ ) difference was not large. In addition to the linear fitting of 2015, two kinds of fitting

equations had  $R^2$  above 0.7; with the highest value reaching 0.77, showing that there was a close relationship between the soil TP and AP contents and that the soil AP content was strongly influenced by the soil TP content. With increasing soil TP, the soil available P increased gradually.

### Relationship between soil TP and AP and hay yield of alfalfa

To understand the relationship between TP and AP content in soil and hay yield of alfalfa in the year of planting, the TP and AP contents in soil were fitted with the alfalfa hay yield. The two-year test results (Fig. 3) showed that there were good linear relationships between the AP and TP contents and alfalfa hay production, with fitting coefficients ( $R^2$ ) less than 0.7 for the TP content and those greater than 0.7 for the AP content, indicating that soil AP content contributes more to alfalfa hay yield than soil TP content.

### Discussion

Irrigation has an important effect on the hay yield of alfalfa of different stubble heights. Research shows that the hay yield of alfalfa increases primarily linearly with increasing irrigation (Grimes *et al.* 1992). The WUE of alfalfa first increases and then decreases with increasing irrigation rate (Song *et al.* 2019). The annual WUE of alfalfa is the highest under suitable water supply conditions, while the annual WUE of alfalfa is the lowest under sufficient water supply conditions (Sun *et al.* 2018). This study showed that an increased amount of irrigation was beneficial to the formation of alfalfa plantings and hay yield, but when the irrigation reached a certain amount, the yield increase effect was not obvious (Table 2). At the early stage of alfalfa growth, under drought conditions, increasing irrigation can promote the water absorption and plant growth of alfalfa (Zhang *et al.* 2017), mainly because excessive irrigation increases alfalfa lodging, which is not conducive to alfalfa photosynthesis or dry matter accumulation, and ultimately reduces the hay yield of alfalfa.

Fertilization is one of the key measures to improve the forage yield of alfalfa. Studies show that fertilization can not only improve the nutritional quality but also increase the growth rate and plant height of alfalfa, thus improving the hay yield of alfalfa (Fan *et al.* 2016). The results of this study showed that the application of P significantly increased the hay yield of alfalfa in the year of planting, but when the application of P was too high, the hay yield did not increase significantly (Table 2). This result may occur because the P fertilizer applied promoted the accumulation of dry matter to a certain extent but a decrease in dry matter was caused by excessive P application, mainly because alfalfa plants have a certain "threshold" range of P uptake and utilization; when a certain threshold is reached, it can effectively promote the growth of alfalfa (Colomb *et al.* 2007; Bai *et al.* 2013), while absorbing excess or greatly exceeding the maximum of absorption, and the P content of alfalfa plants decreases (Berg *et al.* 2018). Additionally, P can promote the growth of the plant root system, improve the root water absorption ability, increase hay yield and improve WUE (Fang *et al.* 2010). In our study, when irrigation rate and P were applied at 4500 m<sup>3</sup> ha<sup>-1</sup> and 100 kg ha<sup>-1</sup>, respectively, the WUE and AEPF of alfalfa improved (Table 3), and the hay yield showed the same pattern (Table 2). As a consequence, reasonable coupling of water and P increased the absorption of water and P by alfalfa and then increased the hay yield.

The application of P had an important effect on the TP and AP content in different soil layers. Research shows that P is one of the most important elements for plant growth. The P for plant growth mainly comes from soil, and inorganic P in soil mainly depends on the P absorbed by plants. Additionally, the availability of inorganic P of different forms in soil to plants is different (Gu *et al.* 2018). The results showed that the TP increased with increasing P application and irrigation (Table 4), while the AP increased first and then decreased (Table 5). This result may be due to the spatial variability and vertical migration of soil P and the diffusion of P promoted by water. In addition, this result may be related to the low content of AP in soil and the short movement distance of this type of P, generally approximately 3–5 cm (Batool *et al.* 2015).

In our study, under the same irrigation amount and the same P application, the contents of TP and AP in the 0–20 cm soil layers were generally the highest (Table 4 and 5), possibly because P has an obvious "surface agglomeration" phenomenon (Lombi *et al.* 2004; Fraser *et al.* 2019) that makes the content of TP and AP in the 0–20 cm soil layer significantly greater than that in the 40–60 cm soil layer. Because the content of soil AP was greatly affected by the content of soil TP (Zhao *et al.* 2018), there was a linear relationship between these parameters (Fig. 2). With increasing TP, the AP gradually increases.

In conclusion, the contents of TP and AP in the water-phosphorus coupled treatment were significantly higher than those in the other non-phosphorus treatments and decreased

gradually with increasing soil depth, indicating that water-phosphorus coupling had an impact on the soil P content and then affected the absorption of P by the alfalfa roots. Research shows that alfalfa roots are mainly concentrated in the 20–60 cm soil layer, of which 20–40 cm soil layer roots account for 35.8% of the total root system (Jiang *et al.* 2017). Therefore, alfalfa topdressing should be applied as deep as possible to meet the soil nutrient needs of deep alfalfa roots (20–60 cm).

## Conclusion

Appropriate irrigation rates and phosphorus levels of 4500 m<sup>3</sup> ha<sup>-1</sup> and 100 kg ha<sup>-1</sup>, respectively, significantly improved the hay yield, WUE and AEPF of alfalfa in the planting year. The contribution rates of AP to hay yield were the largest and could be used as indicators of growth traits.

## Acknowledgements

The research was supported by the National Natural Science Foundation of China (31660693), the Project Funded by the China Postdoctoral Science Foundation (2018T111120, 2017M613252), the Youth Innovation Talent Cultivation Program of Shihezi University (CXRC201605) and the China Agriculture Research System (CARS-34).

## References

- Altinok S, E Yurtseven, S Avci, HS Öztürk, MF Selenay (2015). The effects of different irrigation water salinities and leaching ratios on green and dry forage yields of alfalfa (*Medicago sativa* L.). *Agric For* 61:85–90
- Bai Z, H Li, X Yang, B Zhou, X Shi, B Wang (2013). The critical soil P levels for crop yield, soil fertility and environmental safety in different soil types. *Plant Soil* 372:27–37
- Batool K, QU Khan, R Naz, MJ Khan, OU Sayal, A Bashir, A Latif (2015). Source and rate of inorganic P fertilizer affecting soil phosphatase enzymes, yield and P-uptake of chillies. *Plant Soil Environ* 34:27–33
- Berg WK, S Lissbrant, SM Cunningham, JJ Volenec (2018). Phosphorus and potassium effects on taproot C and N reserve pools and long-term persistence of alfalfa (*Medicago sativa* L.). *Plant Sci* 272:301–308
- Colomb B, P Debaeke, C Jouany, JM Nolot (2007). Phosphorus management in low input stockless cropping systems: Cropland soil responses to P regimes in a 36-year experiment in southern France. *Eur J Agron* 26:154–165
- Fan JW, YL Du, BR Wang, NC Turner, T Wang, LK Abbott, K Stefanova, KHM Siddique, FM Li (2016). Forage yield, soil water depletion, shoot nitrogen and phosphorus uptake and concentration, of young and old stands of alfalfa in response to nitrogen and phosphorus fertilisation in a semiarid environment. *Field Crops Res* 198:247–257
- Fang Y, BC Xu, NC Turner, FM Li (2010). Does root pruning increase yield and water use efficiency of winter wheat? *Crop Pasture Sci* 61:899–910
- Fiasconaro ML, Y Gogorcena, F Muñoz, D Andueza, M Sánchez-Díaz, MC Antolín (2012). Effects of nitrogen source and water availability on stem carbohydrates and cellulosic bioethanol traits of alfalfa plants. *Plant Sci* 191:16–23

- Fosu-Mensah BY, M Mensah (2016). The effect of phosphorus and nitrogen fertilizers on grain yield, nutrient uptake and use efficiency of two maize (*Zea mays* L.) varieties under rain fed condition on Haplic Lixisol in the forest-savannah transition zone of Ghana. *Environ Syst Res* 5:5–22
- Fraser TD, DH Lynch, IP O'Halloran, RP Voroney, MH Entz, KE Dunfield (2019). Soil phosphorus bioavailability as influenced by long-term management and applied phosphorus source. *Can J Soil Sci* 99:292–304
- Geisseler D, WR Horwath, RG Joergensen, B Ludwig (2010). Pathways of nitrogen utilization by soil microorganisms - A review. *Soil Biol Biochem* 42:2058–2067
- Grimes DW, PL Wiley, WR Sheesley (1992). Alfalfa yield and plant water relations with variable irrigation. *Crop Sci* 32:1381–1387
- Gu YJ, CL Han, JW Fan, XP Shi, M Kong, XY Shi, KHM Siddique, YY Zhao, FM Li (2018). Alfalfa forage yield, soil water and P availability in response to plastic film mulch and P fertilization in a semiarid environment. *Field Crops Res* 215:94–103
- Ijaz M, S Hussain, S Ul-Allah, A Nawaz, A Sattar, A Sher, TA Yasir, M Hussain (2018). Timing of phosphorus and boron application affects seed yield, oil contents and profitability of canola under an arid climate. *Intl J Agric Biol* 20:1745–1750
- Ismail SM, MH Almarshadi (2013). Maximizing productivity and water use efficiency of alfalfa under precise subsurface drip irrigation in arid regions. *Irrig Drain* 62:57–66
- Jiang L, Y Zheng, GJ Liu, B Wang, CY Tian (2017). Study on root distribution characteristics and soil physical and chemical properties of apricot and alfalfa under orchard intercropping mode. *Acta Bot Bor-Occident Sin* 37:2489–2495
- Li Y, HY Huang, Y Wang, QH Yuan (2018). Transcriptome characterization and differential expression analysis of disease-responsive genes in alfalfa leaves infected by *Pseudopeziza medicaginis*. *Euphytica* 214:126–127
- Lombi E, MJ McLaughlin, C Johnston, RD Armstrong, RE Holloway (2004). Mobility and ability of phosphorus from granular and fluid monoammonium phosphate differs in a calcareous soil. *Soil Sci Soc Amer J* 68:682–689
- Lu RK (2000). *Methods of soil agricultural chemical analysis*. China Agricultural Science and Technology Press, Beijing, China
- Rahmana MA, K Yong-Gooa, A Iftekhara (2016). Proteome analysis of alfalfa roots in response to water deficit stress. *J Integr Agric* 15:1275–1285
- Rehim A, M Hussain, S Ahmad, S Noreen, H Dogan, M Zia-ul-Haq, S Ahmad (2016). Band application of phosphorus with farm manure improves phosphorus use efficiency, productivity and net returns of wheat on sandy clay loam soil. *Turk J Agric For* 40:319–326
- Saeed IAM, AH El-Nadi (1997). Irrigation effects on the growth, yield, and water use efficiency of alfalfa. *Irrig Sci* 17:63–68
- Singh M (1999). Effect of irrigation and nitrogen on herbage, oil yield and water use of lemongrass (*Cymbopogon flexuosus*) on alfisols. *J Agric Sci* 132:201–206
- Song T, FY Xu, W Yuan, YJ Zhang, TY Liu, MX Chen, QJ Hu, Y Tian, WF Xu, HJ Zhang (2018). Comparison on physiological adaptation and phosphorus use efficiency of upland rice and lowland rice under alternate wetting and drying irrigation. *Plant Growth Regul* 86:195–210
- Song YG, LV Jiao, ZQ Ma, W Dong (2019). The mechanism of alfalfa (*Medicago sativa* L.) response to abiotic stress. *Plant Growth Regul* 89:239–249
- Sun B, YZ Gao, HJ Yang, ZJ Li (2018). Performance of alfalfa rather than maize stimulates system phosphorus uptake and over yielding of maize/alfalfa intercropping via changes in soil water balance and root morphology and distribution in a light chernozemic soil. *Plant Soil* 439:145–161
- Zhang G, C Liu, C Xiao, R Xie, B Ming, P Hou, G Liu, W Xu, D Shen, K Wang, S Li (2017). Optimizing water use efficiency and economic return of super high yield spring maize under drip irrigation and plastic mulching in arid areas of China. *Field Crops Res* 211:137–146
- Zhang QB, L Yu, WH Lu, CH Ma, HX He (2016). Optimal irrigation regime improving yield and quality of alfalfa in year of sowing. *Trans Chin Soc Agric Eng* 32:16–22
- Zhao W, C Song, P Zhou, JY Wang, F Xu, F Ye, XC Wang, WY Yang (2018). Effects of phosphorus application amount and phosphorus application depth on phosphorus utilization rate and phosphorus loss risk in maize and soybean intercropping system. *Chin J Appl Ecol* 29:1205–1214