



# Irrigation and nitrogen fertilization influence on alfalfa yield, nutritive value, and resource use efficiency in an arid environment

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## ABSTRACT

In arid regions, water and nitrogen are the two most limiting factors for sustainable pastoral production systems. In this study, we determined the potential effects of irrigation and nitrogen application on forage yield, nutritive values, and resource use efficiency of alfalfa in arid agro-climatic conditions. Field experiments were carried out using three irrigation regimes (W1, 300; W2, 450; and W3, 600 mm) and three nitrogen application rates (N1, 150; N2, 225; and N3, 300 Kg N ha<sup>-1</sup>). Alfalfa seeds were sown in fall 2014, uniformly managed for crop establishment, and subjected to different treatments in spring 2015–2016. Results indicated that irrigation, nitrogen, and their interaction (W × N) significantly ( $P \leq 0.05$ ) affected alfalfa forage yield, quality, irrigation water use efficiency (IWUE), and nitrogen use efficiency (NUE). Forage yield followed an increasing trend with increasing the irrigation amount at each harvest in both growing seasons. However, crude protein (CP), relative feed values (RFV), and IWUE were significantly decreased while neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were increased at the high irrigation level (W3). The maximum forage yield was achieved with W3 treatment (25.9 and 23.1 t ha<sup>-1</sup>), followed by W2 (25.8 and 21.7 t ha<sup>-1</sup>) in 2015 and 2016. In both years, the highest IWUE (57.3 and 48.2 kg mm<sup>-1</sup>), CP (16.1 % and 17.7 %), and RFV (197.1 % and 186.2 %) values were achieved with W2 treatment. Moreover, increasing N application resulted in a linear decline in alfalfa forage yield, nutritive quality, and resource use efficiency. The maximum forage yield (24.4 and 21.2 t ha<sup>-1</sup>), CP (15.5 % and 16.4 %), RFV (210.3 % and 198.8 %), IWUE (54.3 and 47.0 kg mm<sup>-1</sup>), and NUE (169.9 and 141.2 kg kg<sup>-1</sup>), and lower ADF (25.7 % and 24.9 %) and NDF (31.0 % and 33.1 %) was obtained with application of 150 kg N ha<sup>-1</sup> in 2015 and 2016. The regression equations of irrigation and nitrogen application indicated a quadratic relationship between yield and forage nutritive values. Overall, 450 mm irrigation coupled with 150 kg N ha<sup>-1</sup> (W2N1) showed the most promising effects in terms of achieving optimal forage yield consistent with enhanced forage nutritive values, and greater resource use efficiency of alfalfa in the arid region of North China.

## 1. Introduction

Alfalfa (*Medicago sativa* L.) is an important perennial forage crop for livestock production systems due to its high nutritional value and desirable agricultural traits (Darapuneni et al., 2020; Gao et al., 2020; McDonald et al., 2021). Moreover, it is a high-yielding forage crop that can provide multiple harvests during the same growing season (Wang et al., 2015; Xiao et al., 2015). Because of its adaptation to a wide range of climatic conditions, alfalfa is considered a high-value cash crop, and a major source for sustaining the livestock industry and livelihood of the

subsistence farmers in arid and semiarid regions (Darapuneni et al., 2020; Fan et al., 2016; Gu et al., 2018). For often, the crop is regarded as “the queen of forages” and is cultivated over four million hectares throughout Northern and Northwestern China (Wang et al., 2021). However, water scarcity and low soil fertility are the major impediments to sustainable pasture production and livestock expansion in these regions (Liu et al., 2021; Wang et al., 2021).

In arid and semiarid environments, irrigation water is a major limiting factor for crop growth (Gu et al., 2018; Ismail and Almarshadi, 2013), where evapotranspiration often exceeds the received

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precipitation (Djaman et al., 2020b). Although alfalfa is more drought-tolerant than most forage legumes (Yu et al., 2018), its growth, dry biomass, and forage quality are greatly influenced by water deficiency (Antolín et al., 1995; Li and Su, 2017; Liu et al., 2021). Water deficit conditions impair the  $N_2$  fixation in legumes, leading to N deficiency (Hungria and Vargas, 2000; Sprent, 1976; Yu et al., 2018), decreasing plant N acquisition by limiting the mineralization and transport of N from bulk soil to rhizosphere in dry soil (Gao et al., 2020; Kunrath et al., 2018; Vasileva et al., 2006). Therefore, supplemental irrigation is an effective approach for achieving greater alfalfa biomass in arid regions. However, excessive irrigation may damage alfalfa stands due to salinization in arid and semi-arid regions and increase water loss (Liu et al., 2021; Saeed and El-Nadi, 1997; Wang et al., 2021), which will further increase the pressure on regional natural water resources. Given the increasing irrigation water scarcity, high irrigation costs, and need of ensuring future water supplies, it is critical to identify the optimal irrigation recommendations that could result in higher yields and efficient use of water to meet the required demands and reduce the cost of alfalfa production in arid regions.

Nitrogen (N) is essential for normal crop growth and improved biomass because of its major role in regulating C assimilation (Gao et al., 2020; Lu et al., 2021). In recent years, with increasing feed demands for livestock production in China, excessive N fertilization to boost yield potential has become a common practice for the majority of alfalfa growers. However, the available literature regarding the effects of N application to increase alfalfa productivity is highly controversial. In general, nitrogen fertilization is considered less important for legume crops because of their high potential for atmospheric N fixation which mostly negates the need for synthetic fertilization (Elgharably and Benes, 2021; Haki et al., 2016; Zhu et al., 1998). According to Oliveira et al. (2004), even recommended N application ( $45 \text{ kg ha}^{-1}$  after each harvest) reduced biological N fixation but had no effect on alfalfa forage yield when compared to control treatment without mineral N. Nevertheless, several studies pointed out that the symbiotic  $N_2$  fixation, activity, and nodulation stability may vary over crop growth seasons (Elgharably and Benes, 2021; Hungria and Vargas, 2000), and N fertilizer can benefit crop growth during periods when biological nitrogen fixation is down (Hannaway and Shuler, 1993), after harvest and at low soil N availability period (Raun et al., 1999). Therefore, many researchers support the concept of a starter N application at the re-greening stage and after harvests to promote early plant development and avoid retention of root development (Hartwig and Soussana, 2001; Raun et al., 1999; Vasileva and Pachev, 2015). In addition, the availability of soil N to plants is dependent on soil water status (Antolín et al., 1995; Gao et al., 2020), hence, excessive N application under water deficit conditions may not be conducive. Previous studies on the interplay of water and nitrogen emphasized a reduction in WUE induced by N deficit, and the reciprocal impact of water deficit on the N economy of crops (Gonzalez-Dugo et al., 2010; Kunrath et al., 2018). Therefore, matching N fertilization to irrigation amount is important for improving forage yield and quality while enhancing resource use efficiency in arid climatic conditions.

Besides high yields, excellent forage nutritive value is the most desirable goal because of its direct impact on the profitability of forage production and the livestock enterprises it supports (Haki et al., 2021; Xiao et al., 2015). Forages with high crude protein (CP) contents are considered to have high nutritional values and are valuable for increasing dairy production and quality (McDonald et al., 2021; Zhang et al., 2020). On contrary, more fiber contents such as acid detergent fiber (ADF) and nutrient detergent fiber (NDF) reduce forage quality (Adjesiwor et al., 2017; Mao et al., 2018). In general, stems have comparatively higher NDF and ADF ratios and lower CP contents than other plant parts (Lemaire and Belanger, 2019; Mao et al., 2018). Irrigation and fertilizer management practices influence the stem to sheath ratios, leaf blades, and plant maturity (Gao et al., 2020; Ismail and Almarshadi, 2013), and would directly affect the fiber and CP contents.

Previously, studies reported that boosting forage yields through intensive irrigation and fertilizers may be compromised by a loss in nutritive values (Haki et al., 2021; Liu et al., 2021; Zhang et al., 2020).

Given the available inconsistent results of previous studies and the lack of information about a suitable irrigation-nitrogen recommendation for alfalfa production in arid regions of North China, this study aims to identify an appropriate irrigation and N management practice that can improve forage yield consistent with enhanced forage quality, and resource use efficiency of alfalfa. This study will help in better understanding of physiological regulation mechanisms and will provide new insights into the related forage yield, IWUE, and NUE of alfalfa under different irrigation and N treatments in arid agro-climatic conditions.

## 2. Materials and methods

### 2.1. Experimental site description

The field experiments were conducted in 2015 and 2016 at the Linze Grassland Experimental Station of Lanzhou University, Zhangye ( $103^{\circ} 05' \text{ E}$ ,  $38^{\circ} 38' \text{ N}$ , 1390 m above sea level), located in the Hexi Corridor, Gansu Province, Northwest China. The region has a continental arid climate with abundant sunlight, high evaporation, and critically scarce rainfall. The inland arid area of the research station is a secondary salinized meadow and crop production mainly relies on supplemental irrigation. Frosts are common between September and April and the region enjoys a frost-free period of approximately 175 days, and annual sunshine accounts for 3000 h. The regional average annual precipitation in the last twenty years (1997–2017) was 110 mm. The amount of precipitation was only 114 and 74 mm in 2015 and 2016, respectively. The average annual temperature was  $5.8^{\circ} \text{ C}$ , with a maximum temperature of  $22.8^{\circ} \text{ C}$  in July, and a minimum temperature of  $-11.2^{\circ} \text{ C}$  in January. Fig. 1 depicts monthly precipitation and mean temperature data acquired from a local meteorological station located 800 m away from the experimental fields.

According to the USDA soil taxonomy, soil of the experimental site is classified as aquisalids. Soil properties (upper 30 cm soil profile) measured before the experiment were as follows; pH 8.5, average field capacity (gravimetric; %) 25 %, bulk density  $0.93 \text{ g cm}^{-3}$ , organic matter  $9.34 \text{ g kg}^{-1}$ , available nitrogen  $38.7 \text{ mg kg}^{-1}$ , phosphorus  $20.3 \text{ mg kg}^{-1}$ , and potassium was  $104.5 \text{ mg kg}^{-1}$ .

### 2.2. Experimental design and treatments management

Alfalfa stand was established in 2014, with a seeding rate of  $22 \text{ kg ha}^{-1}$ , and row spacing of 20 cm. The experiment was organized in a factorial design with three irrigation regimes as main plots and three nitrogen application rates as subplots. The irrigation regimes were 300 (W1), 450 (W2), and 600 mm (W3), and the nitrogen application rates included 150 (N1), 225 (N2), and 300 (N3)  $\text{kg ha}^{-1}$ . In this way, the experiment comprised of nine different treatments and there were three replicates per treatment. Water flow meters (Woltman WP) were used to measure the amount of irrigated water applied to each plot. The first surface irrigation was applied at the re-greening stage (30 %), while the second (35 %) and third irrigations (35 %) were applied shortly after the previous harvest during each crop growing season (Table S1). The irrigation timings were similar to local farmers' practices. Moreover, local practiced N application rates by alfalfa growers are about  $300 \text{ kg ha}^{-1} \text{ year}^{-1}$ . Urea (46 % N) was used as a nitrogen source and fertilizer was applied in split doses (60 % at re-greening and 40 % at second harvest). Each plot was 10 m long and 10 m wide (area =  $100 \text{ m}^2$ ), separated by a 1.2 m wide isolation belt, and a ridge was placed between plots. To prevent lateral infiltration, the partitioning ridges of each subplot were covered with an impervious plastic film membrane. A total of 27 plots with a total area of  $2700 \text{ m}^2$  were used in the experiment. The same alfalfa plots dedicated to specific irrigation and nitrogen treatments were used during both crop growing years (2015–2016). Regardless of

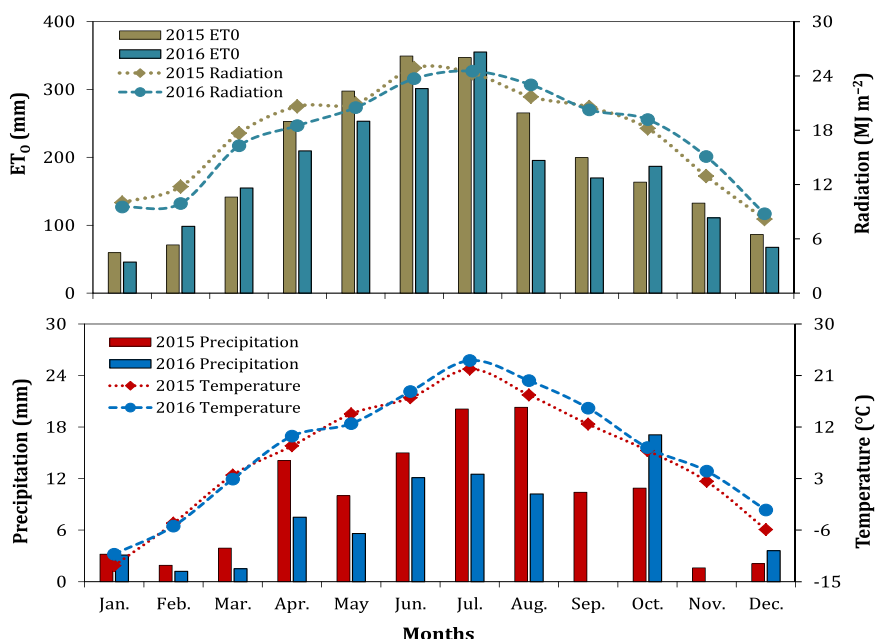


Fig. 1. Monthly average values of air temperature, solar radiation, and cumulative values of reference evapotranspiration (ET<sub>0</sub>) and precipitation at the experimental site during 2015 and 2016.

treatment, all plots received uniform crop management practices such as weeding and plant protection measures.

### 2.3. Plant sampling, measurement, and calculations

Alfalfa was harvested at the early flowering stage (10 % blooming), and a total of six harvests were obtained each year. The first harvest was acquired on April 21 and 24, the second harvest on May 24 and 22, the third harvest on June 22 and 24, the fourth harvest on July 23 and 24, the fifth harvest on August 25 and 27, and the sixth harvest on September 27 and 29 in 2015 and 2016, respectively (Table S1). At each harvest, three representative quadrats samples (1 m × 1 m) were randomly selected at the center of each plot and clipped to a height of about 5 cm for measuring alfalfa forage yield. Forage dry biomass was determined after oven-drying the samples at 75 °C until they reached a constant weight. Hay yield was determined using dry matter (Fan et al., 2016). The last harvest of each season was commenced before the first week of October to ensure adequate accumulation of carbohydrate reserves for winter survival and regrowth.

Dried plant samples were ground into fine powder to pass through a 0.04 in. screen and used for nutritive analyses. Total N contents were determined by the standard Kjeldahl method (FOSS Kjeltac™ 8400 instrument) and crude protein contents (CP, %) was estimated as N% × 6.25 (Ferreira et al., 2015). Neutral detergent fiber (NDF, %) and acid detergent fiber (ADF, %) were determined using ANKOM2000 Automated Fiber Analyzer following the procedure of Van Soest et al. (1991). Relative feed value (RFV) was calculated from dry matter digestibility (DMD) and dry matter intake (DMI) using ADF (%) and NDF (%), respectively (Ferreira et al., 2015):

$$RFV = \frac{DMI \times DMD}{1.29} \tag{1}$$

$$DMI = \frac{120}{NDF (\% DM)} \tag{2}$$

$$DMD = 88.9 - 0.779 \times ADF (\% DM) \tag{3}$$

Irrigation water use efficiency (IWUE) refers to crop economic yield (kg of alfalfa) relative to the amount of irrigation water (mm) provided.

Irrigation water use efficiency (kg mm<sup>-1</sup>) of alfalfa was calculated according to Eq. (4):

$$Irrigation\ water\ use\ efficiency(IWUE) = \frac{Y_{DM}}{I_A} \tag{4}$$

Where Y<sub>DM</sub> is the total alfalfa yield (kg ha<sup>-1</sup>) and I<sub>A</sub> is the amount of applied irrigation (mm).

Nitrogen use efficiency (NUE) refers to the ratio of aboveground biomass to nitrogen supplied. The agronomic nitrogen use efficiency (kg yield kg<sup>-1</sup> of N applied) of alfalfa was calculated using Eq. (5):

$$Nitrogen\ use\ efficiency(NUE) = \frac{Y_{DM}}{N_A} \tag{5}$$

Where Y<sub>DM</sub> is the total alfalfa yield (kg ha<sup>-1</sup>) and N<sub>A</sub> is the amount of nitrogen applied (kg ha<sup>-1</sup>).

### 2.4. Statistical analyses

Analysis of variance (ANOVA) for the two-year data was performed using the General Linear Model procedures in the SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA). In this study, irrigation and nitrogen were considered as fixed and main factors, year as repetitive factor, and replication as a random factor. The interaction effects, wherever found significant were also calculated and presented. Tukey's significant difference test was used to compare the significant differences among the treatment means. The relationships of forage yield and qualitative indexes with treatments under different years were checked for normality, and linear and nonlinear regression analyses were performed. Figures were constructed with Excel 2010 (Microsoft Corp., USA) and Origin 9.1 (Origin Lab Corp., USA).

## 3. Results

### 3.1. Precipitation and air temperature during crop growing seasons

Monthly mean precipitation varied significantly during the two alfalfa-growing seasons. Total rainfall received by alfalfa crop during the first growing season (10th March to 27th September 2015) was 94 mm,

however, it received only 49 mm in the following season (12th March to 29th September 2016) (Fig. 1). In 2015, 70.1 % of the rainfall occurred between June and September, however in 2016, 70.4 % of the rainfall occurred between June and August, with no rainfall occurring in September. Moreover, around 60–68 % of rainfall occurred in events of < 5 mm, too little to be effectively utilized by crops and the annual total precipitation was mainly dependent on a few large rainstorms with over 10 mm of rainfall. Monthly mean temperatures did not differ significantly between the two alfalfa-growing seasons, except for July to September in 2016, when mean temperature was relatively higher than in 2015 (Fig. 1). The minimum mean temperatures observed throughout the alfalfa growing season were 7.9 °C and 8.4 °C in October, while the maximum mean temperatures were 22.1 °C and 23.6 °C in July 2015 and 2016, respectively.

### 3.2. Effects of irrigation and nitrogen application on forage yield

Results indicate significant ( $P \leq 0.05$ ) effects of the year (Y), irrigation (W), and nitrogen (N) treatments on alfalfa forage yield (Table S2). Moreover, interaction effects among year, irrigation, and nitrogen were also significant (Table S2). Initially, with the progression in harvest numbers, alfalfa yield followed an increasing trend and maximum yields were achieved at the third harvest, thereafter declining gradually in all treatments during both years (Fig. 2). Forage yield showed a linear increase with the irrigation amounts, and W3 treatment resulted in a greater yield than other irrigation treatments at each harvest. On the other hand, nitrogen application under irrigation regimes followed a different trend for alfalfa forage yield. Increasing N application under the W1 irrigation level increased forage yield at each harvest. However, increasing N application under W2 and W3 irrigation levels were associated with a decline in forage yield at each harvest (Fig. 2).

Seasonal alfalfa yield among different treatments varied from 13.6 to 29.7 t ha<sup>-1</sup> in 2015, and from 12.7 to 25.6 t ha<sup>-1</sup> in 2016 (Table 1). Forage yield in 2015 was greater by 14.4 % than in 2016. Among irrigation treatments, the W3 treatment achieved the highest seasonal forage yields of 25.9 and 23.1 t ha<sup>-1</sup> in 2015 and 2016, which were greater by 76.3 % and 74.9 % compared to W1 treatment, respectively. Remarkably, forage yield obtained with W2 and W3 treatments was significantly similar in 2015, however, the yield of W2 treatment was lower than W2 treatment in 2016 (Table 2). Among nitrogen treatments, N1 resulted in the highest annual forage yield of 24.4 and 21.2 t ha<sup>-1</sup> in

2015 and 2016 (Table 2), which were greater by 6.6 % and 7.0 % than that of N2 treatment, and 28.5 % and 24.1 % than N3 treatment, respectively. Analysis of the irrigation and nitrogen interactive treatments showed that W2N1 and W3N1 treatments achieved the highest annual forage yield of 28.7 and 29.7 t ha<sup>-1</sup> in 2015, and 25.2 and 25.6 t ha<sup>-1</sup> in 2016, whereas, the lowest yields obtained were 14.9 and 12.7 t ha<sup>-1</sup> with W1N1 treatment, respectively (Table 1). Statistically, no significant difference between W2N1 and W3N1 treatments was observed in both years.

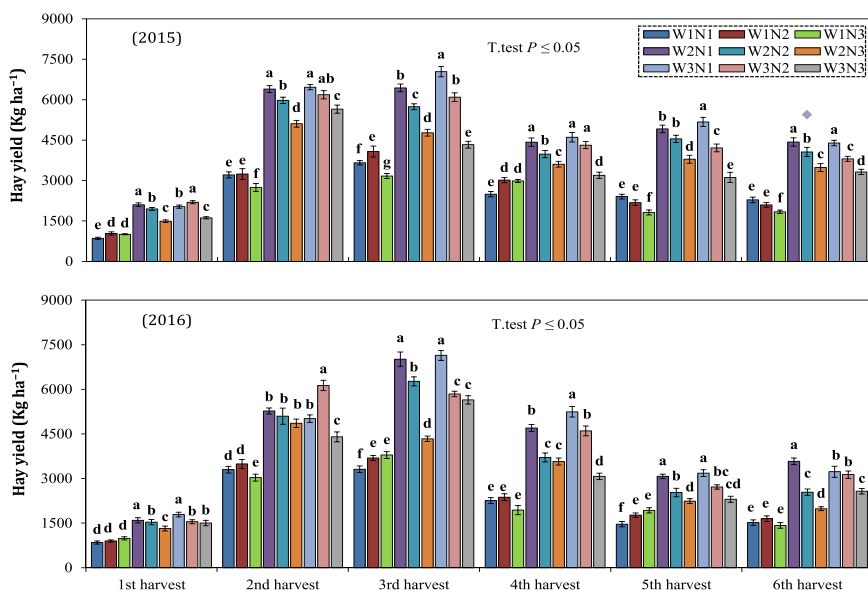
### 3.3. Effects of irrigation and nitrogen application on crude protein

Crude protein (CP) content was significantly affected ( $P \leq 0.01$ ) by year, irrigation, nitrogen treatments, and their interaction effects except for  $Y \times W \times N$  (Table S2). Unlike the forage yield, CP content of alfalfa initially increased with increasing the irrigation amount from W1 to W2, but it sharply declined with W3 at each harvest (Fig. 3). Nitrogen rates followed different trends for CP under different irrigation levels at different alfalfa harvests. In general, increasing the nitrogen application rate under W1 irrigation linearly increased the CP at each harvest (except for the 6th harvest) in both years (Fig. 3). Under the W2 irrigation level, increasing nitrogen from N1 to N2 increased the CP contents at the first, second, and third harvests, but CP was decreased with N3 treatment. Moreover, under W3 irrigation, CP decreased with increasing applied N application throughout the growth period.

Among irrigation treatments, the highest seasonal mean CP contents were achieved with W2 treatment (16.1 % and 17.7 % in 2015 and 2016 (Table 2). Compared to W2, CP yield with W3 treatment decreased by 16.4 % and 22.6 % and was even lower than that of the W1 treatment during 2015 and 2016. In addition, the seasonal CP content of alfalfa with the N1 treatment was greater than that of N2 and N3 treatments during 2015, but the difference between N1 and N2 treatments was not significant in 2016 (Table 2). Among the interaction effects, W2N1 treatment (18.4 % and 18.7 %) yielded the greatest CP, followed by W2N2 (15.7 % and 18.6 %), and W1N3 (16.8 % and 17.3 %), while the lowest CP contents were achieved with W3N2 (13.8 % and 13.5 %), and W3N3 (10.9 % and 11.3 %) treatments in 2015 and 2016, respectively (Table 1).

### 3.4. Effects of irrigation and nitrogen application on relative feed value

The relative feed value (RFV) of alfalfa forage was significantly



**Fig. 2.** Effect of irrigation (W) and nitrogen (N) treatments on forage yield of alfalfa at different harvests in 2015 and 2016. Data are presented as the mean  $\pm$  SD ( $n = 3$ ). Vertical bars with different letters at each harvest indicate significant differences among treatment means based on Tukey's significant difference test. W1, W2, and W3 represent irrigation amounts of 300, 450, and 600 mm, while N1, N2, and N3 represent nitrogen application rates of 150, 225, and 300 kg ha<sup>-1</sup>, respectively.

**Table 1**

Combined effects of irrigation (W) and nitrogen (N) treatments on seasonal total forage yield, mean crude protein, relative feed values (RFV), neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents of alfalfa in 2015 and 2016.

Year	Treatments		Hay yield (t DM ha <sup>-1</sup> )	Crude protein (%)	Relative feed value (%)	Neutral detergent fiber (%)	Acid detergent fiber (%)
2015	W1	N1	14.9de	12.3f	224.5a	29.2e	24.5e
		N2	15.6d	15.3cd	201.0b	32.1d	26.6d
		N3	13.6e	16.8b	182.5d	34.8bc	28.6c
	W2	N1	28.7a	18.4a	220.5a	30.0e	25.1e
		N2	26.3b	15.7c	195.8bc	32.4d	28.7c
		N3	22.2c	14.2de	174.9de	35.5b	30.6b
	W3	N1	29.7a	15.6c	186.0cd	34.1c	27.6cd
		N2	26.8b	13.8e	170.5e	35.3b	31.3ab
		N3	21.2c	10.9g	157.7f	38.4a	32.5a
2016	W1	N1	12.7g	14.1d	213.7a	31.1e	23.5f
		N2	13.9f	15.9c	202.2b	32.0e	25.5de
		N3	13.1fg	17.3b	186.7c	33.95d	27.3c
	W2	N1	25.2a	18.7a	207.2ab	32.1de	24.4ef
		N2	21.5c	18.6a	182.1cd	35.7c	26.1cd
		N3	18.3e	15.9c	169.3e	36.5bc	29.4b
	W3	N1	25.6a	16.4c	175.4de	35.9c	26.8cd
		N2	24.0b	13.5d	159.5f	38.2b	29.8b
		N3	19.8d	11.3e	151.9f	40.0a	31.4a

Data are presented as the means of three replicates (n = 3). Different lowercase letters indicate significant differences among treatment means based on Tukey's significant difference test (P ≤ 0.05). Treatments names are similar to those described in Fig. 2.

**Table 2**

Effects of irrigation (W) and nitrogen (N) treatments on seasonal total forage yield, mean crude protein, relative feed values (RFV), neutral detergent fiber (NDF), and acid detergent fiber (ADF) content of alfalfa in 2015 and 2016.

Treatments	2015				
	Hay yield (t DM ha <sup>-1</sup> )	Crude protein (%)	Relative feed value (%)	Neutral detergent fiber (%)	Acid detergent fiber (%)
W1	14.7b	14.8b	202.7a	32.0b	26.5c
W2	25.8a	16.1a	197.1b	32.6b	28.1b
W3	25.9a	13.5c	171.4c	36.0a	30.5a
N1	24.4a	15.5a	210.3a	31.0c	25.7c
N2	22.9b	14.9b	189.1b	33.3b	28.8b
N3	19.0c	14.0c	171.7c	36.3a	30.6a
Treatments	2016				
	Hay yield (Kg DM ha <sup>-1</sup> )	Crude protein (%)	Relative feed value (%)	Neutral detergent fiber (%)	Acid detergent fiber (%)
W1	13.2c	15.8b	200.9a	32.3c	25.4c
W2	21.7b	17.7a	186.2b	34.7b	26.6b
W3	23.1a	13.7c	162.3c	38.1a	29.3a
N1	21.2a	16.4a	198.8a	33.1c	24.9c
N2	19.8b	16.0a	181.3b	35.3b	27.1b
N3	17.1c	14.8b	169.3c	36.8a	29.3a

Data are presented as the means of three replicates (n = 3). Different lowercase letters indicate significant differences among treatment means based on Tukey's significant difference test (P ≤ 0.05). Treatments names are similar to those described in Fig. 2.

affected (P ≤ 0.01), by irrigation, nitrogen, and the W × N interaction (Table S2). The RFV values decreased gradually following the number of alfalfa harvests in both crop growing seasons (Fig. 4). At each harvest, RFV values remained unchanged with increasing irrigation from W1 to W2, however, the values declined sharply at W3 irrigation treatment. Moreover, RFV values showed a declining tendency with increasing nitrogen application at each harvest. Among the irrigation treatments, the highest mean annual RFV values were achieved with W1 (202.7 % and 200.9 %), followed by W2 treatment (197.1 % and 186.2 %) in 2015 and 2016, respectively (Table 2). Analysis across N treatments showed the highest mean RFV values with N1 treatment (210.3 % and 198.8 %), followed by N2 treatment (189.1 % and 181.3 %) in 2015 and 2016,

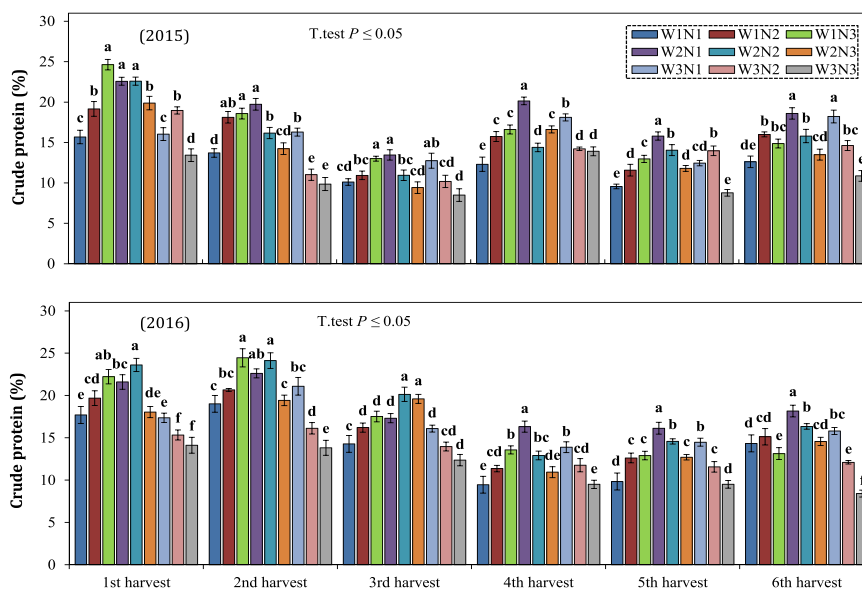
respectively. Considering the combined effect of irrigation and nitrogen, the mean RFV values of all harvests were highest with W1N1 (224.5 % and 213.7 %) and W2N1 (220.5 % and 207.2 %) treatments, while the lowest values were achieved with W2N3 (174.9 % and 169.3 %), W3N2 (170.5 % and 159.5 %), W3N3 (157.7 % and 151.9 %) treatments (Table 1). The mean RFV values of W1N1 and W2N1 treatments were greater by 26.2 – 28.4 % and 22.4 – 26.1 % over that of W2N3, greater by 31.7 – 34.0 % and 29.3 – 30.0 % over W2N3, and 40.7 – 42.4 % and 36.4 – 39.8 % over that of W3N3 treatment, respectively.

### 3.5. Effects of irrigation and nitrogen application on acid detergent fiber and neutral detergent fiber content

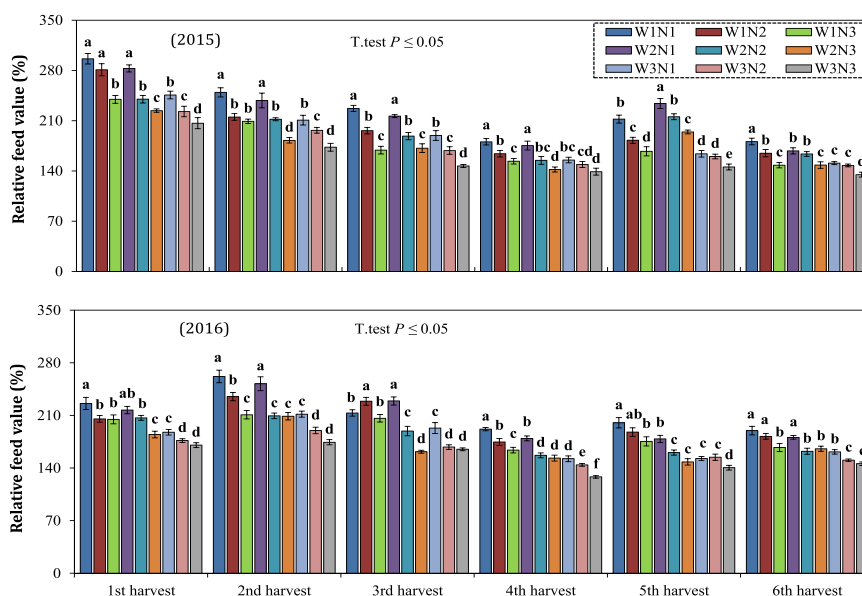
Irrigation and nitrogen treatments and their interaction showed significant effects (P ≤ 0.05) on acid detergent fiber (ADF) and neutral detergent fiber (NDF) content of alfalfa (Table S2). The ADF and NDF contents increased gradually with the progression of the crop growing season and were comparatively greater at later harvests (Tables 3 and 4). The seasonal mean ADF and NDF contents increased linearly as the irrigation amount increased, with the greatest values observed with W3 treatment at each harvest (Table 2). The mean ADF and NDF values of W3 treatment were greater by 14.7 – 15.2 % and 12.3 – 17.8 % compared to that of W1, and greater by 8.2 – 10.0 % and 7.6 – 10.4 % compared to W2 treatment in 2015–2016, respectively. Similarly, increasing nitrogen application rates linearly increased ADF and NDF contents and the highest values were achieved with N3 treatment in both years. The ADF and NDF content in W3 treatments were greater by 17.8 – 18.9 % and 11.3 – 16.8 % compared to N1 treatment, respectively. Among the irrigation and nitrogen interactive treatments, the highest mean ADF and NDF contents were achieved with W3N3 (31.3–32.5% and 38.4–40.0 %) and W3N2 (29.8–31.3 % and 35.4–38.2 %) treatments, while the lowest contents were observed with W1N1 (23.5–24.5 % and 29.2–31.1 %) and W2N1 (24.4–25.1 % and 30.0–32.1 %) treatments in 2015–2016 (Table 1).

### 3.6. Effects of irrigation and nitrogen application on irrigation water use efficiency

Irrigation water use efficiency (IWUE) of alfalfa was significantly affected by year, irrigation, and nitrogen treatments. All of the interaction effects of treatments and year were significant (Table S2). Across the main treatment effects, IWUE exhibited an initial increase and later a



**Fig. 3.** Effect of irrigation (W) and nitrogen (N) treatments on the crude protein content of alfalfa at different harvests in 2015 and 2016. Data are presented as the mean  $\pm$  SD (n = 3). Vertical bars with different letters at each harvest indicate significant differences among treatment means based on Tukey’s significant difference test. Treatments names are similar to those described in Fig. 2.



**Fig. 4.** Effect of irrigation (W) and nitrogen (N) treatments on relative feed values of alfalfa at different harvests in 2015 and 2016. Data are presented as the mean  $\pm$  SD (n = 3). Vertical bars with different letters at each harvest indicate significant differences among treatment means based on Tukey’s significant difference test. Treatments names are similar to those described in Fig. 2.

decreasing tendency with increasing irrigation amount (Fig. 5). The highest mean IWUE achieved was 57.3 and 48.2 kg mm<sup>-1</sup> with W2 treatment, while the lowest was 43.2 and 38.5 kg mm<sup>-1</sup> with W3 treatment in 2015 and 2016, respectively. The IWUE in W3 was even lower than that of W1 treatment. On the other hand, IWUE tended to decline with increasing applied nitrogen application rate under the same irrigation level (Fig. 5). The highest mean IWUE was achieved with N1 treatment (54.3 and 47.0 kg mm<sup>-1</sup>), while that of N2 and N3 treatments were decreased by 26.0 % and 30.8 % in 2015, and 26.0 and 28.6 % in 2016 compared to N1 treatment, respectively. Among the irrigation and nitrogen interaction effects, IWUE of alfalfa was highest with W2N1 (63.8 and 56.1 kg mm<sup>-1</sup>), followed by W2N2 (58.6 and 47.8 kg mm<sup>-1</sup>) treatment in 2015 – 2016 (Fig. 5). When compared to that of W1N1, W2N3, W3N1, and W3N3 treatments, the mean IWUE of W2N1

treatment was greater by 28.5 – 32.5 %, 29.0 – 37.9 %, 28.8 – 31.5 %, and 70.0 – 80.3 % in 2015 – 2016, respectively.

### 3.7. Effects of irrigation and nitrogen application on nitrogen use efficiency

The increase in irrigation amount resulted in an increase in NUE at all N application rates, owing to an increase in forage yield (Fig. 6). The highest NUE values obtained were 129.3 and 114.4 kg kg<sup>-1</sup> from plots provided with maximum irrigation level (W3), while the lowest values of 71.4 and 63.3 kg kg<sup>-1</sup> were achieved with low irrigation level (W1) in 2015 and 2016, respectively. On contrary, the NUE of alfalfa linearly decreased with increasing N application rate at the same irrigation level. The N1, N2, and N3 treatments achieved NUE values of 162.9, 101.9,

**Table 3**

Effects of different irrigation (W) and nitrogen (N) treatments on neutral detergent fiber content of alfalfa at each harvest in 2015 and 2016.

Year	Treatments		Neutral detergent fiber (NDF, dry matter %)					
			1st	2nd	3rd	4th	5th	6th
2015	W1	N1	22.5d	26.5e	28.7d	33.9d	29.9de	33.3d
		N2	23.3d	29.6d	32.1c	37.1c	32.9c	37.4c
		N3	26.7bc	30.4d	36.5b	39.2b	36.5b	39.5bc
	W2	N1	23.5d	27.6e	23.0e	34.8d	26.7f	37.5c
		N2	26.6bc	30.1d	33.3c	38.1bc	28.2ef	37.8c
		N3	29.1a	34.1b	35.9b	41.5a	31.3cd	41.1ab
	W3	N1	25.9c	30.5d	33.5c	38.9bc	35.7b	38.7c
		N2	28.4ab	32.2c	36.6b	38.3bc	37.3b	39.3bc
		N3	30.4a	35.9a	40.7a	41.7a	40.0a	42.0a
2016	W1	N1	29.6d	24.6c	30.9cd	32.5 f	33.4e	35.7f
		N2	32.6c	27.1d	27.3f	34.7de	35.9d	36.2f
		N3	32.7c	29.3c	29.7de	35.6cd	36.9cd	38.6cd
	W2	N1	31.5cd	25.8e	27.6ef	33.6ef	36.6d	37.5de
		N2	32.4c	30.4de	33.1bc	37.7b	39.5bc	41.1ab
		N3	35.1b	29.9c	36.6a	37.0bc	41.9ab	38.4cd
	W3	N1	35.1b	29.9c	32.6c	37.1bc	40.7b	40.1bc
		N2	36.7ab	32.9b	35.2ab	38.7b	40.3b	42.7a
		N3	37.5a	35.0a	36.8a	41.9a	43.5a	43.5a

Data are presented as the means of three replicates ( $n = 3$ ). Different lowercase letters within the same harvest indicate significant differences among treatment means based on Tukey's significant difference test ( $P \leq 0.05$ ). Treatments names are similar to those described in Fig. 2.

**Table 4**

Effects of different irrigation (W) and nitrogen (N) treatments on acid detergent fiber content of alfalfa at each harvest in 2015 and 2016.

Year	Treatments		Acid detergent fiber (ADF, dry matter %)					
			1st	2nd	3rd	4th	5th	6th
2015	W1	N1	22.3b	23.0d	24.2e	29.7c	26.6e	31.0c
		N2	23.8b	26.3bc	27.1bc	30.0bc	31.2cd	29.1 cd
		N3	26.1a	26.3bc	29.0b	31.1bc	29.8d	33.4b
	W2	N1	22.5b	23.6d	24.7de	30.0bc	27.8e	27.2e
		N2	26.2a	26.0bc	27.4bc	32.7ab	30.4cd	28.8de
		N3	25.8a	28.2ab	29.0b	32.7ab	30.2cd	30.0 cd
	W3	N1	26.1a	25.3cd	26.7cd	30.8bc	33.4ab	33.5b
		N2	26.8a	26.8bc	29.0b	35.3a	31.8bc	34.1ab
		N3	27.5a	28.5a	31.6a	34.2a	33.8a	35.9a
2016	W1	N1	21.8c	25.2de	23.2e	20.7d	21.9e	28.3e
		N2	23.4ab	26.3cd	28.1bc	23.2bc	21.1e	30.6d
		N3	21.8c	28.8ab	29.6bc	25.0ab	24.9c	33.7 cd
	W2	N1	19.7d	24.5e	26.8d	20.6d	23.9de	31.0d
		N2	21.7c	26.5cd	27.7cd	22.2cd	26.7bc	32.6 cd
		N3	24.9ab	27.9bc	32.5a	26.4a	28.6ab	36.0b
	W3	N1	23.2bc	26.9cd	27.4cd	24.7ab	28.4ab	36.2b
		N2	24.1ab	27.9bc	32.5a	25.6a	28.2ab	37.2b
		N3	25.2a	30.0a	30.4ab	26.7a	29.9a	40.0a

Data are presented as the means of three replicates ( $n = 3$ ). Different lowercase letters within the same harvest indicate significant differences among treatment means based on Tukey's significant difference test ( $P \leq 0.05$ ). Treatments names are similar to those described in Fig. 2.

and  $63.4 \text{ kg kg}^{-1}$  in 2015, and 141.2, 87.9,  $56.9 \text{ kg kg}^{-1}$  in 2016, respectively (Fig. 6). When compared to N1, the NUE of N2 and N3 treatments was lower by 37.5 % and 61.1 % in 2015 and 37.7 % and 59.7 % in 2016, respectively. Irrigation and nitrogen interaction also significantly affected NUE in both years. Among all the treatments, W2N1 ( $191.4$  and  $168.2 \text{ kg kg}^{-1}$ ) and W3N1 ( $198.1$  and  $170.6 \text{ kg kg}^{-1}$ ) treatments resulted in the highest NUE, while the lowest NUE was achieved with W1N3 ( $45.2$  and  $43.7 \text{ kg kg}^{-1}$ ), W2N3 ( $74.2$  and  $61.0 \text{ kg kg}^{-1}$ ), and W3N3 ( $70.8$  and  $66.0 \text{ kg kg}^{-1}$ ) treatment in 2015 and 2016, respectively (Fig. 6).

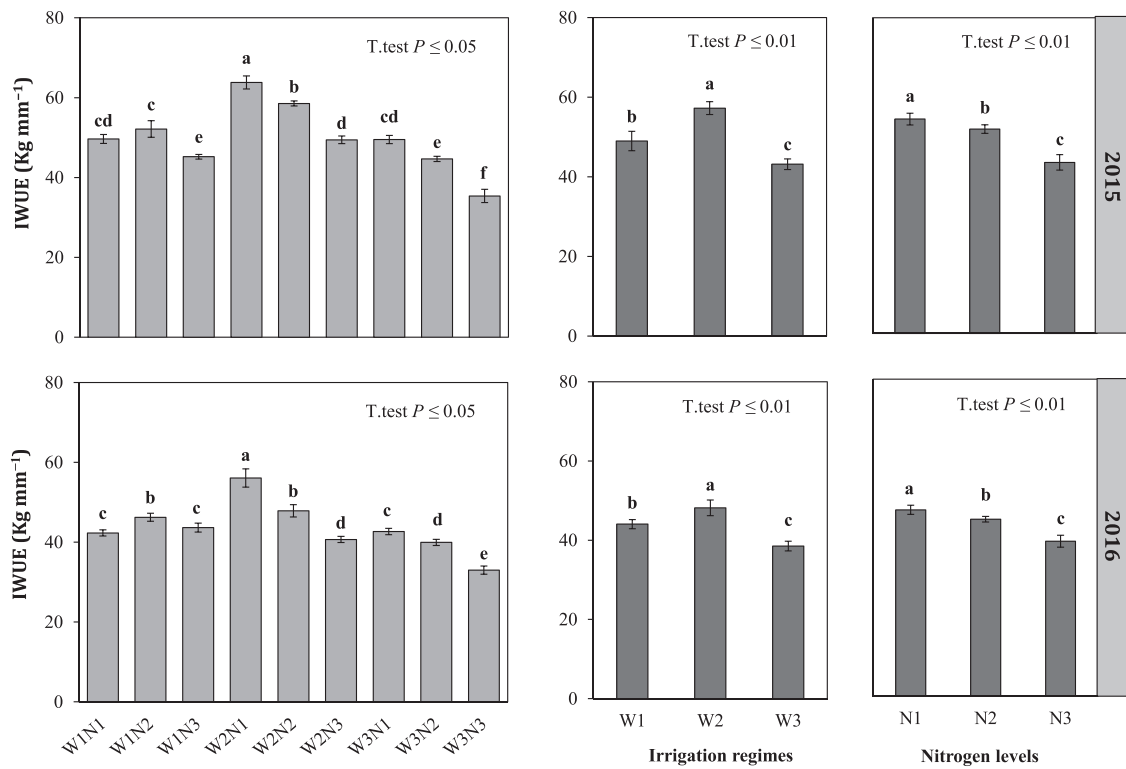
### 3.8. Relationship of measured indexes, irrigation, and nitrogen treatments

The regression analysis revealed a parabolic association of alfalfa forage yield ( $R^2 = 0.98$ ), crude protein contents ( $R^2 = 0.99$ ), NUE ( $R^2 = 0.99$ ), and IWUE ( $R^2 = 0.95$ ) with the applied irrigation amounts (Fig. 7). In addition, a linear and positive relationship existed between NDF ( $R^2 = 0.95$ ) and ADF ( $R^2 = 0.96$ ) with the irrigation regimes. On the other hand, linear and negative relation existed between alfalfa yield ( $R^2$

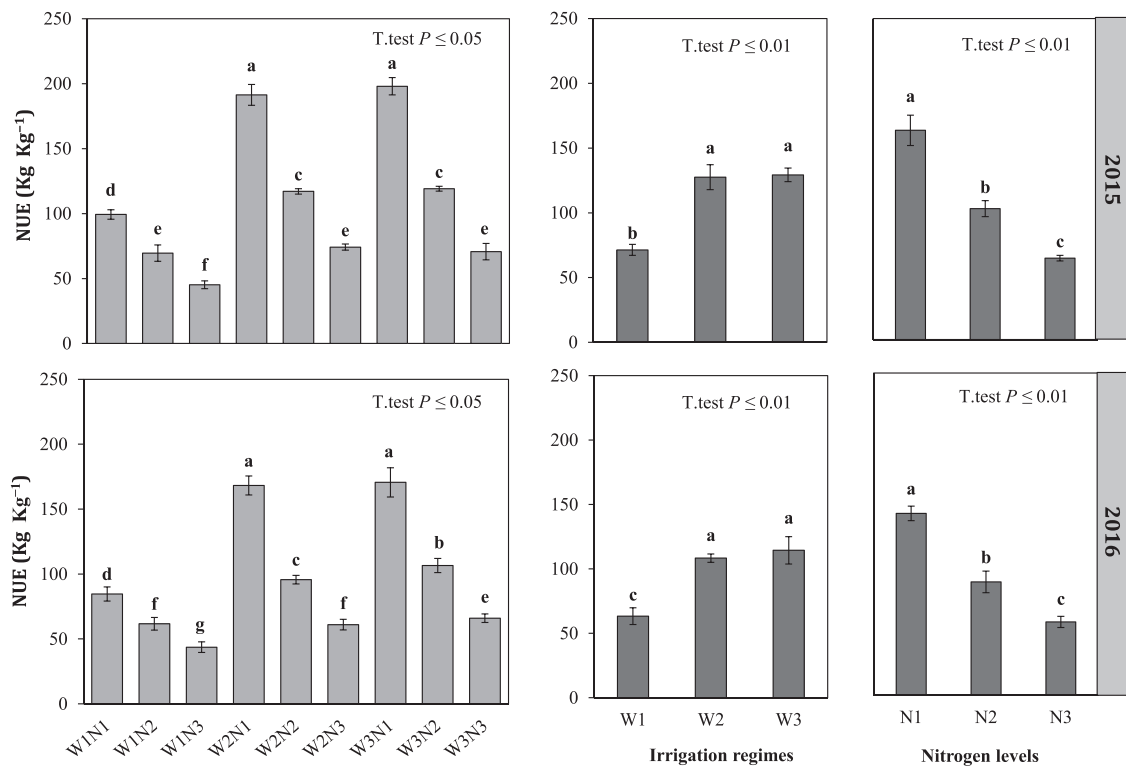
$= 0.95$ ), CP content ( $R^2 = 0.94$ ), IWUE ( $R^2 = 0.90$ ), and NUE ( $R^2 = 0.98$ ) with the nitrogen application rates. However, ADF and NDF contents showed significant and positive correlations with nitrogen rates and the coefficient determination values ( $R^2$ ) were 0.98 and 0.95, respectively (Fig. 7). In addition, our results showed parabolic relationships of CP, RFV, ADF, and NDF with forage yield (Fig. 8). With the increase in forage yield, the CP content and RFV initially decreased and then increased, while the ADF and NDF first increased and then decreased (Fig. 8).

## 4. Discussion

In arid and semi-arid regions of North China, the cultivation of crops on degraded and low fertile soils accompanied by scarce rainfall and water shortages often results in lower forage yields (Xiao et al., 2015; Yu et al., 2018). In these regions, supplemental irrigation and fertilization play a key role in sustaining the growth and production of forage crops (Liu et al., 2021; Lu et al., 2021; Wang et al., 2021). In general, high irrigation amounts are believed to increase forage yield and productivity



**Fig. 5.** Effect of irrigation (W) and nitrogen (N) treatments on irrigation water use efficiency (IWUE) of alfalfa in 2015 and 2016. Data are presented as the mean  $\pm$  SD (n = 3). Vertical bars with different letters indicate significant differences among treatment means based on Tukey's significant difference test. Treatments names are similar to those described in Fig. 2.



**Fig. 6.** Effect of irrigation (W) and nitrogen (N) treatments on nitrogen use efficiency (NUE) of alfalfa in 2015 and 2016. Data are presented as the mean  $\pm$  SD (n = 3). Vertical bars with different letters indicate significant differences among treatment means based on Tukey's significant difference test. Treatments names are similar to those described in Fig. 2.



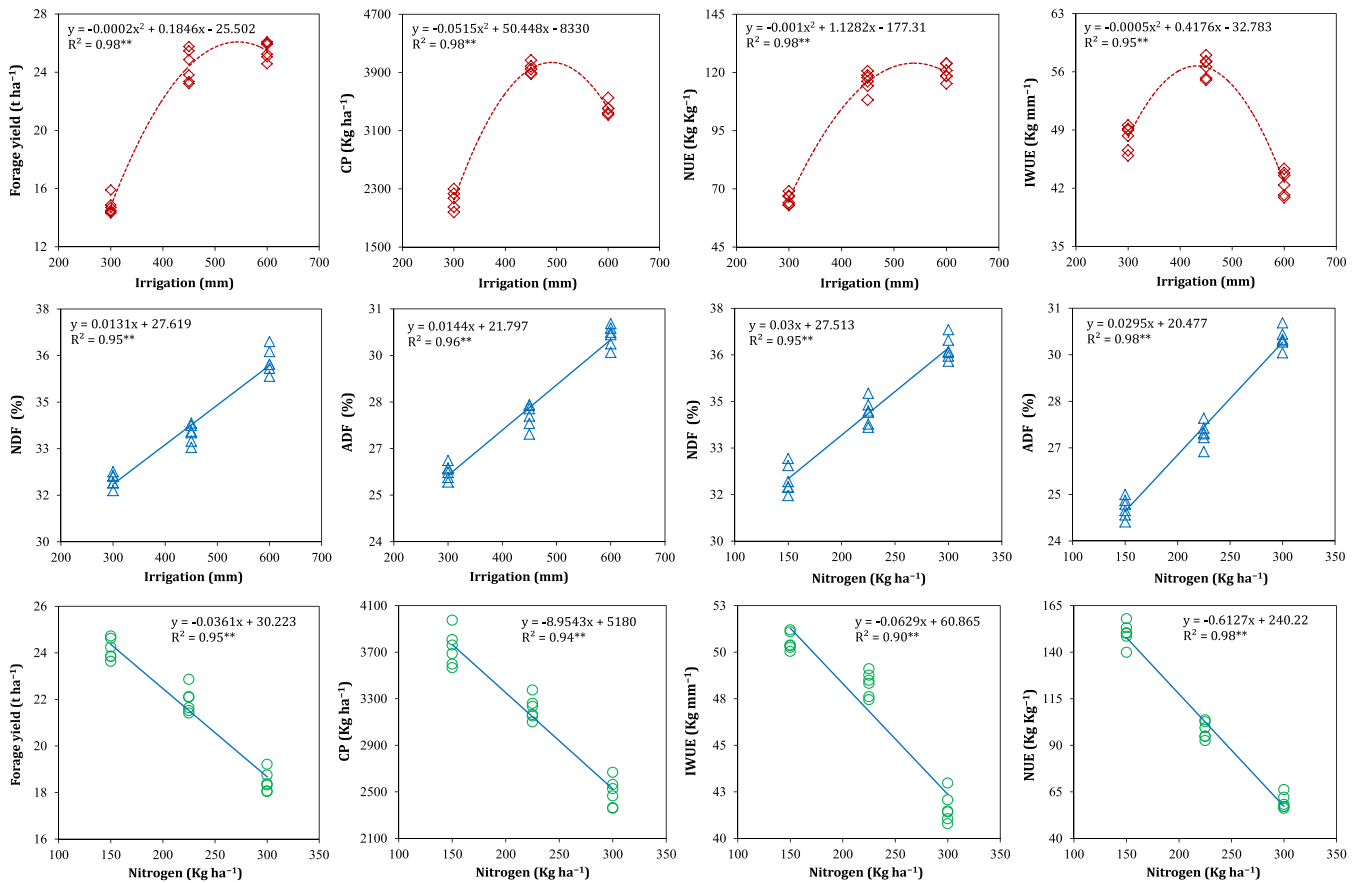


Fig. 7. Relationship of forage yield, crude protein (CP), irrigation water use efficiency (IWUE), nitrogen use efficiency (NUE), acid detergent fiber (ADF), and neutral detergent fiber (NDF) with irrigation and nitrogen treatments. Note: \*\* indicates the significant level at  $P \leq 0.01$ . The X and Y axes were adjusted to minimize the graph's blank areas and highlight the relationship.

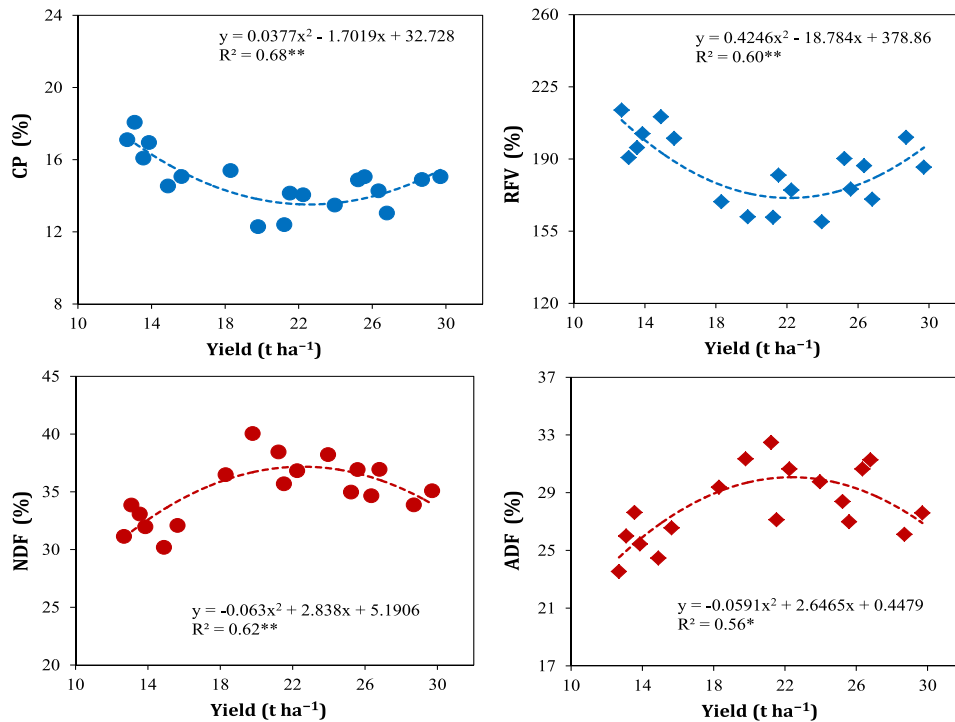


Fig. 8. Correlation of forage yield with crude protein (CP), relative feed values (RFV), acid detergent fiber (ADF), and neutral detergent fiber (NDF) of alfalfa. Note: \* and \*\* indicates the significant level at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively. The X and Y axes were adjusted to minimize the graph's blank areas and highlight the relationship.

in arid and semiarid regions (Djaman et al., 2020b; Fan et al., 2011; Li and Su, 2017). Two-year data from our present experiment also revealed an increase in alfalfa forage yield with increasing irrigation amounts. This increase in forage yield is attributed to enhanced nutrients and water acquisitions, improved photosynthetic capacity, and leaf expansion with irrigation treatments (Ferreira et al., 2015; Xiao et al., 2015), resulting in greater biomass accumulation. Alfalfa yield achieved in this study was among the high range values reported from the arid and semiarid regions of China (Fan et al., 2016; Liu et al., 2021; Wang et al., 2021). In 2015, medium (W2, 450 mm) and high irrigation (W3, 600 mm) treatments showed statically similar seasonal yields but W3 resulted in higher yields compared to other irrigation treatments in 2016. In explanation, the precipitation in 2015 was comparatively higher (94 mm), therefore sufficient soil water was available for crop growth, and increasing irrigation beyond 450 mm had no significant effect on further improving forage yield. While precipitation was comparatively lower (49 mm) in 2016, increasing irrigation volume was critical to meet the crop water demands, and therefore linearly increased alfalfa forage yield. Correlation analysis depicted a significant and strong relationship ( $R^2 = 0.98$ ) between forage yield and irrigation regimes. Li and Su (2017a) described the increase in annual alfalfa forage yield as a function of irrigation amount, and reported forage yield of 11.6–18.6 t ha<sup>-1</sup> with the increase in seasonal irrigation in Northern China. However, Djaman et al. (2020b) perceived a third-order polynomial relationship of alfalfa yield with the amount of irrigation applied in semiarid climatic conditions in USA. Meanwhile, Hanson et al. (2008) reported a curvilinear relationship between alfalfa yield and the amount of applied irrigation, pointing out that this relationship varies with regrowth cycles and is mostly dependent on soil moisture content. Another study showed that irrigation throughout the season had a marginal advantage on alfalfa forage yield over partial irrigation in a semiarid subtropical environment (Darapuni et al., 2020). The differences in these reported studies are attributed to variation in climatic conditions, as well as the magnitude and distribution of precipitation at the experimental sites, which influenced the relationship between irrigation and alfalfa yield. Our research location had a severe dry climatic condition; therefore, supplemental irrigation is particularly critical to crop productivity in this arid region.

The positive relationship of growth and biomass with soil-available nitrogen has been widely recognized in forage crops (Raun et al., 1999; Xie et al., 2015; Zhu et al., 1998). However, the majority of agricultural production systems lack sufficient amounts of soil available N to support crop growth and thus fertilizer application is essential for achieving optimal yields and quality forages. The available literature is inconsistent regarding the effects of N application on legume forage yield, particularly alfalfa. Several studies have found no benefit from nitrogen fertilization to legume crops because of no significant increase in biomass yield or quality (Fan et al., 2011; Oliveira et al., 2004; Xie et al., 2015). In contrast, few studies reported an increase in alfalfa forage yields with nitrogen application when compared to no N treatment (Fan et al., 2016; Lee and Smith, 1972; McDonald et al., 2021; Raun et al., 1999). Our results indicated positive effects of low nitrogen application (150 kg ha<sup>-1</sup>) on the alfalfa biomass accumulation but high nitrogen rates (225 and 300 kg ha<sup>-1</sup>) declined the alfalfa dry matter yield. Since the available soil nitrogen at the experimental sites was comparatively lower (38.7 mg kg<sup>-1</sup>), therefore 150 kg N ha<sup>-1</sup> fertilization was beneficial in improving leaf chlorophyll contents and photosynthetic capacity (Fan et al., 2016; Gao et al., 2020), thereby regulating the dry matter accumulation and resulting in higher alfalfa yields. On the other hand, a decrease in forage yield with higher nitrogen rates might be linked with their inhibitory effects on root growth and development (Li et al., 2018), root nodulation, and nodule configuration (Oliveira et al., 2004; Zhu et al., 1998), consequently decreasing the soil water and nutrients uptake. The inhibition of nitrogen-fixing capacity of symbiotic rhizobium association decreased yield, and yield-related traits with the addition of nitrogen fertilizer have been previously reported in

legume crops (Hamilton et al., 1991; Reinprecht et al., 2020; Xie et al., 2015; Zhu et al., 1998). Based on our results, we hypothesize that there exists a certain threshold for the chemical fertilization absorption by alfalfa, below this threshold, fertilizers can promote growth and development, while fertilizer exceeding the maximum absorption would negatively impact growth and development and reduces forage yield, as validated by previous studies (Fan et al., 2016; Zhang et al., 2020).

For often, the first alfalfa harvest is considered a major determinant of seasonal forage yields, because of its benefit from a longer growing period and greater thermal units resulting in higher biomass accumulation (Djaman et al., 2020a). Djaman et al. (2020b) reported a decrease in alfalfa forage yield from first to fourth harvests and yield at each harvest accounted for 31.4–39.6 %, 23.8–24.2 %, 17.6–21.6 %, and 18.6–23.2 % in 2013 and 2014 growing seasons. Similarly, Li and Su (2017) observed that the first harvest contributed 35–50 % of the total seasonal yield. In our study, the measured forage yield for the first harvest was significantly lower (6.9–7.2 %), reached the maximum at the second (23.5–22.6 %) and third harvests (22.8–26.9 %), and thereafter declined gradually in the later harvests during both crop growing seasons. The first harvest in our study did not receive a longer growth period and the growth cycle for each harvest was more or less identical, which could be a possible reason for yield differences as reported in previous studies. In addition, the difference in timings of irrigation and nitrogen application might be associated with the resulting lower yield at first harvest and greater yield at subsequent harvests in our study. Consistently, a previous study also reported lower yields at early harvests, the exception being when irrigation was provided earlier (Darapuni et al., 2020). The observed trend is distinctive because biomass accumulation in perennial forages is mainly allocated for root growth establishment at the re-greening stage (Jing et al., 2020; Liu et al., 2021). Once the plant has been established and sufficient new foliage is available, the efficient acquisition/utilization of resources (light, water, and nutrients) maximize the photosynthetic capacity at subsequent stages and begins to accumulate more dry matter back in the storage organs, resulting in higher yields (Jing et al., 2020). Jing et al. (2014) and Djaman et al. (2020b) described the early-season regrowth of perennial forages as a reserve-dependent growth stimulated by the accumulation and remobilization of non-structural carbohydrates. Whereas in later stages of the season, the shortened sunshine length in autumn and lower soil water availability also results in less dry matter accumulation, and most of the dry matter is transferred to root system for overwintering, as reported in previous studies (Jing et al., 2020; Orloff et al., 2005).

Crude protein content (CP), relative feed values (RFV), neutral detergent fiber (NDF), and acid detergent fiber (ADF) directly reflect the nutritional quality of forages (Hakl et al., 2021; McDonald et al., 2021; Zhang et al., 2020). In the present study, forage nutritive quality initially increased with increasing irrigation from 300 to 450 mm and later decreased with 600 mm irrigation at each harvest. The decrease in forage quality was associated with a decline in CP contents and increased ADF and NDF contents at the highest irrigation amount. A parabolic association of CP contents with irrigation and a linear relationship of ADF and NDF content with irrigation were perceived in the present study. The increase in irrigation amount reportedly increases stem to leaf ratio and accelerates crop maturity which contributes to decreasing the CP contents and increasing stem fiber contents (Islam et al., 2012; Liu et al., 2021; Xiao et al., 2015). Previously, the nutritive quality of perennial forages was found to be higher under reduced irrigation than those grown under well-watered conditions (Li and Su, 2017; Wang et al., 2021). Among nitrogen treatments, forage nutritive quality was optimal at 150 kg N ha<sup>-1</sup>. Ensuring adequate nitrogen fertilization has been shown to improve forage biomass by regulating leaf to stem ratio which also leads to improved overall forage nutritive values (Hakl et al., 2021). Appropriate nitrogen application has been reported to improve soil N supply capacity (Fan et al., 2016), root growth (Li et al., 2018), stimulate the soil microbial biomass and

enzyme activities, and increase the  $N_2$  fixation (Geisseler et al., 2010). Therefore, we conclude that enhanced N uptake by roots, in turn, increased the shoot N concentration, improving the synthesis of amino acids and subsequently the protein contents of alfalfa (Gao et al., 2020). Moreover, our results revealed the negative impact of high nitrogen rates on nutritive indices of alfalfa. CP contents and RFV were the lowest while ADF and NDF contents were the highest at 300 kg N application. The decrease in CP contents and RFV value with higher nitrogen application could be endorsed to reduced transport efficiency rates of resources. Because high mineral N rate restricts root system nodules development and decreases  $N_2$  fixation of legumes (Oliveira et al., 2004; Hannaway and Shuler, 1993; Xie et al., 2015), which in turn decreases the distribution of belowground resources (water and nutrients absorption), thus limiting CP synthesis. Another possible reason for lower forage quality at high irrigation and nitrogen application is their interactive impact on increasing the cell wall component and fiber quantities, as was evident by higher ADF and NDF contents in our present study. A similar relationship was previously reported in different forage crops (Balabanli et al., 2010; Islam and Garcia, 2012; Liu et al., 2021).

The IWUE and NUE are the major indicators describing the relationship between harvest yield and resource consumption and are used to identify rational irrigation and N amounts to maximize the crop's economic benefits (Darapuni et al., 2020; Islam and Garcia, 2012). In arid regions, improving IWUE is a desirable objective for maintaining sustainable alfalfa production because of high alfalfa water requirements and water deficit conditions (Xiao et al., 2015). Our results depicted an initial increase and later decrease in IWUE with increasing the irrigation amounts, while a linear decrease with increasing the applied nitrogen rates. These results signify that IWUE does not persistently improve with increasing irrigation amounts in arid regions. A similar trend of IWUE with a higher irrigation amount has been shown in alfalfa (Djaman et al., 2020b; Liu et al., 2021). Deficit irrigations have been reported to reduce water consumption without compromising the final yields and so improve the IWUE to various degrees depending on the agronomic conditions and crop species (Ismail and Almarshadi, 2013; Xiao et al., 2015). The estimated IWUE values perceived in our study ranged from 35.4 to 63.8 kg mm<sup>-1</sup> in 2015, and 33.0–56.1 kg mm<sup>-1</sup> in 2016, which were closer to the previously reported values in arid regions of China (Liu et al., 2021). A greater IWUE in 2015 was mainly due to better growing conditions that resulted in about 14.4 % higher forage yield than that in 2016. Also, irrigation requirement was greater in 2016, mainly due to a warmer growing season in comparison to 2015. At the same nitrogen level, IWUE was higher with W2 and lowest with W3 treatment, because the extent of yield increase was not significant while the degree of water increment was higher in W3 treatment. Previously, Payero et al. (2008) reported that IWUE is a function of irrigation and explained that the declining tendency of IWUE with irrigation is only expected in areas with positive dryland yield (yield without irrigation), however, an increase in IWUE with irrigation would be expected when no dryland yield can be obtained without irrigation.

Nitrogen is an integral component of chlorophyll and its deficiency would compromise the plants' photosynthetic efficiency and assimilation of photosynthates (Elgharably and Benes, 2021; Zhang et al., 2020). The effects of nitrogen application on yield and NUE have been widely reported in several forage crops (Dong et al., 2005; Islam et al., 2012; Islam and Garcia, 2012; Vasileva and Pachev, 2015). The relation between nitrogen application, resource use efficiency, and biomass vary between  $N_2$  fixing species and species relying solely on mineral N. Findings from our present study portrayed higher NUE at 150 kg ha<sup>-1</sup> N, while a sharp decline in NUE with further increasing N application rate. A negative correlation ( $R^2 = 0.98$ ) was detected between NUE and nitrogen application amount. The decline in NUE in our study was directly associated with the decrease in alfalfa forage yield under high nitrogen fertilization. These findings advocate that NUE of alfalfa does not continually improve with increasing nitrogen rates, and instead of

applying a high amount of fertilizer, an appropriate amount is more beneficial to improving productivity and hence the NUE. A suitable nitrogen application has been reported to promote root architecture and root activity (Li et al., 2018), enhancing resource acquisitions that regulate photo-assimilates distribution in the aboveground plant parts, and subsequently improve the yield and resource use efficiency of alfalfa (Vasileva and Pachev, 2015). The decrease in NUE of alfalfa with increasing N application is attributed to the inhibitive effects of nitrogen on root development and root nodulation in alfalfa, decreasing the nutrient and water uptake and affecting the yield. These conclusions are validated by research findings reported in legume crops (Oliveira et al., 2004; Islam and Garcia, 2012; Xie et al., 2015).

## 5. Conclusions

Results from the present study depicted that increasing the irrigation amount improved alfalfa forage yield but at the cost of reduced nutritional values. Although W3 treatment resulted in the highest forage yield, it was not significantly different from that of W2 treatment in 2015. On the other hand, increasing nitrogen application sharply declined the yield and quality of alfalfa forage. In addition, the irrigation and nitrogen interaction analysis showed the W2N1 and W3N1 as the best treatment combinations that resulted in high forage yield, crude protein, relative feed values, nitrogen, and water use efficiency of alfalfa. However, W2N1 can be recommended to strive for higher alfalfa production and forage quality while saving irrigation water in arid regions compared to W3N1. For future research, we suggest focusing on irrigation–nitrogen management strategies at different plant growth stages as well as at different localities with variable precipitation patterns to address how climatic variation could affect the effectiveness of irrigation–nitrogen coupling on forage yield and quality. More specific guidelines will help alfalfa growers in maximizing resource use efficiencies and profitability in arid forage production systems.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2022.108587.

## References

- Adjesiwor, A.T., Islam, M.A., Zheljzkov, V.D., Ritten, J.P., Garcia y Garcia, A., 2017. Grass-legume seed mass ratios and nitrogen rates affect forage accumulation, nutritive value, and profitability. *Crop Sci.* 57, 2852–2864.
- Antolín, M.C., Yoller, J., Sánchez-Díaz, M., 1995. Effects of temporary drought on nitrate-fed and nitrogen-fixing alfalfa plants. *Plant Sci.* 107, 159–165.
- Balabanli, C., Albayrak, S., Yüksel, O., 2010. Effects of nitrogen, phosphorus and potassium fertilization on the quality and yield of native rangeland. *Turk. J. Field Crop* 15, 164–168.

- Darapuni, M.K., Lauriault, L.M., Vanleeuwen, D.M., Angadi, S.V., 2020. Influence of irrigation regimes on alfalfa dry matter yield and water productivity in a semiarid subtropical environment. *Irrig. Drain.* 69, 1063–1071.
- Djaman, K., Owen, C., Koudahe, K., O'Neill, M., 2020a. Evaluation of different fall dormancy-rating alfalfa cultivars for forage yield in a semiarid environment. *Agronomy* 10, 146.
- Djaman, K., Smeal, D., Koudahe, K., Allen, S., 2020b. Hay Yield and water use efficiency of alfalfa under different irrigation and fungicide regimes in a semiarid climate. *Water* 12, 1721.
- Dong, S.K., Yang, Z.F., Long, R.J., Hu, Z.Z., Kang, M.Y., 2005. Effect of N fertilizer on the productivity and nutritive values of perennial grass mixtures in the alpine region of Qinghai-Tibetan Plateau, China. *Can. J. Plant Sci.* 85, 361–368.
- Elgharably, A., Benes, S., 2021. Alfalfa biomass yield and nitrogen fixation in response to applied mineral nitrogen under saline soil conditions. *J. Soil Sci. Plant Nutr.* 21, 744–755.
- Fan, F., Xu, S.J., Zhang, Q.G., He, F.Y., Hou, M.H., Song, X.F., 2011. Study on relativity between alfalfa's yields and nutrition constituent effect of nitrogen, phosphorus and potassium fertilizer. *Soil Fertil. Sci. China* 2, 51–56.
- Fan, J.W., Du, Y.L., Wang, B.R., Turner, N.C., Wang, T., Abbott, L.K., Stefanova, K., Siddique, K.H.M., Li, F.M., 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. *F. Crop. Res.* 198, 247–257.
- Ferreira, J., Cornacchione, M., Liu, X., Suarez, D., 2015. Nutrient composition, forage parameters, and antioxidant capacity of Alfalfa (*Medicago sativa*, L.) in response to saline irrigation water. *Agriculture* 5, 577–597.
- Gao, L., Su, J., Tian, Q., Shen, Y., 2020. Contrasting strategies of nitrogen absorption and utilization in alfalfa plants under different water stress. *J. Soil Sci. Plant Nutr.* 20, 1515–1523.
- Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B., 2010. Pathways of nitrogen utilization by soil microorganisms - a review. *Soil Biol. Biochem.* 42, 2058–2067.
- Gonzalez-Dugo, V., Durand, J.L., Gastal, F., 2010. Water deficit and nitrogen nutrition of crops. a review. *Agron. Sustain. Dev.* 30, 529–544.
- Gu, Y.J., Han, C.L., Fan, J.W., Shi, X.P., Kong, M., Shi, X.Y., Siddique, K.H.M., Zhao, Y.Y., Li, F.M., 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.
- Hakl, J., Kunzová, E., Konečná, J., 2016. Impact of long-term organic and mineral fertilization on lucerne forage yield over an 8-year period. *Plant, Soil Environ.* 62, 36–41.
- Hakl, J., Kunzová, E., Tocauerová, Š., Menšík, L., Mrázková, M., Pozdíšek, J., 2021. Impact of long-term manure and mineral fertilization on yield and nutritive value of lucerne (*Medicago sativa*) in relation to changes in canopy structure. *Eur. J. Agron.* 123, 126219.
- Hamilton, S.D., Chalk, P.M., Smith, C.J., Hopmans, P., 1991. Effect of N fertilizer rate on the estimation of N<sub>2</sub> fixation by isotope dilution. *Soil Biol. Biochem.* 23, 1105–1110.
- Hannaway, D.B., Shuler, P.E., 1993. Nitrogen fertilization in Alfalfa production. *J. Prod. Agric.* 6, 80–85.
- Hanson, B.R., Bali, K.M., Sanden, B.L., 2008. Irrigating alfalfa in arid regions. *Irrig. Alfalfa Manag. Mediterr. Desert Univ. Calif. Agric. Nat. Resour. Publ.* 372.
- Hartwig, U.A., Soussana, J.-F., 2001. Ecophysiology of symbiotic N<sub>2</sub> fixation in grassland legumes. *Grassl. Sci. Eur.* 6, 23–26.
- Hungria, M., Vargas, M.A.T., 2000. Environmental factors affecting N<sub>2</sub> fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crops Res.* 65, 151–164.
- Islam, M.R., Garcia, S.C., 2012. Effects of sowing date and nitrogen fertilizer on forage yield, nitrogen- and water-use efficiency and nutritive value of an annual triple-crop complementary forage rotation. *Grass Forage Sci.* 67, 96–110.
- Islam, M.R., Garcia, S.C., Horadagoda, A., 2012. Effects of irrigation and rates and timing of nitrogen fertilizer on dry matter yield, proportions of plant fractions of maize and nutritive value and in vitro gas production characteristics of whole crop maize silage. *Anim. Feed Sci. Technol.* 172, 125–135.
- Ismail, S.M., Almarshadi, M.H., 2013. Maximizing productivity and water use efficiency of alfalfa under precise subsurface drip irrigation in arid regions. *Irrig. Drain.* 62, 57–66.
- Jing, Q., Bélanger, G., Qian, B., Baron, V., 2014. Timothy yield and nutritive value with a three-harvest system under the projected future climate in Canada. *Can. J. Plant Sci.* 94, 213–222.
- Jing, Q., Qian, B., Bélanger, G., VanderZaag, A., Jégo, G., Smith, W., Grant, B., Shang, J., Liu, J., He, W., Boote, K., Hoogenboom, G., 2020. Simulating alfalfa regrowth and biomass in eastern Canada using the CSM-CROPGRO-perennial forage model. *Eur. J. Agron.* 113, 125971.
- Kunrath, T.R., Lemaire, G., Sadras, V.O., Gastal, F., 2018. Water use efficiency in perennial forage species: Interactions between nitrogen nutrition and water deficit. *Field Crops Res* 222, 1–11.
- Lee, C., Smith, D., 1972. Influence of nitrogen fertilizer on stands, yields of herbage and protein, and nitrogenous fractions of field-grown alfalfa. *Agron. J.* 64, 527–530.
- Lemaire, G., Bélanger, G., 2019. Allometries in plants as drivers of forage nutritive value: a review. *Agriculture* 10, 5.
- Li, M., Wang, Y., Adeli, A., Yan, H., 2018. Effects of application methods and urea rates on ammonia volatilization, yields and fine root biomass of alfalfa. *Field Crops Res* 218, 115–125.
- Li, Y., Su, D., 2017. Alfalfa water use and yield under different sprinkler irrigation regimes in North arid regions of China. *Sustain* 9, 1380.
- Liu, M., Wang, Z., Mu, L., Xu, R., Yang, H., 2021. Effect of regulated deficit irrigation on alfalfa performance under two irrigation systems in the inland arid area of midwestern China. *Agric. Water Manag.* 248, 106764.
- Lu, J., Tian, H., Zhang, H., Xiong, J., Yang, H., Liu, Y., 2021. Shoot-soil ecological stoichiometry of alfalfa under nitrogen and phosphorus fertilization in the loess plateau. *Sci. Rep.* 11, 1–10.
- Mao, X., Li, Q., Ren, L., Bai, W., Zhang, W.H., 2018. Application of molybdenum fertilizer enhanced quality and production of alfalfa in northern China under non-irrigated conditions. *J. Plant Nutr.* 41, 1009–1019.
- McDonald, I., Baral, R., Min, D., 2021. Effects of alfalfa and alfalfa-grass mixtures with nitrogen fertilization on dry matter yield and forage nutritive value. *J. Anim. Sci. Technol.* 63, 305–318.
- Oliveira, W.S., Oliveira, P.P.A., Corsi, M., Duarte, F.R.S., Tsai, S.M., 2004. Alfalfa yield and quality as function of nitrogen fertilization and symbiosis with *Sinorhizobium meliloti*. *Sci. Agric.* 61, 433–438.
- Orloff, S., Putnam, D., Hanson, B., Carlson, H., 2005. Implications of deficit irrigation management of alfalfa, in: *Proceedings, California Alfalfa and Forage Symposium, Visalia, CA. Davis.* 12–14.
- Payero, J.O., Tarkalson, D.D., Irmak, S., Davison, D., Petersen, J.L., 2008. Effect of irrigation amounts applied with subsurface drip irrigation on corn evapotranspiration, yield, water use efficiency, and dry matter production in a semiarid climate. *Agric. Water Manag.* 95, 895–908.
- Raun, W.R., Johnson, G.V., Phillips, S.B., Thomason, W.E., Dennis, J.L., Cossey, D.A., 1999. Alfalfa yield response to nitrogen applied after each cutting. *Soil Sci. Soc. Am. J.* 63, 1237–1243.
- Reinprecht, Y., Schram, L., Marsolais, F., Smith, T.H., Hill, B., Pauls, K.P., 2020. Effects of nitrogen application on nitrogen fixation in common Bean production. *Front. Plant Sci.* 1172.
- Saeed, I.A.M., El-Nadi, A.H., 1997. Irrigation effects on the growth, yield, and water use efficiency of alfalfa. *Irrig. Sci.* 17, 63–68.
- Sprent, J.I., 1976. Water deficits and nitrogen-fixing root nodules, in: *Water deficits and plant growth.* 4, 291–315.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.
- Vasileva, V., Pachev, I., 2015. Nitrogen use efficiency and life cycle of root nodules in Alfalfa after different mineral fertilization and soil cultivation. *Glob. J. Environ. Sci. Manag.* 1, 333–339.
- Vasileva, V., Kostov, O., Vasilev, E., 2006. Development of lucerne (*Medicago sativa* L.) treated with mineral fertilizer and manure at optimal and water deficit conditions. *Commun. Agric. Appl. Biol. Sci.* 71, 5–17.
- Wang, Q., Song, X., Li, F., Hu, G., Liu, Q., Zhang, E., Wang, H., Davies, R., 2015. Optimum ridge-furrow ratio and suitable ridge-mulching material for Alfalfa production in rainwater harvesting in semi-arid regions of China. *Field Crops Res.* 180, 186–196.
- Wang, Y., Liu, C., Cui, P., Su, D., 2021. Effects of partial root-zone drying on alfalfa growth, yield and quality under subsurface drip irrigation. *Agric. Water Manag.* 245, 106608.
- Xiao, Y., Zhang, J., Jia, T.T., Pang, X.P., Guo, Z.G., 2015. Effects of alternate furrow irrigation on the biomass and quality of alfalfa (*Medicago sativa*). *Agric. Water Manag.* 161, 147–154.
- Xie, K.Y., Li, X.L., He, F., Zhang, Y.J., Wan, L.Q., David, B.H., Wang, D., Qin, Y., Gamal, M.A.F., 2015. Effect of nitrogen fertilization on yield, N content, and nitrogen fixation of alfalfa and smooth bromegrass grown alone or in mixture in greenhouse pots. *J. Integr. Agric.* 14, 1864–1876.
- Yu, M., Tian, Y., Gao, Q., Xu, X., Wen, S., Fan, Z., Li, X., Gong, J., Liu, Y., 2018. Nonlinear response of lucerne (*Medicago sativa*) biomass and biological nitrogen fixation to different irrigations and sowing modes. *Appl. Soil Ecol.* 125, 257–263.
- Zhang, Q., Liu, J., Liu, X., Sun, Y., Li, S., Lu, W., Ma, C., 2020. Optimizing the nutritional quality and phosphorus use efficiency of alfalfa under drip irrigation with nitrogen and phosphorus fertilization. *Agron. J.* 112, 3129–3139.
- Zhu, Y., Sheaffer, C.C., Vance, C.P., Graham, P.H., Russelle, M.P., Montealegre, C.M., 1998. Inoculation and nitrogen affect herbage and symbiotic properties of annual medicago species. *Agron. J.* 90, 781–786.