agronomy

MDPI

Article

Alfalfa (*Medicago sativa* L.) Nitrogen Utilization, Yield and Quality Respond to Nitrogen Application Level with Center Pivot Fertigation System

Yunling Wang [1,2,†], Maona Li [3,†], Jiali Guo [1] and Haijun Yan [1,4,*]

[1] College of Water Resources and Civil Engineering, China Agricultural University, Beijing 100083, China; wangyunling@cau.edu.cn (Y.W.); s20223091907@cau.edu.cn (J.G.)
[2] College of Animal Science and Technology, Hebei Agricultural University, Baoding 071001, China
[3] College of Grassland Science and Technology, China Agricultural University, Beijing 100083, China; limaona2020@cau.edu.cn
[4] Engineering Research Center of Agricultural Water-Saving and Water Resources, Ministry of Education, Beijing 100083, China
* Correspondence: yanhj@cau.edu.cn
† These authors contributed equally to this work.

Abstract: Nitrogen (N) application with a center-pivot fertigation system is commonly used for alfalfa production in the North China Plain, with its barren soil, but improper N application has resulted in lower N use efficiency and even negative effects on yield and quality. The effects of N application levels on soil NO_3^--N content, nitrogen utilization, yield and quality of alfalfa at various cuttings and established years were evaluated under sprinkler fertigation to optimize the N application schedule. Four N application levels of 0, 12.5, 25 and 37.5 kg N ha^{-1} (N0, N1, N2 and N3) for each cutting were applied to alfalfa at the early vegetative stage from the first to third year after establishment. The results showed that the variation in soil NO_3^--N content was mainly concentrated in the topsoil (0–40 cm) after N application via sprinkler fertigation. N uptake amount was dramatically improved through the N application for one-year-old alfalfa. Compared to the yield under the N0 treatment, the N application significantly improved the yield of alfalfa at the first two cuttings in the first year. The N use efficiency generally reached a high value with a low N application level. N application had an insignificant effect on the alfalfa quality classification during the three years. For obtaining high yield, quality and N use efficiency of alfalfa planted in the North China Plain, the N application level is recommended as 12.5 kg N ha^{-1} at the first cutting and 37.5 kg N ha^{-1} at the second cutting for one-year-old alfalfa.

Keywords: alfalfa; nitrogen fertilization; yield; crude protein content; relative feed values; nitrogen use efficiency

check for updates

Citation: Wang, Y.; Li, M.; Guo, J.; Yan, H. Alfalfa (*Medicago sativa* L.) Nitrogen Utilization, Yield and Quality Respond to Nitrogen Application Level with Center Pivot Fertigation System. *Agronomy* **2024**, *14*, 48. https://doi.org/10.3390/agronomy14010048

Academic Editor: Koki Toyota

Received: 26 October 2023
Revised: 13 December 2023
Accepted: 20 December 2023
Published: 23 December 2023

1. Introduction

With the development of the dairy industry, the demand for forage with high quality is increasing. Alfalfa (*Medicago sativa* L.), a forage crop high in protein, is one of the major forage crops that are extensively planted in China. Based on the support of policies accelerating the development of alfalfa production, the alfalfa planting area has expanded further to 2206.67 thousand ha [1]. As a high-quality forage, alfalfa has high nitrogen (N) content. The critical concentration of N in alfalfa plants, the minimum N content in shoots required to produce the maximum aboveground biomass at a given time, ranges from 2.7% to 3.3% [2], with the highest concentration of nitrogen reaching 3.97% [3]. Being a leguminous crop, alfalfa can assimilate atmospheric N_2 through biological nitrogen fixation (BNF). Annual N_2 fixation can reach 50–450 kg ha^{-1}, and about 30–80% of plant N comes from fixed N [4]. However, alfalfa roots and nodules are not fully developed in the early stage, and the development of active nodules takes 2–4 weeks after planting, even when rhizobium is inoculated [5,6]. Therefore, a large N demand cannot be satisfied through N_2 fixation at the early stage [7]. Applying modest N fertilizer to alfalfa is beneficial to obtain a higher yield and quality in areas with low soil nitrate content and soil organic matter or in the seeding year [4,8].

The impacts of N application on alfalfa yield and quality vary in different growth stages, planting years and soil fertility [9–11]. Generally, N fertilization could improve alfalfa yield in the seeding year with an application level of 40–80 kg N ha^{-1} yr^{-1} [9]. Especially under the condition of low soil fertility, N application could increase alfalfa yield in the first year after established [4]. Some reports also show that the carbon requirements of the nodules could not be fulfilled after alfalfa shoot cutting, and N_2 fixation was reduced [12]. Nitrogen plays a dominant role in regulating carbon assimilation [13], and appropriate application of N fertilizer at this stage could improve N_2 fixation and alfalfa yield [7,14]. However, He et al. [15] reported that N application had no significant effect on the yield of alfalfa in the seeding year and would increase weed yield. Moreover, higher N application levels (225–300 kg N ha^{-1} yr^{-1}) could reduce the alfalfa yield in the first year of planting [16] because higher N application levels have an inhibitory effect on the root growth and development, nodulation and configuration [17–19]. Many studies have shown that N application had no significant effect on the yield of well-established alfalfa [11,18,20], as 70–80% of N demand could be satisfied through N_2 fixation [21]. Meanwhile, Fan et al. [10] found that N application could improve the yield of both young and old alfalfa stands owing to the absence of nodulation. Generally, N application has a significant effect on the alfalfa quality, which could increase the total nitrogen content, nitrate nitrogen and ammonium nitrogen content, and crude protein content (CP) but reduce the neutral detergent fiber (NDF) and acid detergent fiber (ADF) [22–24]. N fertilization could increase crude protein by improving non-protein nitrogen (NPN) that can be degraded to ammonia in the rumen [25,26]. Ammonia is a source for microbial protein synthesis, but excess ammonia is absorbed through the rumen wall and largely excreted as urea in the urine [27]. This could increase the metabolic burden on animals and have a negative impact on the environment. Therefore, animal scientists do not recommend abundant N application to increase the N content in alfalfa forage. However, some researchers have also reported that N application had no significant effect on the alfalfa quality during the first year of establishment [28] and even reduced CP and relative feed values (RFV) with high N application levels [16]. RFV is considered a crucial parameter for alfalfa quality evaluation [29]. Generally, RFV has a negative relationship with ADF and NDF [30]. Overall, the response of alfalfa yield and quality to N application is complicated. The optimal N application level for alfalfa is difficult to be consistent under different conditions.

Nitrogen fertilizer, as one of the most important agricultural inputs, plays an important role in improving crop yield. Annual N fertilizer consumption in China accounted for more than 35% of the total N fertilizer consumption of the world and about 60% of the whole fertilizer consumption in China. However, nitrogen use efficiency (NUE) of crop production in China ranged from 20% to 40% in 2017 [31], which was lower than that in Europe (52%) and the United States of America (68%) [32]. A large amount of N fertilizer has been severely lost due to improper fertilization methods. In alfalfa production, center-pivot irrigation systems have been widely used all over the world [33] because of their advantages, such as large coverage area, low labor costs, high irrigation efficiency, easy fertigation and high application uniformity [34,35]. In recent years, the fertigation method using center-pivot irrigation systems, particularly in the application of urea solutions, has been applied increasingly in China [17]. Compared with furrow irrigation, water could be saved 60–72% with a center-pivot sprinkler fertigation system [36]. Meanwhile, N fertilizer could be saved by 78% in sandy loam soil or 52% in sandy soil with fertigation, compared to that with the traditional fertilization method [37]. Since the end of the 20th century, the center-pivot sprinkler fertigation system has been popularized and applied in the United States and could reduce N leaching loss and improve N use efficiency [34,38]. Owing to the high degree of automation for sprinkler fertigation, N fertilizer could be supplied for alfalfa in each cutting rather than being totally applied at the beginning of the growing season. N demand for alfalfa during different cuttings might be various, but the same N application for each cutting was common in alfalfa production. Therefore, optimization of the N application schedule for alfalfa in different cuttings and established years should be further conducted.

The objectives of this study were (1) to evaluate the effects of four N application levels on the dry matter yield, CP content, RFV and N utilization of alfalfa in different cuttings and years with sprinkler fertigation and (2) to optimize the N application schedule for alfalfa planted in the North China Plain.

2. Materials and Methods

2.1. Experimental Site

The field experiments were conducted at the China Agricultural University Experimental Station from 2016 to 2018. The station is located in the North China Plain in Zhuozhou, Hebei Province (39°27′ N, 115°51′ E), and has a typical temperate sub-humid continental monsoon climate with a summer precipitation pattern (Figure 1). During the experimental period, the average total precipitation was 440.0 mm, 56.61% of which fell between July and August. The average monthly air temperature, wind speed, solar radiation and relative humidity during the three years recorded by a weather station (Watchdog 2000, Spectrum Technologies Inc., Haltom City, TX, USA) installed at the experimental site, are presented in Figure 1. Air temperature and solar radiation both increased first and then decreased with the increase of the month. The relative humidity reached a high level from July to October. Wind speed was faster during March and April (Figure 1). The soil type is classified as EutyicCambisols (World Reference Base (WRB) 2014) and is mainly sandy soil. Other physicochemical properties of the root zone soil (0–80 cm) are shown in Table 1. Overall, soil properties in the field were relatively homogeneous. Soil fertility was relativel y lower, especially the soil organic matter and nitrate nitrogen content. Seeds of the alfalfa cultivar WL363HQ were sown on 28 August 2015, with a row spacing of 30 cm and a sowing rate of 22.5 kg ha^{-1}. All the alfalfa seeds were purchased from the RYTWAY seed company (Beijing, China) and were produced in America. Meanwhile, all the seeds were coated and inoculated with rhizobium during production. Before alfalfa was established, the previous crops were two species of annual gramineous forage grasses (tall fescue (*Festuca arundinacea* Schreb.) and smooth bromegrass (*Bromus inermis* Leyss.)). Meanwhile, diammonium phosphate (N ≥ 18% and P_2O_5 ≥ 46%) of 150 kg ha^{-1} and potassium sulphate (K_2O ≥ 52%) of 100 kg ha^{-1} were applied before alfalfa seeding. During the period of experiments, imazethapyr aqueous solution (5%) was used in weed management. Lambda-cyhalothrin and acetamiprid were used in pest control. During the branching stage at the 1st cutting in 2017, superphosphate (P_2O_5 ≥ 14%) of 300 kg ha^{-1} and potassium sulphate (K_2O ≥ 52%) of 120 kg ha^{-1} were applied to all treatments. In 2016–2018, regrowth of alfalfa began on 29 March, 1 April and 5 April, respectively. Alfalfa was harvested at the early flowering stage (10% of alfalfa in the field bloomed) and four times each year. The harvest dates are shown in Table 2.

Table 1. Soil physicochemical properties at different soil depths in the field at the beginning of the experiment. (mean ± SD; n = 12).

Soil Depth (cm)	Bulk Density (g cm^{-3})	Field Capacity (cm^3 cm^{-3})	Soil pH	Available Phosphorus Content (mg kg^{-1})	Available Potassium Content (mg kg^{-1})	Organic Matter (g kg^{-1})	Nitrate Nitrogen Content (mg kg^{-1})	Ammonium Nitrogen Content (mg kg^{-1})
0–20	1.62 ± 0.06	0.25 ± 0.01	8.32 ± 0.11	45.2 ± 5.97	80.87 ± 8.65	9.84 ± 0.82	4.91 ± 0.68	2.70 ± 0.32
20–40	1.58 ± 0.09	0.20 ± 0.04	8.34 ± 0.10	56.9 ± 6.46	70.93 ± 7.08	10.37 ± 1.05	3.25 ± 0.46	1.68 ± 0.53
40–60	1.57 ± 0.04	0.23 ± 0.03	8.29 ± 0.11	3.53 ± 1.34	70.98 ± 10.94	8.29 ± 0.91	2.18 ± 0.65	1.67 ± 0.63
60–80	1.59 ± 0.05	0.16 ± 0.04	8.35 ± 0.09	11.50 ± 3.64	55.96 ± 6.07	8.35 ± 1.62	2.15 ± 0.37	1.44 ± 0.69

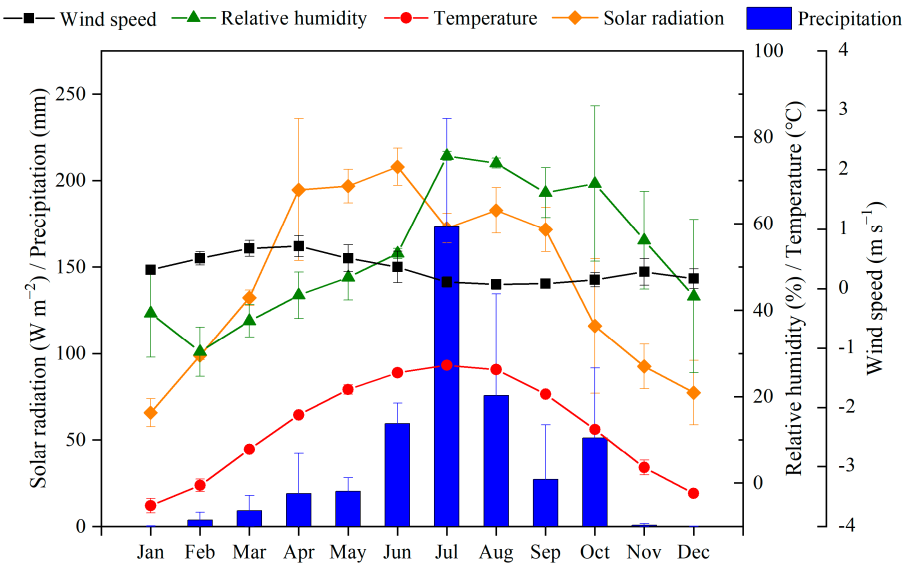

Figure 1. Average monthly weather data during 2016–2018 at the experimental station in the North China Plain.

Table 2. The irrigation amount, fertilizer application date and harvest date during the three years.

Year	Cuttings	Irrigation Amount (mm)	Fertilizer Application Date	Harvest Date
2016	1st cutting	192.0	4 April	26 May
	2nd cutting	68.0	4 June	6 July
	3rd cutting	51.0	18 July	17 August
	4th cutting	78.0	27 August	1 October
2017	1st cutting	165.0	1 April	19 May
	2nd cutting	129.0	4 June	5 July
	3rd cutting	39.0	19 July	14 August
	4th cutting	42.5	26 August	29 September
2018	1st cutting	95.0	12 April	24 May
	2nd cutting	126.0	5 June	4 July
	3rd cutting	12.0	19 July	13 August
	4th cutting	80.0	26 August	28 September

2.2. Experimental Design

To determine the effects of N application level on alfalfa yield, quality and N utilization, the field experiments were conducted based on a single-factor experimental design. Four N application levels, set at 0, 12.5, 25.0 and 37.5 kg N ha^{-1} for each cutting (N0, N1, N2 and N3), were applied to the alfalfa through a center-pivot sprinkler fertigation system. Particularly, no additional N fertilizer was applied, and only the initial soil N content was available for alfalfa under N0 treatment. Urea (N $\geq$ 46%) was used as nitrogen fertilizer in this study. During the three years, alfalfa was harvested four times during the growing season in this region. All of the treatments had three repetitions with a plot size of 7 m $\times$ 10 m, and the layout is shown in Figure 2. The fertilizer treatments were all conducted during the period from regrowth to branching of alfalfa during each cutting. The dates for fertilizer application during the three years are shown in Table 2.

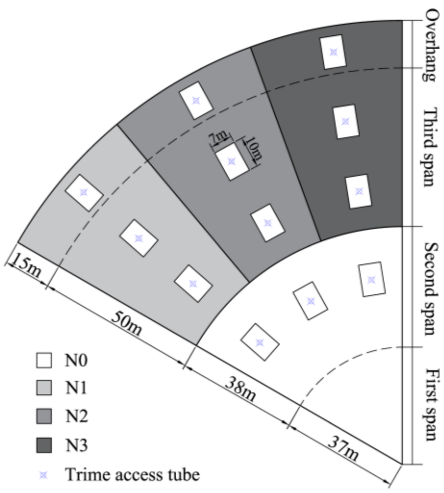

Figure 2. Distribution of experimental plots under the center pivot irrigation system. N0, N1, N2, and N3 represent the N application levels of 0.0, 12.5, 25.0, and 37.5 kg N ha^{-1} for each cutting, respectively.

2.3. Fertigation System and Irrigation Management

During the experimental period, water and fertilizer were supplied to alfalfa through a center-pivot irrigation system. The center pivot was composed of three spans and an overhang, with a total length of 140 m. The lengths of the first, second and third span and overhang were 37, 38, 50 and 15 m, respectively. The sprinklers used in this center pivot were the Nelson R3000, assembled with pressure regulators of 0.138 MPa (20 psi) (Nelson Irrigation Corp., Walla Walla, WA, USA). To realize the opening and closing of individual sprinklers, solenoid valves were installed at the connection between each drop hose and lateral pipe. Additionally, a fertigation system consisting of a piston injection pump and a fertilizer storage tank was equipped for the center pivot. The urea solution, mixed uniformly in the storage tank, was injected steadily into the center pivot through the pump and sprayed into the field with the irrigation water. The radial uniformity coefficients of water and fertilizer application with the whole center pivot were up to 89.84% and 88.77%, respectively [39]. During fertilization, sprinklers in the first and second spans were all closed, and only those in the third span and overhang were open. Different N application levels for the N1–N3 treatments were achieved by adjusting the fertilizer concentration in the storage tank, and the volume of fertilizer solution applied to the three N treatments was the same. Generally, fertilization for the three N treatments could be completed within 2 h. After fertilization, different irrigation amounts were immediately supplemented for N0 and the other N treatments to guarantee the same total irrigation amount for all treatments, which was determined based on crop water requirement. The desired water application in specific spans could be realized by changing the center pivot's travel speed and controlling the opening and closing of the sprinklers by turning the electric solenoid valves on or off. As previously mentioned, the irrigation amounts for different treatments were the same and calculated as the difference between the accumulated crop evapotranspiration (ET_c) and the accumulated effective precipitation ($\geq$5 mm). Crop evapotranspiration (ET_c) was calculated using reference evapotranspiration (ET_o) and crop coefficients (K_c). ET_o was calculated from the weather data using the Penman–Monteith equation. Daily K_c was first generated through FAO-56 and then adjusted with the actual lengths of the growing period and weather data in the experimental station [40]. During the experiment, weather data, including solar radiation, wind speed, temperature, relative humidity and precipitation at 2 m height, were collected every 0.5 h with a weather station (Watchdog 2000, Spectrum Technologies Inc., USA) installed at the experimental site. Irrigation was performed for all plots when the average soil water content at the soil depth of 0–40 cm reached as low as 60% of the field water capacity. An irrigation amount of 20 mm was applied for alfalfa in the first irrigation event of the first cutting during the three years to ensure the regrowth of alfalfa. The total irrigation amounts during the whole alfalfa growing season were 389.0, 375.5 and 313.0 mm for 2016, 2017 and 2018, respectively. Irrigation amounts for each cutting during the three years are shown in Table 2.

2.4. Measurement Parameters

2.4.1. Soil Water Content

Soil water content generally varied with irrigation, rainfall and crop water use. To determine the irrigation time based on the soil water content in this experiment, soil moisture at the depths of 0–20 cm, 20–40 cm, 40–60 cm and 60–80 cm were measured using a TDR Trime-tube system (Trime-T3 TDR, IMKO Ltd., Ettlingen, Baden-Württemberg, Germany) 1 d before and after irrigation during the entire growth period in the three years.

2.4.2. Soil Nitrate Nitrogen Content

N fertilization and plant nutrient uptake could change the soil nitrogen content. Particularly, soil NO_3^--N content is much higher than soil NH_4^+-N content in dryland. To assess the effects of N application on soil NO_3^--N content, soil samples were collected at soil depths of 0–20 cm, 20–40 cm, 40–60 cm and 60–80 cm once 1 or 2 days before fertigation, once within 10 days after fertigation, and once at the end of each cutting in 2016–2018. In 2017–2018, additional measurements of soil NO_3^--N content were conducted once at each growing stage. Soil nitrate-nitrogen content was determined using an AA3 continuous flow analyzer (Bran + Luebbe, Norderstedt, Schleswig-Holstein, Germany).

2.4.3. Total Nitrogen Content of Alfalfa

During the experiments, the total nitrogen content of alfalfa shoots for each cutting was measured using the semi-micro Kjeldahl method for nitrogen determination. All alfalfa shoots for each sample were dried at 105 °C for 0.5 h and then at 75 °C for 48 h. The dried shoots were ground, sifted and boiled in H_2SO_4-H_2O_2. Finally, the total nitrogen content of the plants was determined using a semi-automatic Kjeldahl nitrogen analyzer (K1305A, Shengsheng, Shanghai, China). Nitrogen use efficiency was calculated based on N application level, total nitrogen content and dry matter yield of alfalfa. The formulas are as follows [41]:

$$N_{uptake} = TN \times Y_{DM}, \tag{1}$$

$$\text{ANR} = \frac{(N_x - N_0)}{N_{level}} \tag{2}$$

and

$$\text{NAE} = \frac{(Y_x - Y_0)}{N_{level}}, \tag{3}$$

where N_{uptake} is the total N uptake of alfalfa, kg ha^{-1}; TN is the total nitrogen content, kg kg^{-1}; Y_{DM} is the dry matter yield of alfalfa, kg ha^{-1}; ANR is the apparent recovery of N fertilizer, kg kg^{-1}; N_x is the total N uptake of alfalfa shoot with N application, kg ha^{-1}; N_0 is the total N uptake of alfalfa shoot without N application, kg ha^{-1}; N_{level} is the N application level, kg ha^{-1}; NAE is the agronomic efficiency of N fertilizer, kg kg^{-1}; Y_x is the dry matter yield of alfalfa with N application, kg ha^{-1}; Y_0 is the dry matter yield of alfalfa without N application, kg ha^{-1}.

2.4.4. Yield and Quality of Alfalfa

During the measurements of alfalfa yield, fresh alfalfa plants from three squares (1 m × 1 m) in each plot were cut to a stubble height of 5 cm and weighed as fresh weight. Some samples randomly collected from the fresh alfalfa in each square were dried at 105 °C for 0.5 h and then at 75 °C for 48 h. The ratio of dry weight to fresh weight ($R_{d/w}$) of the sample was calculated. Finally, the dry matter yield of alfalfa per unit area was determined as the product of $R_{d/w}$ and the fresh weight of each square and used as the main yield parameter for further analysis. In addition, after drying, alfalfa samples from each square were further smashed to analyze the content of crude protein (CP) and the relative feed value (RFV). The CP content of alfalfa was measured through near-infrared reflectance spectroscopy (NIR-TR-3750, Foss NIR Systems, Inc., Stockholm, Sweden) based on the model proposed by NIRS Forage and Feed Testing Consortium (NIRSC) [42]. RFV was calculated using the following equations [29]:

$$\text{RFV} = \frac{DDM(\%DM) \times DMI(\%BW)}{1.29}, \tag{4}$$

$$DMI(\%BW) = \frac{120}{NDF(\%DM)} \tag{5}$$

and

$$DDM(\%DM) = 88.9 - 0.779 \times ADF(\%DM), \tag{6}$$

where RFV is the relative feed value, and the unit is the percent of dry matter (%*DM*); *DDM* is the digestible dry matter, %*DM*; *DMI* is the dry matter intake, and the unit is the percent of body weight (%*BW*); *NDF* is the neutral detergent fiber, % *DM*; *ADF* is the acid detergent fiber, %*DM*. Meanwhile, the *NDF* and *ADF* of alfalfa were also measured through near-infrared reflectance spectroscopy. According to the hay grading standard for legumes proposed by the American Forage and Grassland Council [29], the alfalfa quality was graded.

2.5. Statistical Analysis

The linear and quadratic regression analyses of total N uptake, dry matter yield, ANR, NAE and quality of alfalfa with N application level were performed, and the significance of the regression function was investigated through an F test (evaluated as significant at the $p < 0.05$ level). The regression analysis and graphs were performed with Origin Pro2022 software (OriginLab, Northampton, MA, USA). In the figures, no regression curve and equation indicated that relationships between parameters and N application level were insignificant.

3. Results

3.1. Temporal and Spatial Variations of Soil Nitrate N Content

During the experimental period in 2016–2018, soil NO_3^--N content at a depth of 0–40 cm reached a high value and showed great changes over time (Figure 3). Differently, soil NO_3^--N content at depths of 40–80 cm remained relatively stable throughout the growing season. At the beginning of the growing season in 2016, soil NO_3^--N content under different treatments was relatively low, ranging from 0.74 to 5.18 mg kg^{-1}. After the first and third N applications, soil NO_3^--N content at different depths did not noticeably increase. After the second N application, soil NO_3^--N content at a depth of 0–20 cm significantly improved under all treatments, up to 13.88–39.43 mg kg^{-1}. Under the treatments of N2 and N3, soil NO_3^--N content at a depth of 20–40 cm also increased markedly. After the fourth N application, soil NO_3^--N contents at depths of 0–20 cm and 60–80 cm increased under N1–N3 treatments. However, soil NO_3^--N content was reduced to the initial level at the end of each cutting. During the years 2017 and 2018, soil NO_3^--N content was measured once at each growing stage. At the beginning of the experiments in 2017, soil NO_3^--N content at a depth of 0–20 cm increased up to 3.93–21.96 mg kg^{-1}. Under treatments with or without N application, soil NO_3^--N content at a depth of 0–20 cm increased first and then decreased after fertigation or irrigation in 2017 and 2018. In 2018, the soil NO_3^--N content at a depth of 0–20 cm even reached up to 73.29 mg kg^{-1}. Overall, the maximum increment in soil NO_3^--N content (0–20 cm) one week after fertigation reached 94.53, 118.50 and 114.26 kg ha^{-1} for N1, N2 and N3 treatments, respectively, which were much higher than the amount of N application. The increase in soil NO_3^--N content was insignificant after fertigation at the third cutting during the three years due to rainy weather. Meanwhile, soil NO_3^--N content at a depth of 60–80 cm under N2 and N3 treatments increased at the second cutting in 2016, the third cutting in 2018, and the fourth cutting during the three years, which might also be due to heavy rainfall. In general, a significant reduction in soil NO_3^--N content at a depth of 0–20 cm occurred during the late vegetative stage and bud stage of alfalfa. At the end of the growing season in 2018, the soil NO_3^--N content at a depth of 0–80 cm increased by 35.28, 22.03, 24.19 and 9.13 kg ha^{-1} under treatments of N0, N1, N2 and N3, respectively, compared to that at the beginning of the growing season in 2016.

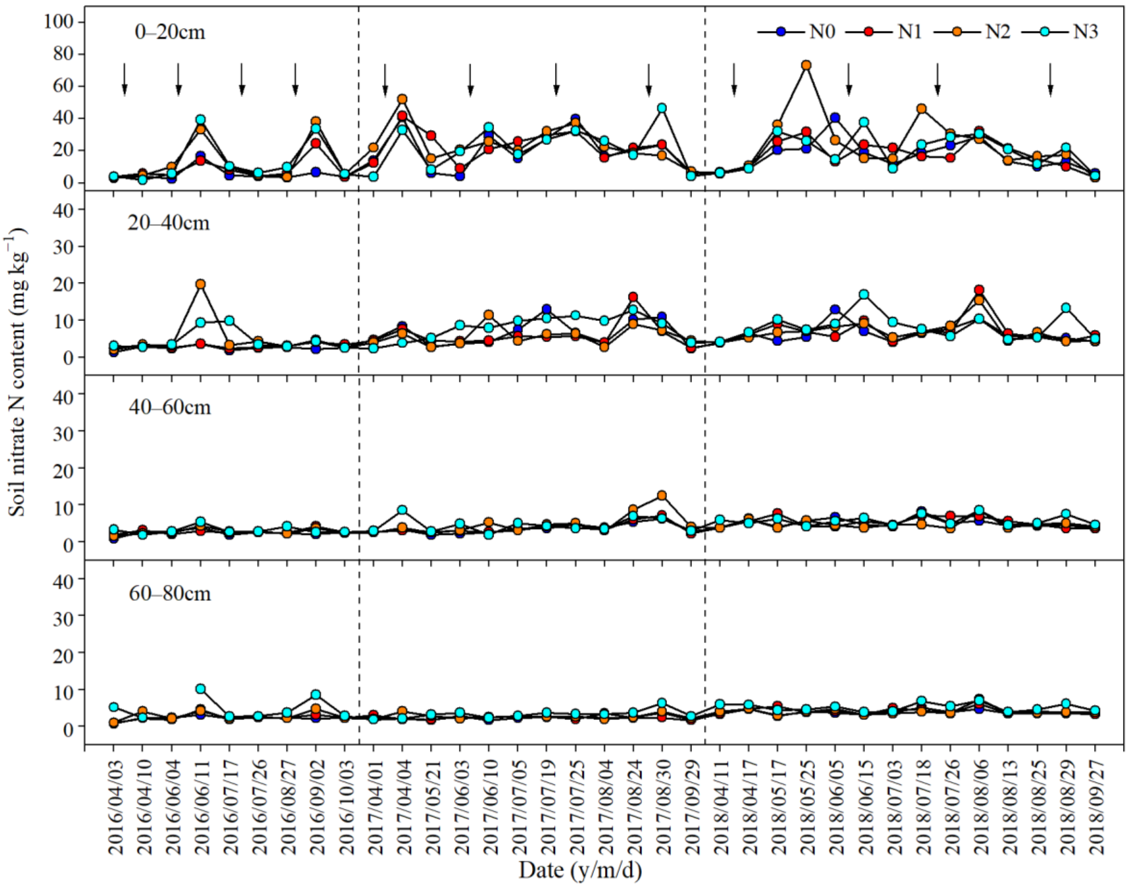

Figure 3. Spatiotemporal variation of soil NO_3^--N content under different treatments in 2016–2018. N0, N1, N2 and N3 represent N application levels of 0.0, 12.5, 25.0 and 37.5 kg N ha^{-1} for each cutting, respectively. Arrows represent the N application events.

3.2. Nitrogen Uptake by Alfalfa Shoot

N uptake by alfalfa shoot generally decreased with stand age and cutting (Figure 4). The average annual N uptake under different treatments for one-, two- and three-year-old alfalfa were 441, 413 and 314 kg ha^{-1}, respectively. The proportion of N uptake for each cutting to annual N uptake during the three years averaged 33.3%, 32.8%, 20.0% and 13.9% for the first to fourth cutting, respectively. In 2016, the total N uptake amount ranged from 386 to 497 kg ha^{-1}, which had a remarkable linear and positive relationship with the N application level (R^2 = 0.99). Especially in the first two cuttings, the N uptake amount had a prominent relationship with the N application level. A significant linear relationship was found between the N application level and the N uptake amount of the first cutting (R^2 = 0.96) (Figure 4b). N uptake amount of the first cutting ranged from 116 to 176 kg ha^{-1} and increased with the N application level. In the second cutting, regression analysis of N uptake amount and N application level showed a significant parabolic relationship with an upward opening (R^2 = 0.99). N uptake amount under N3 treatment reached the highest value of 191 kg ha^{-1}, which was 34.5% higher than that under N0 treatments. N uptake amount of the third and fourth cuttings had no evident relationship with the N application level. In the third cutting, the N uptake of alfalfa increased first and then decreased with the N application level. N uptake amount under the N2 treatment was the highest, with values of 87 kg ha^{-1} and 20.4% higher than that under the N0 treatment. In the fourth cutting, N uptake amounts under different treatments were very similar, ranging from 54 to 61 kg ha^{-1}. In 2017, no significant relationship existed between the total N uptake amount for the whole growing season or each cutting and N application level (Figure 4a,c). The total N uptake amount of alfalfa for the whole growing season with N application was 406–439 kg ha^{-1}, which was 3.9–12.4% higher than that under N0 treatment. In 2018, a significant parabolic relationship with a downward opening was observed between the total N uptake amount and N application level. The total N uptake amount under the N3 treatment was up to 327 kg ha^{-1}, which was 12.7% higher than that under the N0 treatment (Figure 4a). Meanwhile, the N application level only had a prominent relationship with the N uptake amount in the fourth cutting (Figure 4d). In the fourth cutting, a significant parabolic relationship with an upward opening existed between the N uptake amount and the N application level. N uptake amount under N3 treatment was the highest and 27.7% higher than that under N0 treatment.

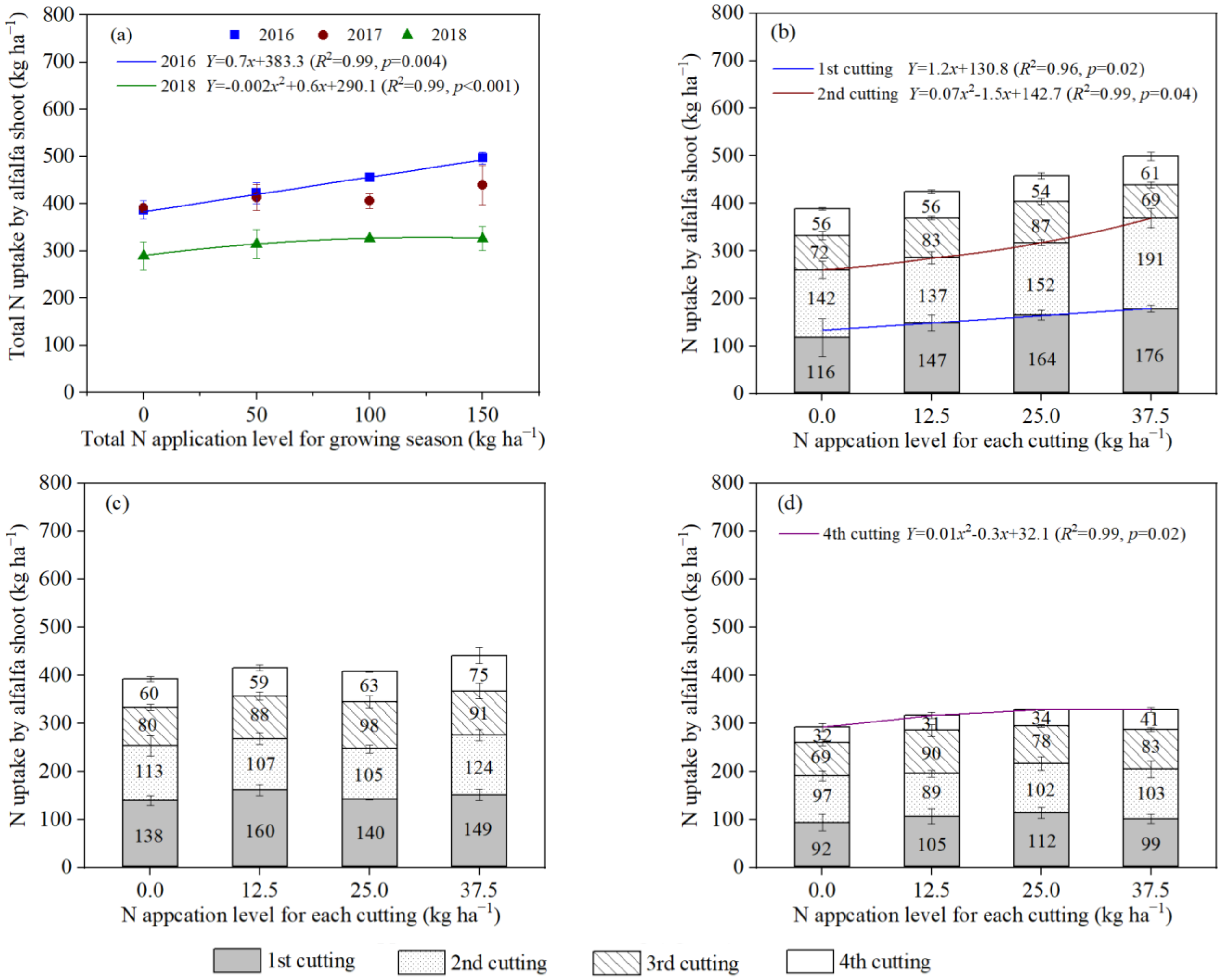

Figure 4. Total N uptake amount of alfalfa shoot for the whole growing season during the three years (**a**) and each cutting in 2016 (**b**), 2017 (**c**) and 2018 (**d**) under different N application levels. Linear or quadratic regression curves and equations indicated that the relationship between N uptake amount and N application level was significant.

3.3. Dry Matter Yield of Alfalfa

During the three years, alfalfa yield gradually reached a high value in the first two years and decreased with cutting (Figure 5). The alfalfa yield of the first and second cuttings was relatively higher, which could reach 63.16–70.33% of the annual yield. Overall, the N application level had a significant parabolic relationship with a downward opening with the annual yield for the three years (Figure 5a). Compared with the N0 treatment, total alfalfa yield could be dramatically increased through N application. In 2016, the average annual yield increased with N application and reached the highest value of 14,368.85 kg ha^{-1} with N3 treatment, which was 41.37% higher than that under treatment without N application. The annual yield was increased by 24.16% and 23.72% under the N1 and N2 treatments, respectively, compared to the yield of the N0 treatment. In 2017, compared to the alfalfa annual yield of the N0 treatment with an average value of 12,849.58 kg ha^{-1}, the alfalfa annual yield under N1, N2 and N3 treatments increased by 12.52%, 6.72% and 13.78%, respectively. In 2018, the annual yield with N application was 12.53–15.67% higher than that under N0 treatment. On the whole, the annual yield reached the highest value of 12,849.58–14,619.74 kg ha^{-1} in 2017. Compared with the annual yield in 2016 and 2017, it was reduced by 8.99–28.22% in 2018, which was mainly due to the emergence of low air temperature (average daily temperature < 8 °C, the minimum air temperature reaching −1.8 °C) during spring green-up stage and serious insect attacks in the third and fourth cuttings. Furthermore, N application level had a significant relationship with the alfalfa yield of the first, second and third cuttings in the first year (Figure 5b). In the first and third cutting, a significant parabolic relationship with a downward opening existed between alfalfa yield and N application level ($p < 0.01$). Alfalfa yield of the first cutting increased with N application level with a decreasing slope and reached the highest under N3 treatment. Compared with yield under N0 treatment, it was improved by 42.4–75.3% with N application. In the second cutting, alfalfa yield had a prominent linear and positive relationship with the N application level. Alfalfa yield could be increased by 44.9% under the N3 treatment compared to that under the N0 treatment. The alfalfa yield of the third cutting increased first and then decreased with the N application level. A similar variation tendency and the relationship between yield and N application level was also found at the third cutting in 2017 and 2018 (Figure 5c,d). Compared to that under N0 treatment, the alfalfa yield of the third cutting could be improved by N1 or N2 treatment in 2016–2018 with the maximum proportion of 19.0%, 15.3% and 7.4%, respectively. N application level had no remarkable relationship with the alfalfa yield of the fourth cutting. In 2017 and 2018, the N application level had no significant relationship with the alfalfa yield in the first and second cuttings. In contrast to that under N0 treatment, alfalfa yield increased by less than 15.9% with nitrogen application at the two cuttings of the two years (Figure 5c,d). Alfalfa yield of the fourth cutting had a significant parabolic relationship with the N application level over the two years. Meanwhile, alfalfa yield increased with the N application level and got the highest value under N3 treatment, but only 273.9–394.5 kg ha^{-1} (22.3–26.9%) higher than that under N0 treatment.

3.4. Nitrogen Utilization of Alfalfa

The relationships between the apparent recovery of N fertilizer (ANR) and agronomic efficiency of N fertilizer (NAE) for alfalfa with the N application level varied in different cuttings and years (Figure 6). In 2016, a significant linear and negative relationship between the N application level and ANR was observed at the third cutting ($R^2 = 0.584$). The ANR of alfalfa under different treatments was relatively higher, with an average value of 1.61–2.41 kg kg^{-1} in the first cutting, but the ANR of the fourth cutting could be negative under the treatments of N1 and N2. Additionally, the N application level only had a significant linear and negative relationship with the NAE of alfalfa in the first and third cuttings. In the two cuttings, the NAE decreased with the N application level and reached the highest with an average value of 121.15 kg kg^{-1} and 36.20 kg kg^{-1} under N1 treatment in the first and third cuttings, respectively. In the second and fourth cuttings, NAE decreased first and then increased with the N application level. The NAE of N2 treatment was generally low and even negative in the fourth cutting. In 2017, the N application level only had prominent linear and negative relationships with the ANR and NAE in the first cutting, and the ANR and NAE of the N1 treatment were significantly higher than those of other treatments. During the second and fourth cuttings, the negative ANR appeared with N1 and N2 treatments. The NAE of the N2 treatment was also the lowest, except for that of the third cutting. The NAE with N3 treatment was the lowest in the third cutting but the highest in the fourth cutting. In 2018, the N application level had linear and negative relationships with the ANR and NAE at the third cutting. The ANR and NAE of N1 treatment were generally higher in the first and third cuttings. Variously, a noteworthy linear and positive relationship between N application level and ANR existed in the fourth cutting. In the second and fourth cuttings, ANR under N1 treatment appeared to have a negative value. Overall, ANR and NAE were relatively higher in 2016 and were generally up to a high value in the first cutting. In addition, the ANR and NAE of N1 or N2 treatments appeared to have a negative value in the second and fourth cuttings, which revealed that the yield or N uptake of alfalfa was not improved through N application and was even reduced compared to that under treatment without N application.

3.5. Quality of Alfalfa

In the first established year of alfalfa (2016), N application generally had an insignificant relationship with the CP of alfalfa (Figure 7). In general, the CP decreased first and then increased with the N application level. The CP of alfalfa under N0 treatment remained at a high level at different cuttings. N application did not significantly improve the CP of alfalfa. N application level only had a significant parabolic relationship with the RFV in the third cutting in 2016. The RFV generally decreased first and then increased with the N application level. In the first, second and fourth cuttings, RFV was high under N0 and N2 treatments and higher than that under N1 and N3 treatments. In the third cutting, the RFV under N3 treatment reached a high level with a mean value of 174.82%, which was higher than that under other treatments. In 2017, relationships between the N application level and the RFV and CP of alfalfa were insignificant in the first three cuttings but remarkable in the fourth cutting. In the fourth cutting, the CP had a significant linear and positive relationship with the N application level. The CP reached the highest value, averaging 25.3% under N3 treatment, which was higher than that under other treatments. The average RFV under N3 treatment was up to 180.12%, which was 10.2%, 16.8% and 17.4% higher than those under N0, N1 and N2 treatments, respectively. In 2018, the N application level only had notable parabolic relationships with the RFV and the CP in the third cutting. In the third cutting, the CP and RFV increased first and then decreased with the N application level, reaching a high level under N1 and N2 treatments. Overall, the CP of alfalfa during the three years ranged from 19.3% to 28.7%. Based on the hay grading standard for legumes according to the CP content, alfalfa in this experiment could be classified as premium-grade grass. While classifying grass grade according to the RFV, alfalfa in 2016 could be generally graded as the premium, and only that under the N1 treatment in the third cutting as the first-grade grass. In 2017 and 2018, only alfalfa in the fourth cutting reached the premium grade standard. Except for the alfalfa of the second cutting in 2017, which was classified as the second-grade standard, alfalfa in the other cuttings in 2017 and 2018 could be classified as first-grade grass. Overall, alfalfa quality in 2016 was better than that in 2017 and 2018. Compared to the N0 treatment, the N application did not significantly improve the CP of alfalfa in 2016 and increased the RFV or CP only at the third and fourth cuttings in 2016–2018 but did not affect the quality classification of alfalfa.

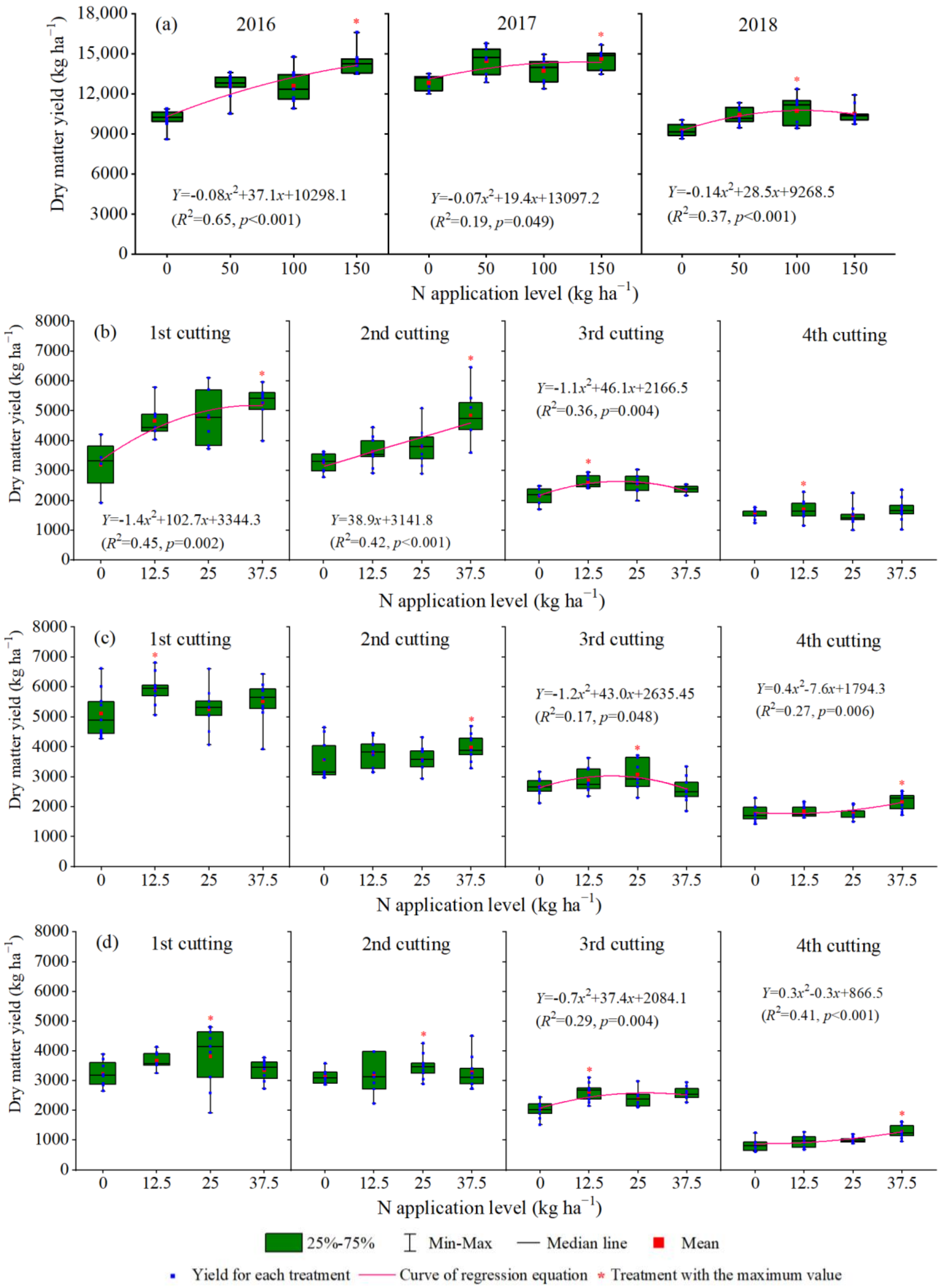

Figure 5. Dry matter yield of alfalfa under different N application levels for each cutting in 2016 (**a**), 2017 (**b**) and 2018 (**c**), as well as the annual yield of the three years (**d**).

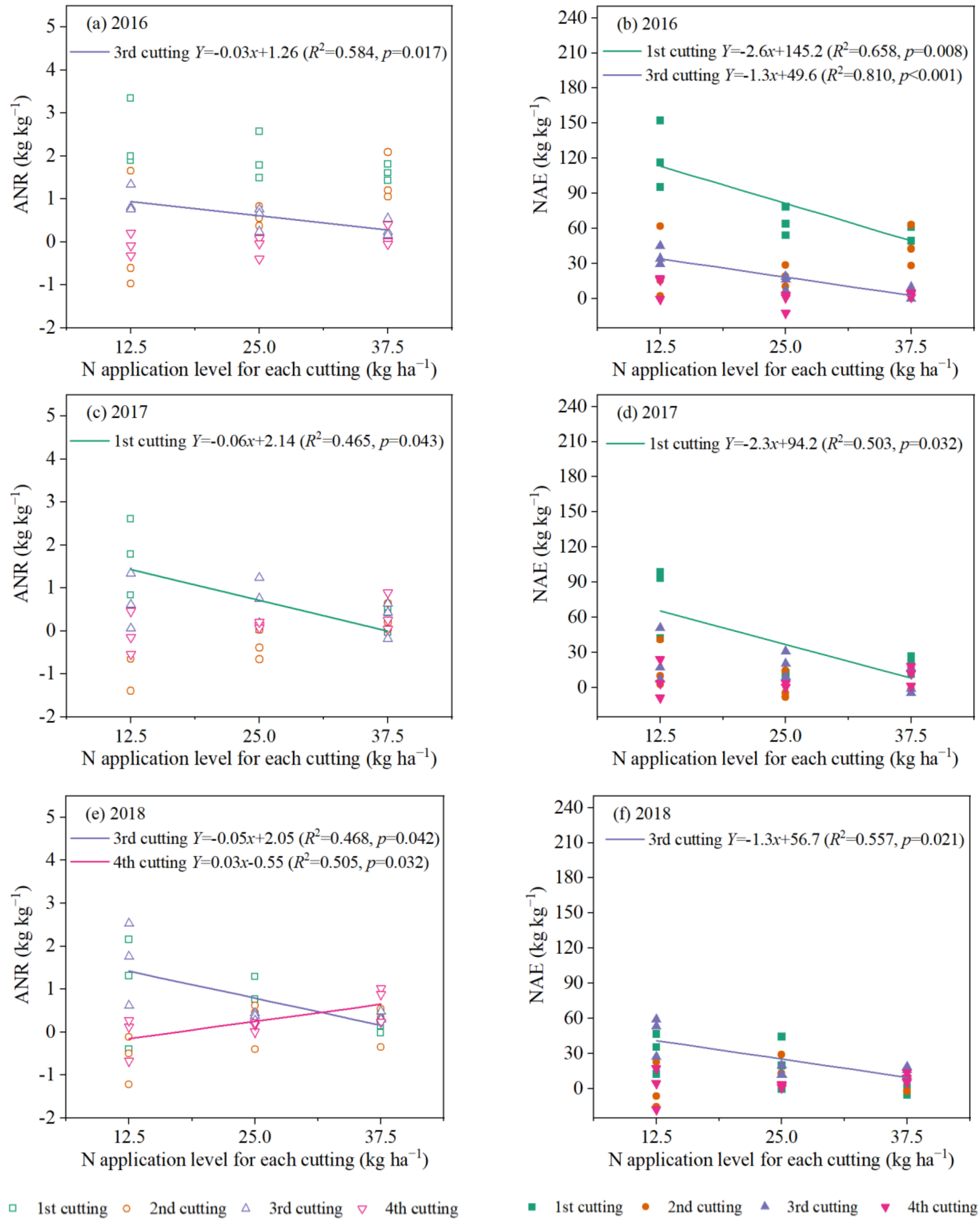

Figure 6. Apparent recovery of N fertilizer (ANR) (**a**,**c**,**e**) and agronomic efficiency of N fertilizer (NAE) (**b**,**d**,**f**) under different N application levels in 2016 (**a**,**b**), 2017 (**c**,**d**) and 2018 (**e**,**f**). Linear regression curves and equations indicated that the relationship of ANR and NAE with N application level was significant.

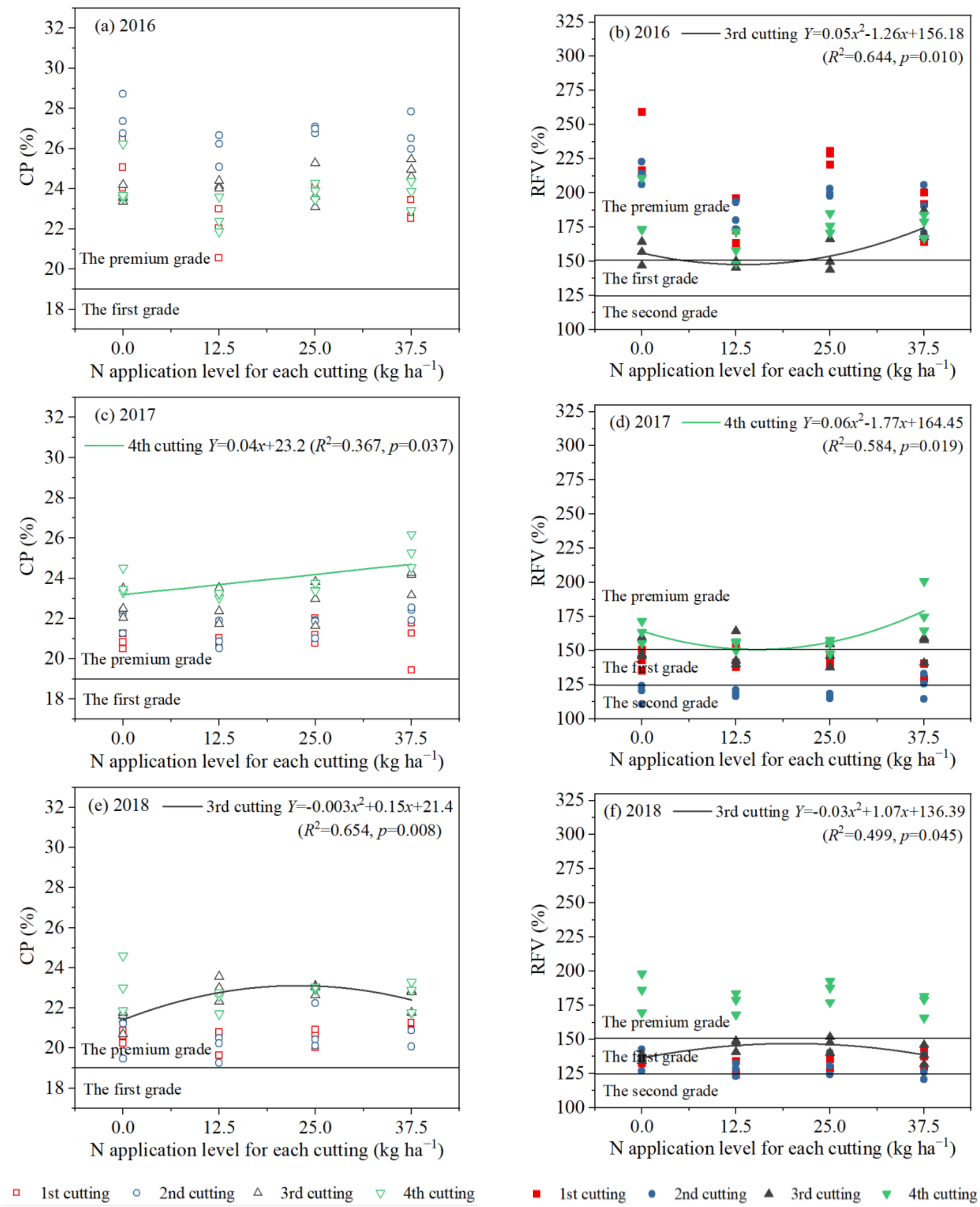

Figure 7. The crude protein (CP) (**a**,**c**,**e**) and the relative feed value (RFV) (**b**,**d**,**f**) of alfalfa under different N application levels in 2016 (**a**,**b**), 2017 (**c**,**d**) and 2018 (**e**,**f**). Linear or quadratic regression curves and equations indicated that the relationship of CP and RFV with N application level was significant.

4. Discussion

Changes in soil nitrogen content mainly appeared at a depth of 0–40 cm after N application. Soil NO_3^--N content at 40–80 cm only increased under N2 and N3 treatments during the rainy season (from June to September). The N applied using sprinkler fertigation did not result in serious N leaching. Similar to our results, many researchers have indicated that soil NO_3^--N content was higher at a depth of 0–40 cm, and N leaching could be reduced under sprinkler fertigation [43,44]. Meanwhile, N leaching could be increased through N application level [45,46], thus soil NO_3^--N content at the deep soil layer was improved under a higher N application level, and no significant N leaching occurred due to the comparatively lower N application level (12.5–37.5 kg N ha^{-1}) in our study. The increment of soil NO_3^--N accumulation was much higher than the N application amount, which might be due to the N input from N mineralization and N_2 fixation [47]. The gross N immobilization level could be 18–119 mg kg^{-1} per day in drying–rewetting soils under sprinkler irrigation [48,49]. The amount of N_2 fixation for alfalfa fields could reach the value of 200–500 kg N ha^{-1} yr^{-1} [50,51]. In addition, soil NO_3^--N accumulated amount for the soil layer of 0–80 cm was increased under all treatments, and the increment reached the highest value under the N0 treatment after the third year of alfalfa production. This indicates that planting alfalfa could improve soil nitrogen content due to N_2 fixation. During the three years, N uptake by alfalfa shoot ranged from 290 to 497 kg ha^{-1} yr^{-1} under different N application levels, which was similar to the results reported by Wu et al. [52]. In the first year after alfalfa was established, the total N uptake amount of alfalfa significantly increased with N application level due to the weak roots and lower ability to fix N, which was consistent with the results reported by Fan et al. [10]. However, in contrast to their results [10], N application level had no significant influence on N uptake for alfalfa established for two and three years in this study. This might be because no irrigation was applied in their experiments, but irrigation was applied based on the soil moisture in our study. Suitable water application with sprinkler irrigation could improve N_2 fixation and mineralization [47], which could satisfy N uptake and impair the effect of N application for older alfalfa.

The effects of N application on the yield and quality of alfalfa were complex and depended on soil conditions, stand ages and weather conditions [53]. In this study, the relationship between alfalfa yield and N application level could be mostly quantified through quadratic regression. Yield, in the first year, responded strongly to the N application. Compared to the yield under N0 treatment, N application could significantly improve alfalfa yield at the first and second cuttings, with the highest proportion of 44.9% and 75.3% under an N application level of 37.5 kg ha^{-1}, respectively, but only 8.7–19.0% at the third and fourth cuttings in the first year after planting. Similar to our study, results reported by Elgharably and Benes [7] also showed that the increase of yield through N application at the first cutting was higher than that at the third cutting. This might be because the lower nodule number restricted the N_2 fixation and total N derived from the atmosphere [7]. In the second and third years, the yield improved through nitrogen application, which was less than 15.9% of that under N0 treatment at the first and second cuttings but could reach approximately 30% at the third and fourth cuttings. The first two cuttings occurred in the spring and early summer in the North China Plain, where abundant nodules in two-year-old and three-year-old alfalfa, along with suitable temperature and soil moisture, likely enhanced N_2 fixation. However, the period of the third cutting entered the summer with hot and rainy weather, and the fourth cutting occurred during the autumn with low temperatures. Soil temperatures that are too high or too low and waterlogging can reduce N_2 fixation [5,54]. Consequently, the advantage of N application in improving yield was greater at the third and fourth cuttings of two-year- and three-year-old alfalfa in this study. It indicated that N application could improve alfalfa yield when the N_2 fixation was limited. N application might also be recommended for the legume in cold regions, where N_2 fixation could be restricted by cold stress [55,56]. The annual yield of alfalfa for the three years increased with N application compared to yields without N application. This aligns with findings from other studies, indicating that nitrogen application can enhance alfalfa yield during its initial establishment period [4,10]. Although many studies have indicated that N application is not recommended for old alfalfa stands [18,20], applying a small amount of N fertilizer to two- or three-year-old alfalfa stands could increase annual yield due to poor soil fertility in the experimental site. However, N application in the third cutting generally did not increase soil NO_3^- -N content and N leaching loss owing to heavy rain. Meanwhile, the improvement of yield through N application was relatively small at the fourth cutting, suggesting that N application might not be economical. Therefore, N application was not recommended for alfalfa at the third and fourth cuttings in the second and third years despite the active effect on alfalfa yield. The influence of N application level on N use efficiency was significant only in some cuttings in the three years, and N use efficiency generally decreased with N application level. The relationship between ANR/NAE and N application level could be quantified using a linear regression. This was consistent with the conclusions of other researchers [16]. The significantly higher ANR and NAE were only obtained with a nitrogen application level of 12.5 kg N ha^{-1} in the first cutting of the first and second years and the third cutting of the first and third years. Different from the yield, the negative effect of N application on the CP content and RFV of alfalfa appeared at the first and second cuttings of the first year after establishment, and CP and RFV under treatment without N application were generally up to the highest value. It accorded with the result proposed by Feng et al. [30] that alfalfa yield is inversely proportional to the quality. This might be because higher N application during the early growth stage of alfalfa could accelerate cell wall components and fiber quantities, resulting in a reduction in the CP and RFV of alfalfa [57,58]. In the second and third years, the N application improved the CP or RFV during the last two cuttings. The relationship between CP/RFV and N application level could be mostly quantified using quadratic regression. However, N application had an insignificant influence on alfalfa quality classification, which might be due to the fact that the alfalfa variety planted in this study had the advantages of large leaves, slim stems and good palatability [59]. Consequently, a nitrogen application level of 12.5 kg N ha^{-1} is recommended for alfalfa at first cutting in the first year to obtain a higher yield and N use efficiency. Meanwhile, due to the insignificant difference in N use efficiency under the three N application levels and the higher yield and good quality noticed under the N3 treatment, a nitrogen application level of 37.5 kg N ha^{-1} is recommended for alfalfa of the second cutting in the first year. For the two-year-old and three-year-old alfalfa, nitrogen application is not recommended for any cutting, considering economic and environmental factors.

5. Conclusions

After nitrogen fertilizer was applied through sprinkler fertigation, the variation in soil NO_3^--N content was mainly concentrated around the soil depth of 0–40 cm. Fluctuation in soil NO_3^--N content at a depth of 40–80 cm only appeared with an N application level of 25.0–37.5 kg N ha^{-1} during the rainy season. After three years of planting alfalfa, soil NO_3^--N content could be increased. During the three years, N uptake by alfalfa shoot for each year ranged from 290 to 497 kg ha^{-1}. N application only had a noteworthy positive influence on the N uptake amount of one-year-old alfalfa. N application could improve the annual yield of alfalfa during the three years, especially in the first year. Compared to the yield under the N0 treatment, the N application significantly improved the alfalfa yield at the first and second cuttings in the first year after establishment. In the second and third years, the N application level had a significant relationship with the yield at the third and fourth cuttings, and the yield could be increased through N application. The N use efficiency of alfalfa at the first and third cuttings showed significant linear and negative correlations with the N application level and reached the highest under an N application level of 12.5 kg N ha^{-1} during the three years. Mostly, the N application level had no remarkable relationship with the N use efficiency during the other cuttings. Compared to alfalfa quality under N0 treatment, N application had negatively affected the alfalfa quality in the first year but had a positive influence during the second and third years. However, the N application had minimal effect on the quality classification of alfalfa during the three years. Overall, the response to N application was strongest for alfalfa yield, followed by ANR and NAE, as well as quality. For obtaining the high yield, quality and N use efficiency of alfalfa planted in the North China Plain with sub-humid climate, N application levels of 12.5 and 37.5 kg N ha^{-1} are recommended for alfalfa of the first and second cuttings, respectively, and alfalfa at the third and fourth cuttings does not require N application in the first year after establishment. N application is not recommended for alfalfa in the second and third year after establishment.

Author Contributions: During this study, H.Y. and Y.W. conceived and designed the experiments; Y.W. and M.L. performed experiments; Y.W., M.L. and J.G. analyzed the data; Y.W., M.L. and H.Y. wrote and revised the manuscript. Overall, Y.W. and M.L. equally contributed to this paper and should be regarded as co-first authors. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially supported by the National Key R&D Program of China (2022YFD1300804), the China Agriculture Research System of MOF and MARA (CARS-34), and the National Natural Science Foundation of China (51939005).

Data Availability Statement: All data generated or analyzed during this study contained within this article and are available upon reasonable request.

Conflicts of 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.

References

1. National Animal Husbandry Station of China. *Forage Statistics in China*; China Agriculture Press: Beijing, China, 2021; p. 70.
2. Darrell, A.M. *Forage Crops*; McGRAW-HILL Book Company: New York, NY, USA, 1984; p. 172.
3. Ntuall, W.F. Effect of N, P, and S fertilizers on alfalfa grown on three soil types in northeastern Saskatchewan. II. Nitrogen, P and S uptake and concentration in herbage. *Agron. J.* **1985**, *77*, 224–228. [CrossRef]
4. Hannaway, D.B.; Shuler, P.E. Nitrogen Fertilization in Alfalfa Production. *J. Prod. Agric.* **1993**, *6*, 80–85. [CrossRef]
5. Liu, Y.; Wu, L.; Baddeley, J.A.; Watson, C.A. Models of Biological Nitrogen Fixation of Legumes. A Review. *Agron. Sustain. Dev.* **2011**, *31*, 155–172. [CrossRef]
6. FAO. *Legume Inoculants and Their Use*; FAO Fertilizer and Plant Nutrition Service; FAO: Rome, Italy, 1984.
7. Elgharably, A.; Benes, S. Alfalfa biomass yield and nitrogen fixation in response to applied mineral nitrogen under saline soil conditions. *J. Soil Sci. Plant Nut.* **2021**, *21*, 744–755. [CrossRef]
8. Helalia, A.M.; Al-Tapir, O.A.; Al-Nabulsi, Y.A. The influence of irrigation water salinity and fertilizer management on the yield of Alfalfa (*Medicago sativa* L.). *Agric. Water Manag.* **1996**, *31*, 105–114. [CrossRef]
9. Bélanger, G.; Richards, J.E. Dynamics of biomass and N accumulation of alfalfa under three N fertilization rates. *Plant Soil* **2000**, *219*, 177–185. [CrossRef]
10. Fan, J.; Du, Y.; Wang, B.; Turner, N.C.; Wang, T.; Abbott, L.K.; Stefanova, K.; Siddique, K.H.M.; Li, F. 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.* **2016**, *198*, 247–257. [CrossRef]
11. Pucek, T.R.; Pyš, J.B. The nutritive value and yield of alfalfa in relation to nitrogen fertilization on sulphur industry reclaimed lands. *J. Agron. Crops Sci.* **1996**, *177*, 295–303. [CrossRef]
12. Aranjuelo, I.; Molero, G.; Erice, G.; Aldasoro, J.; Arrese-Igor, C.; Nogués, S. Effect of shoot removal on remobilization of carbon and nitrogen during regrowth of nitrogen-fixing alfalfa. *Physiol. Plant* **2015**, *153*, 91–104. [CrossRef] [PubMed]
13. Gao, L.; Su, J.; Tian, Q.; Shen, Y. Contrasting strategies of nitrogen absorption and utilization in alfalfa plants under different water stress. *J. Soil Sci. Plant Nut.* **2020**, *20*, 1515–1523. [CrossRef]
14. Raun, W.R.; Johnson, G.V.; Phillips, S.B.; Thomason, W.E.; Dennis, J.L.; Cossey, D.A. Alfalfa yield response to nitrogen applied after each cutting. *Soil. Sci. Soc. Am. J.* **1999**, *63*, 1237–1243. [CrossRef]
15. He, F.; Xie, K.; Li, X. Effect of nitrogen fertilizer and seeding rate on yield of alfalfa and weeds. *Pol. J. Environ. Stud.* **2018**, *27*, 647–653. [CrossRef]
16. Kamran, M.; Yan, Z.; Jia, Q.; Chang, S.; Ahmad, I.; Ghani, M.U.; Hou, F. Irrigation and nitrogen fertilization influence on alfalfa yield, nutritive value, and resource use efficiency in an arid environment. *Field Crops Res.* **2022**, *284*, 108587. [CrossRef]
17. Li, M.; Wang, Y.; Adeli, A.; Yan, H. Effects of application methods and urea rates on ammonia volatilization, yields and fine root biomass of alfalfa. *Field Crops Res.* **2018**, *218*, 115–125. [CrossRef]
18. Oliveira, W.S.D.; Oliveira, P.P.A.; Corsi, M.; Rodrigo, F.; Duarte, S.; Tsai, S.M. Alfalfa yield and quality as function of nitrogen fertilization and symbiosis with *Sinorhizobium meliloti*. *Sci. Agric.* **2004**, *61*, 433–438. [CrossRef]
19. Zhu, Y.; Sheaffer, C.C.; Vance, C.P.; Graham, P.H.; Russelle, M.P.; Montealegre, C.M. Inoculation and nitrogen affect herbage and symbiotic properties of annual medicago species. *Agron. J.* **1998**, *90*, 781–786. [CrossRef]
20. Xie, K.; Li, X.; He, F.; Zhang, Y.; Wan, L.; David, B.H.; Wang, D.; Qin, Y.; Gamal, M.A.F. 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.* **2015**, *14*, 1864–1876. [CrossRef]
21. Wivstad, M.; Mårtensson, A.M.; Ljunggren, H.D. Field measurement of symbiotic nitrogen fixation in an established lucerne ley using 15N and an acetylene reduction method. *Plant Soil* **1987**, *97*, 93–104. [CrossRef]

22. Zhao, J.; Huang, R.; Wang, X.; Ma, C.; Li, M.; Zhang, Q. Effects of combined nitrogen and phosphorus application on protein fractions and nonstructural carbohydrate of alfalfa. *Front. Plant Sci.* **2023**, *14*, 1124664. [CrossRef] [PubMed]
23. Chase, L.E.; Long, T.A.; Washko, J.B.; Baumgardt, B.R. Effect of nitrogen fertilization on constituents of alfalfa. *J. Dairy. Sci.* **1976**, *59*, 170–174. [CrossRef]
24. Cherney, D.J.R.; Cherney, J.H.; Siciliano-Jones, J. Alfalfa composition and in sacco fiber and protein disappearance as influenced by nitrogen application. *J. Appl. Anim. Res.* **1995**, *8*, 105–120. [CrossRef]
25. Berça, A.S.; Cardoso, A.D.S.; Longhini, V.Z.; Tedeschi, L.O.; Boddey, R.M.; Reis, R.A.; Ruggieri, A.C. Protein and carbohydrate fractions in warm-season pastures: Effects of nitrogen management strategies. *Agronomy* **2021**, *11*, 847. [CrossRef]
26. Vanderwalt, J.G. Nitrogen-metabolism of the ruminant liver. *Aust. J. Agric. Res.* **1993**, *44*, 381–403. [CrossRef]
27. Lapierre, H.; Lobley, G.E. Nitrogen recycling in the ruminant: A review. *J. Dairy Sci.* **2001**, *84*, 223–236. [CrossRef]
28. Justes, E.; Thiebeau, P.; Avice, J.C.; Lemaire, G.; Volenec, J.J.; Ourry, A. Influence of summer sowing dates, N fertilization and irrigation on autumn VSP accumulation and dynamics of spring regrowth in alfalfa (*Medicago sativa* L.). *J. Exp. Bot.* **2002**, *53*, 111–121. [CrossRef]
29. Rohweder, D.A.; Barnes, R.F.; Jorgensen, N. Proposed hay grading standards based on laboratory analyses for evaluating quality. *J. Anim. Sci.* **1978**, *47*, 747–759. [CrossRef]
30. Feng, Y.; Shi, Y.; Zhao, M.; Shen, H.; Xu, L.; Luo, Y.; Liu, Y.; Xing, A.; Kang, J.; Jing, H.; et al. Yield and quality properties of alfalfa (*Medicago Sativa* L.) and their influencing factors in China. *Eur. J. Agron.* **2022**, *141*, 126637. [CrossRef]
31. Chen, B.; Ren, C.; Wang, C.; Duan, J.; Reis, S.; Gu, B. Driving forces of nitrogen use efficiency in Chinese croplands on county scale. *Environ. Pollut.* **2023**, *316*, 120610. [CrossRef]
32. Zhang, X.; Davidson, E.A.; Mauzerall, D.L.; Searchinger, T.D.; Dumas, P.; Shen, Y. Managing nitrogen for sustainable development. *Nature* **2015**, *528*, 51–59. [CrossRef]
33. Li, Y.; Su, D. Alfalfa water use and yield under different sprinkler irrigation regimes in north arid regions of China. *Sustainability* **2017**, *9*, 1380. [CrossRef]
34. Gascho, G.J.; Hook, J.E.; Mitchell, G.A. Sprinkler-applied and side-dressed nitrogen for irrigated corn grown on sand. *Agron. J.* **1984**, *76*, 77–81. [CrossRef]
35. O'Shaughnessy, S.A.; Evett, S.R.; Andrade, M.A.; Workneh, F.; Price, J.A.; Rush, C.M. Site-specific variable-rate irrigation as a means to enhance water use efficiency. *Trans. ASABE* **2016**, *59*, 239–249. [CrossRef]
36. Schepers, J.S.; Varvel, G.E.; Watts, D.G. Nitrogen and water management strategies to reduce nitrate leaching under irrigated maize. *J. Contam. Hydrol.* **1995**, *20*, 227–239. [CrossRef]
37. Walter, C.B. Impact of residual soil nitrate on in-season nitrogen applications to irrigated corn based on remotely sensed assessments of crop nitrogen status. *Precis. Agric.* **2005**, *6*, 509–519. [CrossRef]
38. Stone, K.C.; Camp, C.R.; Sadler, E.J. Corn yield response to nitrogen fertilizer and irrigation in the Southeastern Coastal Plain. *Appl. Eng. Agric.* **2010**, *26*, 429–438. [CrossRef]
39. Yan, H.; Ma, J.; Wang, Z. Design and field experiment on fertilizer injection device in center pivot irrigation system. *Trans. Chin. Soc. Agric. Mach.* **2015**, *46*, 100–106, (In Chinese with English Abstract).
40. Allen, R.G.; Pereira, L.S.; Raes, D.; Smith, M. Crop evapotranspiration: Guidelines for computing crop water requirements. In *Irrigation and Drainage Paper No. 56*; United Nations FAO: Rome, Italy, 1998; pp. 24–28.
41. Congreves, K.A.; Otchere, O.; Ferland, D.; Farzadfar, S.; Williams, S.; Arcand, M.M. Nitrogen use efficiency definitions of today and tomorrow. *Front. Plant Sci.* **2021**, *12*, 637108. [CrossRef]
42. Shenk, J.S.; Westerhaus, M.O. The application of near infrared reflectance spectroscopy (NIRS) to forage analysis. In *Forage Quality, Evaluation, and Utilization*; Fahey, G.C., Jr., Ed.; JohnWiley & Sons: Hoboken, NJ, USA, 1994; pp. 406–449. [CrossRef]
43. Yang, W.; Jiao, Y.; Yang, M.; Wen, H.; Gu, P.; Yang, J.; Liu, L.; Yu, J. Minimizing soil nitrogen leaching by changing furrow irrigation into sprinkler fertigation in potato fields in the Northwestern China Plain. *Water* **2020**, *12*, 2229. [CrossRef]
44. Lv, G.; Kang, Y.; Li, L.; Liu, S. Nutrient distribution, growth, and water use efficiency in maize following winter wheat irrigated by sprinklers or surface irrigation. *Irrig. Drain.* **2011**, *60*, 338–347. [CrossRef]
45. Li, Y.; Huang, G.; Chen, Z.; Xiong, Y.; Huang, Q.; Xu, X.; Huo, Z. Effects of irrigation and fertilization on grain yield, water and nitrogen dynamics and their use efficiency of spring wheat farmland in an arid agricultural watershed of Northwest China. *Agric. Water Manag.* **2022**, *260*, 107277. [CrossRef]
46. Liu, X.; Ju, X.; Zhang, F.; Pan, J.; Christie, P. Nitrogen dynamics and budgets in a winter wheat–maize cropping system in the North China Plain. *Field Crops Res.* **2003**, *83*, 111–124. [CrossRef]
47. Barakat, M.; Cheviron, B.; Angulo-Jaramillo, R. Influence of the irrigation technique and strategies on the nitrogen cycle and budget: A review. *Agric. Water. Manag.* **2016**, *178*, 225–238. [CrossRef]
48. Bengtsson, G.; Bengtson, P.; Månsson, K.F. Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio andmicrobial activity. *Soil Biol. Biochem.* **2003**, *35*, 143–154. [CrossRef]
49. Pulleman, M.; Tietema, A. Microbial C and N transformations during drying and rewetting of coniferous forest floor material. *Soil Biol. Biochem.* **1999**, *31*, 275–285. [CrossRef]
50. Issah, G.; Schoenau, J.J.; Lardner, H.A.; Knight, J.D. Nitrogen fixation and resource partitioning in alfalfa (*Medicago sativa* L.), cicer milkvetch (*Astragalus cicer* L.) and sainfoin (*Onobrychis viciifolia* Scop.) using ^{15}N enrichment under controlled environment conditions. *Agronomy* **2020**, *10*, 1438. [CrossRef]

51. Walley, F.L.; Tomm, G.O.; Matus, A.; Slinkard, A.E.; van Kessel, C. Allocation and cycling of nitrogen in an alfalfa-bromegrass sward. *Agron. J.* **1996**, *88*, 834–843. [CrossRef]
52. Wu, W.; Liu, M.; Wu, X.; Wang, Z.; Yang, H. Effects of deficit irrigation on nitrogen uptake and soil mineral nitrogen in alfalfa grasslands of the inland arid area of China. *Agric. Water Manag.* **2022**, *269*, 107724. [CrossRef]
53. Wang, L.; Xie, J.; Luo, Z.; Niu, Y.; Coulter, J.A.; Zhang, R.; Li, L. Forage yield, water use efficiency, and soil fertility response to alfalfa growing age in the semiarid Loess Plateau of China. *Agric. Water Manag.* **2021**, *243*, 106415. [CrossRef]
54. Havelka, U.D.; Boyle, M.G.; Hardy, R.W.F. Biological nitrogen fixation. In *Nitrogen in Agricultural Soils*; Stevenson, F.J., Ed.; ASA: Madison, WI, USA, 1982; pp. 365–422.
55. Thurston, C.L.; Grossman, J.M.; Fudge, R.; Maul, J.E.; Mirsky, S.; Wiering, N. Cold stress reduces nodulation and symbiotic nitrogen fixation in winter annual legume cover crops. *Plant Soil* **2022**, *481*, 661–676. [CrossRef]
56. Alexandre, A.; Oliveira, S. Response to temperature stress in rhizobia. *Crit. Rev. Microbiol.* **2012**, *39*, 219–228. [CrossRef] [PubMed]
57. Liu, M.; Wang, Z.; Mu, L.; Xu, R.; Yang, H. Effect of regulated deficit irrigation on alfalfa performance under two irrigation systems in the inland arid area of midwestern China. *Agric. Water Manag.* **2021**, *248*, 106764. [CrossRef]
58. Islam, M.R.; Garcia, S.C. 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.* **2012**, *67*, 96–110. [CrossRef]
59. Li, Y.; Wu, F.; Shi, S.; Bai, X. Evaluation on production and nutritional value of 13 introduced alfalfa cultivars in Hexi corridor of Gansu Province. *Agric. Res. Arid Areas* **2019**, *37*, 119–129. (In Chinese with English Abstract)