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Effect of Nitrogen Application and Cutting Frequency on the Yield and Forage Quality of Alfalfa in Seasonal Cultivation

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Abstract: Although nitrogen application and cutting frequency (CF) are two important factors affecting forage productivity and quality, their effects on alfalfa (*Medicago sativa* L.), particularly in humid areas, remain less understood. Here, we investigated the fertilization and cutting regimes for seasonal alfalfa cultivation in humid areas in southern China. Treatments performed over a 2-year period were of a split-plot design with four N application rates (60, 120, 180, and 240 kg N ha^{-1}) and three CFs (five, four, and three times.). After cutting, forage components, yield, and quality were measured. In both 2-year cutting cycles, the effects of N application × CF interactions on forage yield and quality were non-significant. N application and CFs influenced plant height, mass shoot^{-1}, leaf area shoot^{-1}, and shoots plant^{-1}. CF had remarkable effects on forage quality under different N applications, with forage cut five times having the best nutritive value and quality. However, neutral and acid detergent fiber contents were lower than when cutting three times, and produced the lowest yields. Forage cut four times had the highest in vitro digestible dry matter. In conclusion, to obtain high yields and desirable quality, the application of 180 kg N ha^{-1} and cutting three to four times in spring could be a suitable strategy for alfalfa forage production during seasonal cultivation in humid areas of southern China.

Keywords: alfalfa; seasonal cultivation; nitrogen; cutting frequency; forage yield and quality

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1. Introduction

Alfalfa (*Medicago sativa* L.), which is noted for its high protein content, is considered the most important forage crop worldwide. Seasonal cultivation is a rapid forage cultivation method used in short-term fallow fields during the winter and spring seasons, and in recent years has been widely practiced in southern China. Factors that have contributed to this trend are as follows. (i) With the adjustment of agricultural structure in southern China, many farmlands remain fallow during winter after planting rice. Seasonal cultivation can optimize and take full advantage of the remaining land allocation [1]. (ii) With rapid economic development, the scarcity of forage has tended to restrict livestock husbandry, and most of the total forage consumed in China is imported. The seasonal cultivation of alfalfa is considered a fundamental method to alleviate this shortfall [2]. (iii) In subtropical areas, the summer climate is hot and humid, conditions that are unsuitable for alfalfa growth. Seasonal cultivation of alfalfa can contribute to avoiding this problem and reduces the competition with weeds during the seedling stage [3]. (iv) Alfalfa cultivation occurs in a rotation system with good nitrogen (N) fixation, which is beneficial for the subsequent growth of crops such as rice and corn [4]. Hence, seasonal cultivation, particularly that of alfalfa, is destined to become a major objective for sustaining the further development of forage production in southern China.

The cultivation of forage alfalfa is of particular importance in agriculture, in that by fixing N_2, these plants contribute to replenishing the stocks of soil N, and also serve as an important source of feedstuff for livestock and poultry. The response of alfalfa to N fertilization has received considerable scientific attention. Biological N_2 fixation and N fertilizers are the two main sources that meet the nitrogen requirements for high yields. Many studies on N_2 fixation in alfalfa have shown that it is difficult to draw a general conclusion regarding the response of alfalfa to N fertilization [5,6], with some research providing evidence indicating that the use of N fertilizer at seeding may not be justified [7] and will promote weeds and influence crop growth, thereby subjecting alfalfa seedlings to excessive interspecific competition. In addition, the early application of small amounts of nitrogen fertilizer or superfluous application during the growth period often results in the suppression of both root nodule establishment and subsequent activity [8]. According to Lindström [9], prior to establishment of the legume plant–rhizobia symbiosis, host plant development and N-fixing activity could be inhibited by moderate or high levels of combined N. Conversely, a considerable volume of research on alfalfa has indicated that combined N application can have beneficial effects with respect to enhancing forage yield [10–13]. During the initial rapid growth period of alfalfa and after cutting, the application of N in an appropriate environment should theoretically increase yield by promoting shoot re-growth and nutrient accumulation. In this regard, Nuttall [14] found that compared with no N application, a single application (45 kg N ha^{-1}) during early spring promoted a significant increase in alfalfa dry matter production, although this practice was deemed to be uneconomical. Likewise, post-cutting, alfalfa should retain a canopy that enables full light interception, and N application should increase the storage of N in leaves to maintain the photosynthetic apparatus for converting incoming radiation to new biomass and, eventually, forage yield. Furthermore, N fertilization can improve the quality of alfalfa hay, although the increment is sometimes limited and the practice uneconomical [15,16].

The frequency with which alfalfa is cut is an important factor that influences both crop productivity and forage quality, and numerous studies have reported that excessive cutting reduces alfalfa yield [17,18]. Frequent cutting invariably shortens the alfalfa re-growth period, thereby leading to a reduction in shoot mass and resulting in a low dry matter (DM) yield, and it also contributes to a reduction in the survival of alfalfa shoots [19]. It has been established that maximum DM yields can be obtained by combining a low frequency of cutting with a long intervening period between cuts, which could be attributable to the fact that depending on the specific environment and management, frequently harvested alfalfa plants may not be able to replenish the carbohydrates required for new growth, and excessive cutting may even cause fatal damage to the root stubble [20]. The nutrient content of feed is largely dependent on the growth stage when plants are cut and the time of harvest. The quality of alfalfa forage declines markedly with advancing maturity and is associated with an increase in the stem to leaf ratio. A moderate frequency of cutting may thus be more beneficial, in that it tends to be conducive to the production of tender alfalfa plants with increased forage crude protein (CP) and crude ash contents, lower crude fiber contents, and consequently higher quality [21,22].

Numerous previous studies have examined the effects of different N applications and cutting frequencies (CF) [23,24]. However, with respect to humid environments, comparatively little is known regarding the field yield and forage quality of alfalfa in response to different N application and CF. Previous studies have shown that in humid environments, such as those characterizing southern China, high precipitation and the clayey loam and acidic soil may lead to the abnormal growth of alfalfa root nodules and low availability of soil N, and, under these conditions, the amounts of N fixed by alfalfa are insufficient to compensate for the N removed from the field with the harvesting of shoots [25,26]. Accordingly, the application of N fertilizer may be necessary to promote alfalfa seedling development and re-growth after successive cuttings [11,14]. In previous studies, we evaluated the maximal herbage production of alfalfa in response to cutting two times (the general condition) above ground in southern China [27]. However, under the favorable temperature and moisture conditions during spring in this area, alfalfa grows rapidly, and, consequently, a low frequency of cutting would be unable to prevent alfalfa from lodging, resulting in advanced maturation, and would potentially lead to the development of root rot. Accordingly, the establishment of appropriate cutting schedules is required to guarantee sufficient alfalfa yields and quality. Our primary objectives in this study were (i) to evaluate the effect of N application and CF on alfalfa productivity and forage quality, and (ii) to characterize forage yield components and the nutritive composition of alfalfa in response to different CFs under different N applications to obtain the optimum alfalfa yields and quality. Given that there is currently limited information to guide the development of fertilization and defoliation in moist areas, such as southern China, the study was conducted to determine the response of alfalfa to N fertilization and different cutting frequencies to establish an appropriate regime for the seasonal production of alfalfa forage in humid areas.

2. Materials and Methods

2.1. Experimental Site and Meteorological Information

Field studies were conducted in 2013 and 2014 to determine the response of alfalfa to N fertilization and CF during seasonal cultivation (Figure 1). The experiment was carried out at the Jiangsu Academy of Agricultural Sciences (118°46′ E 32°03′ N). The region is characterized by a subtropical monsoon climate, with a mean annual precipitation of 1100 mm and an average temperature of 15.7 °C (Figure 2). Precipitation during the growth stage (from March to May) in 2013 exceeded that during the same period in 2014. The soil in this area is a free-draining sand-clay loam; the soil properties are presented in Table 1. The field used for the purposes of this study was previously under corn cultivation.

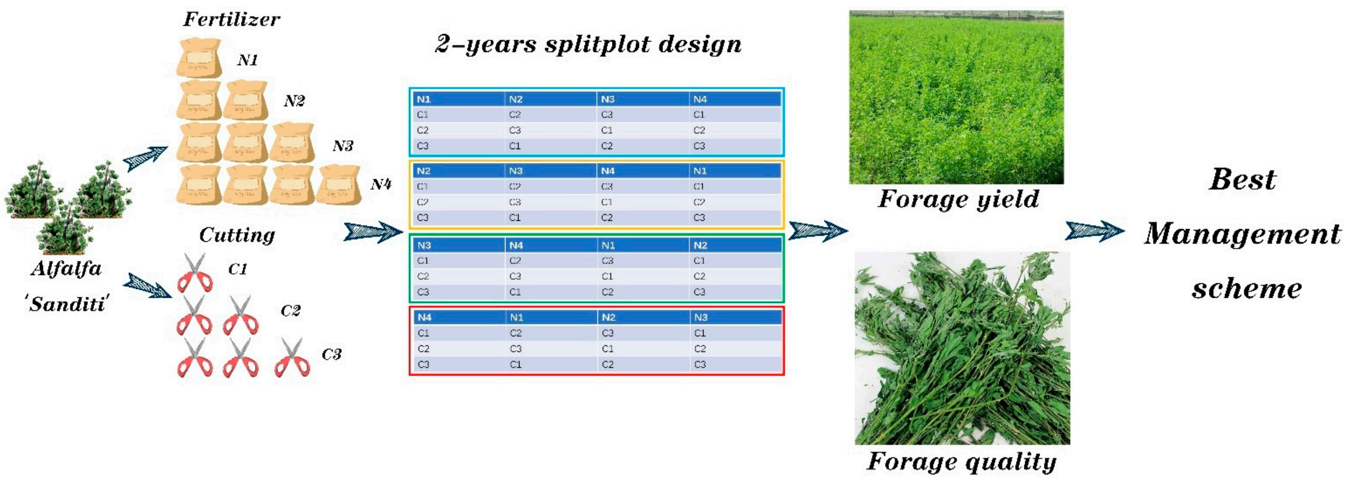

Figure 1. Experimental design for the whole study.

Table 1. Soil physiochemical properties.

Field Soil Properties	JAAS [a]	JAAS [b]
Organic matter (%)	2.26	2.43
pH	6.97	6.89
Total nitrogen (%)	0.119	0.173
Available nitrogen (mg kg^{-1})	6.68	7.34
Available phosphorus (mg kg^{-1})	68.60	78.69
Available potassium (mg kg^{-1})	117.54	120.56

[a] Jiangsu Academy of Agricultural Sciences Site A. [b] Jiangsu Academy of Agricultural Sciences Site B.

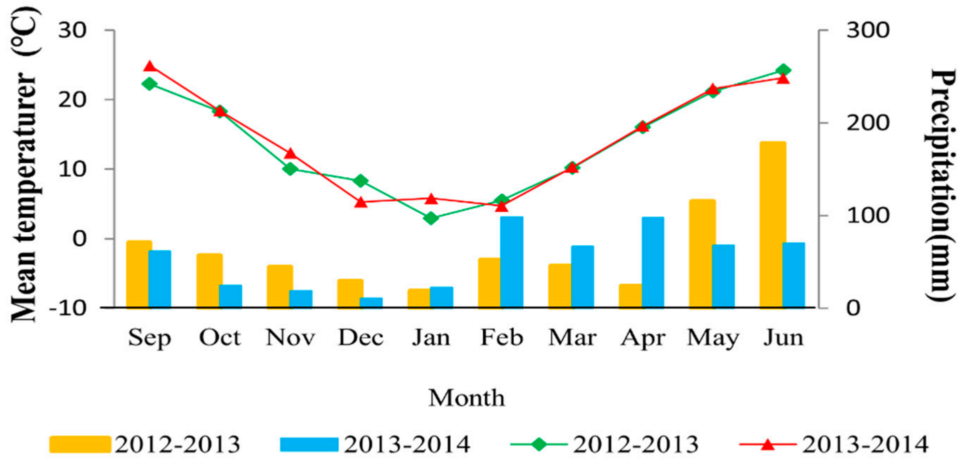

Figure 2. Mean temperature and precipitation monthly in experimental period of September–June from 2012−2014.

2.2. Experimental Design

The field experiment was based on a split-plot design with 12 plots in each of 3 blocks. The main plot was nitrogen applications, and the subplot was cutting regimes. Each plot was 10 m^2 (2 m × 5 m) in area. Alfalfa (cv. Sanditi, Barenbrug Beijing International Grass Co., Ltd., Beijing, China) was sown on 25 September 2012, and 28 September 2013, for each year of experiments, in 5 m long rows with an inter-row spacing of 0.3 m. The seeding rate was 22.5 kg ha^{-1}. The area surrounding the experimental plots was sown with the guard rows to protect the experimental rows from other factors. The experimental period extended from September to June of the following year, with cutting treatments being performed from April to June. During this stage, we assessed CFs involving five, four, and three shoot re-growth cycles of 15, 20, and 30 days, respectively. Nitrogen fertilizer [urea (46%N)] was applied at 60, 120, 180, or 240 kg N ha^{-1}, with 10% of the fertilizer being applied during the initial phase of growth in early spring (air temperature approximately 5 °C), and with the application of the remainder being split equally after each cutting (Figures 3, S1 and S2). Herbicide application was scheduled each year prior to sowing.

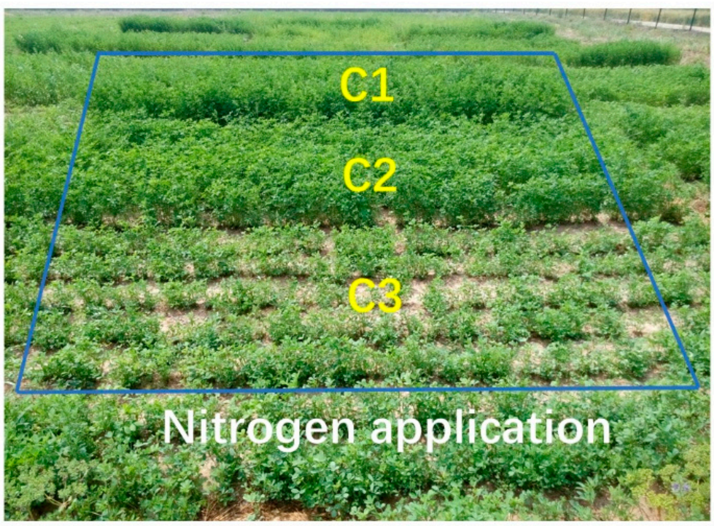

Figure 3. Experiment profile of N application and cutting frequency in the field.

2.3. Sample Collection

Plants in each plot were hand-harvested, leaving cut plants with 5 cm of stubble. Plant height, shoot $plant^{-1}$ (number of branches), and fresh weight were measured immediately after harvesting in the field. Alfalfa samples collected from a 1 m^2 area in each plot were returned to the laboratory. Leaf area plant^{-1} was measured from five plants per treatment using an LI−3000C Portable Leaf Area Meter. Forage dry matter yield (kg DM ha^{-1}) was calculated using samples dried to a constant weight in a forced-air oven at 60 °C. Five of the harvested plants were divided into leaves and stems and dried to calculate mass shoot^{-1} and stem/leaf ratio. Total forage yield was calculated as the sum of each cutting harvest in both years.

2.4. Chemical Analyses

The remainder of samples were dried to a constant weight at a temperature of 65 °C in an aerated oven, and then ground through a 1 mm sieve for compositional analysis. Crude protein (CP) content was determined using the Kjeldahl method [28]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined as described by Van Soest et al. [29]. The in vitro dry matter digestibility (IVDMD) of each treatment was analyzed based on a two-stage fermentation technique described by Tilley and Terry [30], as modified by Goto and Minson [31].

In vitro digestibility of dry matter (IVDDM) was calculated using the following formula:

$$\text{IVDDM} = \text{DM} \times \text{IVDMD}$$

Relative feed value (RFV) was calculated using the following formulae [32]:

$$\text{Dry Matter Intake (DMI) (\%)} = 120/(\text{NDF})$$

$$\text{Dry Matter Digestibility (DMD) (\%)} = 88.9\text{–}0.779\ (\text{ADF})$$

$$\text{RFV} = \text{DMI} \times \text{DMD}/1.29$$

2.5. Statistical Analysis

The study was conducted over two consecutive years (2013 and 2014). Split-plot analyses of variance (ANOVA) were carried out to test the effects of nitrogen application rate and cutting regime on the agronomy trait, forage yield, and nutritive paraments of alfalfa. The main plot factor was the nitrogen application rate. The cutting regime, as the subplot factor, was completely randomized. The differences between the means of alfalfa agronomy characteristic and nutritive values were tested using the least significant difference (LSD) test at the $p < 0.05$ level. All statistical analyses were performed using SPSS version 17.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Forage Yield Components

In both study years, the effects of N application × CF interaction on forage yield component parameters were found to be non-significant ($p > 0.05$) (Table 2). In general, height plant^{-1} made the most substantial contribution to forage yield. For all cutting treatments, we found that in both years, alfalfa height plant^{-1} increased gradually with an increase in N application rate, reaching a maximum value of 57.81 cm in response to the application of 180 kg N ha^{-1}, although we detected a significant decrease between the effects of 180 and 240 kg N ha^{-1} in 2013 ($p < 0.05$) (Table 2). Similarly, we detected no significant difference in height plant^{-1} among N application treatments under different cutting schedules in 2014 ($p > 0.05$). CF had a significant effect on height plant^{-1} ($p < 0.05$). Shoots collected from plots cut three times were found to be significantly taller than those collected from plots cut four or five times, indicating that a higher frequency of cutting results in an insufficiently long interval for re-growth. Significant interactions were found for Year × N application for height plant^{-1}.

Table 2. Plant height, mass shoot^{-1}, and leaf area shoot^{-1} of alfalfa under different nitrogen application and cutting frequency in 2013 and 2014.

Nitrogen Application (kg N ha^{-1})	Cutting Frequency	Plant Height (cm)		Leaf Area Shoot^{-1} (cm^2)		Mass Shoot^{-1} (g DM)	
		2013	2014	2013	2014	2013	2014
60	Three	53.12 ± 1.26 b	58.82 ± 3.26 a	310.22 ± 20.36 a	395.96 ± 11.78 a	0.956 ± 0.13 ab	0.967 ± 0.09 ab
	Four	40.86 ± 2.30 cd	42.27 ± 1.36 b	207.79 ± 18.32 bc	315.89 ± 14.76 b	0.613 ± 0.08 c	0.652 ± 0.12 c
	Five	34.98 ± 1.25 f	35.59 ± 2.54 c	178.42 ± 8.78 c	253.52 ± 25.36 d	0.579 ± 0.11 c	0.639 ± 0.07 c
120	Three	55.62 ± 3.87 ab	62.04 ± 1.21 a	350.86 ± 15.36 a	400.62 ± 27.32 a	1.119 ± 0.20 a	1.130 ± 0.15 a
	Four	41.35 ± 4.21 c	43.87 ± 0.99 b	242.73 ± 6.87 b	319.08 ± 19.12 bc	0.685 ± 0.15 bc	0.737 ± 0.06 bc
	Five	35.35 ± 2.65 f	37.42 ± 1.54 c	210.09 ± 22.58 bc	283.19 ± 12.34 cd	0.583 ± 0.04 c	0.656 ± 0.14 c
180	Three	57.81 ± 3.20 a	65.59 ± 2.65 a	369.08 ± 19.63 a	418.85 ± 37.12 a	1.161 ± 0.09 a	1.172 ± 0.20 a
	Four	42.50 ± 1.36 c	46.51 ± 1.87 b	250.36 ± 20.68 b	333.79 ± 21.78 b	0.744 ± 0.15 bc	0.754 ± 0.07 bc
	Five	37.25 ± 0.98 ef	36.92 ± 3.54 c	219.34 ± 15.87 bc	292.44 ± 18.78 bcd	0.620 ± 0.23 c	0.669 ± 0.17 c
240	Three	54.03 ± 2.98 b	64.89 ± 2.87 a	365.85 ± 9.87 a	416.32 ± 23.78 a	1.040 ± 0.14 a	1.051 ± 0.17 a
	Four	39.34 ± 2.12 de	45.90 ± 3.78 b	248.98 ± 19.78 b	317.83 ± 34.78 bc	0.692 ± 0.07 bc	0.724 ± 0.09 bc
	Five	36.21 ± 1.38 f	36.56 ± 1.87 c	217.57 ± 31.25 bc	286.01 ± 10.74 bcd	0.557 ± 0.15 c	0.649 ± 0.14 c
Year (Y)		**		*		NS	
Nitrogen application (NA)		**		NS		*	
Cutting frequency (CF)		***		***		***	
Y × NA		**		NS		NS	
Y × CF		NS		NS		NS	
NA × CF		NS		NS		NS	
Y × NA × CF		NS		NS		NS	

Means with different lowercase letters were significantly different at $p < 0.05$. NS, non-significant at 0.05 level of probability; * significant at the 0.05 level of probability; ** significant at the 0.01 level of probability; *** significant at the 0.001 level of probability.

Alfalfa forage yield can also be described in terms of the product of three other plant components, namely leaf area plant^{-1}, mass shoot^{-1}, and shoot plant^{-1}. For each of the three cutting schedules, we found that although plants in plots treated with high N application had greater leaf area plant^{-1} than those receiving a low rate of N application, the effects of N were not significant in either year ($p > 0.05$) (Table 2). Irrespective of N application, cutting three times resulted in a larger leaf area plant^{-1} than in the other two CF treatments, whereas cutting five times resulted in the smallest area ($p < 0.05$).

Mass shoot^{-1} has been established to be the most important component of forage yield and can be reflected by height plant $^{-1}$ and leaf area plant^{-1}. In both years, we detected a significant effect of CF on mass shoot^{-1} from three cuts for each N application treatment ($p < 0.05$), with a maximum value of 1.161 g and 1.172 g DM being recorded in plots receiving application of 180 kg N ha^{-1} in 2013 and 2014, respectively (Table 2). Cutting three times resulted in the highest mass shoot^{-1}, cutting five times the lowest, and cutting four times resulted in an intermediate mass shoot^{-1}. In 2013, the values in the plots cut three times averaged 50.56% and 65.18% higher than in those cut four and five times, respectively, whereas, in 2014, the values were 56.41% and 82.76% higher, respectively. However, in both years, we detected no significant difference between four and five cuts in plots treated with 60 kg N ha^{-1} ($p > 0.05$).

Shoot plant^{-1} is positively associated with forage yield, and, in the present study, we found that the number of shoots in alfalfa plants increased with increasing N fertilization, with the number in plants treated with 180 kg N ha^{-1} being significantly higher than those in plants treated with 60 kg N ha^{-1} in both years ($p < 0.05$) (Table 3). However, we detected no significant difference ($p > 0.05$) between plants receiving the 180 and 240 kg N ha^{-1} treatments in 2014. CF was found to have a significant effect on shoot plant^{-1}; however, the values obtained showed an opposite trend to those recorded for the other three yield components, with a greater frequency of cutting giving rise to a larger number of shoots per plant, with the highest number of 5.09 in 2013 and 5.23 in 2014 being recorded in plots cut five times. In 2013, we detected no significant differences in the number of shoots per plant in 60 and 120 kg N ha^{-1} plots between different cutting times ($p > 0.05$), whereas no significant differences were detected between 180 and 240 kg N ha^{-1}-fertilized plots between different cutting times in 2014 ($p > 0.05$).

Table 3. Shoots plant^{-1} and dry matter yield of alfalfa under different nitrogen application and cutting frequency in 2013 and 2014.

Nitrogen Application (kg N ha^{-1})	Cutting Frequency	Shoots Plant^{-1}		DM Yield (t ha^{-1})	
		2013	2014	2013	2014
60	Three	3.55 ± 0.50 e	4.17 d ± 0.41 d	10.02 ± 0.87 bc	10.62 ± 0.65 cde
	Four	3.67 ± 0.28 e	4.53 ± 0.83 bcd	9.18 ± 0.41 cd	10.43 ± 0.36 de
	Five	4.12 ± 0.27 de	4.89 ± 0.34 ab	8.45 ± 0.41 d	9.89 ± 0.71 e
120	Three	3.99 ± 0.15 cd	4.39 ± 0.35 cd	10.37 ± 0.62 abc	10.93 ± 0.36 bcd
	Four	4.32 ± 0.26 cd	4.85 ± 0.41 abc	9.82 ± 0.41 c	10.95 ± 1.04 bcd
	Five	4.41 ± 0.41 bcd	5.10 ± 0.82 a	9.27 ± 0.36 cd	10.58 ± 0.64 cde
180	Three	4.47 ± 0.52 bc	4.86 ± 0.66 abc	11.44 ± 0.74 a	12.02 ± 0.87 a
	Four	4.91 ± 0.32 ab	5.08 ± 0.36 a	11.09 ± 0.65 ab	11.73 ± 0.63 ab
	Five	5.09 ± 0.47 a	5.23 ± 0.54 a	9.59 ± 0.36 cd	10.79 ± 0.74 bcde
240	Three	4.35 ± 0.32 cd	4.86 ± 0.18 abc	11.19 ± 0.85 ab	11.56 ± 0.41 abc
	Four	4.40 ± 0.64 bcd	5.03 ± 0.91 a	10.40 ± 0.95 abc	11.45 ± 0.61 abcd
	Five	5.00 ± 0.52 ab	5.21 ± 0.61 a	9.18 ± 1.05 cd	10.49 ± 0.44 de
Year (Y)		**		NS	
Nitrogen application (NA)		**		***	
Cutting frequency (CF)		***		***	
Y × NA		NS		NS	
Y × CF		NS		NS	
NA × CF		NS		*	
Y × NA × CF		NS		NS	

Means with different lowercase letters were significantly different at $p < 0.05$. NS, non-significant at 0.05 level of probability; * significant at the 0.05 level of probability; ** significant at the 0.01 level of probability.; *** significant at the 0.001 level of probability.

3.2. Dry Matter Yield

The effect of the N application × CF interaction on forage DM yield was not significant in either year ($p > 0.05$) (Table 3). However, we detected differences in alfalfa dry matter yield for the N application rate combined with each CF ($p < 0.05$). With an increase in N application, DM yield was enhanced gradually from 60 from 180 kg N ha^{-1} among all CF treatments, but subsequently declined slightly at a N application of 240 kg N ha^{-1}. Among all CF treatments, the highest DM yields were obtained in response to the 180 kg N ha^{-1} treatment, with values of 11.44, 11.09, and 9.59 t ha^{-1} being obtained in plots cut three, four, and five times in 2013, and corresponding values of 12.02, 11.73, and 10.79 t ha^{-1} were obtained in 2014. The observed pattern of DM yields was found to be comparable to those of forage yield components, with the CF-dependent alfalfa DM yield coinciding with the height plant^{-1}, mass shoot^{-1}, and leaf area plant^{-1}. In each year, the CF effect was significant ($p < 0.05$), with cutting three times resulting in the highest DM yield, and cutting five times resulting in the lowest yield.

3.3. CP Content

The forage DM nutritive values of alfalfa grown in plots receiving different N applications under the three CFs are presented in Tables 4 and 5. The CP content of forage is among the most important criteria used to evaluate forage quality. We found that the N application × CF interaction had no significant effect on alfalfa CP content ($p > 0.05$), and there were no significant ($p > 0.05$) differences among the different N application treatments (Table 4). However, the recorded values of CP content showed an increasing trend with an increasing N application rate, with the highest values being obtained in plots treated with 240 kg N/ha under each of the three CF schedules. Similarly, we detected no significant differences among the three CFs with respect to CP content ($p > 0.05$), although overall, irrespective of N application, cutting five times resulted in a higher CP content than the other two CF treatments, whereas cutting three times resulted in the lowest CP content.

Table 4. Crude protein, IVDMD, and IVDDM of alfalfa under different nitrogen application and cutting frequency in 2013 and 2014.

Nitrogen Application (kg N ha^{-1})	Cutting Frequency	Crude Protein (%)		IVDMD (%)		IVDDM (t ha^{-1})	
		2013	2014	2013	2014	2013	2014
60	Three	18.68 ± 0.65	18.35 ± 0.35	49.79 ± 2.85 d	52.95 ± 2.84 b	4.89 ± 0.22 d	5.69 ± 0.08 d
	Four	18.69 ± 0.32	18.76 ± 0.33	56.40 ± 1.84 ab	58.33 ± 1.45 a	5.24 ± 0.12 bcd	6.13 ± 0.11 abcd
	Five	19.95 ± 0.45	19.58 ± 0.47	56.42 ± 0.98 ab	58.83 ± 1.62 a	4.83 ± 0.14 d	5.87 ± 0.08 cd
120	Three	19.13 ± 0.35	18.94 ± 0.68	50.45 ± 1.74 d	53.51 ± 0.85 b	5.18 ± 0.09 cd	5.92 ± 0.07 bcd
	Four	19.23 ± 0.23	19.53 ± 0.74	57.11 ± 0.98 ab	58.97 ± 2.01 a	5.76 ± 0.11 abc	6.48 ± 0.13 ab
	Five	19.34 ± 0.62	19.94 ± 0.69	58.11 ± 1.54 a	59.17 ± 1.05 a	5.37 ± 0.21 bcd	6.32 ± 0.08 abc
180	Three	19.41 ± 0.41	19.09 ± 0.52	52.12 ± 0.84 cd	53.79 ± 0.88 b	5.92 ± 0.14 ab	6.29 ± 0.04 abc
	Four	19.59 ± 0.22	19.81 ± 0.71	56.33 ± 0.74 ab	58.57 ± 1.54 a	6.33 ± 0.09 a	6.70 ± 0.15 a
	Five	19.58 ± 0.74	19.94 ± 0.26	58.40 ± 1.76 a	60.26 ± 1.26 a	5.71 ± 0.07 abc	6.57 ± 0.21 a
240	Three	19.47 ± 0.25	19.24 ± 0.81	51.65 ± 2.03 cd	54.15 ± 2.01 b	5.69 ± 0.11 abc	6.18 ± 0.07 abcd
	Four	19.61 ± 0.61	20.11 ± 0.15	54.25 ± 1.74 bc	58.87 ± 0.99 a	5.70 ± 0.13 abc	6.63 ± 0.06 a
	Five	19.71 ± 0.46	20.14 ± 0.26	57.35 ± 0.84 a	59.55 ± 1.65 a	5.38 ± 0.08 bcd	6.31 ± 0.17 abc
Year (Y)		**		*		NS	
Nitrogen application (NA)		NS		NS		**	
Cutting frequency (CF)		NS		***		***	
Y × NA		NS		NS		NS	
Y × CF		NS		NS		NS	
NA × CF		NS		NS		NS	
Y × NA × CF		NS		NS		NS	

Means with different lowercase letters were significantly different at $p < 0.05$. NS, non-significant at 0.05 level of probability; * significant at the 0.05 level of probability; ** significant at the 0.01 level of probability; *** significant at the 0.001 level of probability.

Table 5. NDF, ADF, and RFV of alfalfa under different nitrogen application and cutting frequency in 2013 and 2014.

Nitrogen Application (kg N ha^{-1})	Cutting Frequency	NDF (%)		ADF (%)		RFV	
		2013	2014	2013	2014	2013	2014
60	Three	49.50 ± 2.53 a	46.71 ± 1.87 a	25.18 ± 2.13 a	23.91 ± 1.87 a	166.91 ± 2.26 b	176.68 ± 3.25 b
	Four	43.61 ± 1.84 b	41.29 ± 2.36 b	24.41 ± 2.03 c	23.58 ± 1.56 ab	189.36 ± 3.85 a	199.96 ± 3.14 a
	Five	43.28 ± 2.63 b	40.30 ± 2.15 b	22.61 ± 1.58 cde	22.16 ± 1.54 cde	190.72 ± 6.25 a	204.82 ± 2.65 a

Table 5. *Cont.*

Nitrogen Application (kg N ha^{-1})	Cutting Frequency	NDF (%)		ADF (%)		RFV	
		2013	2014	2013	2014	2013	2014
120	Three	49.31 ± 1.36 a	45.90 ± 3.05 a	24.24 ± 2.03 ab	23.02 ± 0.88 abc	167.41 ± 9.56 b	180.08 ± 4.63 b
	Four	43.53 ± 3.06 b	40.96 ± 0.99 b	23.28 ± 0.74 cd	22.79 ± 1.36 bcd	189.61 ± 5.52 a	201.49 ± 2.56 a
	Five	43.02 ± 1.41 b	40.29 ± 0.21 b	21.54 ± 1.54 de	21.87 ± 2.01 def	191.89 ± 7.26 a	204.95 ± 4.32 a
180	Three	48.87 ± 1.84 a	45.37 ± 1.25 a	23.07 ± 1.87 b	22.58 ± 2.58 bcd	168.99 ± 5.26 b	182.09 ± 3.68 b
	Four	43.07 ± 2.41 b	40.23 ± 1.63 b	22.68 ± 1.35 cde	22.47 ± 1.41 cd	191.66 ± 4.15 a	205.15 ± 2.98 a
	Five	42.64 ± 2.64 b	39.75 ± 0.78 b	20.69 ± 2.54 e	21.10 ± 1.63 f	193.61 ± 8.78 a	206.22 ± 1.89 a
240	Three	49.14 ± 1.87 a	45.94 ± 1.12 a	24.79 ± 0.96 ab	22.89 ± 0.74 abcd	168.09 ± 3.65 b	179.75 ± 2.74 b
	Four	42.78 ± 0.98 b	40.56 ± 2.13 b	22.63 ± 1.74 cde	22.22 ± 1.63 cde	192.92 ± 4.25 a	203.60 ± 3.58 a
	Five	42.73 ± 1.21 b	40.04 ± 1.54 b	22.12 ± 2.01 de	21.37 ± 2.45 ef	193.24 ± 2.36 a	207.68 ± 5.85 a
Year (Y)		*		*		NS	
Nitrogen application (NA)		NS		*		NS	
Cutting frequency (CF)		***		***		***	
Y × NA		NS		NS		NS	
Y × CF		NS		NS		NS	
NA × CF		NS		NS		NS	
Y × NA × CF		NS		NS		NS	

Means with different lowercase letters were significantly different at $p < 0.05$. NS, non-significant at 0.05 level of probability.; * significant at the 0.05 level of probability.; ** significant at the 0.01 level of probability.; *** significant at the 0.001 level of probability.

3.4. IVDMD and IVDDM

With respect to alfalfa IVDMD and IVDDM, we detected no significant effect of the N application × CF interaction ($p > 0.05$) (Table 4). Similarly, N application had no significant effect on alfalfa IVDMD ($p > 0.05$). Among the cutting schedules, we found that in both years, cutting three times was associated with the lowest IVDMD, whereas IVDMD for forage cut five times was found to be significantly higher than that obtained for the other two CFs in 2013 ($p < 0.05$). Additionally, IVDMD for forage cut four and five times was significantly higher than for three cuts in 2014 ($p < 0.05$), but there was no significant difference between them. IVDDM values were calculated based on the IVDVD and DM yields, and with the exception of four cuts in the 180 and 240 kg N ha^{-1} treatments, alfalfa forage IVDMD showed a trend similar to that observed for DM yield, whereas in response to all CF treatments, the values increased significantly ($p < 0.05$) up to N application of 180 kg N ha^{-1}. Nevertheless, in contrast to the trend in DM yield, in both years, cutting four times resulted in the highest IVDDM, given that this treatment contributed to a higher forage DM yield and moderate IVDVD (Tables 2 and 3), being on average 6.36% and 8.14% higher than that obtained with three and four cuts, respectively, in 2013, and 7.75% and 3.50% higher, respectively, in 2014 (Table 4).

3.5. NDF, ADF, and RFV

Other important quality characteristics of forage include the concentrations of NDF and ADF. In this study, we found that the NDF content of alfalfa in plots treated with 180 kg N ha^{-1} was slightly lower than that recorded in plots fertilized at other rates of N application under each CF (Table 5). In both years, however, we detected no significant differences among the different N application treatments ($p > 0.05$). NDF values were significantly higher in plots cut three times ($p < 0.05$), whereas there were no significant differences ($p > 0.05$) in the NDF contents of alfalfa cut four and five times during the 2 years. In 2013 and 2014, cutting five times produced alfalfa plants with the lowest NDF content 42.64% and 39.75%, respectively, at a N application of 180 kg N ha^{-1}. With respect to ADF, we observed a trend similar to that of NDF, namely a significant reduction

($p < 0.05$) in response to an increase in N application from to 60 to 180 kg N ha^{-1} for all three CF treatments, followed by a subsequent slight increase in response to N application to 240 kg N ha^{-1} (Table 5). In both 2013 and 2014, the lowest ADF contents (20.69% and 21.10%, respectively) were recorded in plots treated with 180 kg N ha^{-1}. Furthermore, in both years, the ADF content was found to be significantly influenced by CF ($p < 0.05$), with a three-cut schedule resulting in the highest ADF content, whereas cutting five times produced plants with the lowest ADF content, and cutting four times produced plants with intermediate values.

RFV is an index that is used to predict the intake and energy value of forage and is derived from the NDF and ADF contents (DDM and DMI). In both years, we found that the N $\times$ CF interaction had no significant effect on forage RFV ($p > 0.05$) (Table 5). Although there was an increasing trend in RFV with an increasing N application rate, N fertilization had no significant effects on alfalfa forage RFV. Similarly, CF had significant effects on alfalfa forage RFV under different N applications ($p < 0.05$); significantly higher RFVs were obtained in treatments with four and five cuts than in those with three cuts, reaching values of 193.24 and 207.68, respectively, under 240 kg N ha^{-1} treatment.

4. Discussion

1. Although the temperature profiles of the two study years were essentially similar, rainfall was relatively higher in the spring of 2014 than in that of 2013, which might have contributed to better alfalfa re-growth in 2014. Contrastingly, the summer of 2013 was somewhat wetter than that of 2014, and the associated waterlogging decreases photosynthesis in many plant species and leads to the development of leaf injury symptoms, such as wilting and chlorosis [33], and thus may have had an undesirable influence on forage growth and nutritional quality [34]. Consequently, this may have accounted for the slightly higher alfalfa yield and quality parameters obtained in 2014.
2. Under seasonal cultivation, alfalfa grows rapidly in spring and summer, and an appropriate frequency of cutting can contribute to preventing plant lodging and over-maturity. The findings of some studies have accordingly indicated that low rates of N application following each alfalfa cut can promote increases in yield. In this study, we found that the N $\times$ CF interaction had no significant effect with respect to annual alfalfa yield. However, we established that N application may influence the potential yields obtained using different CFs, and that the enhancement in yield obtained in response to N application rate was attributable to increases in the parameters height plant^{-1}, shoot plant^{-1}, and leaf area plant^{-1}. These findings are consistent with those reported by Cherney and Duxbury [35], who found that an increase in the availability of inorganic N resulted in linear increases in alfalfa shoot weight, height, and number, whereas Barber et al. [36] reported that the application of N can remobilize alfalfa shoot growth. We similarly found that the responses of alfalfa yield to N fertilization in the range of 60 to 240 kg N ha^{-1} were largely linear. In this regard, Feigenbaum and Hadas [13] have shown that the application of 100 kg N ha^{-1} ammonium sulfate after the first cut in spring led to an increase in alfalfa yields, whereas Hojjati et al. [37] found that compared with a no-N control treatment, the application of N contributed to increases in the above-, below-ground, and whole plant weights of alfalfa, and that applying N at 30 kg ha^{-1} was as effective as 60 kg ha^{-1} in enhancing growth. Given that cutting results in a decline in N_2 fixation, this raises the question as to whether this practice limits alfalfa regrowth, and, if so, whether alfalfa plants require supplementary N fertilization to ensure maximum productivity [11]. In this regard, Raun et al. [38] and Jenkins and Bottomley [39] and have suggested that low N rates could be applied to alfalfa following each cutting to increase alfalfa yield at either the final or penultimate harvest. The application of N after each harvest has been found to promote leaf development in alfalfa, giving rise to a higher leaf area plant^{-1}, as well as promoting shoot growth, thereby maximizing the interception of light by leaves. In addition, by promoting alfalfa photosynthesis, N contributes to enhancing

the synthesis and availability of carbon-assimilating enzymes [40]. However, given that it has been established that excessive N application (e.g., 240 kg N ha^{-1}) does not promote additional increases in alfalfa yield, this would imply that alfalfa plants may not require supplementary subsurface N accumulation to achieve higher production [41,42].

3. Compared with the application of N, we found that in both study years, the frequency of cutting had a more appreciable influence on alfalfa yield. Specifically, for each N application treatment, we detected a decline in the yield components height $plant^{-1}$, leaf area $plant^{-1}$, and mass $shoot^{-1}$ in response to an increase in the frequency with which plots were cut, thereby resulting in lower forage yield, which was consistent with the findings of previous studies [43,44]. It has been suggested that such reductions in yield components could be ascribed to reductions in the amounts of energy captured during photosynthesis, particularly under conditions of a shorter re-growth period, and a reduction in taproot organic reserves [45]. In contrast to Ventroni et al. [46], who observed a reduction in potential alfalfa shoots $plant^{-1}$ when cut at high frequency compared with plants that were cut at a lower frequency, we found that more frequent cutting promoted an increase in the number of growth tillers, accordingly, resulting in a high shoot $plant^{-1}$. However, although frequent cutting increases the number of growth points, it also diminishes shoot mass and results in no net change in forage yield, and, in the present study, we obtained the highest yields at the lowest cutting frequency. Cutting inevitably leads to a decline in alfalfa photosynthetic capacity, particularly in the earliest initiated leaves after cutting, which, together with subsequent reductions in canopy expansion rates, diminishes forage yield [47]. In addition, a high cutting frequency influences the total forage yield and limits the recovery capacity of alfalfa. It has long been suggested that providing an adequate recovery period between defoliations and cutting at a low frequency can increase DM yield [19,20]. In the present study, we found that the low alfalfa yield obtained when cutting five times may have been attributable to a reduction in plant mass, resulting in smaller and weaker plants that are more susceptible to pests, diseases, temperature stress, and competition from weeds.
4. Forage quality and yield have often been reported to be negatively correlated. In this study, we established that although N application had a favorable effect on alfalfa forage quality, these effects tended to be limited, and there were no significant differences among the assessed fertilization schedules. The CP content of forage is among the most important criteria used to evaluate forage quality, and is associated with the leafiness of plants, which can be increased in response to the application of N. Actually, alfalfa shoot CP content was sensitive to N fertilizer application. Even a low N fertilizer application rate (<30 kg ha^{-1}) led to a similar increase in shoot CP concentration [41], but sometimes an excessive application of N fertilizers results in a significant reduction in protein N in order to increase free amino acids and nonprotein N [48]. Under all CF treatments, we found that alfalfa CP content increased slightly with an increase in the rate of N application between 60 and 240 kg N ha^{-1}. According to Woelfel and Poulton [16], N application has little effect on herbage CP content, and, similarly, Dent and Aldrich [49] found that by applying 63.50 kg N ha^{-1}, the mean CP content of 13 varieties of grasses was 34.1% compared with the 33.2% when no N was applied. Digestibility, measured as in vitro digestible DM, is an important component of forage quality, and over 60% of the variation in animal productivity is explained by the amount of forage consumed [50]. We found that IVDMD and IVDDM were not significantly affected by the rate of N application, and similar differences were observed with respect to the contents of NDF and ADF, which are important parameters of forage DM nutrition [51]. These fibers are measures of cell wall components and are closely correlated with the rates of ruminant digestion. In the present study, we observed declines in the contents of NDF and ADF with increasing N application up to 180 kg N ha^{-1}. Demment et al. [15] reported perceptible reductions in levels of the alfalfa fiber components NDF, ADF, and CEL in an NH_4NO_3-dependent

environment, and there was no clear increase in RFV, as neither the contents of NDF or ADF showed a significant variation in response to different rates of N application.

5. Generally, it has been established that cutting is an effective strategy for enhancing alfalfa quality, and the response of forage quality to CF observed in the present study is consistent with that reported by other researchers. For example, Brink and Markten [52], Sheaffer et al. [53], and Xu [54] have shown that the frequency with which alfalfa is cut is an important factor that influences forage quality. However, although the frequency of cutting influences the CP content of alfalfa, the response is not always significant or consistent. We found that the frequency of cutting had no significant effect on the mean alfalfa CP content. With regards to differences in the application of N, we observed that the lowest and highest frequencies of cutting produced the lowest and highest CP contents, respectively. During the latter stages of growth with advancing maturity, alfalfa cell wall constituents (cellulose, hemicellulose, and lignin) increase by 0.16% of the dry matter per day, which has the effect of reducing forage digestibility [55]. However, although cutting at an earlier growth stage produces forage with higher digestibility and crude protein content, it results in a lower total yield. In this regard, we found that cutting four times resulted in the highest IVDDM with a moderate DM yield and IVDMD. Prolonging the cutting interval results in an accumulation of cellular structural carbohydrates with advancing maturity, whereas an increase in CF has the effect of reducing the contents of NDF and ADF [22]. However, we detected only slight reductions in the NDF and ADF contents of alfalfa cut four and five times, with no significant difference between the two treatments. The RFV value has been extensively used to rank fodder for sale, and, consequently, it is necessary to determine a suitable harvest time to ensure fodder of high quality [56]. We found that compared with three cuts, the RFV value of alfalfa was significantly higher in the forage cut four and five times, although there was no significant difference between the latter two CFs. Accordingly, consistent with previous research, we demonstrated that an increase in the frequency of cutting potentially contributes to a better quality of alfalfa hay.

5. Conclusions

In southern China, seasonal cultivation is considered an effective agricultural strategy for reducing the scarcity of forage. The findings of this study indicate that in response to N application after successive cuts, alfalfa can be successfully grown and harvested via seasonal cultivation in clayey soils under humid conditions. Although we detected no significant interaction between the frequency with which alfalfa was cut and the rate of N application, N fertilization was found to stimulate the re-growth of alfalfa after cutting and contributed to enhancing forage yield and quality. The use of N fertilizer in certain programs, as recommended for the same soil and climatic conditions found in the study area, appears to be a feasible means of promoting alfalfa growth and thus the development of animal husbandry. We established that a high frequency of cutting resulted in low forage yield, although there was an improvement in alfalfa forage quality, characterized by a considerable IVDDM. On the basis of our findings, we propose that the application of 180 kg N ha^{-1} in conjunction with three to four cuts could be an appropriate regime for a high-yielding production of high-quality alfalfa under seasonal cultivation. However, given that the selection of appropriate rates of N application and cutting times are influenced by economics at the time of production, it is imperative that such expenses are minimized as much as possible to maintain farmer profitability and sustainable production. Further field trials are required to determine alfalfa nitrogen-use efficiency (NUE) and to refine the rates of N application.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13051063/s1, Figure S1: The experimental site with the alfalfa crops; Figure S2: The experimental site with the alfalfa crops.

Author Contributions: K.Z. and Y.S. carried out the experimental design. K.Z. and H.Q. performed the experiments. K.Z. and Y.L. (Yan Li) and prepared the manuscript and coordinated its revision. K.Z., C.Z., Y.L. (Yonglong Li) and Y.S. read and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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