https://doi.org/10.17221/457/2023-PSE

Fertilisation and environmental factors affect the yield and quality of alfalfa in China

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Citation: Li S., Liu Z.Q. (2024): Fertilisation and environmental factors affect the yield and quality of alfalfa in China. Plant Soil Environ., 70: 276–286.

Abstract: Alfalfa (*Medicago sativa* L.) is a superior-quality perennial legume forage crop cultivated in China. However, fertiliser applications and the environmental factors affecting alfalfa yield and quality have not been well documented. In this study, we conducted a meta-analysis using a dataset from 105 studies published between 2003 and 2023 to explore the effects of fertiliser application and environmental factors on the yield and quality of alfalfa. The results showed that compared to the non-fertiliser control levels, fertiliser application increased alfalfa yield by 24.61% and improved the quality of alfalfa by increasing crude protein by 11.63% and decreasing acid detergent fibre by 7.69% and neutral detergent fibre by 6.76%. Alfalfa yield and the crude protein effect size increased with increasing altitude but decreased with increasing latitude based on fertiliser application. The acid detergent fibre and neutral detergent fibre effect size were positively correlated with mean annual temperature and mean annual precipitation. In conclusion, applying fertiliser is a productive approach to enhance the yield and grade of alfalfa, but environmental factors have an effect. This study provides comprehensive information on fertiliser applications and environmental factors that affect alfalfa yield and quality. These results provide insight into further improving alfalfa yield and quality and contribute to the development of alfalfa.

Keywords: grassland; soil fertility; nutrient; precipitation; production; heterogeneity

Alfalfa (*Medicago sativa* L.) is a high-quality forage legume, often called the king of forages, that has been cultivated on more than 5.467×10^5 ha in China since 2020 (Wang and Xu 2021). The cultivated area of alfalfa has been expanding with the development of animal husbandry and the demand for returning farmland to grassland (Li 2002, Wang and Xu 2021). However, the yield and quality of alfalfa in China fall short of meeting the escalating demands of the livestock industry (Zhang et al. 2009, Wang and Xu 2021). The self-sufficiency rate of high-quality alfalfa in China was 64% during 2012–2020 (Jin et al. 2021), while imports of alfalfa hay grew from 0.44×10^6 to 1.36×10^6 tons (Wang and Zhong 2021). Consequently, evaluating how to expand the yield and quality of alfalfa in China is important for the flourishing animal husbandry industry.

The yield and quality of alfalfa are closely related to soil fertility. Reasonable fertilisation management and timely supplements of nutrients deficient in the soil (Bahulikar et al. 2020, Wang et al. 2021) increase the yield and quality of alfalfa (Wei et al. 2018). Fertilisation is a direct approach to improve the yield and quality of alfalfa (Fang et al. 2021, Wan et al. 2022). Many studies have explored the effects of fertiliser applications on the yield and quality of alfalfa (Fang et al. 2021, Wan et al. 2022). However, the results have varied substantially or were contradictory due to differences in cuttings, cultivars, water supply, soil, and environmental factors.

Applying fertiliser significantly increases alfalfa quality (Wan et al. 2022), and alfalfa crude protein (CP), acid detergent fibre (ADF), and neutral detergent fibre (NDF) reflect its nutritional quality

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(Robinson 1999, Marković et al. 2009, Acharya et al. 2020). Applying nitrogen (N) fertiliser increases alfalfa CP content (Zhang et al. 2020). Applying phosphorus (P) fertiliser increases CP content and reduces alfalfa's ADF and NDF contents (Lissbrant et al. 2009, Macolino et al. 2013, Yu et al. 2018). Applying potassium (K) fertiliser due to a large amount of stem growth leads to an increase in ADF and NDF content and further reduces the quality of alfalfa (Lissbrant et al. 2009, Jungers et al. 2019). Another study showed that K fertiliser has no significant effect on ADF or NDF of alfalfa (Macolino et al. 2013). Different fertilisers present different results, so further research is needed to explore the optimal approach.

Many studies have reported how environmental factors affect the yield and quality of alfalfa, such as soil, light, precipitation, and temperature, and how these factors potentially influence the growth and development of alfalfa, resulting in differences in yield and quality (Pembleton et al. 2011, Baslam et al. 2014, Gardarin et al. 2014, Lee et al. 2017). For example, some studies have shown that as temperature increases, dry matter production increases (Sanz-Sáez et al. 2012, Liang et al. 2013, Dellar et al. 2018), while other studies have reported a negative relationship between yield and temperature (Smith 1970, Jacob et al. 2020). Additionally, the yield and quality of alfalfa respond to environmental factors differently under different conditions (Santos et al. 2020, Tlahig et al. 2021). Most previous studies were based on site-specific experiments in which only certain conditions were considered. Research on specific locations has limited the significance of revealing the general effect of environmental factors on alfalfa production and quality.

These conflicting findings on the effects of fertiliser application and environmental factors on alfalfa yield and quality have confused alfalfa farmers. Thus, to support the expansion of animal husbandry and improve the production capacity of alfalfa, further research must be conducted on how different fertiliser applications and environmental factors affect the yield and quality of alfalfa on a large spatial scale. Our study aims to (1) conduct a pairwise meta-analysis to determine the effect of fertiliser application on alfalfa yield and quality and (2) examine the effects of environmental factors on the alfalfa yield and quality in China.

MATERIAL AND METHODS

Data collection. We searched the Web of Science and the China National Knowledge Infrastructure (CNKI) (between January 1, 2000, and September 5, 2023) using the following keywords: "alfalfa", "*Medicago sative*", "fertilize", "fertilization", "fertilizer", "yield", "quality", and "China". We screened the articles to determine whether the studies met our inclusion criteria: (1) alfalfa was the only plant cultivated in the field; (2) included yield or quality data from control and fertilised treatments; and (3) located in mainland China. In each study, the mean and standard deviation (SD) or standard error (SE) of the yield and quality, as well as the sample size (*n*), were obtained from the control and fertilisation treatments. If only the SE were provided, the SD was calculated as the SE outcome multiplied by the sample size's square root. Missing SDs were calculated using 0.1 times the mean of the dataset (Luo et al. 2006). Data were extracted from graphs using "WebPlotDigitizer 4.6" (Burda et al. 2017). We also extracted the corresponding climate (mean annual temperature (MAT) and mean annual precipitation (MAP)) and altitude data from the WordClim website (http://worldclim.org/version2) if not provided by the study.

The final dataset consisted of 105 studies from 79 Chinese sites (Figure 1): 3 040 pairs of observations on alfalfa yield, 1 743 pairs of observations on alfalfa CP, 1 206 pairs of observations on alfalfa ADF, and 1 212 pairs of observations on alfalfa NDF. The dataset included 82 peer-reviewed publications, 19 Master's theses, and four doctoral dissertations. Fertiliser types: N, nitrogen; P, phosphorus; K, potassium; M, organic fertiliser; NP, combined application of nitrogen and phosphorus; NK, application of nitrogen and phosphorus; PK, application of phosphorus and potassium; NPK, application of nitrogen, phosphorus, and potassium; F + M, application of organic and inorganic fertilisers. The environmental factors included MAT, MAP, altitude, and latitude.

Data analysis. We calculated the log-response ratios (lnRR) for each study as the effect size for our meta-analysis (Hedges et al. 1999). We calculated lnRR for each study using:

$$\text{lnRR} = \ln\frac{Y_t}{Y_c} \quad (1)$$

Where: Y_t and Y_c – mean values of the treatment and control groups, respectively. The variance (υ) of each effect size was calculated as follows:

$$v = \frac{S_t^2}{N_t Y_t^2} + \frac{S_c^2}{N_c Y_c^2} \quad (2)$$

Where: N_t – sample size of the treatment; N_c – sample size of the control group; S_t – standard deviation of the treatment; S_c – standard deviation of the control group.

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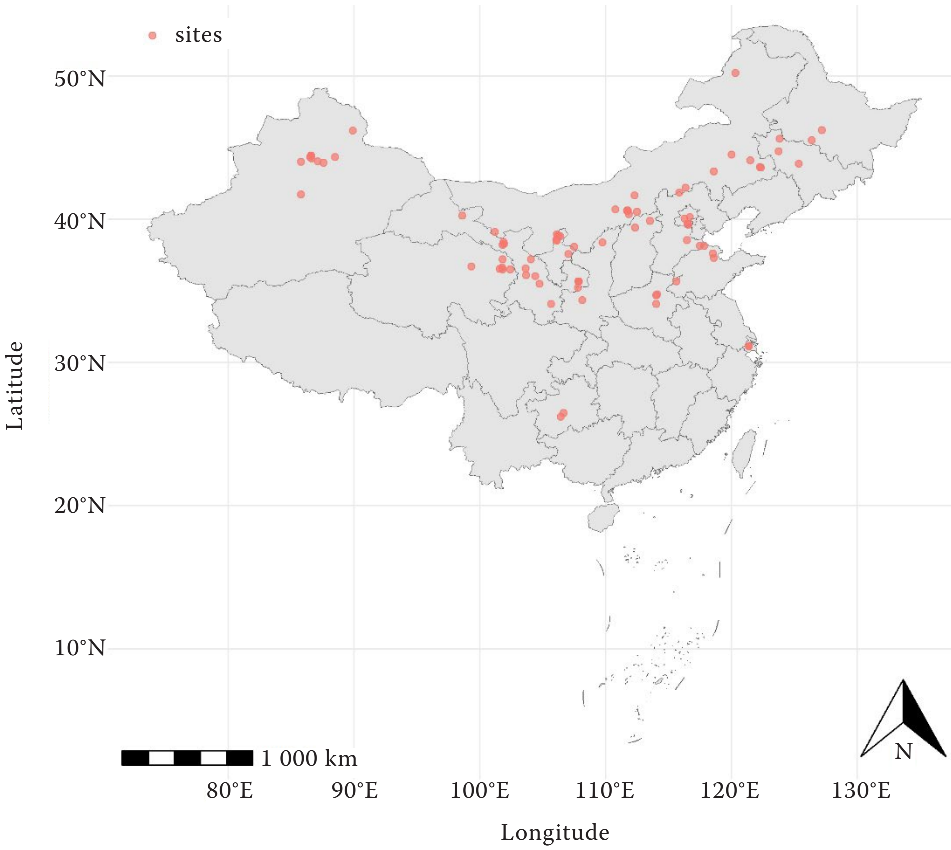

Figure 1. A map of the geographical location of studies included in the meta-analyses

The percent change was computed as follows:

$$\text{change rate} = (\exp(\ln RR) - 1) \times 100\% \quad (3)$$

A random-effects model was used to assess the overall responses. Then, we used mixed-effects meta-regressions and included MAT, MAP, altitude, and latitude as moderators for each response variable. For the meta-regressions, total heterogeneity was partitioned into the variance influenced by the moderators (Q_m, Q-statistic, which renders information on whether the moderator illuminates any significant heterogeneity in the data) and the residual error variance (Q_e). The Q_m-statistic is a Wald-type test of model coefficients, and a significant Q_m-statistic predicates that the moderators contributed to the heterogeneity in the effect size (Viechtbauer 2010).

Egger's regression was used to test for potential publication bias in each variable (Egger et al. 1997). Alfalfa yield had no publication bias. CP, ANF, and NDF had light bias risk in this study, but the data were almost symmetrically distributed around the mean effect size, and the data were highly accurate, presenting a random sampling error (Figure 2) (Liu 2011).

R version 4.2.2 (R Core Team, 2022) was applied for all statistical analyses, and the effect size and publication bias was estimated using the R package "metafor". A *P*-value < 0.05 was considered significant.

RESULTS

The effect of fertilisation on alfalfa yield and quality. Overall, fertilisation significantly increased the yield of alfalfa (Figure 3, Table 1). The increase in yield was the highest after applying organic fertiliser, reaching 80.40%, followed by the F + M and NP treatments, which were 49.18% and 37.71%, respectively (Table 1). Alfalfa CP also increased significantly due to fertilisation. The NP, F + M, and N treatments increased CP by 18.53, 17.35, and 16.18% (Table 1). In contrast, fertilisation significantly decreased alfalfa ADF and NDF. Alfalfa ADF decreased by 18.13% under the F + M treatment and 12.19% under the NP treatment (Table 1). Alfalfa NDF decreased 18.13% under the F + M treatment, compared to single N, P, and K fertiliser applications, which decreased less than combined NP, NK, PK, and NPK fertiliser applications (Table 1).

Alfalfa yield in response to environmental factors. Fertilisation significantly positively affected total alfalfa yield; however, it was highly variable among experiments (Qt = 135 474.67, *P* < 0.0001). Altitude explained 4.72% of the variation in the alfalfa yield

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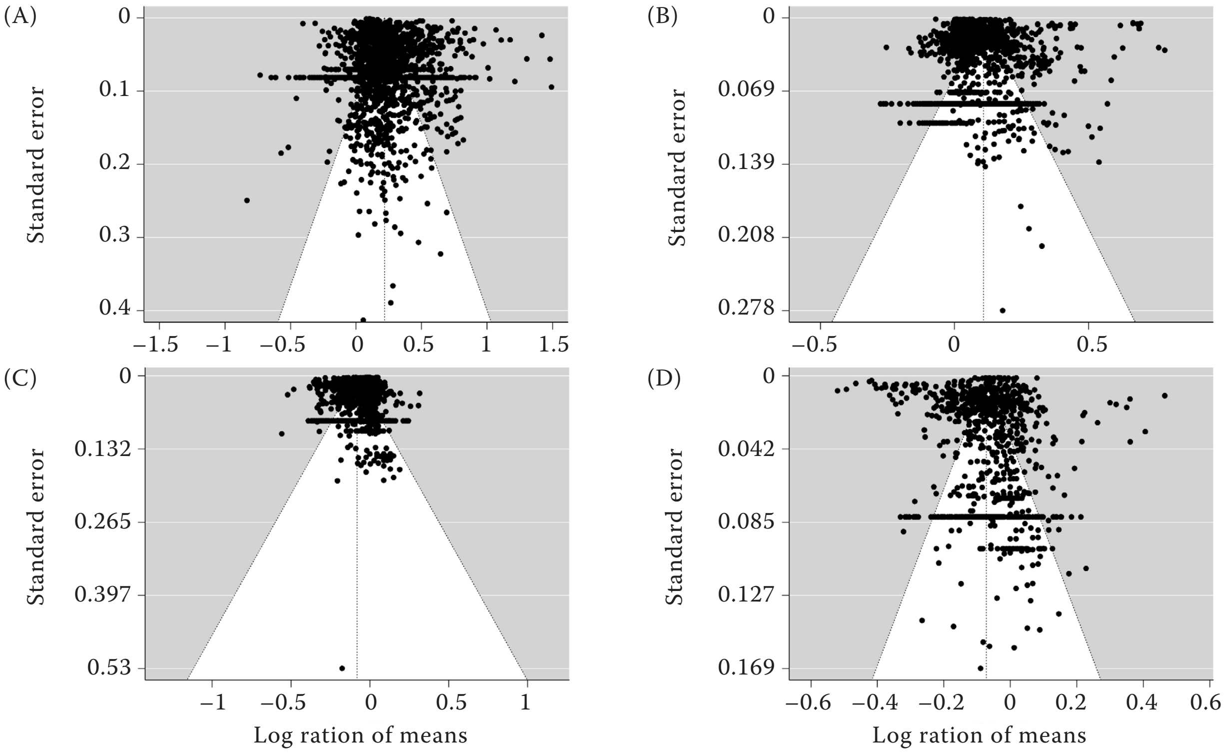

Figure 2. Funnel plot of standard error of (A) yield; (B) crude protein; (C) acid detergent fibre, and (D) neutral detergent fibre against response variable Ln(R). The vertical line represents the mean log response ratio Ln(R) estimated

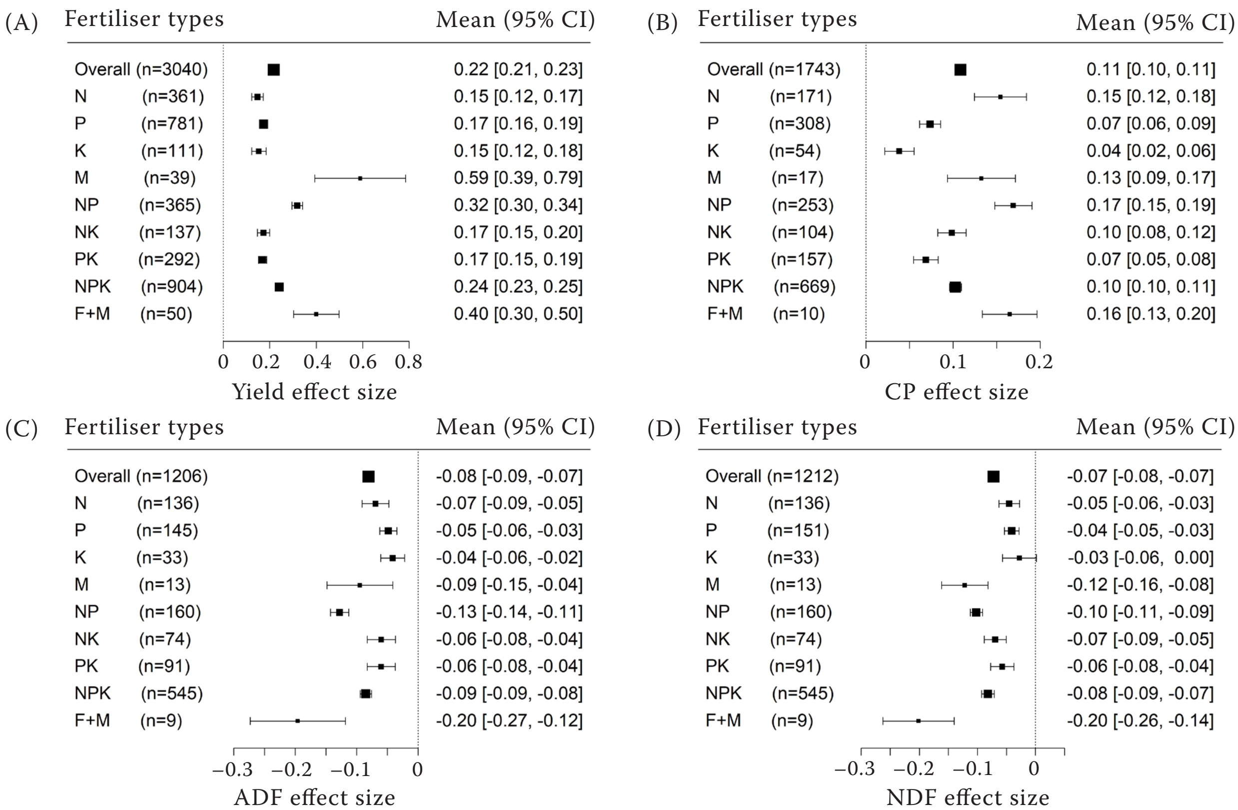

Figure 3. The effect of fertilisation on the yield and quality of alfalfa. (A) Yield effect size; (B) crude protein (CP) effect size; (C) acid detergent fibre (ADF) effect size, and (D) neutral detergent fibre (NDF) effect size. *n* – number of observations; the dotted line represents zero; M – organic fertiliser; F – inorganic fertiliser

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Table 1. The rate of change in alfalfa yield and quality

Fertiliser types	Yield change (%)	CP change (%)	ADF change (%)	NDF change (%)
Overall	24.61	11.63	–7.69	–6.76
N	16.18	16.18	–6.76	–4.88
P	18.53	7.25	–4.88	–3.92
K	16.18	4.08	–3.92	–2.96
M	80.40	13.88	–8.61	–11.31
NP	37.71	18.53	–12.19	–9.52
NK	18.53	10.52	–5.82	–6.76
PK	18.53	7.25	–5.82	–5.82
NPK	27.12	10.52	–8.61	–7.69
F + M	49.18	17.35	–18.13	–18.13

CP – crude protein; ADF – acid detergent fibre; NDF – neutral detergent fibre; M – organic fertiliser; F – inorganic fertiliser

effect size (Figure 4A). Latitude predicted 4.36% of the variance in the alfalfa yield effect size (Figure 4B). The MAT and MAP effects were relatively weak at 0.85% and 0.18%, respectively (Figure 4C–D).

Alfalfa CP in response to environmental factors. As expected for a biological meta-analysis, significant residual heterogeneity was detected in the random-effects meta-analysis for the alfalfa CP dataset (Qt =

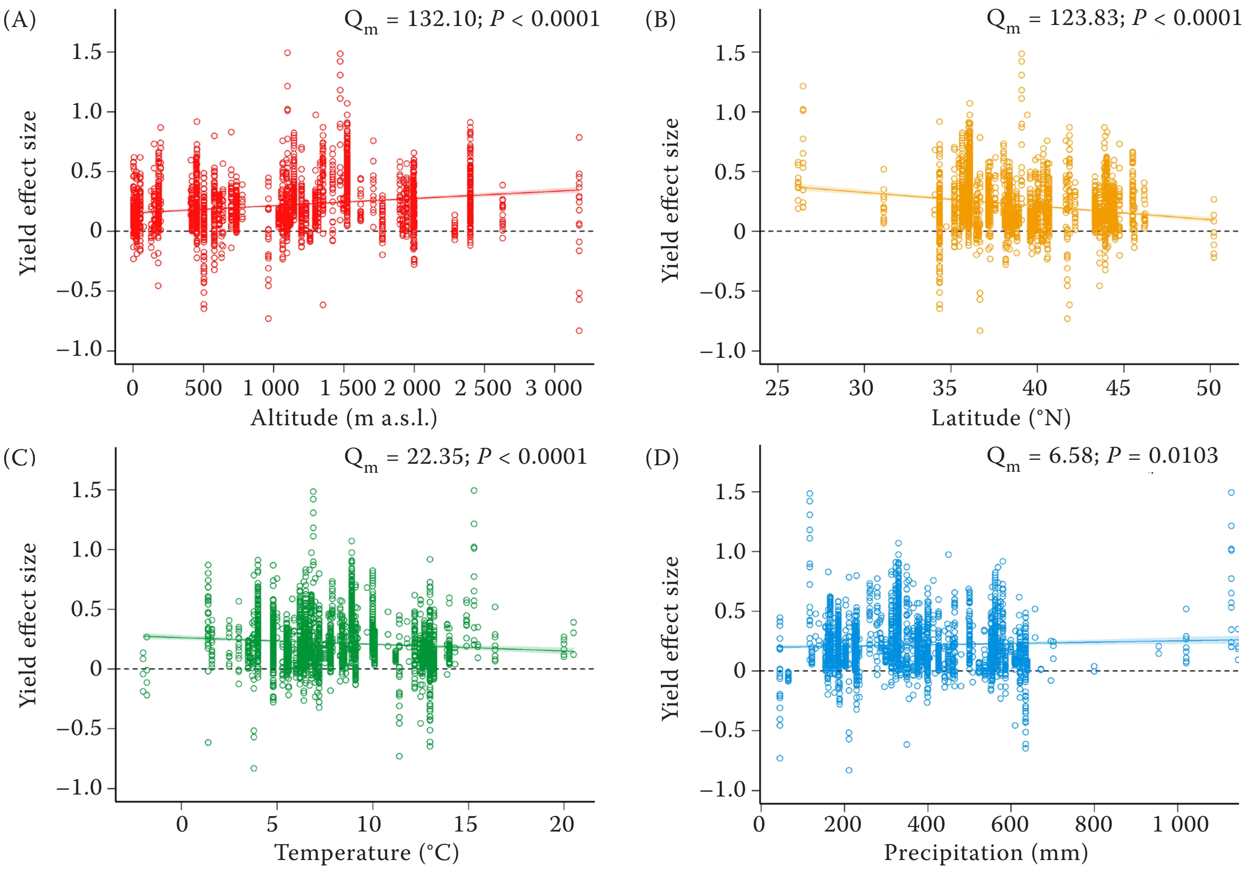

Figure 4. Alfalfa yield in response to environmental factors. (A) Alfalfa yield in response to altitude; (B) alfalfa yield in response to latitude; (C) alfalfa yield in response to mean annual temperature, and (D) alfalfa yield in response to mean annual precipitation. Q_m – Q-statistic, which renders information on whether the moderator illuminates any significant heterogeneity in the data

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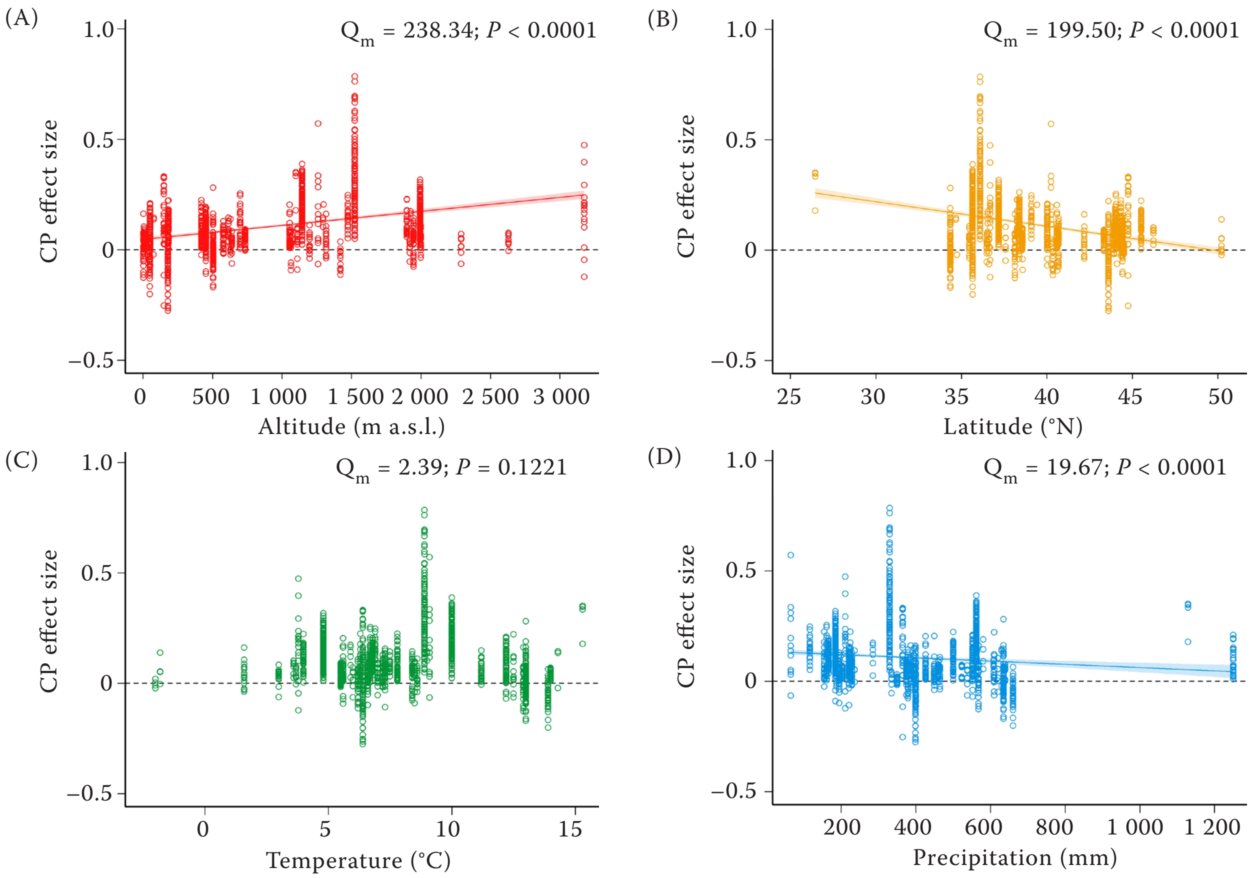

Figure 5. Alfalfa crude protein (CP) effect size in response to environmental factors. (A) Alfalfa crude protein effect size in response to altitude; (B) alfalfa crude protein effect size in response to latitude; (C) alfalfa crude protein effect size in response to mean annual temperature, and (D) alfalfa crude protein effect size in response to mean annual precipitation. Q_m – Q-statistic, which renders information on whether the moderator illuminates any significant heterogeneity in the data

234 985.63, $P < 0.0001$), which we tried to explain with different moderators. Altitude explained 12.58% of the variation in the alfalfa CP effect size (Figure 5A). Latitude predicted 11.21% of the variance in the alfalfa CP effect size (Figure 5B). MAT had no significant effect on total alfalfa CP, and the MAP effect was relatively weak at 1.13% (Figure 5C–D).

Alfalfa acid detergent fibre in response to environmental factors. Fertilisation was highly effective against alfalfa ADF, and the effectiveness of the defence varied substantially across studies (Qt = 44 891.55, $P < 0001$). Altitude had no significant effect on total alfalfa ADF (Figure 6A), and the effect of latitude was relatively weak at 1.79% (Figure 6B). MAT and MAP predicted 6.69% and 4.33% of the variance in the alfalfa ADF effect size (Figure 6C–D).

Alfalfa neutral detergent fibre in response to environmental factors. Fertilisation significantly negatively affected total alfalfa NDF; however, it was highly heterogeneous among experiments (Qt = 149 195.10, $P < 0.0001$). Altitude had no significant effect on total alfalfa NDF (Figure 7A), and the MAP effect was relatively weak at 2.33% (Figure 7D). MAT and latitude predicted 6.69% and 4.33% of the alfalfa NDF effect size variance, respectively (Figure 7B–C).

DISCUSSION

This study explored the effect of fertilisation on the yield and quality of alfalfa in China. All fertiliser applications led to a significant increase in alfalfa yield (Figure 3A). Our research also showed that M applications achieved the largest production increases. Organic fertilisers have complete nutrients, are long-lasting, rich in organic colloids, and easily form a positive soil structure and improve soil fertility (Xie et al. 2015). Applying the NPK combination led to a greater increase in yield compared with the

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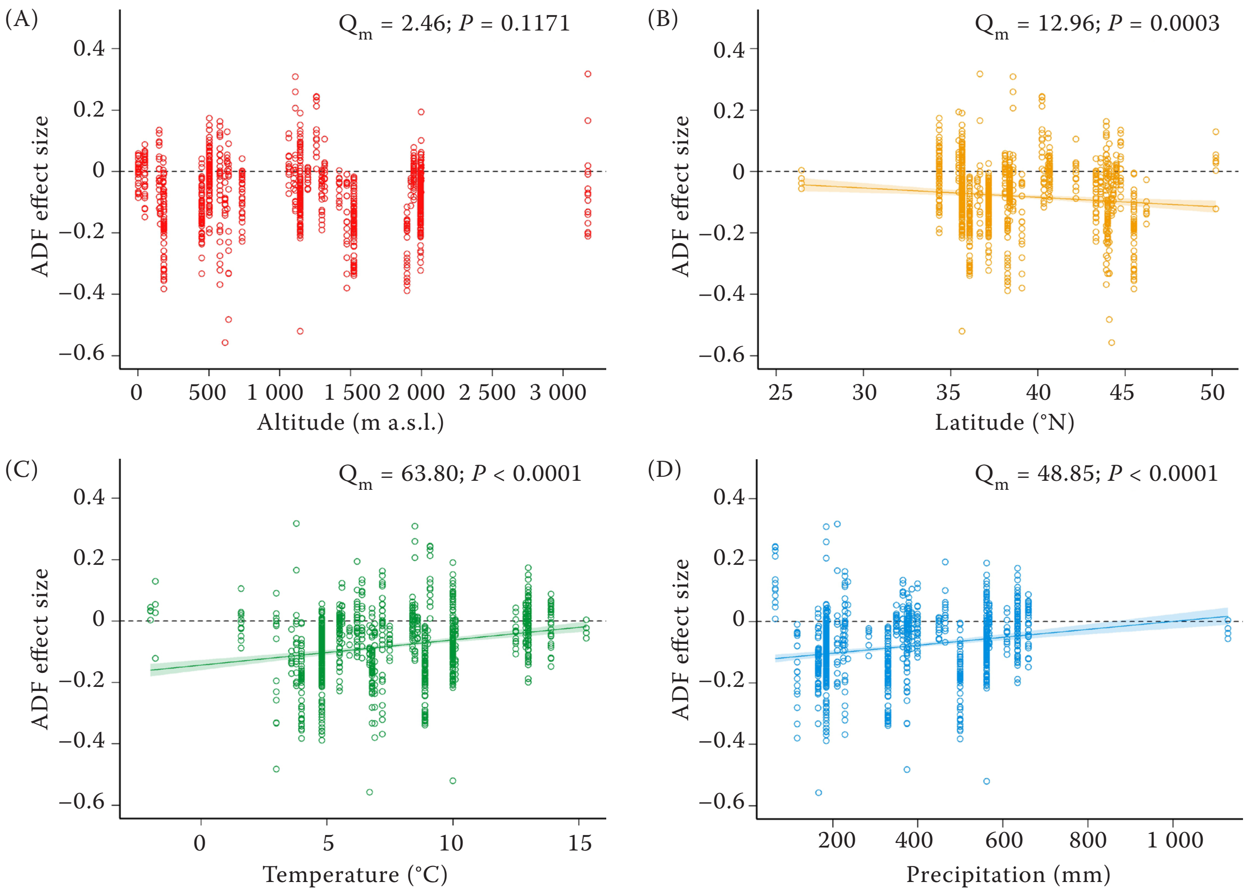

Figure 6. Alfalfa acid detergent fibre (ADF) effect size in response to environmental factors. (A) Alfalfa acid detergent fibre effect size in response to altitude; (B) alfalfa acid detergent fibre effect size in response to latitude; (C) alfalfa acid detergent fibre effect size in response to mean annual temperature, and (D) alfalfa acid detergent fibre effect size in response to mean annual precipitation. Q_m – Q-statistic, which renders information on whether the moderator illuminates any significant heterogeneity in the data

individual N, P, and K applications, which agreed with Cai et al. (2021) and Wan et al. (2022). The combined application of NPK fertiliser promotes cold resistance, root activity, and photosynthesis in alfalfa (Qamar et al. 2005, Tautges et al. 2018, Jungers et al. 2019), thereby further significantly increasing alfalfa production (Xiao et al. 2016, Zhang et al. 2020). A combined application of nitrogen and phosphorus also has a significant effect. NP affects the growth of alfalfa leaves and roots, changes photosynthesis and water use efficiency (Li et al. 2012, Wang et al. 2013), and promotes nitrogen fixation levels (Qi et al. 2013), thereby further increasing plant growth and yield. Applying organic fertiliser provides abundant nutrients for alfalfa and improves soil physicochemical properties and biological activities (Hu et al. 2007). Therefore, the combination of organic and chemical fertilisers promoted interactions between different nutrient elements and enhanced the increase in alfalfa yield.

As alfalfa is a perennial nitrogen-fixing legume plant, nitrogen is the main nutrient element during growth and synthesises amino acids and proteins (Liu et al. 2017). Our results show little difference in the higher yield between applying nitrogen and applying the combination of NK and PK. Although alfalfa is an N-fixing crop, the main alfalfa-producing area in China is in the northern region, where soil nutrient content is low, and soil water holding capacity is poor (He et al. 2014). Applying nitrogen fertiliser achieves higher average yields, improving alfalfa hay quality while increasing hay yield by 12.4–22.5% annually (Mao et al. 2018). The yield of alfalfa tends to increase first and then decrease with the increase in nitrogen fertiliser (Li et al. 2022), mainly because adding nitrogen at high nitrogen levels inhibits the

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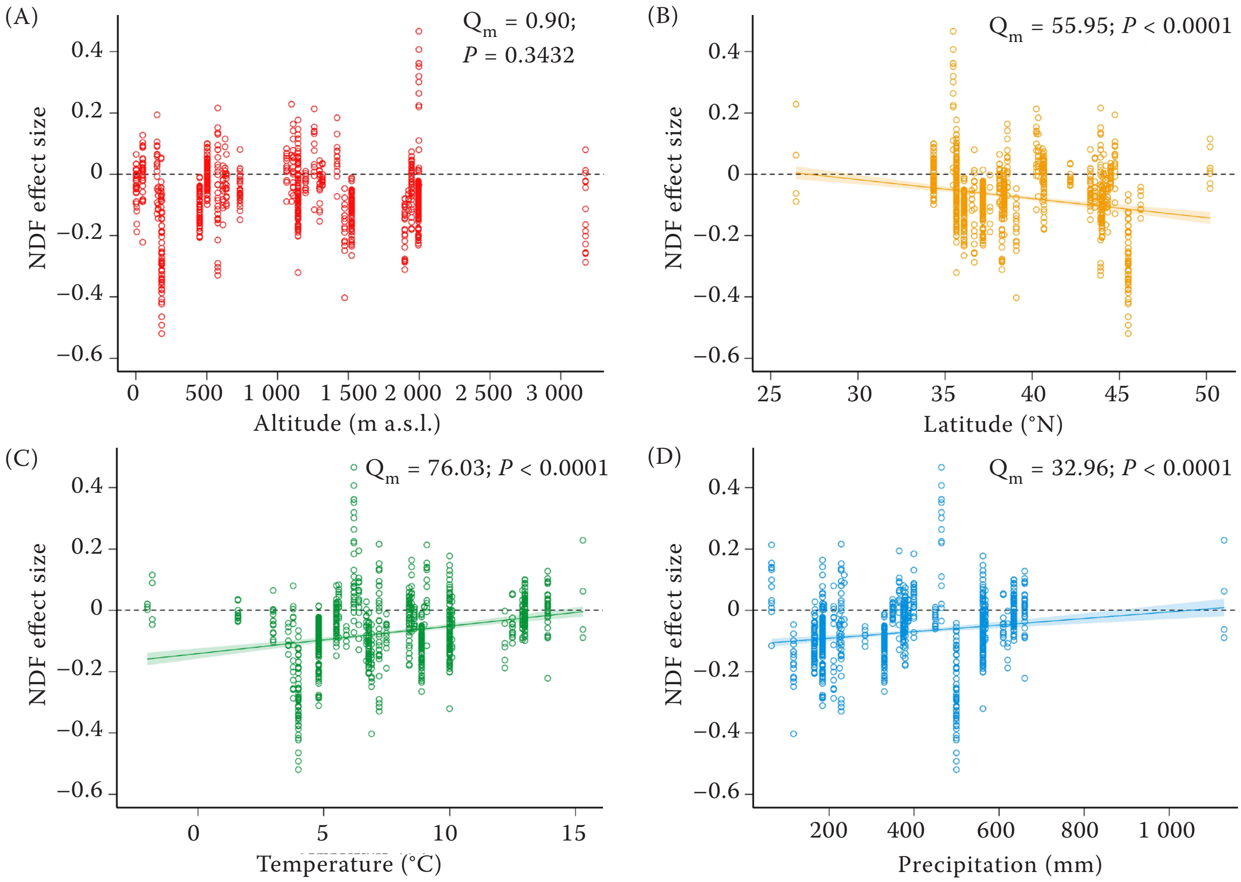

Figure 7. Alfalfa neutral detergent fibre (NDF) in response to environmental factors. (A) Alfalfa neutral detergent fibre in response to altitude; (B) alfalfa neutral detergent fibre in response to latitude; (C) alfalfa neutral detergent fibre in response to mean annual temperature; and (D) alfalfa neutral detergent fibre in response to mean annual precipitation. Q_m – Q-statistic, which renders information on whether the moderator illuminates any significant heterogeneity in the data

synthesis of nitrogenase and the growth of root nodules, which reduces the ability of nitrogenase to fix nitrogen (Bansal et al. 2014), and decreases alfalfa yield under a high nitrogen level. Therefore, a reasonable application of nitrogen fertiliser helps improve the alfalfa yield.

Applying fertiliser significantly ameliorated the CP content of alfalfa (Figure 3B). Compared with P or K applications, N applications reported greater CP, as N directly stimulates key N metabolic enzymes and further promotes protein synthesis (Shah et al. 2017). Phosphorus increases N fixation efficiency and assists with N accumulation and CP synthesis in alfalfa (Macolino et al. 2013). Thereby, a combined application of NP fertiliser promotes alfalfa CP. K fertiliser activates various enzymes and promotes the absorption, assimilation, and utilisation of P by alfalfa (Wang et al. 2020). N and P fertiliser applications promote plant metabolism but decrease cell wall and lignin concentrations (Parsons et al. 2009). Applying N and P fertilisers in this study significantly decreased alfalfa ADF and NDF concentrations. K improved the cell wall concentrations and increased stem fibre digestibility and cell wall composition and concentration (Lamb et al. 2012); therefore, the effect of K was not as significant. Organic fertiliser provided abundant nutrients for the growth of alfalfa and significantly decreased alfalfa ADF and NDF concentrations due to the large amounts of N and P. However, a study in North America indicated that fertilisation with P, and especially K, increased forage yield and resulted in slight decreases in forage nutritive value (Lissbrant et al. 2009). Another European study also showed that intensive mineral fertilisation applied over successive years in a crop rotation reduced the forage nutritive value of lucerne

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under increased forage productivity (Hakl et al. 2021). The above two studies indicated that higher NDF and lower digestibility with increasing nutrient supply and lower NDF under higher fertilisation cannot be automatically expected (Lissbrant et al. 2009, Hakl et al. 2021). This is slightly inconsistent with the results of this study, possibly due to the fact that alfalfa is mainly grown in areas with lower soil nutrients in northern China. Thus, fertilisation increased the yield and quality of alfalfa.

Climate has a tremendous effect on large-scale forage production patterns (Liu et al. 2021, Wang and Zhong 2021). China is located in the northern hemisphere, and as latitude decreases (i.e., from north to south), temperature and precipitation gradually increase (Xiang et al. 2023). As altitude increases, temperature and precipitation decrease (Zhou et al. 2023). Our findings demonstrate that yield and CP effect size increased in alfalfa along the altitude gradient. Low-altitude areas may be more restricted by N and P, while high-altitude areas may be more restricted by P and K (Wang et al. 2023). Low-fertility soils supplemented with fertiliser exhibit accelerated alfalfa growth and significantly improved yield. N, P, and K help improve CP content (Macolino et al. 2013, Shah et al. 2017, Wang et al. 2020). The ADF content and NDF effect size value were not significantly related to altitude, indicating that altitude does not affect fibre content. Plant growth at low latitudes was more affected by soil P than at mid-high latitudes, whereas plant growth at mid-high latitudes was more affected by soil N than at low latitudes (Tian et al. 2018, Yan et al. 2018). Our study site was located in a mid-latitude region, although yield, CP, ADF, and the NDF effect size had significantly negative effects with latitude, fertiliser applications appropriate for local conditions, such as excessive N fertiliser, may increase non-protein N content and further reduce alfalfa CP content, as well as reduce N fixation capacity and yield of alfalfa (Bahulikar et al. 2020). MAT and MAP in this research do not reduce too much heterogeneity in alfalfa yield and CP effect size, perhaps due to agricultural management practices, including water supply (Santos et al. 2020), cutting (Hakl et al. 2017), the harvest period (Palmonari et al. 2014), and the growth year (Fan et al. 2016) have generally affected the yield and CP. MAT and MAP account for some of the heterogeneity in ADF and NDF. High precipitation alleviates the water restriction for alfalfa and decreases the fibre concentration (Liu et al. 2018), which was contrary to our results. High alfalfa yields in China are predominantly found in the northern regions, including Northeast China, Xinjiang Province, and the Loess and Inner Mongolian Plateaus, but seldom in southern regions. Conclusively, our results indicate that although environmental factors significantly affect the large-scale patterns of alfalfa quality, they cannot be used solely to predict the development of alfalfa in China, and their synergistic interactions with other factors should be considered.

Acknowledgement. The authors are grateful to several researchers whose published articles were used for this meta-analysis. The authors are grateful to the associate editor and the reviewers for their useful feedback that improved this paper.

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Received: November 18, 2023
Accepted: March 6, 2024
Published online: April 4, 2024