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Uncertainty analysis of critical nitrogen dilution curves for wheat

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A R T I C L E I N F O

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A B S T R A C T

Critical nitrogen (N_c) dilution curves have been widely used for the plant N status diagnosis, N management, crop modeling, and remote sensing. Wheat is a major crop worldwide whose N_c dilution curves developed under different conditions (genotype × environment × management) show large parameter variations. Herein, a dataset of 19 nitrogen fertilizer experiments (n = 656) from five wheat planting sites in China was used to evaluate the uncertainty in these curves and explain the sources of differences using the Bayesian theory method. The uncertainty of the fitted curve decreased with the increase in plant biomass. The parameter A_2 of fitted curves of genotype × environment × management combinations showed a greater variation than A_1, with slight parameter differences among different genotypes and planting sites. The variables related to genotype and growth environment, i.e., maximum N concentration, maximum biomass, and accumulated growing degree days during the vegetative growth period, were the sources of differences in curve parameters. Though the N_c curve differences between genotype × environment × management were statistically significant, the nitrogen nutrition index (NNI) differences remained relatively small. This study provides insights for developing a more diverse N_c dilution curve to aid in optimal nitrogen fertilization management in wheat.

1. Introduction

Wheat (*Triticum aestivum* L.) is a staple food for millions of the world's population. The fast-growing population and the improvements in living standards underscore the need to produce more food to meet people's demands (Ortiz et al., 2008). Nitrogen (N) is the most abundant element used as an artificial fertilizer in agricultural production (Zhao et al., 2007; Dambreville et al., 2008). It has attracted worldwide attention because of its effects on the environment (Reis et al., 2016). Farmers usually apply excessive N fertilizer to ensure high wheat yields (Heffer, 2009). However, excessive N application does not significantly increase wheat yield but rather increases the risk of environmental pollution and resource loss (Chen et al., 2014). Establishing an appropriate crop N nutrition diagnosis method is essential to optimize N fertilizer management by increasing the understanding between crop N nutrition and growth. Crops absorb sufficient N, achieve yield potential, and increase nitrogen recovery efficiency to reduce the negative environmental impact of nitrogen (Zhao et al., 2009).

The establishment of the critical nitrogen (N_c) dilution theory of crops has improved the understanding of crop N nutrition and growth status (Lemaire and Salette, 1984a,b). The power function dilution curve (power function $N_c = A_1 DM^{-A2}$) is used to describe the decreasing crop N concentration with increasing biomass. In the power function, A_1 and A_2 are the curve's estimated parameters; parameter A_1 is the plant N concentration when the above-ground biomass is 1 t ha^{-1}, whereas parameter A_2 controls the slope of the curve. The N nutrition index (NNI, the ratio of plant N concentration to critical N concentration) based on the N_c dilution curve is an effective index that quantifies the N status and fertilization decision of crops. An NNI value of 1 indicates the plant's optimum N status, while an NNI greater than or less than 1 indicates excessive and insufficient N, respectively (Lemaire et al., 2008). Several crops have been evaluated since the concept of plant N_c dilution curve was established, including wheat (Justes et al., 1994; Yue et al., 2012), rice (Sheehy et al., 1998; Ata-Ul-Karim et al., 2013; He et al., 2017),

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maize (Plénet and Lemaire, 1999; Ziadi et al., 2007), oilseed rape (Colnenne et al., 1998), potato (Bélanger et al., 2001). The concept has been widely used to evaluate crop nutrition, growth prediction, and fertilization management (Chen et al., 2021). Improving the prediction of plant nitrogen status in field crops requires reliable estimation of the critical nitrogen curve.

The N_c dilution curve for winter wheat in France (Justes et al., 1994) was $N_c = 5.35\ DM^{-0.44}$. It was thus used in wheat production worldwide (Stockle and Debaeke, 1997; Jeuffroy and Recous, 1999). Since then, several researchers have developed new N_c dilution curves for wheat under different growth conditions and tried to improve the performance of the N_c dilution curve under specific conditions. Yue et al. (2012) established the N_c dilution curve for wheat in the North China Plain to be $N_c = 4.15\ DM^{-0.38}$. Zhao et al. (2020) and Guo et al. (2020) indicated differences in N_c curves under different water conditions after recalibrating the N_c dilution curve using experimental wheat data collected under different irrigation conditions, and Zhao et al. (2020) also showed how to modify N_c curve under different irrigation conditions. Study has also shown that the N_c curves of two wheat genotypes in the same experiment were different (Zhao et al., 2012). These differences indicate that the curve parameters are affected by the growth environment, management, and genotype. Though these differences may not show actual differences under different conditions, they may show errors in model parameter estimations (Chen and Zhu, 2013). Further studies should be conducted to comprehensively explain the changes in N_c dilution curve parameters with genotype × environment × management to aid in developing a universal N status diagnostic tool for wheat. It is particularly crucial to analyze the uncertainties in the parameters of the N_c dilution curves and clarify the source of the difference across different genotypes, environments, and management. Makowski et al. (2020) and Ciampitti et al. (2021) proposed a Bayesian theoretical method to analyze the uncertainty of the N_c dilution curve parameters for major field crops (wheat, maize, and rice). The method allows one-step fitting of these curves directly from the original biomass and N concentration measurements. Based on the method, the confidence interval and parameter distribution of the fitted curve can be calculated and used to compare various crops, genotypes, or planting systems. The method can also estimate the parameter uncertainties.

Herein, the new Bayesian method was used to develop the N_c dilution curve for wheat by analyzing numerous N fertilizer experimental data (biomass and N concentration) during the vegetative growth period of wheat. The study aimed to evaluate the uncertainty of fitted N_c dilution curves and compare the statistical differences of the N_c dilution curve under different planting conditions (genotype × environment × management). It also aimed to analyze the different sources of fitted N_c dilution curve parameters under different planting conditions and explore the influence of curve difference on nitrogen diagnosis.

2. Materials and methods

2.1. Experiment design

Nineteen N fertilizer treatments were carried out in this study during the nine wheat growing seasons across eastern and central China. The study locations included Nanjing (NJ, 118°78′E, 32°04′ N), Rugao (RG, 120°76′E, 32°27′ N), Yizheng (YZ, 119°10′ E, 32°16′ N), Xuzhou (XZ, 117°13′E, 34°47′ N) and Xinxiang (XX, 113°48′E, 35°11′ N). Nine different wheat genotypes (Low-gluten wheat: Ningmai 9 (NM9), Ningmai 13 (NM13), medium-gluten wheat: Yangmai 16 (YM16), Aikang 58 (AK58) and Yangfumai 4 (YFM4), high-gluten wheat: Huaimai 20 (HM20), Yumai 34 (YM34), Xumai 30 (XM30), Jimai 22 (JM22)) were selected for evaluation. All experiments contained at least four N application rates. Each experiment included one or two N application rates considered non-limiting N for wheat growth and several N rates considered limiting N for wheat growth. Wheat was sown at the end of October or early November and matured in June of the following year. Except for the experiment in Xinxiang, which used different N fertilizer topdressing ratios, the other 18 experiments employed 50 % N fertilizer before planting and jointing. Phosphate fertilizers were applied before sowing, whereas 50 % of potassium fertilizers were applied before the sowing and jointing stage. Irrigation and field management followed local recommendations. Table 1 shows the experiment details, whereas Supplementary Table S1 shows the experimental sites and soil data.

2.2. Biomass and shoot N concentration

At least four plant samples for each experiment were obtained during the vegetative growth period to ensure successful curve fitting. The plants were divided into different organs and stored at 105 °C for 30 min to deactivate the tissue's metabolic activity. They were then dried at 80 °C until a constant dry weight was attained, and their weights were subsequently used to calculate the above-ground biomass of wheat. Different wheat organs were also mechanically crushed, passed through a 2 mm sieve, and stored to analyze their N concentration following the Kjeldahl method (Bremner and Mulvancy, 1982).

2.3. Data analysis

This study was based on the hierarchical Bayesian model. It was assumed that a linear-plateau function described the response of crop biomass to N concentration. In contrast, the N_c dilution curve parameters were directly derived from the fitted probability distribution (Makowski et al., 2020). The first level of the model described biomass response to N concentration on a given observation date based on a linear-plateau function. Each observation date corresponded to a specific crop growth stage in a given year, during which stage biomass and N concentration were measured for different N fertilizer levels. The second level of the model described the variability of the linear-plateau function parameters across the observation date using the probability distribution to calculate the N_c dilution curve. The third level described a priori information about parameter values. This study obtained a stable posterior distribution by adjusting the weak priori information range without strongly limiting parameter values, the prior ranges of parameter A_1 and A_2 of each fitted curve were 0–12 and 0–1, respectively.

The classic method for developing N_c dilution curves was applied to obtain a series of critical N concentration points to fit the N_c dilution curve (Lemaire et al., 2008). The N_c point was then corrected to the N_c dilution curve without considering its regression error. The new method does not distinguish between N-limiting and non-N-limiting crop growth treatment nor determines the N_c point (Makowski et al., 2020). All the uncertainties could be estimated and expressed as a probability distribution. They analyzed the uncertainty of any interest quantities. It allowed the user to calculate confidence intervals for the N_c dilution curve and its parameters. In the same line, the Markov chain Monte Carlo Algorithm (MCMC) was employed using the R package Rjags (Plummer, 2017) to estimate the posterior distribution of model parameters. Convergence was achieved after about 50,000 iterations. The first 50,000 iterations were discarded, followed by running the MCMC algorithm for 30,000 iterations. The MCMC algorithm results were then used to calculate the median and 95 % confidence intervals for multiple interest quantities.

The correlation between the curve parameters fitted under different experimental conditions and the genotype, environment and management, including maximum N concentration (Nmax), maximum biomass (DMmax) and days (VPD) in vegetative growth period, accumulated growth degree days (AGDD), rainfall, and photosynthetic effective radiation (PAR) in vegetative growth period, planting density, were comprehensively analyzed to determine the factors of the parameter difference in the N_c dilution curves. In addition, the wheat vegetative growth period refers to the stage from sowing to flowering.

Table 1
Characteristics of field experiments.

Exp. No	Year	Location	Cultivar	N rate (Kg ha^{-1})	N topdressing rate (%)	Sowing date	Plant density ($\times 10^4$ plants ha^{-1})	Row spacing (m)	Sampling date (days after sowing)	Number of data
1	2003–2004	Nanjing	Ningmai 9	0-75-150-225-300	50	Oct 25, 2003	180	0.25	141, 166, 174, 178, 186	30
2	2003–2004	Nanjing	Huaimai 20	0-75-150-225-300	50	Oct 25, 2003	180	0.25	141, 166, 174, 178, 186	30
3	2005–2006	Nanjing	Ningmai 9	0-90-180-270	50	Nov 18, 2005	180	0.25	150, 161, 170, 180	16
4	2005–2006	Nanjing	Yumai 34	0-90-180-270	50	Nov 18, 2005	180	0.25	150, 161, 170, 180	16
5	2007–2008	Nanjing	Ningmai 9	0-90-180-270	50/by plant status	Oct 25, 2007	150	0.25	146, 152, 176, 183, 194	35
6	2009–2010	Yizheng	Yangmai 16	0-75-150-225-300	50	Nov 5, 2009	180	0.25	109, 127, 133, 143, 154, 169, 176	35
7	2009–2010	Yizheng	Ningmai 13	0-75-150-225-300	50	Nov 5, 2009	180	0.25	109, 127, 133, 143, 154, 169, 176	35
8	2010–2011	Yizheng	Yangmai 16	0-75-150-225-300-375	50	Nov 6, 2010	180	0.25	117, 137, 143, 152, 159, 170, 176	42
9	2010–2011	Yizheng	Ningmai 13	0-75-150-225-300-375	50	Nov 6, 2010	180	0.25	117, 137, 143, 152, 159, 170, 176	42
10	2011–2012	Xinxiang	Aikang 58	0-75-150-225-300	65–50-35	Nov 7, 2011	300	0.25	124, 139, 147, 154, 162, 170, 175	91
11	2012–2013	Rugao	Xumai 30	0-75-150-225-300	50	Oct 28, 2012	225	0.25	119, 132, 142, 153, 161, 166	30
12	2012–2013	Rugao	Ningmai 13	0-75-150-225-300	50	Oct 28, 2012	225	0.25	119, 132, 142, 153, 161, 166	30
13	2013–2014	Rugao	Xumai 30	0-75-150-225-300	50	Oct 26, 2013	225	0.25	111, 121, 134, 140, 152, 160, 166, 171, 178	45
14	2013–2014	Rugao	Ningmai 13	0-75-150-225-300	50	Oct 26, 2013	225	0.25	111, 121, 134, 140, 152, 160, 166, 171, 178	45
15	2013–2014	Xuzhou	Xumai 30	0-90-180-270-375	50	Oct 26, 2013	240	0.20	129, 145, 158, 166, 176	25
16	2013–2014	Xuzhou	Jimai 22	0-90-180-270-375	50	Oct 26, 2013	240	0.20	129, 145, 158, 166, 176	25
17	2014–2015	Rugao	Ningmai 13	0-120-225-330	50	Oct 27, 2014	225	0.25	105, 132, 142, 152, 162, 173, 178	28
18	2014–2015	Rugao	Yangfumai 4	0-120-225-330	50	Oct 27, 2014	225	0.25	105, 132, 142, 152, 162, 173, 178	28
19	2014–2015	Rugao	Huaimai 20	0-120-225-330	50	Oct 27, 2014	225	0.25	105, 132, 142, 152, 162, 173, 178	28

$$AGDD = \sum_{1}^{N} \left(\frac{T_{max} + T_{min}}{2} - T_{base} \right) \quad (1)$$

Where, N was the days in vegetative growth period, T_{max} and T_{min} were the highest and lowest temperature of the day respectively, and T_{base} was the lowest temperature at which wheat starts physiological activities, in this study the t_{base} was 0 °C (Gallagher, 1979; Baker et al., 1980).

The root mean square error (RMSE) and normalized root mean square error (NRMSE) were used to compare the NNI calculated using the specific N_c curve and the average N_c curve. The RMSE and NRMSE were calculated using Eq.s (1) and (2), respectively. In the equations, P_i and O_i were the NNI calculated by the specific N_c curve and the average N_c curve, n was the sample size, and S was the mean value for the data.

$$RMSE = \sqrt{\frac{\sum_{i-1}^{n} (P_i - O_i)}{n}} \quad (2)$$

$$NRMSE(\%) = \frac{RMSE}{S} \times 100\% \quad (3)$$

3. Results

3.1. The uncertainty of the N_c dilution curve

Plant N concentration was diluted with the increase of plant biomass. Fig. 1 shows the N_c dilution curve of each genotype. The uncertainty of the N_c dilution curve was dependent on the biomass level. The uncertainty of the curve (the width of the 95 % confidence interval of the fitted curve) decreased as the biomass increased. At a lower biomass level (1 t ha^{-1}), the confidence interval's width ranged between 0.59 % and 2.43 %, while at a higher biomass level (15 t ha^{-1}), the width ranged between 0.17 % and 0.85 %. The relative uncertainty of parameters A_1 and A_2 (the ratio of the 95 % confidence interval width of the curve parameter to the median) was about 0.31 % and 0.47 %, respectively.

The width of the confidence interval decreased to a minimum and remained at a low level when the biomass was about 5 t ha^{-1}. However, the uncertainty of Yumai 34 continued to increase with the increase of biomass after the uncertainty decreased to the minimum (Fig. 2A). The uncertainty of the fitted curve was affected by the biomass level and the size of the experimental dataset (Fig. 2, Table 1). The uncertainty of the N_c dilution curve was reduced by increasing the number of observation dates or experimental treatments. Thirteen nitrogen fertilizer treatments (including five nitrogen levels and three top dressing ratios) and seven observation dates (91 data pairs) were carried out for Aikang 58. Its curve's uncertainty was maintained at a low level. Fewer observation dates or experimental treatments kept the uncertainty (confidence interval width) at a high level. There were only four nitrogen fertilizer treatment experiments (28 data pairs) carried out on Yangfumai 4, while Yumai 34 was observed four times (16 data pairs). The N_c dilution curve's uncertainty level estimated by all experiments was the lowest (Fig. 2A, B). It was maintained at a very low level throughout the vegetative growth period. The confidence interval width stabilized below 0.1 %N when the biomass level exceeded 3.3 t ha^{-1}.

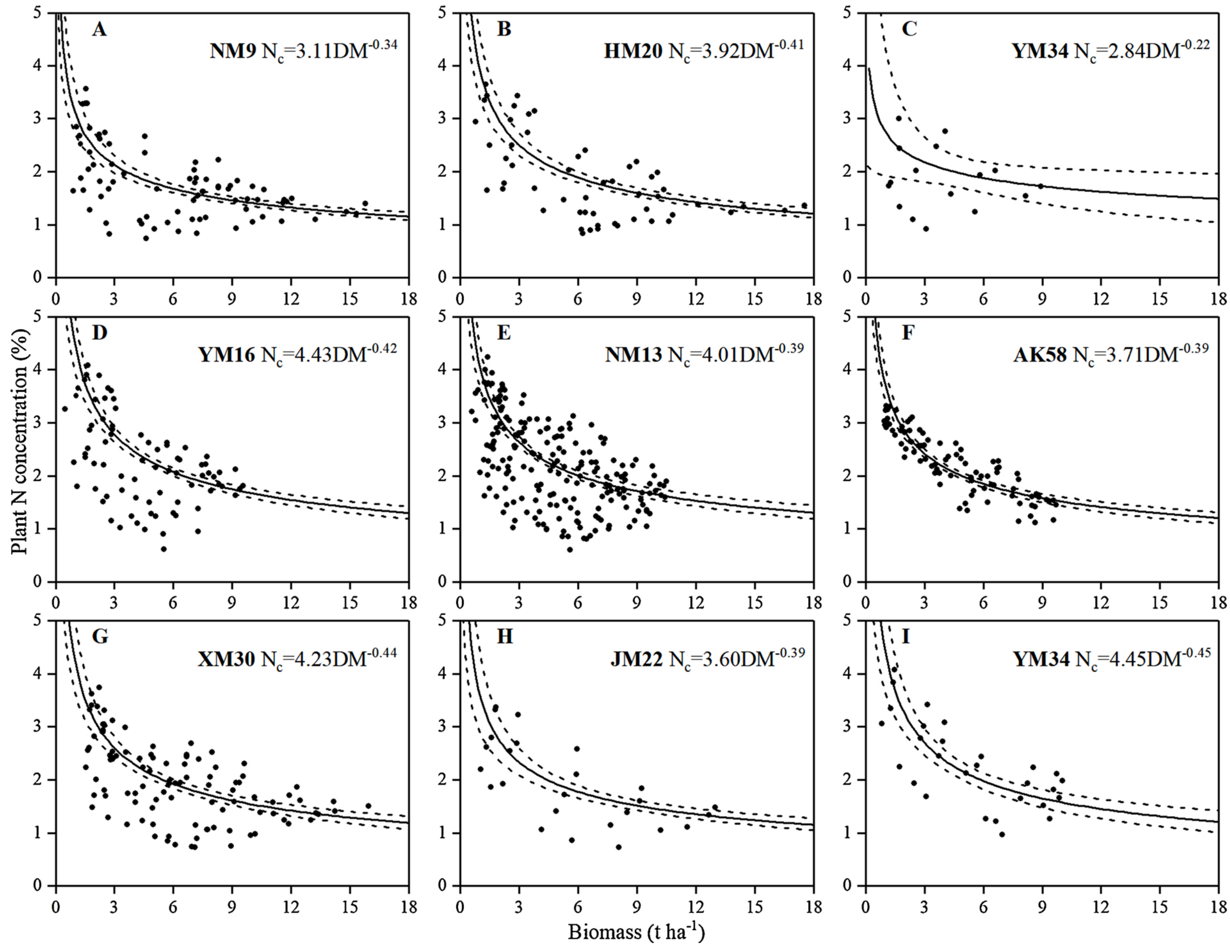

Fig. 1. Relationships between plant N concentration (%N) and plant biomass for each genotype during the vegetative growth period. Solid lines represent the critical N curves and their 95 % credibility intervals (dashed lines) whereas, each circle represents individual observations.

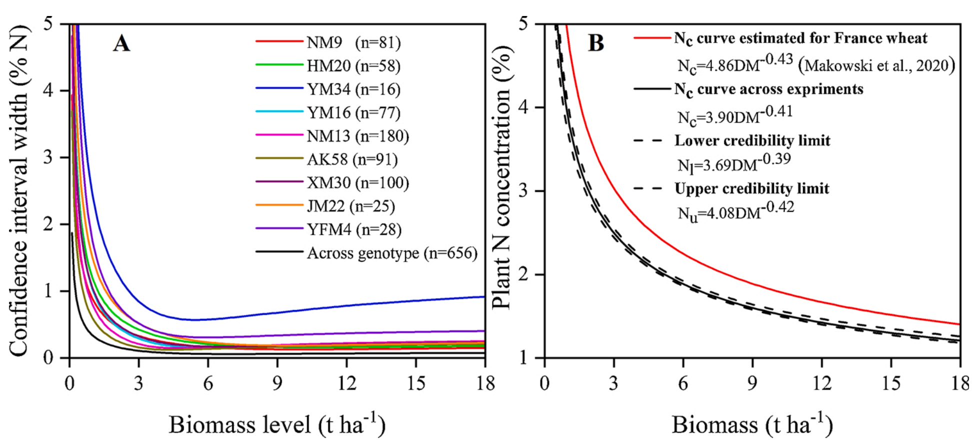

Fig. 2. The relationship between biomass (t ha^{-1}) level and confidence interval width (% N) for each genotype (panel A). Panel B compares the N_c curve across all experiments and the N_c curve of France wheat estimated by Makowski et al. (2020).

3.2. The differences between fitted curves

There were significant differences across different genotypes, planting sites, and years (Fig. 3). Larger values of A_1 and A_2 meant greater initial plant N concentration and dilution rate, respectively. A box plot was established for the parameter distribution of all 19 fitted curves to compare the differences between parameters A_1 and A_2 independently (Fig. 4). The posterior distribution median range of A_1 and A_2 parameters was 2.08–5.47 and 0.20−0.52, respectively. The curve parameter values fitted independently by each experiment fluctuated around the curve parameter values fitted by the hybrid of all experiment data (Fig. 4). The median values of parameters A_1 and A_2 of the hybrid curve were 3.90 and 0.41, respectively. The posterior distribution median of parameters A_1 and A_2 showed a consistent trend of change. The fitted curve with a high A_1 value also has a high A_2 value except for the 2013 RG NM13 experiment.

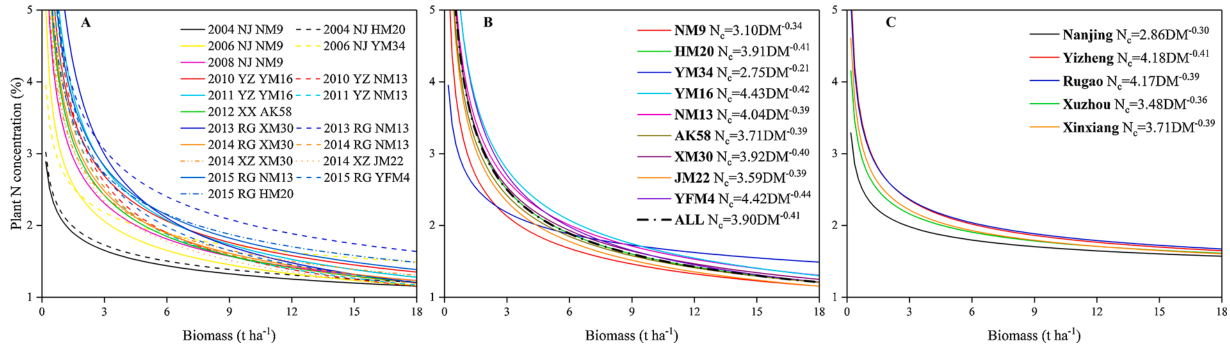

Fig. 3. Relationships between plant N concentration (%N) and plant biomass of each experiment, genotype, and planting site. Each line represents the median of the posterior distribution (panel A, B, C).

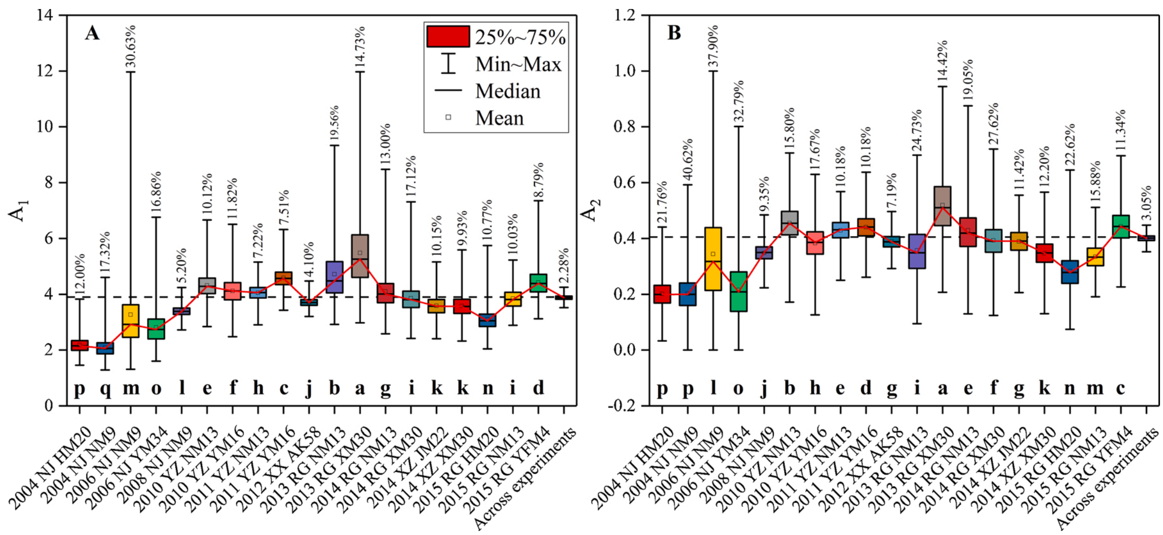

Fig. 4. Box plot of the posterior distribution of curve parameters A_1 (panel A) and A_2 (panel B) for each experiment. The dotted lines represent the posterior distribution median of curve parameters A_1 (y = 3.90, panel A) and A_2 (y = 0.41, panel B). The red lines connect the posterior distribution median of parameters A_1 and A_2 for each experiment, respectively. Different letters represent significant differences (p < 0.05). The percentage represents the variation coefficients of the parameters A_1 and A_2.

Among the 19 independently fitted curves, 2014 XZ JM22 and 2014 XZ XM30, 2014 RG XM30 and 2015 RG NM13 had no significant differences in parameter A_1. However, the remaining 15 fitted curves had significantly different parameter A_1 ($P < 0.05$, Fig. 4). Similarly, most of the fitted curve parameters A_2 were different except in 2004 N J NM9 and 2004 N J HM20, 2011 YZ NM13 and 2014 RG NM13, 2014 XZ JM22 and 2012 XX AK58) (Fig. 4B). These findings indicated that the genotype × growth × management effects significantly affected the fitted curve parameters A_1 and A_2. Notably, a few of the 19 curves herein were planted under the same environment and management, reflecting genotype differences. The fitted curve parameters of different wheat genotypes were also significantly different ($P < 0.05$, Supplementary Fig. 1). Seven cultivars showed significant differences in parameter A_1. However, there were no significant differences in YFM4 and YM16 in parameter A_1. In contrast, all nine cultivars showed significantly different parameter A_2 values. These findings suggested that the environmental × management effect significantly affected both the curve parameters A_2 and A_1. Different planting sites also showed significant differences in parameters A_1 and A_2 ($P < 0.05$, Supplementary Fig. 2).

The variation coefficient (the ratio of the standard deviation to average) between genotype × environment × management effects of parameter A_1 was smaller (CV = 12.47 %) than that of parameter A_2 (CV = 19.09 %) (Fig. 4). This result meant that A_1 was less affected by the comprehensive effect of genotype × environment × management than A_2. Based on the genotype, the curve parameter A_1 (7.96 %) had a smaller variation coefficient than A_2 (11.66 %) (Supplementary Fig. 1). Based on the planting sites, A_1 (5.00 %) and A_2 (7.23 %) showed a smaller variation coefficient (Supplementary Fig. 2). However, the wheat genotype and planting location curves were smaller than those of the combined effect of genotype × environment × management. Generally, the variation coefficient of N_c dilution curve parameter A_2 was higher than that of A_1.

3.3. The source of parameter differences in the N_c dilution curve

Parameter A_1 and A_2 were significantly positively correlated (Fig. 5), Both were affected by genotypes and environment. The Nmax had a highly significant positive correlation with the curve parameters A_1 and A_2, indicating that it determined the A_1 and A_2 curve parameters. Wheat genotypes with high plant N concentration in the early growth period had a higher value of A_1 and A_2 curve parameters. In contrast, the DMmax of wheat showed a significant negative correlation with the A_1 and A_2 curve parameters. Wheat genotypes with high biomass had lower values of parameters A_1 and A_2. Only the AGDD had a significant negative correlation with curve parameters A_1 and A_2. The significant effects of rainfall and PAR on parameters A_1 and A_2 of the fitted curves were not observed.

Nonetheless, the significant relationships between Nmax, DMmax,

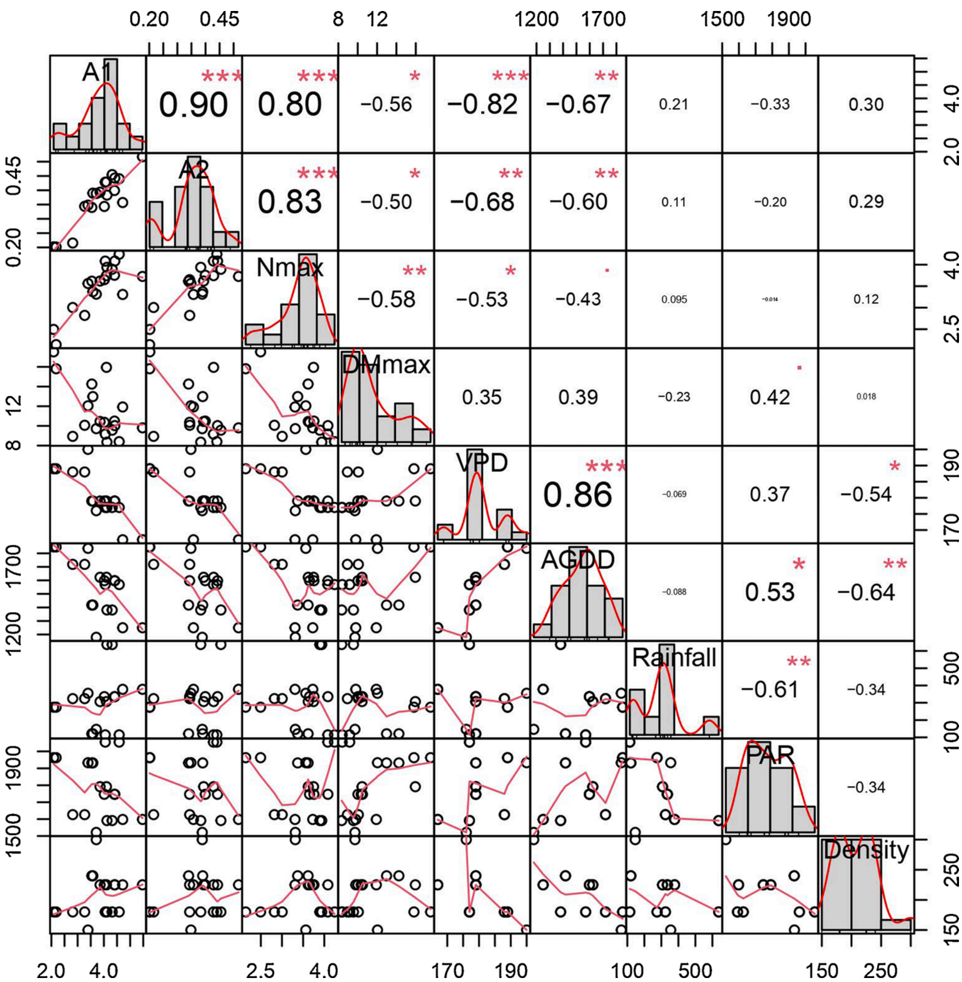

Fig. 5. Scatter graph matrix showing the correlation coefficient and significance between the fitted parameter (A_1 (A1) and A_2 (A2)), maximum N concentration (Nmax), maximum biomass (DMmax), and days (VPD) in vegetative growth period, accumulated growth degree days (AGDD), rainfall, photosynthetic effective radiation (PAR) and planting density, respectively. Larger fonts display larger correlation coefficient values. ***, significant P < 0.001, **, significant P < 0.01, *, significant P < 0.05, ·, significant P < 0.1.

VPD, and AGDD suggests that the plant N concentration in the early growth period was also affected by the growth environment (Fig. 5). The maximum biomass of wheat before flowering was positively affected by PAR. Though the wheat planting density had no significant effect on the fitted curve parameters, it was positively correlated with the vegetative growth days of wheat. Vegetative growth days of wheat with high planting density were longer. Collectively, both the genotype and the environment had a significant influence on the fitted curve parameters. Selected management (planting density) also had a potential influence on the fitted curve parameters.

3.4. Estimation of the nitrogen nutrition index

N_c curves from the genotype × environment × management were statistically different among the 19 experiments. However, the difference was relatively small (Fig. 6), with a low NRMSE (11.52 %) between the NNIs calculated by the average and the specific N_c curves (Fig. 6A). The dynamic change of NNI were shown in Supplementary Fig. 3. These results indicated that the errors could be statistically accepted if the average N_c curve was used for NNI calculation. The deviation between the NNI calculated by most specific curves and the average curve was small except for the NNI calculated in experiments 2004 N J HM20,

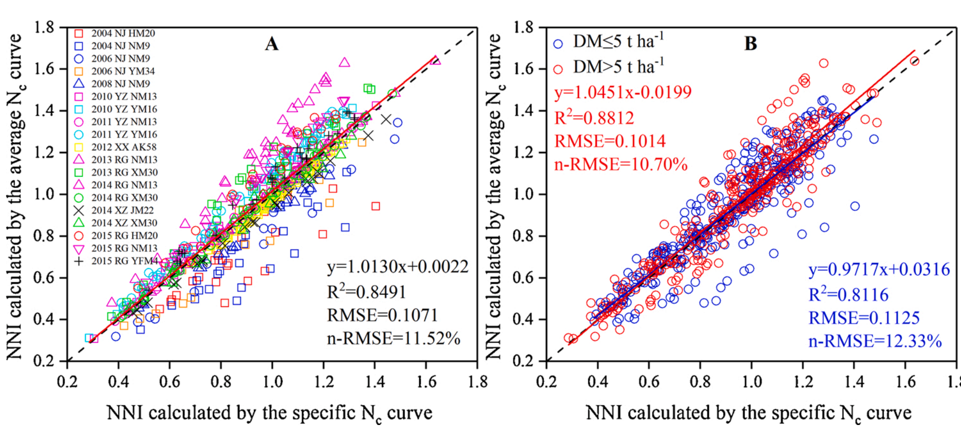

Fig. 6. Comparison of the NNIs calculated using the average and specific N_c curves (panel A: experiment dataset; panel B: biomass level).

2004 N J NM9, and 2013 RG NM13 (Supplementary Fig. 4). The estimated error was slightly lower at higher biomass (DM > 5 t ha^{-1}) compared to the lower biomass (DM < 5 t ha^{-1}), with an NRMSEs of 10.70 % and 12.33 %, respectively (Fig. 6B).

4. Discussion

It is rare to obtain numerous rigorous wheat nitrogen fertilizer experiment data. This study evaluated the biomass and plant N concentration dataset of 19 wheat nitrogen fertilizer treatments from five experimental sites using the recently proposed Bayesian framework (Makowski et al., 2020). The uncertainty of the N_c dilution curve of wheat was dependent on the biomass level, as previously reported by Makowski et al. (2020). The response curve was vertical (a large N concentration associated with very small biomass) at low biomass. Consequently, the N_c value was determined with very low precision, causing parameter A_1 to have high uncertainty. Nonetheless, the uncertainty of N concentration at high biomass became relatively low as the response curve for high biomass became horizontal (a low N concentration associated with large biomass). The high correlation between A_1 and A_2 implies that the corresponding uncertainty in A_2 absorbs a part of the uncertainty in A_1. Besides, the dataset size of the fitted curve (number of experimental treatments × observation times) determined the uncertainty level of the N_c dilution curve of wheat. A larger dataset helped determine the N_c dilution curve and better reflected the actual differences of the N_c dilution curve. Cognizant of this, it is crucial to reduce the uncertainty at low biomass by concentrating more data points at this period or using more effective experimental designs with many replicates to reduce data point scattering (Ratjen and Kage, 2016). Accurate estimation of the N_c dilution curve reflects the true differences rather than measurement and statistical errors. It also determines the sources of differences in the fitted N_c dilution curve (Chen and Zhu, 2013).

Most of the N_c dilution curves were different in the 19 experiments. The different curves reflect the comprehensive effects of genotype × environment × management on relative changes in biomass and N concentration during the wheat growth process. As such, changes in crop genotypes, environment, and management necessitate the reassessment of the N_c dilution curve fitted parameters to reduce the risk of crop N nutrition diagnostic errors and facilitate fertilization management decision making (Yin et al., 2018; Makowski et al., 2020; Zhao et al., 2020). Two processes cause a decrease in crop N concentration. The N in the shaded part of the leaves is transported to the top of the canopy for optimal use, thus reducing the N concentration in the shaded part of leaves. Additionally, the plant keeps growing upward to catch more light, thereby leading to an increased proportion of the plant stem and leaves (Greenwood et al., 1990; Justes et al., 1994). Plants have no competition with each other during their early growth stages under sufficient light. They are mainly characterized by increased metabolic processes and slow nitrogen dilution (Le Bot et al., 1998; Mills et al., 2009). Herein, the curve parameter A_1 only changed slightly, indicating that the N concentration of the same crop genotype in the early growth stages was constant and only slightly affected by the environment × management (Ziadi et al., 2009). The change of N concentration in the early vegetative growth stage of wheat reflects the true genotype difference and environment × management effects. Both parameters A_1 and Nmax were negatively affected by AGDD. The negative effect was attributed to the long overwintering period and different environments that changed the wheat's N concentration. Notably, there were high-nitrogen and low-nitrogen or winter and spring wheat cultivars included in the study. However, the study dataset only considered the N concentration changes when the wheat biomass exceeded 1 t ha^{-1} and ignored the N concentration changes that occurred earlier. Previous studies postulate that wheat genotypes with stronger N uptake capacity tend to have higher parameter A_1 values. Their structural tissues increase rapidly, and the N content of metabolic tissue is diluted, thereby causing a rapid decline in the plant N concentration (Ata-Ul-Karim et al., 2013; Caloin and Yu, 1984). A larger A_1 value translated to a larger A_2 value, higher wheat biomass, and shorter vegetative growth days (Fig. 5). Genotypes with strong N uptake can accumulate more dry matter resulting in higher plant N concentration (Yue et al., 2012).

Parameter A_2 reflected the dilution process of plant N concentration with increasing biomass. It was the specific mode of N concentration dilution. These results suggested that the degree of variation of the fitted curve parameter A_2 was higher than that of parameter A_1, as previously reported by Kage et al. (2002); Sheehy et al. (1998); Ziadi et al. (2007); Justes et al. (1994). The comprehensive effects of genotype × environment × management were slightly larger than those of parameter A_1. The change of N with biomass during wheat growth was easily determined by its planting environment. Both genotype and environment had a slight influence on parameter A_2. Parameter A_2 of the high-biomass wheat genotype curve with higher N concentration was greater. The higher vegetative growth days and AGDD maintained the vegetative growth of wheat. The active biomass accumulation ability delayed plant senescence and reduced the rate of plant N decline. However, more light increased the biomass accumulation in the vegetative growth period of wheat, potentially explaining the parameter differences. Previous studies postulate that the planting density significantly impacts the A_1 and A_2 values of the maize N_c curve (Greenwood et al., 1990; Ziadi et al., 2007). Herein, the planting density lacked a significant influence on the curve. This phenomenon was attributed to wheat having more tillers, which adjust the canopy size based on plant density. The tillers' density at the end of winter potentially affected the N_c curve during the early growth period. Seginer (2004) demonstrated that plant density during the early crop growth period is an important factor in the N dilution process. It explains the cause of the relatively high N concentrations of wheat in France (Justes et al., 1994; Fig. 2B). The uneven distribution of N in the canopy of wheat plants was attributed to the vertical distribution of light in the canopy and crop senescence. Wheat with a higher planting density reaches the biomass of 1 t ha^{-1} earlier, and their vegetative growth time is shorter. They also contain less structural organization (stem, sheath, and leaf veins). Only the AGDD had a significant negative correlation with curve parameters A_1 and A_2. The significant effects of rainfall on parameters A_1 and A_2 on the fitted curves was not determined because all the experiments were under irrigation. During the management of N nutrition during the crop growth period, especially when the crop under N deficiency, the NNI accuracy depends on the uncertainty of the N_c dilution curve. As such, it is crucial to evaluate these differences. Herein, the N_c curve difference between genotype × environment × management was statistically significant, but the NNI differences remained relatively small. The high uncertainty observed during the early growth stages indicates that NNI-based plant diagnosis should be conducted after a plant's physiological development to reduce uncertainty.

Nevertheless, this study was limited by several factors. The new N_c dilution curve evaluation method did not provide adequate information regarding the diagnosis of crop N nutrition and fertilization decision-making based on the uncertainty results. Therefore, the sources of differences in the N_c dilution curve of wheat need to be systematically analyzed to develop a new curve incorporating these differences. Detailed descriptions of the N_c dilution curve under different genotype × environment × management combinations should also be added to expand the application prospects of the N_c dilution curve (Fabbri et al., 2020).

5. Conclusion

This study used the Bayesian theoretical framework to estimate the N_c dilution curve parameters of wheat. A comprehensive analysis of genotype × environment × management was successfully performed to identify the uncertainty and parameter differences of the fitted curves. The biomass level determined the uncertainty of the fitted curve.

Increasing the biomass-N pairs and observations helped to accurately determine the N_c curve by reducing the entire curve's uncertainty level. The variation of the curve parameter A_1 was less than that of A_2 under the combined effect of genotype × environment × management. There were also slight differences in parameters A_1 and A_2 under different genotypes and planting site conditions. Genotype and environmental factors, i.e., the maximum N concentration, maximum biomass, and accumulated growth degree days during the vegetative growth period, were the sources of differences in curve parameters. Though the N_c curve difference between genotype × environment × management was statistically significant, the NNI differences remained relatively small.

Credit authorship contribution statement

BY: Data curation, formal analysis, writing of the draft paper.
XW: Data curation, formal analysis, writing of the draft paper.
GL: Study design, review of the paper.
DM: Data analysis.
QC: Data collection and data curation.
XL: Data collection and data curation.
LL: Review and editing.
BL: Review and editing.
YZ: Study design, review of the paper.
WC: Study design, review of the paper.
LT: Study design, formal analysis, review and editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eja.2021.126315.

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