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□ *The study was conducted at three sites during 2008 and 2009 in the North-East China Plain (NECP). Field experiments consisted of five or six nitrogen (N) fertilization rates (0–350 kg N ha^{-1}). Shoot biomass and N concentration (N_c) of spring maize (*Zea mays* L.) were determined on six sampling dates during the growing season. Nitrogen application rate had a significant effect on aerial biomass accumulation and Nc. As expected, shoot Nc declined during the growing period. A critical N dilution curve ($N_c = 36.5\ W^{-0.48}$) was determined in China, which was a little different from those reported for maize in France and Germany. Besides, the N nutrition index (NNI) calculated from this critical N dilution curve was significantly related to relative grain yield, which can be expressed by a linear with plateau model ($R^2 = 0.66$; $P < 0.001$). NNI can be used as a reliable indicator of the level of N deficiency during the growing season of maize.*

Keywords: critical nitrogen concentration (N_c), nitrogen nutrition index (NNI), spring maize, dry matter

INTRODUCTION

Sustainable agricultural development must address relevant environment challenges while endeavoring to meet growing food demand with high crop yield (Liu et al., 2006). In order to achieve maximum yield, the quantities of nitrogen (N) fertilizer applied was often larger than the quantity strictly required (Ma et al., 2008). But maximum yield does not always correspond to maximum N fertilizer application (Scharf and Lory, 2009). Excessive N fertilization resulted in serious environmental problems because

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of atmospheric, soil and water enrichment with reactive N of agricultural origin (Ju et al., 2009). So it is important to provide farmers with crop N status diagnostic tools in order to decide the rate and the timing of N fertilizer applications. Optimized nutrient management practices that are based on soil testing and yield targets have been developed to provide sustainable crop production and environment protection (He et al., 2009; Cui et al., 2008; Alivelu et al., 2006). However, it has to be kept in mind that estimating the amount of N that is available for crops under filed condition depends on the actual condition for mineralization in the specific year. Moreover, soil-based N management practices have been found to be poorly correlated with optimum N rated currently (Vanotti and Bundy, 1994; Doerge, 2002; Nafziger et al., 2004).

Besides soil-based diagnostic tools, the plant itself is the best indicator for the N supply from the result of the growth period. Plant-based diagnostic methods of N deficiency have been used to improve N management and decrease the risk of N loss to ground and surface waters (Lemaire et al., 2008; Ziadi et al., 2008b; Naud et al., 2009; Vouillot et al., 1999). Previous research has indicated a close relationship between N concentration (Nc) and shoot biomass. The plant-based diagnostic methods of N deficiency can be based on the definition of a critical N concentration, that is, the minimum N concentration required for maximum growth (Ulrich, 1952).

Many studies show that plant nitrogen concentration decreased during the growth cycle, even when there is ample of N. Lemaire and Salette (1984a, 1984b) and Lemaire et al. (1985) demonstrated that for grasses and lucerne the decline in plant N concentration (%N) was related to dry matter accumulation by stand (W) whatever the climatic conditions of the year or the species and genotype. This decline in%N was described by a negative power function called “dilution curve”:

$$\mathrm{N_c} = aW^{-b} \tag{1}$$

When W is expressed in Mg ha^{-1} and N in g kg^{-1}, then coefficient a represents plant N concentration in percent when crop mass is 1 Mg ha^{-1}. Coefficient b is represents the coefficient of dilution which describes the relationship of decreasing N concentration with increasing shoot biomass. Lemaire and Gastal (1997) indicated that every species should have its own critical N dilution curve according to its histological and morphological characteristics. Such an approach has been developed for potato (*Solanum tuberosum* L.) (Greenwood et al., 1990), winter wheat (*Triticum aestivum L.*) (Greenwood et al., 1990), pea (*Pisum sativum* L.) (Ney et al., 1997), rice (*Oryza sativa* L.) (Sheehy et al., 1998), rapeseed (*Brassica napus* L.) (Colnenne et al., 1998), tomato (*Lycopersicon esculentum* Mill.) (Tei et al., 2002), annual ryegrass (*Lolium multiflorum* Lam.) (Marino et al., 2004), linseed (*Linum* *usitatissimum* L.) (Flénet et al., 2006), cotton (*Gossypium* spp.) (Xue et al., 2007), and sprint wheat (Ziadi et al., 2010) and so on.

In maize (*Zea mays* L.), the parameters for this allometric function were estimated by Plénet and Lemaire (2000) ($a = 34.1$ and $b = 0.37$) from data obtained in France using approximately weekly sampling up to 25 d after silking on irrigated maize crops. The similar parameters ($a = 31.4$, and $b = 0.39$) was confirmed by Herrmann and Taube (2004) in Germany and the range of the critical N curve was extended to maturity. Ziadi et al. (2008a) proved the critical N curve from France is valid in eastern Canada and the nitrogen Nutrition Index (NNI) calculated from that curve is a reliable indicator of the level of N stress during the growing season of corn.

Spring maize is widely grown in the North-East China Plain, which is one of the three golden areas of "Corn belt", produced above 30% of the national maize production, and is considered to be the most important cereal production area in China. However, a critical N dilution curve has never been determined for Chinese spring maize. Differences in the N critical curve between species and experiments sites have been reported. So a validation of these parameters for the climatic conditions and corn hybrids of north-east China is therefore required.

Our objectives were: (1) to determine a critical N dilution curve for spring maize in China; (2) to compare this curve with existing critical N dilution curves for spring maize, and (3) to assess the probability of using this critical N curve to estimate the level of nutrition in spring maize.

MATERIALS AND METHODS

Site Description and Treatment

A field experiment was conducted at three sites in Liufangzi (43°34′N, 124°54′E), Taojia (43°39′N, 124°59′E) and Yushu (44°49′N, 126°33′E) in the North-East China Plain (NECP, which is the main production region of maize in China) in 2008 and 2009. A new experimental site was selected each year but with the same county. No irrigation water was supplied during the maize growing season. Site characteristics are presented in Table 1. At all sites the maize hybrid of 'Zhengdan 958' was cultivated, with the plant density of 60,000 plants ha^{-1}.

Treatments consisted of six N applications: 0, 70, 140, 210, 280 and 350 kg N ha^{-1} (in urea form), except the Yushu 2009 site, in which just five N applications were conducted. A randomized complete block design was employed with three replications. To ensure the population density (60,000 plants ha^{-1}) and even distribution of plants in the plot, double sowing and thinning at the 3–5 leaf stage were carried out. All plots were 40m^2 and received 75 kg phosphorus pentoxide (P_2O_5) ha^{-1} (in diammonium phosphate form) and 75 kg dipotassium oxide (K_2O) hm^{-1} (in potassium

TABLE 1 Site characteristics in different years

	2008			2009		
Soil/crop information	Liufangzi	Taojia	Yushu	Liufangzi	Taojia	Yushu
Organic matter (%)	1.89	2.44	2.45	2.0	2.3	2.7
pH	6.2	6.6	5.2	4.9	5.2	5.6
Available N (mg kg^{-1})	128.9	146.9	156.9	118.2	124.2	130.3
Available P (mg kg^{-1})	65.7	68.9	78.3	75.7	16.1	31.8
Available K (mg kg^{-1})	129.2	158	147.4	122.4	199.6	179.9
Precipitation during growing season (mm)	599	536	498	249	250	285
Planting date	2 May	2 May	6 May	30 Apr.	30 April	6 May
Harvesting date	29 Sep.	29 Sep.	6 Oct.	22 Sep.	22 Sep.	6 Oct.

chloride form) as basic fertilizer. At each plot 1/3 N was used as basic fertilizer and 2/3 N was dressed on 8-9 leaf stage.

Sample Collection and Analysis

Five plants per block for each treatment was harvested at five or six grown stages in both years. After drying at 80°C to a constant weight, dry weights of all samples were recorded. Each sample at any stage was ground to pass a 1mm screen. Sample mills were mineralized using sulfuric acid (H_2SO_4)-hydrogen peroxide (H_2O_2) and N was measured using the standard Kjeldahl method (Horowitz, 1970). Grain yield was determined in each plot by harvesting whole plants manually and was adjusted to 14% moisture.

Data Analysis

Shoot biomass data for each sampling date, site and year were subjected to ANOVA using SAS (SAS Institute, Cary, NC, USA), and the Least Significant Difference (LSD) values were presented when the treatment effect had a P value ≤ 0.05. The determination of a critical N curve requires the identification of data points for which N does not limit shoot growth or is not in excess. These data points correspond to a N rate above which shoot biomass does not significantly increase. We used the procedure proposed by Greenwood et al. (1990) and Ziadi et al. (2010) to identify these data points. For each site-year and sampling date, the significantly highest shoot biomass ($P \leq 0.05$) obtained with any rate of N fertilization and the corresponding N concentration were identified and selected. In cases where the highest shoot biomass was obtained with two or more N rates, the lower rate was selected. These data points were then used to determine the relationship between critical N concentration and shoot biomass using an allometric function.

Data points not retained for establishing the parameters of the allometric function were used to test the validity of the critical curve. These data points were characterized as representing limiting and non-limiting N conditions using a method similar to that of Greenwood et al. (1990). Sampling dates were not used to test the validity of the critical N dilution curve if the ANOVA indicated no significant ($P > 0.05$) differences among the N application rates. For the remaining sampling dates, treatments were classified using the LSD test. Treatments with significantly ($LSD_{0.05}$) lower shoot biomass were considered to be limiting, whereas treatments with significantly higher shoot biomass were considered to be non-limiting.

The N nutrition index (NNI) describes the radio between the actual and the critical plant N concentration (N_c) at a particular biomass, an approach previously used for corn (Ziadi et al., 2008b). The relative yield was calculated as the ratio of the grain yield obtained for a given N rate to the highest grain yield across the N application rates. The relative yield was expressed as a function of NNI, and the linear-plateau function was estimated using SAS.

RESULTS

Shoot Biomass and Nitrogen Concentration

Shoot biomass ranged from 0.48 to 22.53 Mg DM ha^{-1} from joining stage to maturity, depending on N application rates, sampling date, site and year (Figure 1). The N application rate affected shoot biomass throughout the growing season, although this effect was not always statistically significant. N concentration in above-ground shoot biomass declined during the growing season and with increasing shoot biomass (Figure 2). A higher N application rate generally resulted in a higher plant N concentration. Nitrogen concentrations varied from a maximum of 38.25 g kg^{-1} DM at Taojia on 27 June 2008 to a minimum of 4.96 g kg^{-1} DM observed at Taojia on 22 September 2009.

Determination of a Critical Nitrogen Dilution Curve

Among all site-years, 26 sampling dates out of 29 met the previously defined statistical criteria (Table 2). Each of these sampling dates provides a critical point of N concentration for a given shoot biomass. As previous researches the critical N dilution curve cannot be applied to low shoot biomass ($<$1 Mg DM ha^{-1}). At these earlier stages of growth, critical N concentration takes a constant value due to the small decline of N_c with increasing shoot biomass and the lack of competition for light among isolated plants (Lemaire and Gastal, 1997; Herrmann and Taube, 2004). So one critical points corresponded to a shoot biomass of < 1 Mg DM ha^{-1} and was exclude. Therefore, 25 critical points, those with a shoot biomass ≥ 1.31 Mg DM ha^{-1}, were used to estimate the parameters of the critical N dilution curve:

$$N_c = 36.5W^{-0.48} \tag{1}$$

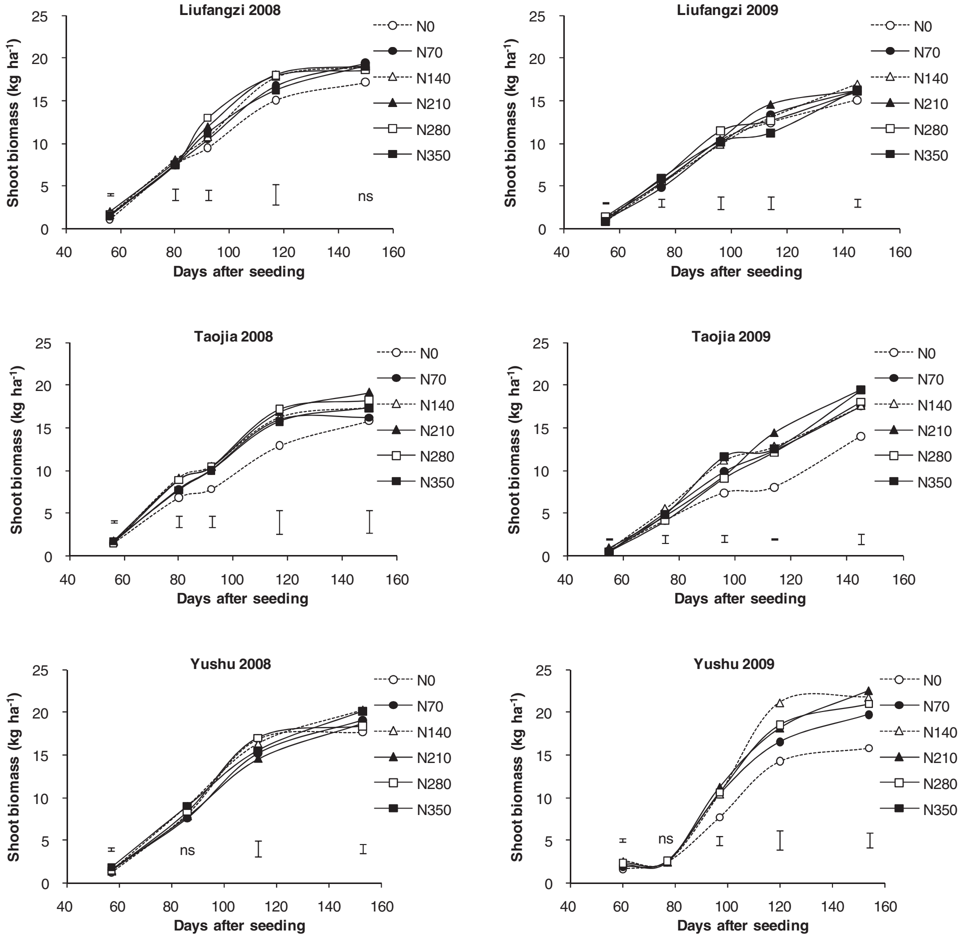

FIGURE 1 Maize shoot biomass on different sampling dates at six site-years. The vertical bars represent LSD values ($P <= 0.05$) at each sampling date, and ns indicates that the LSD was not determined.

Where N_c is the total N concentration in shoots expressed in g kg^{-1} DM, and W is the total shoot biomass expressed in Mg DM ha^{-1}. The model accounted for 81.1% of the total variance. The 95% confidence interval of the mean was 4.4 N g kg^{-1} DM for a shoot biomass of 1.31 Mg DM ha^{-1} and 2.2 N g kg^{-1} DM for a shoot biomass of 21.8 Mg DM ha^{-1} (Figure 3).

Discrimination between Limiting and Non-Limiting Nitrogen Conditions

The data points not retained by statistical criteria defined earlier were used to test the validity of the critical curve. A new selection was operated on these data in order to retain the significantly either N limiting or non-limiting treatments use the method described by Justes et al. (1994). As shown in

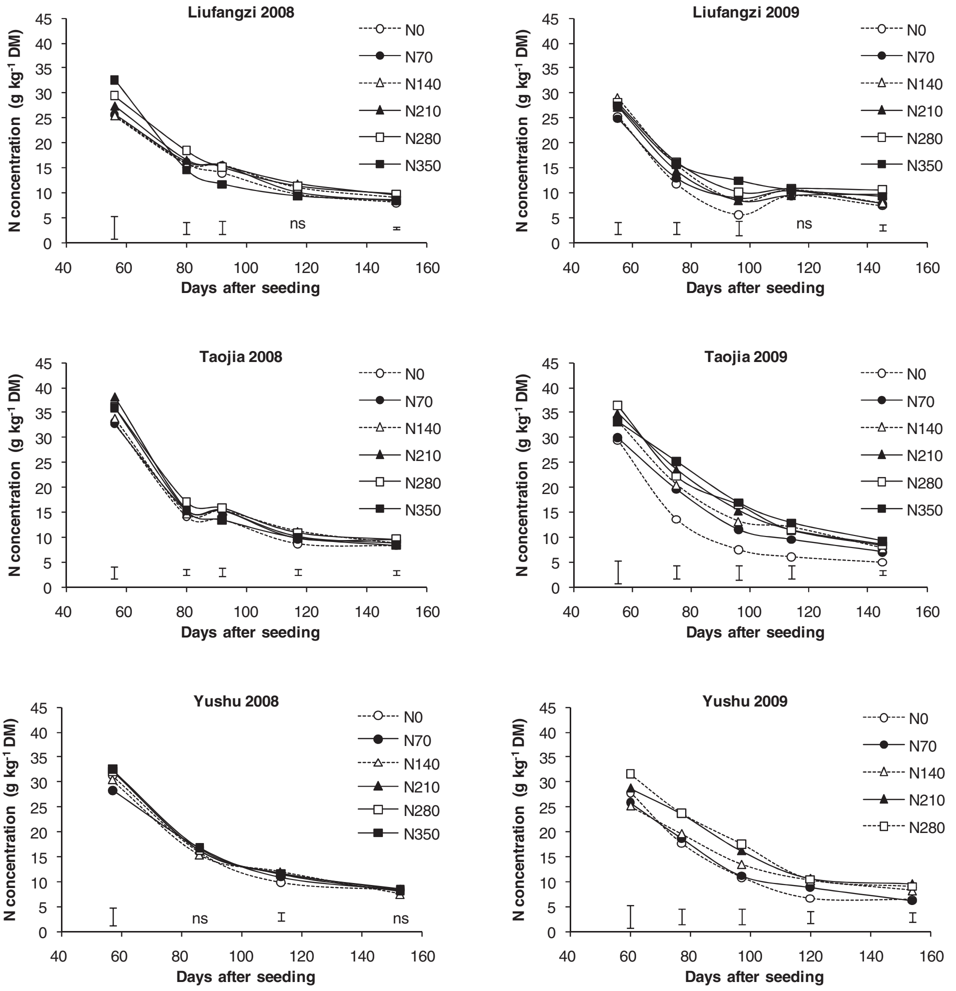

FIGURE 2 Nitrogen (N) concentration of shoot biomass on different sampling dates at six site-years. The vertical bars represent LSD values ($P <= 0.05$) at each sampling date, and ns indicates that the LSD was not determined.

Figure 4, the new critical N dilution curve discriminated between the limiting and non-limiting N conditions. More than 88% of data points identified as limiting N conditions were under the critical N dilution curve. So this partial validation suggests that this critical N dilution curve discriminates efficiently between limiting and non-limiting N conditions. But more validation should be done with a completely independents data.

Minimum and Maximum Nitrogen Concentration

Taking into account all the data from the six experiments in 2008 and 2009, it can be concluded that the N concentration for a given biomass

TABLE 2 Data points selected to establish the critical N dilution curve

Site	Year	Sampling date	Applied N (kg ha^{-1})	Shoot biomass (Mg DM ha^{-1})	Shoot N concentration (g kg^{-1} DM)
Liufangzi	2008	27 June	210	2.02	27.35
Liufangzi	2008	21 July	0	7.47	15.80
Liufangzi	2008	2 Aug.	70	10.45	15.43
Liufangzi	2008	27 Aug.	70	16.76	10.06
Taojia	2008	27 June	70	1.53	32.75
Taojia	2008	21 July	140	9.09	14.75
Taojia	2008	2 Aug.	70	10.05	15.37
Taojia	2008	27 Aug.	70	15.92	9.71
Taojia	2008	29 Sep.	140	17.32	8.72
Yushu	2008	2 July	70	1.53	28.37
Yushu	2008	27 Aug.	0	16.87	9.86
Yushu	2008	6 Oct.	140	20.27	7.53
Liufangzi	2009	24 June	140	1.31	28.97
Liufangzi	2009	14 July	0	5.23	11.71
Liufangzi	2009	4 Aug.	0	10.33	5.57
Liufangzi	2009	22 Aug.	70	13.33	10.34
Liufangzi	2009	22 Sep.	140	16.98	7.98
Taojia	2009	24 June	140	0.79	33.20
Taojia	2009	14 July	70	5.09	19.54
Taojia	2009	4 Aug.	140	11.16	13.22
Taojia	2009	22 Aug.	210	14.42	11.44
Taojia	2009	22 Sep.	210	19.49	8.51
Yushu	2009	5 July	140	2.54	25.16
Yushu	2009	11 Aug.	70	10.41	11.17
Yushu	2009	3 Sep.	140	21.15	10.28
Yushu	2009	7 Oct.	140	21.78	8.26

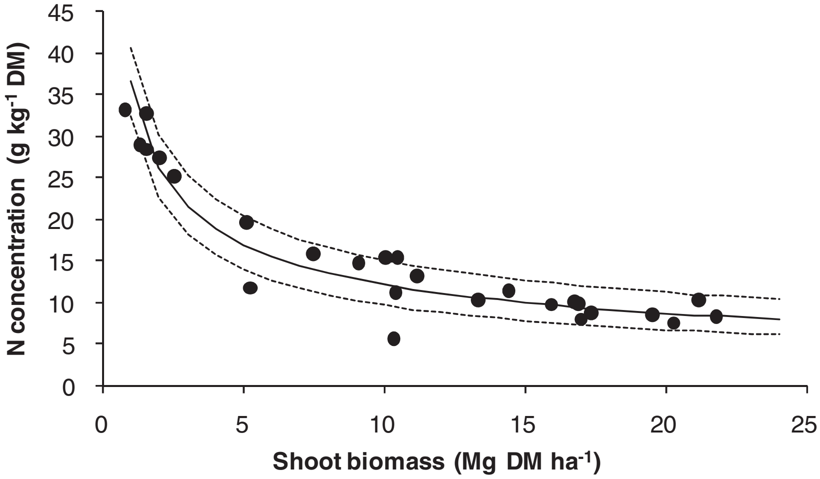

FIGURE 3 Critical nitrogen (N) data points used to define the critical N dilution curve. The solid line represents the critical N dilution curve ($N_c = 36.5W^{-0.48}$; $R^2 = 0.81$) describing the relationship between the critical N concentration and shoot biomass of spring wheat in north-east China. The dotted lines represent the confidence band ($P = 0.95$).

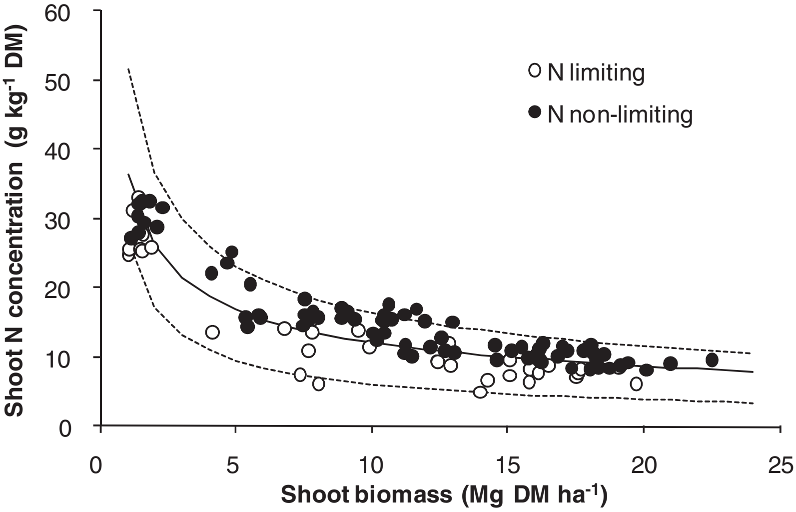

FIGURE 4 Validation of the critical nitrogen (N) curve using data from maize grown under limiting and non-limiting N growing condition. Solid line, critical N curve ($N_c = 36.5W^{-0.48}$) describes the relationship between the critical N concentration and shoot biomass of maize. The dashed line represent the minimum ($N_{min} = 26.9W^{-0.65}$) and maximum ($N_{max} = 51.6W^{-0.50}$) N concentration.

varies greatly with N supply. Using the observed maximum and minimum N concentrations (N_{max} and N_{min}) on each sampling date, two boundary curves were calculated (Figure 4). They correspond to the following equations:

$$N_{min} = 26.9W^{-0.65}\ (R^2 = 0.95) \tag{2}$$

$$N_{max} = 51.6W^{-0.50}\ (R^2 = 0.95) \tag{3}$$

Nitrogen Nutrition Index

We used the critical N dilution curve, defined in Eq. (1) to estimate the N_c for each sampling date and to calculate the NNI. Values of NNI $\geq$ 1.0 indicate that the crop is in a situation of non-limiting N, whereas values of NNI $<$ 1.0 indicate N deficiency. For instance, at Yushu 2009, the data points with N application of 0 and 70 kg ha^{-1} were generally smaller than 1 during the whole growing stage, indicated that N was limiting growth. With 210 and 280 kg N ha^{-1} the data points was above the critical N curve, indicating excessive N nutrition (Figure 5). At Liufangzi 2008, the data points with 350 kg N ha^{-1} were lower than other treatment, indicating N application may not always increase the N concentration in biomass, excessive N also may lead to N concentration decreasing.

The relationship between relative yield and NNI can be expressed by a linear-plateau function, accounted for 66% of the variation. Based on

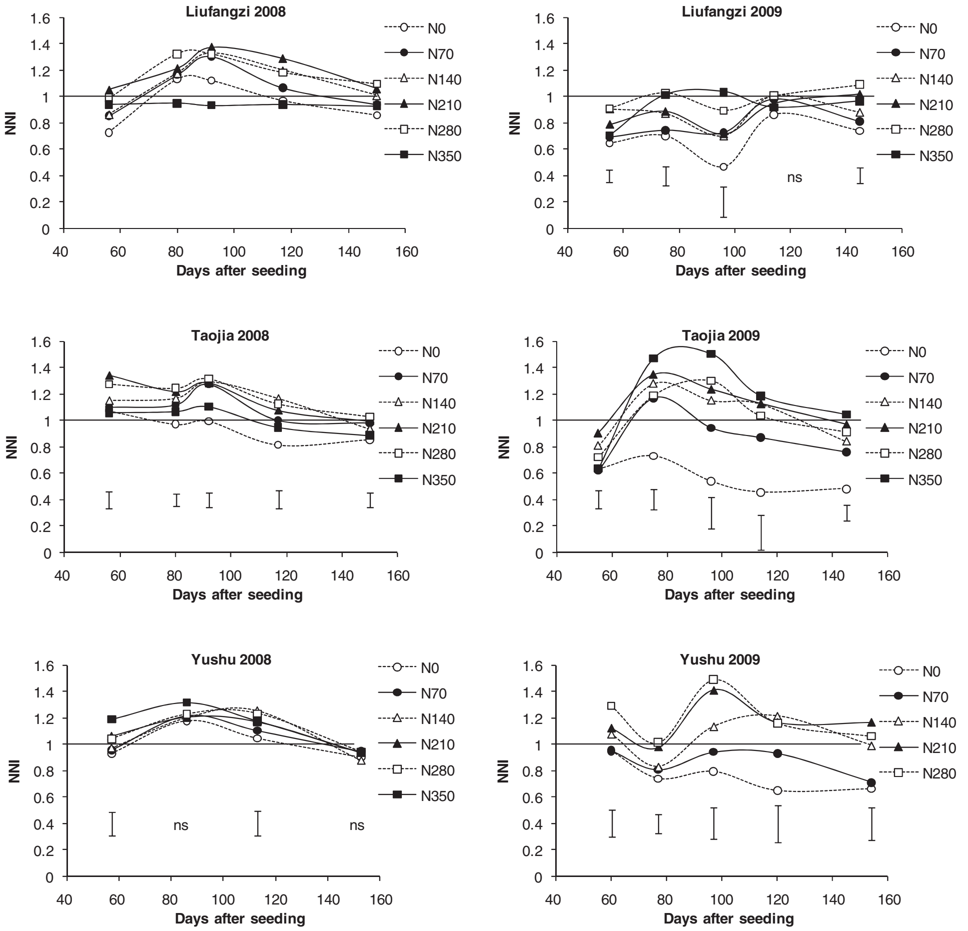

FIGURE 5 Nitrogen nutrition index (NNI) of maize fertilized with various nitrogen (N) application rates in an experiment conducted at six site-years. The NNI is presented for the different sampling dates expressed in days after seeding. The vertical bars represent LSD values ($P <= 0.05$) at each sampling date, and ns indicates that the LSD was not determined.

this relationship, for an NNI > 0.93, the relative grain yield was near 1.0 (Figure 6).

DISCUSSION

The variation in N concentration reduced with time, or advancing maturity, has been reported by many researches (Xue et al., 2007). This decline in N concentration with time, or increasing biomass, is mainly attributed to two reasons. The first one is self-shading of leaves that induced a non-uniform leaf N content from the top canopy layers with high N concentration to the shaded layers with low N concentration (Pons and Pearcy, 1994). The second

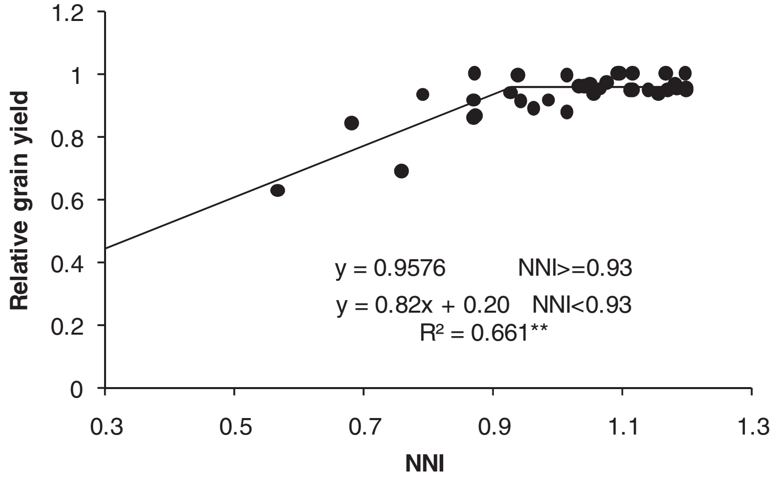

FIGURE 6 Relationship between relative grain yield and the nitrogen nutrition index (NNI) of maize in and experiment conducted at six site-years. The NNI data were averaged over all sampling dates.

one is the increasing in the proportion of plant structural and storage tissues with a lower nitrogen concentration (Caloin and Yu, 1984).

In this study, nitrogen concentrations varied from a maximum of 38.25 g kg^{-1} DM at Taojia on 27 June 2008 to a minimum of 4.96 g kg^{-1} DM observed at Taojia on 22 September 2009. A similar range of N concentration (7-34 g N kg^{-1} DM) was reported by Plénet and Lamaire (2000) for corn grown with different N application rates in France. The range of 9.1 to 38.5 g N kg^{-1} DM for corn was reported in Germany (Herrmann and Taube, 2004). And in eastern Canada the similar range of 6.1 to 38.7 g N kg^{-1} DM was reported by Ziadi et al. (2008a).

Plénet and Lemaire (2000) firstly estimated N dilution curve ($N_c = 34.0W^{-0.37}$) in maize using approximately weekly sampling up to 25 d after silking on irrigated maize. With the similar parameters, Herrmann and Taube (2004) extended the range beyond the growth stage silking plus 25 d until silage maturity ($N_c = 34.1W^{-0.391}$). Most of all data points with a shoot biomass > 1 Mg DM ha^{-1} were located under the critical N dilution curve of Plénet and Lemaire (2000) and Herrmann and Taube (2004), especially at the later stage of growing season (shoot biomass > 14 Mg DM ha^{-1}). In this study, 25 critical points were selected to estimate the parameters of the critical N dilution curve: $N_c = 36.5W^{-0.48}$, accounted for 81% of the variation. For a low biomass (i.e. approximately 1 Mg DM ha^{-1}), the critical N dilution curve of Plénet and Lemaire (2000) and Herrmann and Taube (2004) was slightly lower comparing with our new critical N dilution curve (Figure 7). The dilution parameter *b*, however, has a higher value in our model. As a result, for increasing shoot biomass, N concentration decreased more quickly in our study. The reason for this phenomenon is not clear

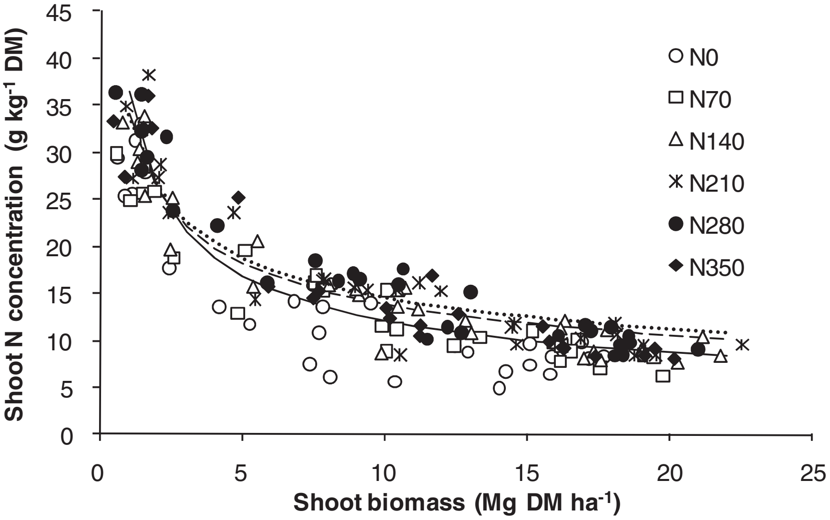

FIGURE 7 Comparison of different critical nitrogen dilution curves for maize. The solid line represents the critical N dilution curve of spring maize ($N_c = 36.5W^{-0.48}$) in Northeast China. The dotted line represents the critical N dilution curve in France (Plenet and Lemaire, 2000) ($N_c = 34.0W^{-0.37}$). The dashed line represents the critical N dilution curve in Germany (Plenet and Lemaire, 2000) ($N_c = 34.1W^{-0.391}$).

and may be related to a lot of factors, including different climate, type of cultivar and field management. For cotton, Xue et al. (2007) reported the slightly difference of critical N dilution model the two sites even in the same country. And Ziadi et al. (2010) indicated there was a big difference between winter wheat and spring wheat. In potatoes grown in Canada, difference also be detected between two cultivars (Bélanger et al., 2001). The difference of plant density between the maize in different region may explain part of the different observed between the critical N curves of previous studies and ours. In the research of maize in France, Germany, and Eastern Canada, the plant densities were 90,000, 100,000 and 79,040 plants ha^{-1}, respectively, which were much higher than in our research (60,000 plants ha^{-1}). In agreement with Ziadi et al. (2010), although only circumstance evidence, the plant density may affect the critical N curve.

According to the two boundary curves, for a shoot biomass of 3 kg DM ha^{-1}, the N concentration can vary by a factor of 2.3. Total N concentration can vary on both sides of the critical dilution curve: by a factor of 1.4 above and 1.6 below. Little bigger variability was reported by Plénet and Lemaire (2000) in France (1.75 above and 1.75 below). The N_{max} curve corresponds to an estimate of the maximum N accumulation capacity in the shoot. In these cases, the N absorption rate would be regulated by mechanisms directly associated with growth or indirectly associated with it via N metabolism (Justes et al. 1994). For the highest shoot biomasses, N_{max} could have been underestimated because at the end of the growing stage it is difficult to maintain high levels of the mineral N availability. In the area between the critical curve and the maximum curve, N absorption would be determined by the N availability in the root medium and not be regulated by the growth rate (Justes et al., 1994). In the area below the critical dilution curve, N absorption would be limited by mineral N availability in the soil which would be determined by growth rate. According to Penning de Vries (1982), N_{min} is the inferior limit at which the metabolism would cease to function.

The NNI values in our study ranged from 0.45 to 1.51. In East Canada, Ziadi et al. (2008a) reported the values ranging from 0.30 to 1.35. With decreasing NNI below 0.93, the relative grain yield decreased. The model of N_c and the resulting NNI, therefore, adequately identified situation in corn making it possible to quantify the level of corn N nutrition. But a major difficulty in using the NNI at the farm level, however, is the need to determine the actual crop mass and its N concentration. Measurement of the total N concentration and the calculation of NNI are costly, time-consuming and therefore not appropriate for standard farm practice. But leaf N concentration has strongest effect on the chlorophyll-meter readings. Ziadi et al. (2008b) indicated that the chlorophyll-meter readings were significantly related to NNI. Nitrogen-dilution curves can be used as a tool for diagnosing the status of N in wheat (Prost and Jeuffroy, 2007), durum wheat (Debaeke et al., 2006), cotton (Xue et al., 2007), rice (Shibu et al., 2010) and so on. Moreover, the relationship between yield and NNI can also be used in the parameterization and validation of growth models for predicting the N response and/or N requirement of plant (Demotes-Mainard and Jeuffroy, 2001).

CONCLUSIONS

A critical N dilution curve ($N_c = 36.5W^{-0.48}$) was developed for spring maize in Northeast China. This curve was a bit different from those developed for maize in France and in Germany. The resulting NNI was calculated from this critical N dilution curve was highly related to relative grain yield. Therefore, the concept of a critical N concentration provides a reference method for assessing the status of N nutrition during crop growth in Northeast China. Besides, this critical N dilution curve can be used as a tool for diagnosing the status of N in maize.

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