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Effects of enhanced atmospheric ammonia on photosynthetic characteristics of two maize (*Zea mays* L.) cultivars with various nitrogen supply across long-term growth period and their diurnal change patterns

L.X. ZHANG[*,**], H. QIANG[**,***], S.Q. LI[*,**,***,+], and X.L. CHEN[**,***]

State Key Laboratory of Soil Erosion and Dryland Farming and College of Life Sciences,
Northwest A & F University, 712100, Yangling, Shaanxi, P.R. China[*]
State Key Laboratory of Soil Erosion and Dryland Farming, Institute of Soil and Water Conservation,
Northwest A & F University and Chinese Academy of Sciences , 712100, Yangling, Shaanxi, P.R. China[**]
College of Resource and Environment, Northwest A & F University, 712100, Yangling, Shaanxi, P. R. China[***]

Abstract

We investigated the effect of enhanced atmospheric ammonia (NH_3) in combination with low and high nitrogen (LN and HN, respectively) growth medium on photosynthetic characteristics of two maize (*Zea mays* L.) cultivars (NE5 with high- and SD19 with low N-use efficiency) across long-term growth period and their diurnal change patterns exposed to 10 nl l^{-1} and 1,000 nl l^{-1} NH_3 fumigation in open-top chambers (OTCs). Regardless of the level of N in medium, increased NH_3 concentration promoted maximum net photosynthetic rate (P_{max}) and apparent quantum yield (AQY) of both cultivars at earlier growth stages, but inhibited P_{max} of NE5 from silking to maturity stage and that of SD19 at maturity stage only above the ambient concentration. Greater positive/less negative responses were predominant in the LN than in the HN treatment, especially for SD19. Dark respiration rate (R_D) remained more enhanced in the LN than in the HN treatment for SD19 as well as increased in the LN while decreased in the HN treatment for NE5 at their silking stage, following exposure to elevated NH_3 concentration. Additionally, enhanced atmospheric NH_3 increased net photosynthetic rate (P_N) and stomatal conductance (g_s) but reduced intercellular CO_2 concentration (C_i) of both cultivars with either the LN or HN treatment during the diurnal period at tasseling stage. The diurnal change patterns of P_N and g_s showed bimodal curve type and those of C_i presented single W-curve type for NE5, when NH_3 concentration was enhanced. As for SD19, single-peak curve type was showed for both P_N and g_s while single V-curve type for C_i. All results supported the hypothesis that appropriately enhanced atmospheric NH_3 can increase assimilation of CO_2 by improving photosynthesis of maize plant, especially at earlier growth stages and after photosynthetic "noon-break" point. These impacts of elevated NH_3 concentration were more beneficial for SD19 as compared to those for NE5, especially in the LN supply environment.

Additional key words: atmospheric ammonia; diurnal time course; maize; nitrogen supply; photosynthetic parameters.

Introduction

At the global scale, among all nitrogen (N) species in the atmosphere and their deposition to terrestrial vegetation and other receptors, NH_3 is considered to be the foremost. It is emitted by a large number of sources such as volatilization from animal waste and synthetic fertilizers, biomass burning, losses from soils under native vegetation and agricultural crops, emissions from human excreta and fossil fuel combustion (Fangmeier *et al.*

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[+] Corresponding author; fax: (+86-29) 87016171; e-mail: lishiqing.8888@yahoo.com.cn

Abbreviations: AQY − apparent quantum yield; C_i − intercellular CO_2 concentration; g_s − stomatal conductance; HN − high nitrogen; LN − low nitrogen; OTCs − open-top chambers; PAR − photosynthetically active radiation; P_{max} − net maximum photosynthetic rate; P_N − net photosynthetic rate; R_D − dark respiration rate; RH − relative humidity; Rubisco − ribulose-1,5-bisphosphate carboxylase/oxygenase.

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1994, Beat and Albrecht 2002). Farming is recognized as a major source of atmospheric NH_3, contributing by 50% to the global NH_3 emissions (Schjoerring 1991). Maize (*Z. mays* L.) is a major provision crop in the north of China, which is frequently subjected to impacts of increased atmospheric NH_3 emission, particularly in fertilizer-enriched field (Li *et al.* 2004b). Atmospheric NH_3 is deposited to soil and water, either by dry deposition of NH_3 or by dry and wet deposition of ammonium (NH_4^+) (Fangmeier *et al.* 1994). Close to its sources, acute exposures to NH_3 can result in visible foliar injury on vegetation. Adverse effects on vegetation occur when the rate of foliar uptake of NH_3 is greater than the rate and capacity for *in vivo* detoxification by the plants (Krupa 2003). On the other hand, as a plant nutrient, plants can utilize atmospheric NH_3 to improve plant growth and/or production if the mole fraction of NH_3 in the atmosphere is greater than the mole fraction of gaseous NH_3 in the substomatal cavity. Much of our current knowledge of the effects of NH_3 on higher plants is predominantly derived from studies conducted in Europe. It was found that atmospheric NH_3 input is closely correlated with the morphology and metabolism of crops (Bohme *et al.* 2003, Li *et al.* 2009).

Atmospheric NH_3 exposure may result in impacts on leaf photosynthesis of plants (Li *et al.* 2004 a, Chen *et al.* 2008). Different studies in relation to effects of enhanced atmospheric NH_3 on photosynthetic characteristics of plants have been carried out and these studies have shown a great diversity of responses, which were closely dependent on cultivar, growth stage, and N rate in medium (Van Hove *et al.* 1989, Wedin and Tilman 1996, Tatsuro *et al.* 2001, Kimball *et al.* 2002, Li *et al.* 2004a, Li *et al.* 2009). Some experts pointed out that increased NH_3 concentration enhanced photosynthesis of *Pinus sylvestris,* poplar trees, and Douglas fir (Van der Eerden *et al.* 1992, Van Hove *et al.* 1989, Van der Eerden and Pérez-Soba 1992), but remained unaffected in leaf photosynthesis of sunflower (*Helianthus annnus* L.) and *Acacia auriculaeform* (Berger *et al.* 1986, Zhao *et al.* 2003). Taken together, R_D of *Populus euramericana* remained unaffected under 73.3–146.5 nl(NH_3) l^{-1} for 6–8 weeks. However, increase by 76% of R_D was found under 351.6 nl(NH_3) l^{-1} for three months. Castro *et al.* (2005) pointed out that atmospheric NH_3 up to 4,000 nl l^{-1} can be regarded as a nutrient for the fast growing of *B. oleracea.* The concentration at which NH_3 changes from being a nutrient to a toxin is not clear-cut, since NH_3 can still be metabolized when plant growth is already affected (Fangmeier *et al.* 1994).

However, the vast majority of studies have been reported for effects of atmospheric NH_3 on photosynthetic characteristics of plant in forest and grassland. Hence, there is little information available in the literature about responses of crops in agriculture field, especially across long-term growth period. The diurnal time courses of photosynthetic parameters of maize plant have not well been elucidated in exposure to enhanced NH_3 (Wedin and Tilman 1996, Tatsuro *et al.* 2001, Kimball *et al.* 2002, Li *et al.* 2004a, Li *et al.* 2004b, Chen *et al.* 2008). Based on such considerations, we hypothesize that higher constitutive photosynthesis induced by enhanced atmospheric NH_3 may provide a mechanism of the improvement of plant growth of different maize cultivar under two levels of N in solution medium. The aim of this work was to investigate how photosynthetic parameters were affected in two maize cultivars exposed to two levels of atmospheric NH_3 concentration and N solution medium across long-term growth period and their diurnal time courses.

Materials and methods

Plant material and experimental design: Solution culture experiments were conducted at Institute of Soil and Water Conservation, Chinese Academy of Sciences (Yangling, P. R. China). Two maize (*Zea mays* L.) cultivars (NE5 with high- and SD19 with low N-use efficiency) were supplied for the present experiments as indication crops by College of Resources and Environment of China Agricultural University and Shiping Academy of Agricultural Sciences of Jilin Province, respectively (Chun 2004).

The open-top chambers (OTCs) used in the experiments (Fig. 1) are containers described by Paul and Bert (1993), initially designed for exposing plants to elevated CO_2 concentrations under close to natural conditions, with a square 1.2 m × 1.2 m base and 1.5 m tall perpendicular glass walls topped by glass quadrilaterals inclined towards the centre in an iron frame (total volume *cca.* 3 m^3). The chambers were each equipped with a fan and an air control system, which included a steel cylinder (inner diameter 600 mm, total length 1,800 mm) containing 95% NH_3. NH_3 was fed from the bottom into the OTCs through a *YQA-441* NH_3 decrement gauge with a pressure range of 0–4 MPa (*Shanghai Shuangying Boat Decompressor Manufacture Co., Ltd.*, Shanghai, China) and *Φ8* constant pressure oxygen pipes. The NH_3 flux was measured using an *LZB-2* flux meter with anticorrosion glass rotameter (measuring range 6–60 ml min^{-1}, rated working pressure ≤ 1 MPa) (*Changzhou Shuangfa Thermal Instrument Co., Ltd.*, Changzhou, China). In addition, air was fed from the bottom of the OTCs using a *ZB-0.10/8* air compressor (air displacement 0.1 m^3 min^{-1}, rated air pressure 0.8 MPa) and *Φ8* constant pressure oxygen pipes (*Shanghai Luodi Air Compressor Co., Ltd.*, Shanghai, China). The air flux was maintained at 1.7 l min^{-1}, as measured by an *LZB-2* flux meter with an anticorrosion glass rotameter (measuring range

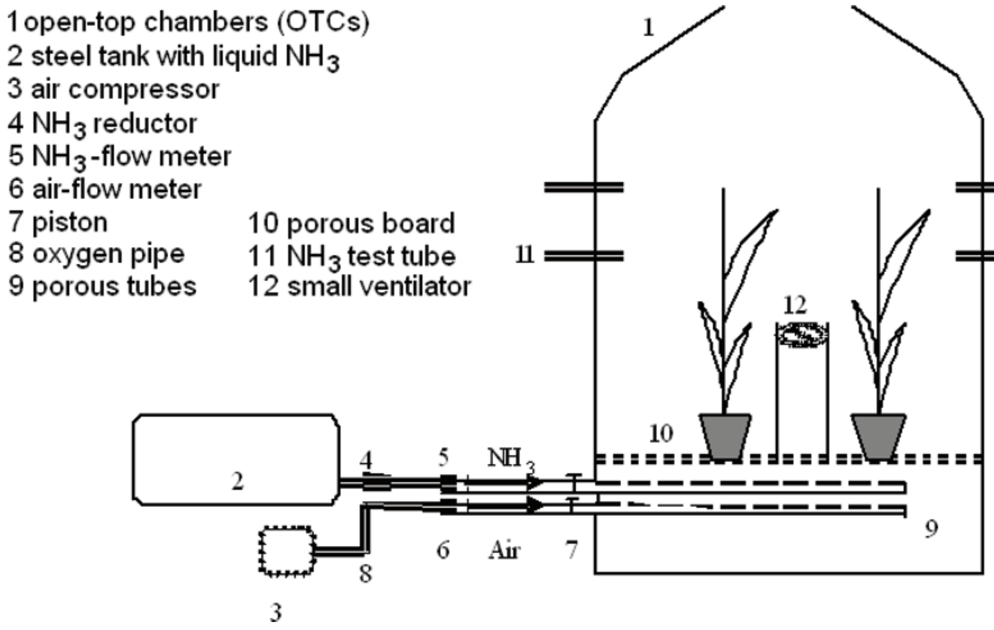

Fig. 1. Open-top chamber (OTC) device

0.25–2.5 m^3 h^{-1}, rated working pressure ≤1 MPa) (*Changzhou Shuangfa Thermal Instrument Co., Ltd.*, Changzhou, China).

The temperature with 25/18°C of average day/night, 60–70% relative humidity (RH) and NH_3 concentration inside each chamber were regulated by passing air (heated and moistened as appropriate) and NH_3 through porous pipes at the bottom of the container, while the fan (providing air speeds of less than 0.5 m s^{-1}) was used to maintain close to uniform distribution of NH_3 and reduced temperature (if necessary) in the chamber. The temperature in the chambers was monitored to verify that the temperature was the same in the chamber before and after NH_3 was supplied, and thus that the effects of the varying NH_3 concentration on the growth performance or photosynthesis of plants were not confounded by variations in temperature.

The NH_3 concentration in each chamber was measured four times per day (at 08:00, 11:00, 14:00, and 17:00 h, respectively) by a *GTL-C* indoor air detector equipped with a *pH618* test pen (NH_3 testing precision, ± 0.01465 nl l^{-1}) (*Shanghai Minyi Electron Co., Ltd.*, Shanghai, China) mounted on a tripod placed in the centre of the chamber before the gas was supplied. On each sampling occasion, 5 ml of NH_3 test reagent was extracted by an injector and injected into a glass bottle for sampling. The glass bottle was immediately plugged and connected to the instrument. During the tests, the flux was adjusted to 2 l min^{-1} and the exhaust time was controlled by the auto-timing device. When the sampling time was complete, the glass bottle was removed, unplugged, the reagent in the glass bottle was poured into the test cup and the test pen was inserted into the cup to measure the NH_3 concentration. Throughout the entire growth period, from 08:00 to 18:00 h every day, the NH_3 concentration in the OTCs used for the background ambient (control) and elevated NH_3 treatments were maintained precisely at 10 nl l^{-1} and 1,000 nl l^{-1}, respectively, by continuously supplying NH_3 and air at appropriate ratios.

Plant growth: Seeds of two maize cultivars were surface-sterilized in 10% H_2O_2 solution for 15 min. After rinsing in distilled water, seeds were imbibed for 12 h and then sown in porcelain trays containing quartz sand. Seeds were germinated in the dark under 23°C covering with clean wet filter papers. When the roots grew to the length of 2–4 cm, the seedlings were transferred and fixed in the holes of styrofoam boards by using absorbent cotton in deionized water in plastic trays in the growth chamber under the conditions with 25/18°C of average day/night temperature, 60–70% RH, and 350 μmol m^{-2} s^{-1} light intensity and 16/8 h of light/dark regime. The solution was replaced once a day and aerated continuously.

PVC pots with a volume of 3,534 cm^3 (inner diameter 15 cm, height 20 cm) were used to contain 3.5 l of nutrient solution for plant growth. NH_3 fumigation and N treatments were initially proceeded on the 3rd day after the seedlings were transferred into OTCs at their three-leaf stage. Split-plot design was used with a total of 8 treatments, of which NH_3 concentration is treated as the main plot factor, N supply level as the subplot factor and cultivar as the sub-subplot factor. The pots with two replicates of NH_3 concentration treatment were placed in 4 OCTs, respectively. Thirty-two pots in each chamber covered eight replicates of two cultivars and two N rates treatments in solution medium. Each treatment was exchanged in the different chambers at certain intervals (10 days) to reduce the test error caused by the chamber difference. Complete nutrient solution (Hoagland and Arnon 1950) was supplied for the present solution culture experiment, which contains all essential minerals for plant growth and all solutions are made up of distilled water. The pH of the nutrient solution was added to emulsion, which was made of higher alcohol to restrain NH_3 change between two phases of gas and liquid, then adjusted to 6.2 (± 0.1). The low- and high N rates in medium were served by 1/9 and 1/3 strength of the complete solution *i.e.* 5.00 and 1.67 mmol l^{-1} nitrate, respectively. Desired N concentrations were maintained by irrigating sufficiently with a new solution. The pot tops were laid with cover boards with small holes for plants fixation with sponge and adhesive tape, and the space between plants and holes was sealed with wax. The solution was aerated without NH_3 for 4 h a day to assure normal growth. The plants of two maize cultivars were cultivated on June 05, 2007 and maintained for 90-day growth period under each treatment. The plant parameters in OTCs were as following: plant height: 130–132 cm; ear height: 60–65 cm; stem diameter: 1.4–1.6 cm; number of leaves: 12–15.

Determination of light-response curves and parameters of diurnal time courses: The second completely developed leaf from the top of plant at jointing and tasseling stages (on day 30 and 42 after transplant, respectively) and leaf in spike at silking and mature stages (on day 68 and 80 after transplant, respectively) were measured from 09:00 to 14:00 h, respectively using *LI-6400* portable photosynthesis system (*LI-COR Inc.*, Lincoln, NE, USA). Two leaves of one plant were determined and repeated three replicates. The RH was maintained at 65% and leaf temperature was 25ºC in the leaf chamber. The setting values of photosynthetically active radiation (PAR) were in turn 2,000; 1,600; 1,400; 1,200; 1,000; 800, 600, 400, 200, 150, 100, 50, and 0 μmol m^{-2} s^{-1} and the ambient CO_2 concentration of 360 μmol mol^{-1} condition. The light-response curves of P_N with light intensity changing in each treatment were simulated referring to the index model (formula 1) of light response of maize by Guo *et al.* (2005). The leaf photosynthetic parameters from light-response curves, *i.e.* P_{max}, AQY, and R_D under each treatment were computed following the above method (Guo *et al.* 2005). The parameters of diurnal time courses at tasseling stage covered P_N, g_s, and C_i, the conditions of PAR of 1,200 μmol m^{-2} s^{-1} and ambient CO_2 concentration of 360 μmol mol^{-1} (Yu *et al.* 2001, Leakey *et al.* 2004).

$$P_N = P_{max}\,(1 - e^{-\frac{AQY\ PAR}{P_{max}}}) - |R_D| \qquad (1)$$

where P_N represents leaf net photosynthetic rate under different PAR.

Statistical analysis: All data were subjected to analysis of variance (*ANOVA*) with *SAS* software package (*SAS Institute Inc*., Cary, NC, USA, 1996). Appropriate standard errors of the means (SE) were calculated for presentation with table and line diagram. The significance of the treatment effect was determined using *F*-test, and to determine the significance of the means, the *Duncan*'s multiple range test was estimated at 5% probability level.

Results

Leaf photosynthetic parameters from light-response curves: In combination with the LN treatment, exposure of the plant to enhanced atmospheric NH_3 concentration promoted an increase in P_{max} of SD19 above the ambient NH_3 concentration by 52.9%, 27.1%, and 14.0% at jointing, tasseling, and silking stages, respectively. No significant impact of high NH_3 treatment was found at mature stage ($p>0.05$). As for NE5 treated with elevated atmospheric NH_3 concentration, P_{max} was increased by 30.7% and 12.2% at jointing and tasseling stages, respectively, unaffected at silking stage ($p>0.05$) , but decreased by 15.0% at mature stage. With respect to the HN treatment, increases of 45.4% and 22.8% in P_{max} of SD19 were obtained at jointing and tasseling stages, respectively, induced by the high NH_3 concentration treatment as compared with low NH_3 treatment. However, nonsignificant impact ($p>0.05$) and decreased effect (by 14% decrease) occurred at silking and mature stages, respectively. With regard to NE5, 21.6% increase, nonsignificant impact ($p>0.05$), 11.9% and 16.4% decreases were observed at jointing, tasseling, silking and mature stages, respectively, when subjected to enhanced NH_3 concentration treatment. P_{max} of NE5 was significantly greater than that of SD19 under both N combined with NH_3 treatments at all growth stages. With the development of plants, P_{max} of both cultivars increased and reached the summit values at tasseling/silking stage and reduced at mature stage in each treatment (Table 1).

AQY was significantly greater in LN-treated plants of both cultivars exposed to increased atmospheric NH_3 than in their counterparts exposed to the ambient concentration (by 52.0% and 29.3% in SD19 together with 23.1% and 16.2 % higher for NE5, respectively) at jointing and tasseling stages. No significant impact of the high NH_3 treatment occurred at silking stage for NE5 and at mature stage for SD19, respectively ($p>0.05$). The positive effects of enhanced atmospheric NH_3 were found at tasseling stage for SD19 (by 12.9% increase) while the negative effects were obtained at mature stage for NE5 (by 15.1% decrease). In contrast, among plants of both cultivars subjected to the HN treatment, AQY of SD19

Table 1. Responses of maximum net photosynthetic rate (P_{max}) in SD19 and NE5 leaves at several key growth stages to enhanced NH_3 concentration (EAN, 1,000 nl l^{-1}) and the ambient NH_3 concentration (control, 10 nl l^{-1}) in the low N- (LN, 1.67 mmol l^{-1}) and high N (HN, 5.00 mmol l^{-1}) supply in the growth medium. *Different letters* show significant differences among means at $p<0.05$. Means ± SD ($n = 4$).

Cultivar		P_{max} [μmol(CO_2) m^{-2} s^{-1}] Jointing stage	Tasseling stage	Silking stage	Mature stage
Low N combined with different NH_3 concentration					
SD19	Control	16.18 ± 0.89^{b}	24.05 ± 1.02^{b}	26.40 ± 1.42^{b}	17.06 ± 1.22^{a}
	EAN	24.63 ± 1.55^{a}	30.57 ± 1.08^{a}	30.09 ± 2.70^{a}	16.04 ± 3.01^{a}
NE5	Control	25.02 ± 2.52^{b}	32.89 ± 2.20^{b}	37.07 ± 4.26^{a}	27.92 ± 2.60^{a}
	EAN	32.69 ± 2.33^{a}	36.91 ± 2.67^{a}	35.96 ± 3.67^{a}	23.73 ± 2.84^{b}
High N combined with different NH_3 concentration					
SD19	Control	19.96 ± 1.03^{b}	25.09 ± 1.22^{b}	27.90 ± 3.33^{a}	22.53 ± 1.30^{a}
	EAN	29.02 ± 0.12^{a}	30.68 ± 1.91^{a}	30.39 ± 3.29^{a}	19.37 ± 2.12^{b}
NE5	Control	28.17 ± 2.03^{b}	35.63 ± 3.49^{a}	38.79 ± 1.24^{a}	29.61 ± 1.89^{a}
	EAN	34.24 ± 1.22^{a}	37.65 ± 1.92^{a}	34.17 ± 2.21^{b}	24.75 ± 2.20^{b}

Table 2. Responses of apparent quantum yield (AQY) in SD19 and NE5 leaves at several key growth stages to enhanced NH_3 concentration (EAN, 1,000 nl l^{-1}) and the ambient NH_3 concentration (control, 10 nl l^{-1}) in the low N- (LN, 1.67 mmol l^{-1}) and high N (HN, 5.00 mmol l^{-1}) supply in the growth medium. *Different letters* show significant differences among means at $p<0.05$. Means ± SD ($n = 4$).

Cultivar		AQY [mol(CO_2)mol^{-1}] Jointing stage	Tasseling stage	Silking stage	Mature stage
Low N combined with different NH_3 concentration					
SD19	Control	0.050 ± 0.001^{b}	0.058 ± 0.007^{b}	0.070 ± 0.004^{b}	0.046 ± 0.004^{a}
	EAN	0.076 ± 0.002^{a}	0.075 ± 0.005^{a}	0.079 ± 0.005^{a}	0.042 ± 0.003^{a}
NE5	Control	0.065 ± 0.002^{b}	0.074 ± 0.005^{b}	0.092 ± 0.006^{a}	0.073 ± 0.006^{a}
	EAN	0.080 ± 0.002^{a}	0.086 ± 0.006^{a}	0.088 ± 0.004^{a}	0.062 ± 0.007^{b}
High N combined with different NH_3 concentration					
SD19	Control	0.060 ± 0.003^{b}	0.068 ± 0.002^{b}	0.077 ± 0.007^{a}	0.059 ± 0.003^{a}
	EAN	0.084 ± 0.003^{a}	0.085 ± 0.005^{a}	0.081 ± 0.008^{a}	0.051 ± 0.004^{b}
NE5	Control	0.075 ± 0.002^{b}	0.086 ± 0.007^{a}	0.096 ± 0.006^{a}	0.082 ± 0.004^{a}
	EAN	0.088 ± 0.004^{a}	0.092 ± 0.006^{a}	0.085 ± 0.007^{b}	0.068 ± 0.005^{b}

Table 3. Responses of dark respiration rate (R_D) in SD19 and NE5 leaves at several key growth stages to enhanced NH_3 concentration (EAN, 1,000 nl l^{-1}) and the ambient NH_3 concentration (control, 10 nl l^{-1}) in the low N- (LN, 1.67 mmol l^{-1}) and high N (HN, 5.00 mmol l^{-1}) supply in the growth medium. *Different letters* show significant differences among means at $p<0.05$. Means ± SD ($n = 4$).

Cultivar		R_D [μmol(CO_2) m^{-2} s^{-1})] Jointing stage	Tasseling stage	Silking stage	Mature stage
Low N combined with different NH_3 concentration					
SD19	Control	1.173 ± 0.100^{a}	2.234 ± 0.190^{a}	2.280 ± 0.212^{b}	1.359 ± 0.110^{a}
	EAN	1.190 ± 0.220^{a}	2.260 ± 0.204^{a}	2.839 ± 0.270^{a}	1.246 ± 0.100^{a}
NE5	Control	1.738 ± 0.120^{a}	3.297 ± 0.230^{a}	3.358 ± 0.190^{b}	1.800 ± 0.102^{a}
	EAN	1.736 ± 0.180^{a}	3.260 ± 0.290^{a}	3.820 ± 0.220^{a}	1.692 ± 0.104^{a}
High N combined with different NH_3 concentration					
SD19	Control	1.477 ± 0.056^{a}	2.151 ± 0.081^{a}	2.640 ± 0.108^{a}	1.523 ± 0.055^{a}
	EAN	1.472 ± 0.059^{a}	2.154 ± 0.087^{a}	3.175 ± 0.092^{b}	1.501 ± 0.067^{a}
NE5	Control	1.744 ± 0.210^{a}	3.379 ± 0.030^{a}	3.599 ± 0.130^{a}	1.890 ± 0.200^{a}
	EAN	1.750 ± 0.320^{a}	3.350 ± 0.270^{a}	3.240 ± 0.210^{b}	1.777 ± 0.210^{a}

and NE5 exposed to elevated atmospheric NH_3 concentration was by 40.0% and 25.0% higher at jointing stage as well as 17.3% and 6.9% (nonsignificant) higher at tasseling stage, respectively. Nonsignificant (p>0.05) and negative effects (by 11.5% decrease) on AQY occurred at silking stage for SD19 and NE5, respectively. As for mature stage, reduction of AQY by 13.6% and 17.1% was recorded for SD19 and NE5, respectively. Cultivar NE5 recorded greater AQY than SD19 under both N combined with NH_3 treatments across the entire growth period. With the development of plants, AQY of both cultivars increased and reached the summit values at tasseling/silking and decreased at mature stage in each treatment (Table 2).

The effects of increased atmospheric NH_3 on R_D of SD19 and NE5 remained nonsignificant at jointing, tasseling, and mature stages under both N treatments (p>0.05). However, the positive effects were found in SD19 at silking stage and the greater impacts were obtained under LN medium (by 24.5% increase) than those under HN medium (by 20.2% increase). As for NE5 at silking stage, 13.7% increase and 10.0% decrease of R_D were found in the plants exposed to LN- and HN treatments, respectively. R_D of NE5 was significantly greater than that of SD19 under both N combined with NH_3 treatments during the entire growth period. With the development of plants, R_D of both cultivars increased and reached the summit values at tasseling/silking stage and reduced at mature stage in each treatment (Table 3).

The diurnal time courses of photosynthesis characters: Responses of the diurnal time courses of P_N, g_s, and C_i induced by the enhanced NH_3 treatment in maize SD19 and NE5 under two levels of N medium are depicted in Figs. 2–4. Increased atmospheric NH_3 concentration imposed a significant enhancement of P_N and g_s of SD19 and NE5 under both N treatments during the diurnal time courses. Above the background NH_3 concentration, P_N and g_s were averagely increased by 18.0% and 30.6% in SD19 as well as 16.4% and 15.5% in NE5 in the LN medium, by 12.3% and 20.4% in SD19 together with 10.0% and 11.2% in NE5 in the HN medium, respectively, when treated with enhanced NH_3 concentration. In addition, the diurnal change patterns of

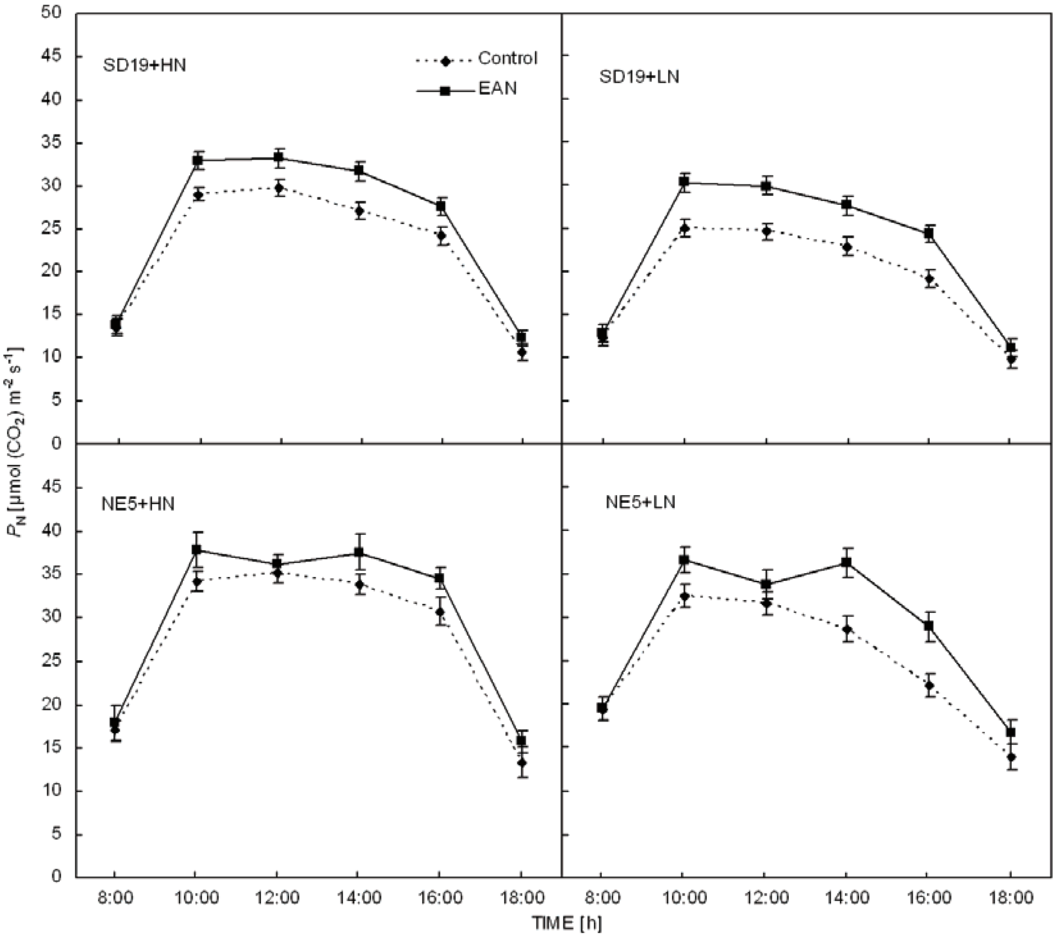

Fig. 2. Responses of net photosynthetic rate (P_N) in SD19 and NE5 leaves during the diurnal time courses to enriched NH_3 concentration (EAN; 1,000 nl l^{-1}) and the ambient NH_3 concentration (control, 10 nl l^{-1}) in the high N-(HN) and low N (LN) supply in the growth medium. Means ± SD, (n = 4).

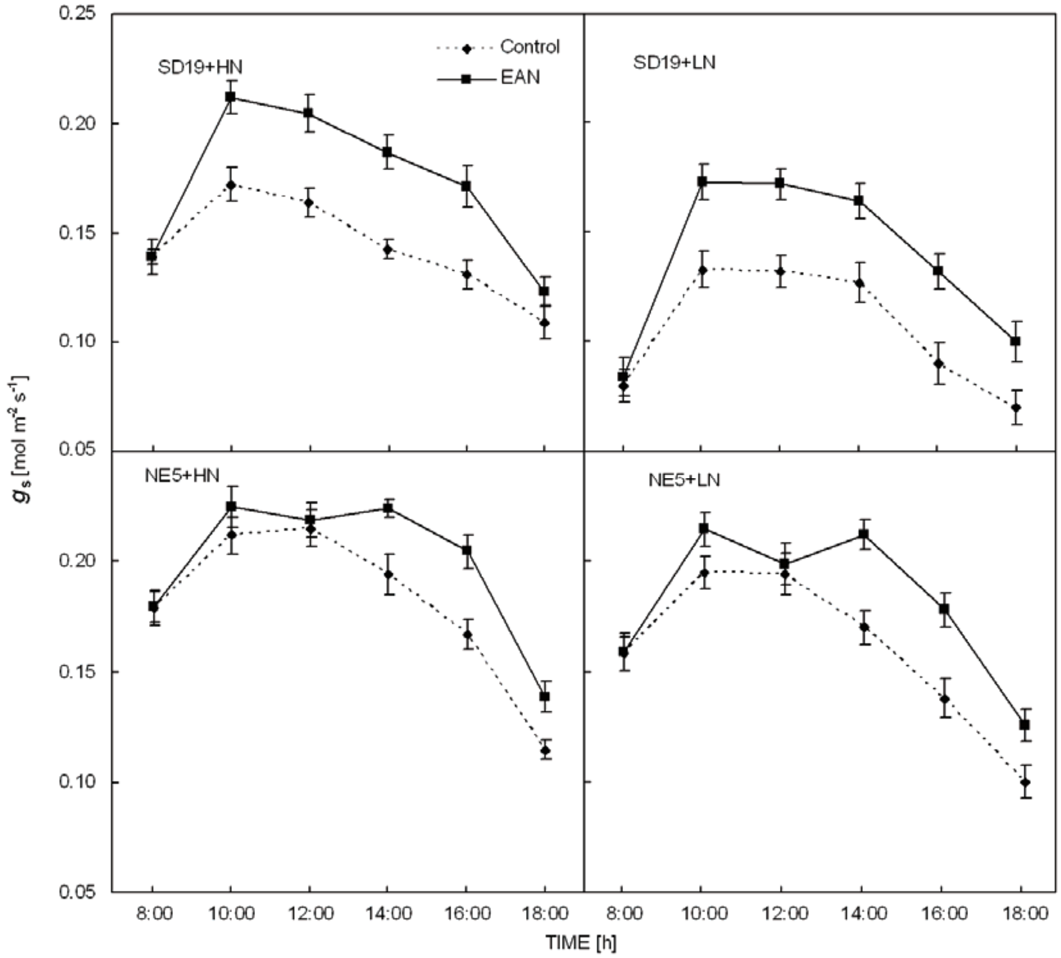

Fig. 3. Responses of stomatal conductance (g_s) in SD19 and NE5 leaves during the diurnal time courses to the enriched NH_3 concentration (EAN, 1000 nl l^{-1}) and the ambient NH_3 concentration (control, 10 nl l^{-1}) in the high N (HN) and low N (LN) supply in the growth medium. Means ± SD, ($n = 4$).

P_N and g_s of SD19 showed unimodal curve type exposure to the ambient and enhanced NH_3 concentrations in combination with the LN- and HN medium. The peak values of P_N and g_s occurred at 10:00 h. With respect to NE5, the diurnal time courses of P_N and g_s under the high NH_3 treatment presented double-peak curve type in both two levels of N medium. The peak value of P_N and g_s reached at 10:00 h, declined at 12:00 h and reached the second summit value at 14:00 h. However, the single-peak curves were still shown under the background NH_3 concentration. The obvious positive effects of enhanced NH_3 concentration on P_N and g_s of both cultivars were found in either the LN- or HN treatment during the course from 10:00 to 16:00 h except at 12:00 h for NE5 (Figs. 2, 3).

The diurnal change patterns of C_i of maize SD19 and NE5 were reversed in relation to P_N. Enhanced NH_3 concentration decreased C_i of maize SD19 and NE5 under both N treatments during the diurnal time courses. In contrast to the background NH_3 concentration, C_i of SD19 and NE5 was averagely reduced by 22.5% and 7.0% in the LN medium, and by 16.5% and 6.1% in the HN medium, respectively, when exposed to the high NH_3 treatment. Moreover, the diurnal time courses of C_i under both NH_3 concentrations in SD19 were single V-curve types in either the LN or HN medium. The lowest C_i of maize SD19 occurred at 10:00 h. The marked decrease responses of P_N were obtained from 10:00 to 16:00 h. The diurnal change patterns under enhanced NH_3 treatment on C_i of NE5 showed single W-curve in whether the LN or HN medium. The two bottom values of C_i occurred at 10:00 and 14:00 h, respectively. The single V-curve was still shown under the background NH_3 concentration. The marked decrease responses of P_N were obtained from 10:00 to 14:00 h except at 12:00 h for NE5 (Fig. 4).

Discussion

The effects of enhanced atmospheric NH_3 on the parameters of light-response curves of two maize cultivar in the LN and HN solution medium across long-term growth period: P_{max} and AQY reflect potential plant photosynthetic ability and the photochemical activity of PSII, respectively. As such, they can be two good parameters of light-response curves for evaluating the responses of crop plant induced by enhanced NH_3 concentration (Tuzet *et al.* 2003, Zhao *et al.* 2003, Guo *et al.* 2005). Some experts pointed out that atmospheric NH_3 concentration induces an enhancement of P_{max} of *P. sylvestris,* poplar trees and Douglas fir (*Pseudotsuga menziesii*) (Van der Eerden *et al.* 1992, Van Hove *et al.* 1989, Van der Eerden and Pérez-Soba 1992). Adverse studies stated that photosynthesis of plants such as sunflower (*H. annnus* L.) and *A. auriculaeform* was almost not affected by atmospheric NH_3 enrichment. However, relevant researches on AQY under enhanced NH_3 treatment are still rare (Berger *et al.* 1986, Reich *et al.* 1998, Zhao *et al.* 2003). Different effects of parameters of light response curves, *i.e.* P_{max} and AQY, induced by enhanced atmospheric NH_3 concentration, may be dependent on plant species and cultivar as well as growth medium environment such as N-supply status. The positive effects of enhanced atmospheric NH_3 on P_{max} and AQY of both maize cultivars became weaker with the development of the plants, and the negative impacts were mainly found at the mature stage. Moreover, the greater impacts of increased NH_3 concentration on P_{max} and AQY were obtained for the low-N-use efficiency cultivar (SD19) than for high-N-use efficiency cultivar (NE5). The inhibition effects of high NH_3 concentration for NE5 occurred earlier (at silking stage) than for SD19 (at mature stage), especially in the HN medium (Tables 1, 2). These findings consequently indicate that a crop cultivar with low-N-use efficiency may have weaker N absorption ability from growth medium. Such atmospheric NH_3 absorption by plant

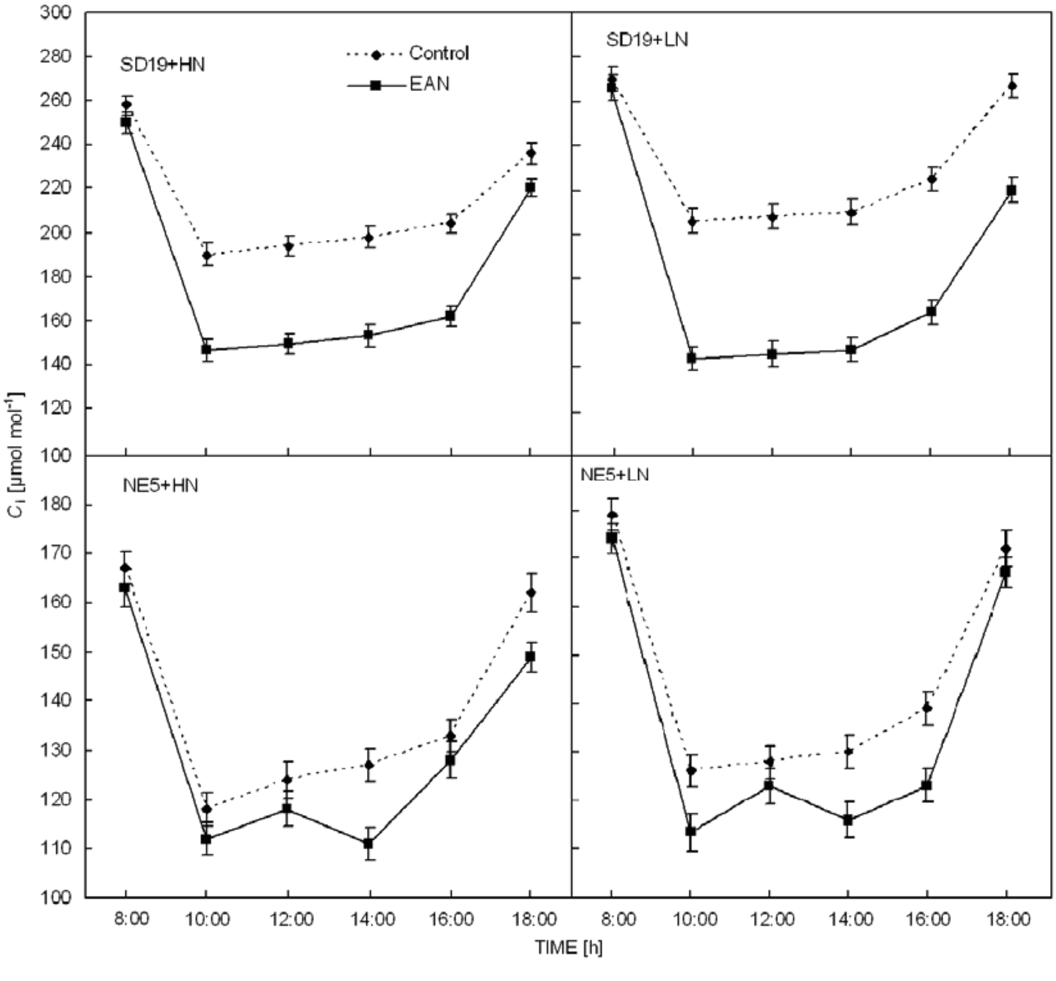

Fig. 4. Responses of intercellular CO_2 concentration (C_i) in SD19 and NE5 leaves during the diurnal time courses to enriched NH_3 concentration (EAN, 1,000 nl l^{-1}) and the ambient NH_3 concentration (control, 10 nl l^{-1}) in the high N (HN) and low N (LN) supply in the growth medium. Means ± SD, ($n = 4$).

canopy might offset the deficiency from root absorption, which showed the stronger use ability of atmospheric NH_3 to produce high photosynthetic ability and photochemistry activity of PSII (Woodin and Farmer 1993, Krupa 2003, Li *et al.* 2004a, Tables 1, 2). Regardless of maize cultivar, more increase/less decrease of P_{max} and AQY due to elevated atmospheric NH_3 concentration was in order of LN medium > HN medium. Nevertheless, NE5 maintained greater P_{max} and AQY than SD19 for each treatment. The possible mechanism might be as follows: relative deficiency of N nutrition of plant is beneficial for the absorption of atmospheric NH_3 through leaves and synthesis of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) which results in the increase of P_{max} (Zhao *et al.* 2003). Under HN medium, excessive NH_3 concentration in cell possibly restrained Rubisco activity which reduces P_{max} and AQY of plants (Van der Eerden *et al.* 1990).

Leaf dark respiration belongs to the most fundamental plant physiological processes and plays a major role in the carbon cycle at scales from the leaf to the globe. Despite its importance, we know far less about local and global patterns of interspecific variation in leaf R_D than for other leaf traits such as P_{max} and AQY (Reich *et al.* 1998). It has been recognized that R_D of plant was affected by an increased atmospheric NH_3 concentration (Fangmeier *et al.* 1994). The present studies point out that the significant positive effects of enhanced atmospheric NH_3 on R_D of both cultivars remained merely at silking stage, especially for SD19 in the LN medium. Cultivar NE5 had greater R_D than SD19 in each treatment. With the development of plants, R_D was markedly increased at tasseling/silking stage and reduced at mature stage (Table 3). These results elucidated that enhanced NH_3 concentration imposed greater effects on R_D of maize plants at silking stage. Berger *et al.* (1986) stated that R_D of *P. euramericana* increased by 76% under 351.6 nl(NH_3) l^{-1} for three months. However, R_D of *P. euramericana* did not increase under 73.3–146.5 nl(NH_3) l^{-1} concentration for 6–8 weeks. Van der Eerden *et al.* (1990, 1992) and Zhao *et al.* (2003) stated that R_D of Taiwan acacia (*Acacia confusa* Merr.) was not affected by NH_3 fumigation regardless of N resource. The effect of NH_3 fumigation was closely dependent on the experimental conditions. Thus we need to study further to clarify the boundary factors for efficient effects on R_D.

The effects of enhanced atmospheric NH_3 on the diurnal time courses of photosynthetic characters: P_N and C_i reflect plant photosynthetic ability and CO_2 assimilation rate, respectively. In addition, g_s influences on exchange of CO_2, water vapour, and sensible heat fluxes between atmosphere and terrestrial ecosystem resulting in the rate of CO_2 fixation in leaf mesophyll tissue. As such, they can be good indicators of diurnal time courses for evaluating the responses of crop plant induced by enhanced NH_3 concentration (Yu *et al.* 2001). Leaves of *P. euramericana* (poplar) showed increased g_s, in particular, P_N and the CO_2 assimilation rate after NH_3 exposure at 146.5 nl l^{-1} for 6 or 8 weeks (Van Hove *et al.* 1989). Increased photosynthesis was also reported for *P. sylvestris* exposed to 351.6 nl(NH_3) l^{-1} (Van der Eerden *et al.* 1990, Van der Eerden and Pérez-Soba 1992). In the present study, it is concluded that increased atmospheric NH_3 concentration imposed a more significant enhancement of P_N and g_s but reduction of C_i in SD19 than that in NE5 under both N treatments in the diurnal time courses. The above effects were more pronounced in the LN treatment than those in the HN treatment. The results here showed that enhanced NH_3 concentration could increase P_N and g_s but reduce C_i of maize SD19 and NE5 (Figs. 2–4). Van Hove *et al.* (1989) assumed that a higher demand for carbon skeletons resulting from NH_3 assimilation was responsible for increased CO_2 fixation and increased g_s to be regulated by C_i, whereas NH_3 itself had no direct influence on stomatal conductance. Thus, NH_3 uptake may cause an autocatalytic increase of additional NH_3 flux into the leaves by inducing stomatal opening *via* the internal CO_2 level, as long as photon flux density is sufficient for equivalent photosynthesis (Van der Eerden and Pérez-Soba 1992).

The diurnal pattern of photosynthetic characteristics of C_4 plant such as maize under the ambient NH_3 concentration generally shows single-peak curve. This diurnal pattern may be affected by plant species and cultivar, concentration of atmospheric CO_2 and NH_3, and N-supplying environment (Tuzet *et al.* 2003). Most researches were conducted on photosynthesis and CO_2 uptake during the diurnal courses under the free-air environment (Tuzet *et al.* 2003, Leakey *et al.* 2004). The responses of diurnal pattern of photosynthetic characteristics under NH_3 enrichment have not been fully understood (Krupa 2003). In the current studies on maize at jointing stage, the authors found that the diurnal change patterns under enhanced NH_3 treatment on P_N and g_s of NE5 showed double-peak curves but C_i presented single W curves in either the LN- or HN medium. As for SD19, the diurnal change patterns of P_N and g_s showed single-peak curves and those of C_i presented single V curves under the ambient and elevated NH_3 concentrations in combination with the LN- and HN treatments. The above similar diurnal change patterns of P_N, g_s, and C_i of SD19 also occurred in NE5 under the ambient NH_3 concentration. The peak value of P_N and g_s but the lowest values of C_i occurred at 10:00 h, declined at 12:00 h and reached the second summit value at 14:00 h in either the LN- or HN medium. It is concluded that the marked increase responses of P_N and g_s but decrease of C_i were obtained in the low-N-use efficiency maize cultivar (SD19) during 10:00–16:00 h. However, for the high-N-use efficiency maize cultivar (NE5), such obvious effects were prohibited by photosynthetic "noon break" at 12:00 h, consequently a nonsignificant impact of increased atmospheric NH_3 concentration on these parameters was observed. Moreover, the significant impacts of enhanced NH_3 treatment, especially, occurred from 14:00 to 16:00 h. The laws of marked increase of P_N and g_s but obvious reduction of C_i showed that the enhanced atmospheric NH_3 might be attributed to induction of stomatal opening to increase exchange of CO_2 and water vapour by declination of stomatal restriction during 14:00–16:00 h. Together with this, photosynthesis in mesophyll cell might be improved under elevated NH_3 concentration at afternoon due to a reduction of nonstomatal restriction, which is closely associated with a crop cultivar and a level of N medium (Yu *et al.* 2001, Li *et al.* 2004a; Li *et al.* 2004b). The consistency of photosynthetic physiological parameters in diurnal time course can be a useful index for atmospheric NH_3-use efficiency for plant growth. Increased atmospheric NH_3 imposed an increase of the fixation of carbon in plants. The modulation of CO_2 concentration in plants might occur through an increase of g_s, which is beneficial for the transmittance and absorption of atmospheric NH_3 (Fangmeier *et al.* 1994).

In summary, we elucidated the modulation mechanism in relation to photosynthesis induced by enhanced atmospheric NH_3 in two contrasted maize cultivars with N-use efficiency under two levels of N solution medium throughout long-term growth period and their diurnal time courses. We propose that enhancing atmospheric NH_3 adjustment might be effective in enhancing the assimilation of CO_2 by improving photosynthesis of maize plant, especially at earlier growth stages and after photosynthetic "midday depression" point of crops, especially for low N-use efficiency cultivars in the low-N-supplying environment and should be an important research direction of plant physiology on atmospheric NH_3 responses in the future.

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