Research Article

Received: 4 December 2012 Revised: 7 February 2013 Accepted article published: 22 March 2013 Published online in Wiley Online Library: 3 May 2013

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6144

Effects of enhanced atmospheric ammonia on physiological characteristics of maize (*Zea mays* L.)

Xiaoli Chen[a,b] and Shiqing Li[a]*

Abstract

BACKGROUND: Elevated atmospheric NH_3 may affect photosynthesis rates and biomass production of crops and the effect may be responsible for the soil nitrogen (N) levels. Plants were exposed to 0 and 1000 nL L^{-1} with and without N (+N and − N) in open-top chambers (OTCs) to investigate effects of atmospheric NH_3 on photosynthetic and chlorophyll fluorescence parameters of maize plants.

RESULTS: At two N levels, NH_3 exposure at 1000 nL L^{-1} led to an increase in plant height, biomass production, net photosynthetic rates (P_n) and stomatal conductance (g_s) compared to ambient NH_3. Exposure to 1000 nL L^{-1} NH_3 resulted in a significantly higher photochemical quenching (q_p) and non-photochemical quenching (q_{np}), while minimal fluorescence (F_o), maximum fluorescence (F_m) and maximum photochemical efficiency (F_v/F_m) were not affected. For shoots, N concentrations for − N-1000 and + N-1000 treatments were 49–50% and 26–30% higher, respectively, than those of − N-0 and + N-0 treatments.

CONCLUSION: No visible damage was observed and plants growing on low soil N took up more leaf-derived N than those fertilised at higher N level. Therefore, atmospheric NH_3 can be considered as a quick fertiliser for crops and should be estimated in a further study with soil N fertilisers in order to reduce the dosage.

Keywords: atmospheric ammonia; nitrogen levels; open-top chambers (OTCs); chlorophyll fluorescence; maize

INTRODUCTION

Atmospheric ammonia (NH_3) can play an important role in agricultural cropping systems. It can be released to or absorbed from the atmosphere by soils and plants in response to fertiliser application practices, by over-fertilisation of nitrogen (N), or by plant N stress.[1] Elevated atmospheric NH_3 may have both positive and negative effects on plants. At lower and more realistic NH_3 concentrations, the extra N input results in a stimulation of photosynthesis and in a higher biomass production.[2,3] These positive effects often coincide with changes in shoot:root ratio and nutrient imbalance.[4,5] Exposure of plants in high atmospheric NH_3 concentrations may affect growth negatively and result in direct toxic effects. Visible injury due to high concentrations of NH_3 has been observed on crop species only in the direct vicinity of sources of NH_3, such as poultry and pig farms.[6]

Positive responses have been documented in crops such as beans (*Phaseolus vulgaris* L.), rice (*Oryza sativa* L.) and winter wheat (*Triticum aestivum* L.).[7,8] An NH_3 stimulation of plant growth in theory requires a corresponding increase in nutrient acquisition to maintain the plant N metabolism to nutrient balance. Plants normally take up nitrogen by the roots.[9] In addition, plant shoots are also able to absorb NH_3 from the atmosphere and use it as an additional nitrogen source.[10–12] The foliar uptake of NH_3 is determined by the diffusive conductance of the stomata and the foliar absorbed NH_3 may be metabolised by the glutaminesynthetase/glutamatesynthase (GS/GOGAT) cycle.[12,13] Very few studies have described responses of crops to both high NH_3 and N levels in a medium. The driving force for NH_3 uptake is its concentration gradient between ambient air and the mesophyll tissue. Plants have a compensation point for NH_3, reflecting the fact that they can both absorb and emit NH_3.[14] Elevated concentrations may significantly contribute to N nutrition, as was shown by Faller in experiments with up to 1.6 mg m^{-3} NH_3.[15] In that case growth of sunflower continued to occur if NH_3 was the only nitrogen source. Effects of chronic exposures to NH_3 on crops have not been studied extensively and crop responses to combinations of N levels and NH_3 have seldom been reported. Much of our current knowledge of the effects of NH_3 on higher plants is predominantly derived from studies conducted on semi-natural vegetation in Europe.[16,17]

This paper presents the results of a sufficient study on the long-term effects of incremental concentrations of NH_3 at two soil N levels on maize (*Zea mays* L.). The maize cultivar with a C_4 pathway was selected because of its high sensitivity to NH_3 exposures.[18] The

* *Correspondence to: Shiqing Li, State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, China. E-mail: sqli@ms.iswc.ac.cn*

a *State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, China*

b *Institute of Water Saving Agriculture in Arid Regions of China, Northwest A&F University, Yangling, Shaanxi 712100, China*

C_4 plants have large reduction potential separated to mesophyll cells due to the special 'flower ring' structure, which is formed by bundle sheath and outer mesophyll cells adjacent to it. This study was conducted in open-top chambers (OTCs). We hypothesised that a high NH_3 concentration could stimulate plant growth and improve N contents of shoots, and the increase effect depends on N levels in the soil. The objectives of this study were (1) to assess the effects of chronic NH_3 exposures on maize growth, as well as photosynthetic and chlorophyll fluorescence parameters of leaves; and (2) to determine the shoot N value and investigate whether an increased N level influences its responses to NH_3.

MATERIALS AND METHODS

Cultivation and experimental design

Spring maize (*Zea mays* L. cv. Hudan No. 9, a local variety) was purchased from the Crop Institute, Shaanxi Province Agricultural Academy of Science and maize seeds were potted (20 cm diameter, 23 cm height) in the six OTCs. Seeds were planted on 1 May 2008 and 2009, respectively, and grown outdoors. After emergence, maize was thinned to one plant per pot. After 3 weeks of sowing, 16 pots were randomly placed in each of six OTCs and exposures started at the tasseling stage on 21 May 2008 and 2009, respectively. Construction and performance of the OTCs were same, as described by Dueck.[19] These PVC pots were filled with 5 kg soil per pot. Plants were potted in typical loess soil of the semi-arid region of the Loess Plateau, i.e. Calcaric Cambisols with a light loamy texture. Soil pH was 7.8, available N (i.e. hydrolytic N, 1 mol L^{-1} NaOH hydrolysis) was 18.7 μg g^{-1}, available P (0.5 mol L^{-1} $NaCO_3$) 4.2 μg g^{-1}, available K (1 mol L^{-1} neutral NH_4OAc) was 128 μg g^{-1} and field water capacity was 20%. The pot with N (+ N) received both 0.2 g pure N (urea) and 0.0655 g pure P kg^{-1} soil (KH_2PO_4), while the pot without N (− N) was only fertilised with 0.0655 g pure P kg^{-1} soil (KH_2PO_4). All fertilisers were mixed into the soil in powder at the beginning of the experiment.

The study was carried out at Yangling, Shaanxi, during April to July 2008 and 2009. The OTCs were 3.0 m long, 3.0 m wide and 2.4 m high. Three OTCs were maintained at an ambient NH_3 concentration of about 0 nL L^{-1} and the other three at an elevated concentration of about 1000 nL L^{-1}. Each of the eight pots of a chamber was used as the sub-plot and two N levels were applied for the high and ambient NH_3 concentration main plots. For practical convenience the high and ambient NH_3 concentration main plots were contiguous. N treatments were randomly assigned to sub-plots in each chamber. The experimental design was a two-factor split plot in randomised complete blocks with NH_3 concentration (0 and 1000 nL L^{-1}) as the main treatment and two N levels (+N and − N) as the sub-treatments. With three replications, there were a total of six OTCs each with two sub-plots for a total of 12 sub-plots. The ambient NH_3 concentration was used as the control although it is recognised that some air still fills in the chambers even without injecting NH_3.

Exposure to ammonia

A constant amount of NH_3 was added to chambers for 12 h day^{-1} during daylight to reach a desired 12 h mean concentration of 1000 nL L^{-1} (elevated NH_3 treatment) and without NH_3 was added to the other chambers (ambient NH_3 treatment). Each chamber was equipped with a fan and an air control system, which included a steel cylinder (inner diameter 600 mm, total length 1800 mm) containing 95% NH_3. During the period from seedling emergence to the end of the experiment, pressurised NH_3 diluted with N_2 (1000 μL L^{-1}) was injected into the incoming air stream and adjusted to desired concentrations by Automated Manifest System (AMS) electronic mass flow controllers. NH_3 concentrations were verified during the experimental period by leading the air stream at a known flow rate into a 1 mmol L^{-1} EDTA solution for 2 h. Aliquots were taken from the initial volume and NH_4^+ was measured colorimetrically at 410 nm (Starrcol SC-60-S; R&R Mechatronics, Hoorn, The Netherlands) with Nessler's reagent A (Merck, KGaA, Germany) mixed 1:1 with 9 mol L^{-1} NaOH (volume ratio).[16] Air temperature did not differ significantly (one-way ANOVA: $F = 0.46$, $P = 0.63$) among the six chambers (25.7 ± 4.3 °C in three 1000 nL L^{-1} NH_3 chambers versus. 24.9 ± 5.2 °C in three control chambers) throughout the experiment, and relative humidity was in a range of 40–50%. The concentration of NH_3 was measured occasionally in each OTC without enhanced NH_3 to ensure the unified growing conditions. The detailed NH_3 control system for two main growing seasons is shown in Fig. 1.

Sampling and measurements

Four plants per treatment were randomly harvested at the end of the silking stage after 65 days of exposure (27 June). Plant height was determined using a steel ruler. After the determination of plant height, the root ball was soaked in tap water until the maize plant roots in each pot were totally isolated from plastered soil before drying. The roots were separated from the shoot and both

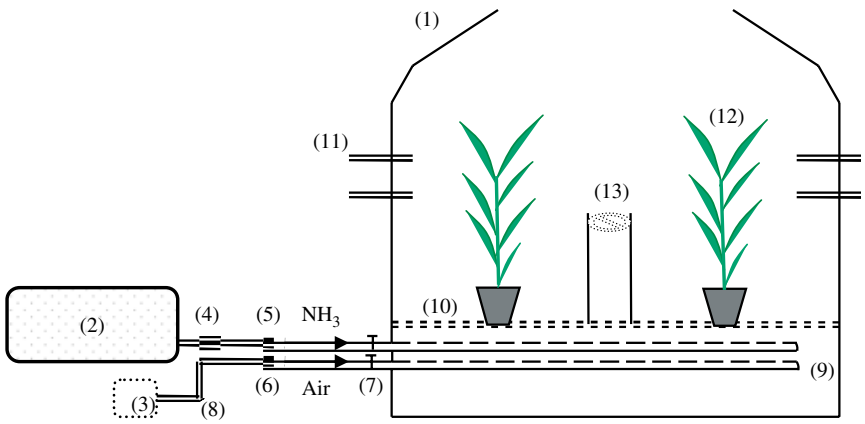

Figure 1. A schematic diagram showing open-top chambers. (1) Open-top chamber (OTC), (2) NH_3 source, (3) air compressor, (4) NH_3 valve, (5) NH_3-flow meter, (6) air-flow meter, (7) piston, (8) oxygen pipe, (9) porous tubes, (10) porous board, (11) NH_3 test tube, (12) maize, (13) small ventilator.

were dried at 60–70 °C for 3 days. The total above-ground biomass in each pot was expressed in terms of grams dry matter per pot. Total N in the maize shoot was determined by the Kjeldahl method using an automatic nitrogen analyser (K2300; FOSS, Shanghai, Sweden). The flag leaf in each plant was determined for the net photosynthetic rates (P_n) and modulated chlorophyll fluorescence. The testing for each leaf was repeated three times. The P_n value was measured from 09:00 to 11:00 hours at silking stage with a portable photosynthesis system (LI-6400; Li-Cor, Lincoln, NE, USA). During the exposure in the OTCs, modulated chlorophyll fluorescence measurements on individual leaves were carried out in order to obtain more information about the action of NH_3 and N on the photosynthetic process at the chloroplast level. These parameters included minimal fluorescence (F_o), maximum fluorescence (F_m), maximum photochemical efficiency (F_v/F_m), photochemical quenching (q_p) and non-photochemical quenching (q_{np}). The measurements were conducted with a pulse amplitude modulated chlorophyll fluorimeter (Imaging-PAM; Portable Chlorophyll Fluorometer, WALZE, Germany). A detailed description of the measurement and the experimental procedure have been given in a previous paper.[20]

Statistical analysis

The treatments consisted of all combinations of two NH_3 levels and two N levels. Treatments were assigned to chambers in a completely randomised design. Assay results of plant tissue samples obtained within a chamber were averaged for use as a chamber replicate value. Data were checked for homogeneity of variance before the statistical analysis. Treatment effects were statistically analysed as a 2 × 2 factorial using analysis of variance techniques (SAS Proc GLM, SAS Systems for Windows, Ver. 9.1; SAS Institute, Cary, NC, USA). Comparisons between the control and the other treatments were made using estimate statements in one-way analysis of variance tests, and multiple comparisons among treatments were performed using the Turkey–Kramer method. Mean values were expressed as LSD means.

RESULTS AND DISCUSSION

Chlorophyll fluorescence

The fluorescence measurements showed no significant main effect of NH_3 on the minimal fluorescence (F_o), the maximum fluorescence (F_m) and maximum photochemical efficiency (F_v/F_m). However, an effect on the photochemical quenching (q_p) and non-photochemical quenching (q_{np}) was observed (Table 1). There were no interactions of year × NH_3 and year × N for the fluorescence parameters, so these interactions were not considered further. Both the year effect and main effect of N were significant for all fluorescence parameters ($P \leq 0.05$) (Table 1). The different behaviour of leaves exposed to 1000 nL L^{-1} NH_3 as compared to leaves exposed to ambient air was evident only in the transition from dark to light. After 150 s at a photon flux density of 35 µmol m^{-2} s^{-1} the differences between the treatments were negligible. In addition, modulated chlorophyll fluorescence measurements on individual leaves were carried out to obtain more information about the action of NH_3 and N on the photosynthetic process at chloroplast level (Table 1). In the present study no effect on the maximum photochemical efficiency (F_v/F_m) was found. Based on this, it can be concluded that the increase in P_n was probably due to an increased activity of Photosystemll (PSII). Meanwhile, leaves exposed to 1000 nL L^{-1} NH_3 had a higher quantum yield for CO_2 fixation than leaves exposed to ambient air. Apparently, leaves treated with elevated NH_3 had a higher efficiency of photosynthesis in normal air when the light intensity was the limiting factor. A further study showed that after transition from dark to light, the actual quantum yield (Φ_{PSII}) of these leaves reached a steady state more rapidly than control leaves (data not shown), suggesting that a more rapid activation of the Calvin cycle occurs after onset of the illumination. Our findings are consistent with the elevated-NH_3 enhancement effect on polar leaves reported by Van Hove *et al*.[3]

Net photosynthesis and stomatal conductance

The ANOVA (Table 2) showed no interaction effects of year × NH_3 and year × N for P_n and g_s, while the interaction between NH_3 × N

Table 1. Effect on chlorophyll fluorescence: analysis of variance (ANOVA) for the years 2008 and 2009 data of maize grown at two NH_3 concentrations (0 and 1000 nL L^{-1}) at two N treatments, with and without N (+N and − N, respectively)

Year	[NH_3] (nL L^{-1})	N level	F_o	F_m	F_v/F_m	q_p	q_{np}
2008	0	−N	0.096[b]	0.412[b]	0.761[a]	0.688[a]	0.602[a]
		+N	0.102[a]	0.451[a]	0.781[a]	0.698[a]	0.618[a]
	1000	−N	0.098[b]	0.414[b]	0.779[a]	0.612[a]	0.524[b]
		+N	0.104[a]	0.455[a]	0.785[a]	0.603[a]	0.589[a]
2009	0	−N	0.083[b]	0.422[b]	0.753[a]	0.643[a]	0.606[a]
		+N	0.103[a]	0.459[a]	0.789[a]	0.690[a]	0.622[a]
	1000	−N	0.086[b]	0.427[b]	0.769[a]	0.621[a]	0.522[a]
		+N	0.105[a]	0.465[a]	0.792[a]	0.632[a]	0.524[a]
ANOVA							
YEAR			<0.01	<0.01	0.05	<0.01	<0.01
[NH_3]			NS	NS	NS	<0.01	0.03
N			<0.01	<0.01	0.03	0.05	<0.01
[NH_3] × N			NS	NS	NS	0.02	0.05
YEAR × [NH_3]			NS	NS	NS	NS	NS
YEAR × N			NS	NS	NS	NS	NS

Values with different lower case letters are significantly different between two treatments.
Mean chlorophyll fluorescence variables on a per plant basis are minimal fluorescence (F_o), maximum fluorescence (F_m), maximum photochemical efficiency (F_v/F_m), photochemical quenching (q_p) and non-photochemical quenching (q_{np}) after exposures to NH_3 for 65 days.
NS, not significant.

Table 2. Effect on growth variables: analysis of variance (ANOVA) for the years 2008 and 2009 data of maize grown at two NH_3 concentrations (0 and 1000 nL L^{-1}) at two N treatments, in the presence and absence of N (+N and − N, respectively)

Year	$[NH_3]$ (nL L^{-1})	N level	Plant height	P_n	g_s	Shoot dry weight*	Root dry weight
2008	0	−N	66.80	18.60[b]	0.17[b]	16.80[b]	3.89[b]
		+N	108.20	23.60[a]	0.19[a]	41.70[a]	5.98[a]
	1000	−N	79.70	22.40[b]	0.20[a]	22.50[b]	4.02[b]
		+N	118.30	26.60[a]	0.21[a]	49.80[a]	6.01[a]
2009	0	−N	64.80	17.80[b]	0.16[b]	17.70[b]	3.82[b]
		+N	112.30	22.90[a]	0.18[a]	43.20[a]	5.96[a]
	1000	−N	81.20	21.60[b]	0.19[b]	23.30[b]	4.10[b]
		+N	120.60	25.70[a]	0.21[a]	48.60[a]	6.05[a]
ANOVA							
YEAR			0.02	0.03	0.05	<0.05	NS
$[NH_3]$			<0.01	<0.01	<0.01	<0.01	NS
N			0.04	0.05	<0.01	<0.01	0.05
$[NH_3] \times N$			0.03	0.04	0.02	<0.01	NS
YEAR × $[NH_3]$			NS	NS	NS	NS	NS
YEAR × N			NS	NS	NS	NS	NS

*Sum of leaf and stem dry weight.
Values with different lower case letters are significantly different among treatments.
Mean growth variables on a per plant basis are plant height (cm), net photosynthetic rates (P_n) (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s) (mmol $m^{-2}As^{-1}$) and dry weight (g $plant^{-1}$) of shoots and roots after exposures to NH_3 for 65 days.

level had a significant effect on P_n and g_s of leaves ($P < 0.05$). A positive effect on maximal photosynthetic rates (P_{max}) was observed for leaves at elevated NH_3 treatments (data not shown). Leaves treated with 1000 nL L^{-1} NH_3 showed a significant increase in net photosynthetic rate (P_n) and stomatal conductance (g_s) as compared to leaves treated with ambient air (Table 2) in 2008 and 2009. P_n means for 0 nL L^{-1} NH_3 increased by 27 and 29% over the − N control of 18.60 and 17.80 μmol CO_2 m^{-2} s^{-1} as the N level increased in 2008 and 2009, respectively. Values for 1000 nL L^{-1} NH_3 increased by 19 and 19% over control of 22.40 and 21.60 μmol CO_2 m^{-2} s^{-1}. The means for − N treatment increased by 20 and 21% over ambient air with increase in NH_3 concentration in 2008 and 2009, respectively. Values for the + N treatment increased by 13 and 12% over control of 23.60 and 22.90 μmol CO_2 m^{-2} s^{-1} (Table 2). Similar results were observed on g_s. The higher P_n of these leaves corresponded to a higher g_s.

The positive effect of the long-term NH_3 exposure on P_n and stomatal conductance of leaves is in accordance with previous results.[20] However, plants supplied with different N levels differed in their photosynthetic response to increased NH_3 in the air. The higher P_n of these leaves corresponded to a higher g_s. Many studies have shown that elevated NH_3 concentrations increased photosynthesis rates and biomass production in higher plants.[12,21,22] In our experiment, elevated NH_3 increased P_n both in the presence and absence of nitrogen in soil during the 2 year experiment (Table 2). We found that the increment in P_n in the absence of N plants exposed to high levels of atmospheric NH_3 was higher than that of the presence of N plants. This suggests that the extra N uptake via the leaves is repressed at high internal N status and becomes depressed when N supplies are limited. At moderate ambient NH_3 concentrations, NH_3 may stimulate photosynthesis most likely by providing a source of nitrogen for synthesis of ribulose 1,5-bisphosphate carboxylase–oxygenase, catalysing the addition of CO_2 to 1,5-bisphosphate in the Calvin cycle.[23]

Plant height and biomass production

For both 0 and 1000 nL L^{-1} NH_3, +N promoted maize plant height and biomass production (Table 2). YEAR, NH_3 and the interaction between $NH_3 \times N$ all significantly influenced plant growth ($P < 0.05$) except for root biomass. After exposures to NH_3 for 65 days, mean plant heights for 1000 nL L^{-1} NH_3 were higher than those for 0 nL L^{-1} for two N levels in each experimental year. In 2008, the means for the − N-1000 and + N-1000 treatments increased by 19 and 9%, respectively, over control of 66.80 and 108.20 cm. The corresponding values were 25 and 7%, respectively, higher than those of − N-0 and + N-0 treatments in 2009. Plant height means for + N level for 0 nL L^{-1} NH_3 increased by 62 and 73%, respectively, compared to − N-0 value of 66.8 and 64.8 cm in 2008 and 2009. For 1000 nL L^{-1} NH_3, plant height showed similar increases in response to N levels. The means for + N treatment were 48 and 49%, respectively, higher than the − N-0 values of 79.70 and 81.20 cm in each experimental year.

Statistical analyses indicated main effect ($P < 0.05$) of NH_3 on shoot biomass rather than root biomass that were assessed at both harvests (27 June and 26 July). Shoot biomass was simulated by enhanced NH_3. Significant NH_3 by N interactions were observed for shoot biomass only on 27 June of both years. The shoots biomass means for − N-1000 treatments were 34 and 32% higher, respectively, than those for − N-0 controls in 2008 and 2009. The corresponding values for + N-1000 treatments were increased by 19 and 13%, respectively, for the + N-0 control. For 0 nL L^{-1} NH_3, the presence of N led to increases in shoot biomass by 1.5 and 1.4% in 2008 and 2009, respectively, compared to the corresponding absence of N (Table 2). Values for 1000 nL L^{-1} NH_3 treatments were 1.2 and 1.1% higher than those for the − N-0. N levels had a significant effect on roots biomass. Averaged values of + N treatments for both years were increased by 55 and 49%, respectively, for 0 and 1000 nL L^{-1} NH_3 treatments compared − N control (Table 2).

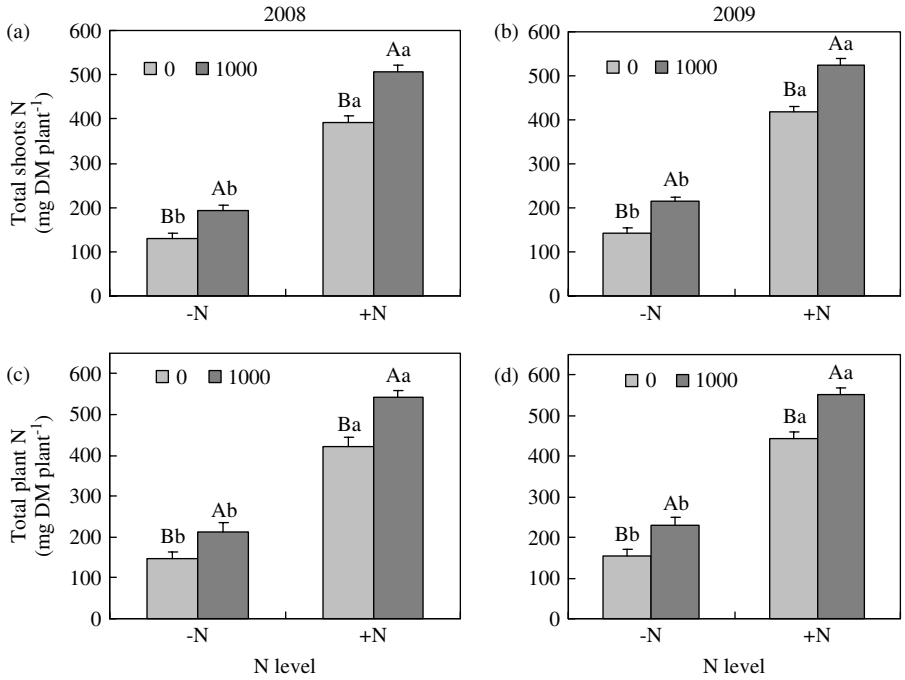

Figure 2. Total N content in shoots dry matter of maize in 2008 (a) and 2009 (b), and total plant N in 2008 (c) and 2009 (d). Plants were exposed for 65 days at two NH_3 concentrations (0 and 1000 nL L^{-1}) at two soil N treatments with and without N (+N and − N). Data represent the mean of four individual plants per treatment ± SD. Different upper case letters indicate significant difference between control and NH_3-exposed plants at the same N level and different lower case letters indicate significant difference between − N and + N treatments at the same NH_3 exposure.

One of the most obvious plant responses following NH_3 assimilation is enhanced growth. In general, biomass production increased with increasing concentrations of NH_3, and growth was stimulated by enhanced NH_3 at both the silking stage (65 days of exposure). The effect of NH_3 on biomass production depended on the atmospheric concentration, and differed between absence and presence of N.[1] In this experiment, shoot biomass rather than root biomass was simulated by enhanced NH_3. The shoots biomass means for − N-1000 treatments were 34 and 32% higher, respectively, than those for − N-0 controls in 2008 and 2009 (Table 2). The result was in agreement with the results reported by Clement *et al*. for winter wheat.[24] The increased growth is mainly due to the shoot growth response. Root growth increases only slightly or at least less than shoot growth, leading to higher shoot:root ratios.[22] Increased shoot:root ratios were observed after NH_3 exposures of 65 days at two N levels (data not shown). Atmospheric NH_3 enters the leaves of higher plants almost exclusively through the stomata. The absorption of NH_3 by plants depends on NH_3 concentration in the atmosphere and the NH_3-use compensation point of plant. If the former exceeds the latter, NH_3 enters into the intercellular space through stomata on the leaf, dissolves in moisture, and is finally assimilated.[25] However, normally, root uptake of NH_3 does not occur since the NH_3 deposited to the soil is readily dissolved to yield NH_4^+.[1] Therefore, NH_3 application is mainly beneficial to the growth of shoot and leaf without N.

Total nitrogen content of shoot

Figure 2 shows the N concentrations in shoots and total biomass responses to the N differences within the OTCs for both levels after 65 days. The total N concentrations in maize shoots and biomass were higher for 1000 nL L^{-1} NH_3 compared to 0 nL L^{-1} NH_3 control at two N levels in either year. In 2008, the shoots and plant total N for − N treatments at 1000 nL L^{-1} NH_3 were increased by 49 and 44%, respectively, compared to 0 nL L^{-1} NH_3 control under − N treatments. For + N treatments, total N measured on 25 June also showed similar increase in response to NH_3 levels. The corresponding means were 30 and 28% higher for + N-1000 than values for + N-0 treatments (Fig. 2). In 2009, the N values of shoots and plant were increased by 50 and 47%, respectively, at elevated NH_3 in the − N treatments, whereas the corresponding values in the + N treatments only increased by 26 and 25%, respectively, over the 0 nL L^{-1} control of 417.86 and 442.89 mg plant^{-1}. The tendency was the same for N treatments in both years (Fig. 2). There were significant effects of N levels on total shoots and plant N at the same NH_3 concentration. A NH_3 × N interaction was also observed for total N content in shoots and plant.

After 65 days exposure, the total shoots N concentration in maize leaves exposed to NH_3 increased by 49% and 50%, respectively, in 2008 and 2009 with respect to ambient air at N absence, while the increase caused by N presence were only 30% and 26% in each year (Fig. 2). This observation implies that a lower uptake of atmospheric NH_3 would be expected for plants fertilised with N than for unfertilised plants, due to the higher compensation point. This finding agrees with the conclusion of Pérez-Soba and Van der Eerden who also found a more marked effect with different N sources on N concentration in needles of Scots pine (*Pinus sylvestris* L.).[23] After 4 months of plant growth they found a 49% increase in the N content of needles by NH_3, while there was only an 8% increase due to fertilisation. The extant nutritional status of the plant may also be of importance in determining rates of uptake of both gaseous and wet-deposited nitrogen. In particular the nitrogen status of the plant seems most relevant in this context. Plants growing on low soil nitrogen take up more leaf-derived nitrogen than those fertilised with higher nitrogen. Wilson suggested that tissue nitrogen status determines whether

a plant can act as a source or sink for wet-deposited nitrogen.[26] If NH_3 was the only nitrogen source, plant growth continued to occur. In experiments with *Lolium multiflorum* (ryegrass), foliar NH_3 uptake at 520 $\mu g\, m^{-3}$ (728 ppb) supplied 47.3% of total plant N at fertilisation with 100 mg $^{15}NO_3$-N kg^{-1} and 35.2% at 200 mg $^{15}NO_3$-N kg^{-1} dry soil.[27] We also found a linear increase in the total plant N as the NH_3 concentration increased. In the present long-term experiment, a significant difference between biomass (Table 2), total N (Fig. 2) and fertiliser N levels after 1000 nL L^{-1} NH_3 exposure was observed.

The present study was focused on investigating the effects of elevated NH_3 on physiological characteristics of maize. The time of NH_3 exposure was only 65 days due to the difficulty of NH_3 concentration. Effects of long-term chronic exposures to NH_3 on crops have not been studied extensively. No studies were found in the literature that examined how soil N fertiliser application would affect crop plant response to atmospheric NH_3 exposure. A further study would be made to obtain the data on soil N in the pots which would gain very much in value. The relationship between leaf nutrition and soil nutrition would be explained. Therefore, the information was worth collecting and would help a great deal in interpreting plant growth and yield results.

CONCLUSION

Ammonia (NH_3) is one of the major air pollutants. Elevated atmospheric NH_3 may affect photosynthesis rates and biomass production of crops and the effect may be responsible for soil nitrogen levels. This experiment demonstrates that spring maize can tolerate high atmospheric NH_3 concentrations. From the results, it is evident that atmospheric NH_3 can act as an N supplement source. With and without N conditions, exposure to elevated NH_3 resulted in an increase in net photosynthetic rates (P_n), indicating that NH_3 was taken up by leaves and metabolised. Meanwhile, the nitrogen status of the plant may be of importance in determining rates of uptake of the extra N. The extra N uptake is repressed at high internal N status and becomes depressed without nitrogen in the soil. However, the physiological basis of atmospheric NH_3 is still largely unknown and the relationship between shoot N and soil N would be worth further investigation, which makes the study of greater value.

ACKNOWLEDGEMENTS

This work is jointly supported by the National Natural Science Foundation of China (No. 31270553), the Ministry of Science and Technology of China ('973' project: Grant No. 2009CB118604), and the '111' Project (No. B12007).

REFERENCES

1 Krupa SV, Effects of atmospheric ammonia (NH_3) on terrestrial vegetation: a review. *Environ Pollut* **124**:179–221 (2003).
2 Van der Eerden LJM and Pérez-Soba M, Physiological responses of *Pinus sylvestris* to atmospheric ammonia. *Trees* **6**:48–53 (1992).
3 Van Hove LWA, Heeres P and Bossen ME, The annual variation in stomatal ammonia compensation point of rye grass (*Lolium perenne* L.) leaves in an intensively managed grassland. *Atmos Environ* **36**:2965–2977 (2002).
4 Van Dijk HFG, De Louw MHJ, Roelofs JGM and Verburgh JJ, Impact of artificial ammonium-enriched rainwater on soils and young coniferous trees in a greenhouse. Part II – effects on the trees. *Environ Pollut* **63**:41–59 (1990).
5 Fangmeier A, Hadwiger A and Vander EJ, Effects of atmospheric ammonia on vegetation – A review. *Environ Pollut* **86**:43–82 (1994).
6 Van der Eerden LJ, Toxicity of ammonia to plants. *Agric Environ* **7**:223–235 (1994).
7 Tonneijck AEG and van Dijk CJ, Responses of bean (*Phaseolus vulgaris* L. cv. Pros) to chronic ozone exposure at two levels of atmospheric ammonia. *Environ Pollut* **99**:45–51 (1998).
8 Hayashi K, Hiradate S, Ishikawa S and Nouchi I, Ammonia exchange between rice leaf blades and the atmosphere: Effect of broadcast urea and changes in xylem sap and leaf apoplastic ammonium concentrations. *Soil Sci Plant Nutr* **54**:807–818 (2008).
9 Glass ADM, Britto DT, Kaiser BN, Kinghorn JR, Kronzucker HJ, Kumar A, *et al*. The regulation of nitrate and ammonium transport systems in plants. *J Exp Bot* **370**:855–864 (2002).
10 Van Hove LWA, Koops AJ, Adema EH, Vredenberg WJ and Pieters GA, Analysis of the uptake of atmospheric ammonia by leaves of *Phaseolus vulgaris* L. *Atmos Environ* **21**:1759–1763 (1987).
11 Lea PJ, Blackwell RD and Joy KW, Ammonia assimilation in higher plants, in *Nitrogen metabolism of plants. Proceedings of the phytochemical society of Europe*, ed. by Mengel K and Pilbeam DJ. Clarendon Press, Oxford, pp. 153–186 (1992).
12 Pérez-Soba M, Stulen I and Van der Eerden LJM, Effects of atmospheric ammonia on the nitrogen metabolism of Scots pine (*Pinus sylvestris*). *Environ Pollut* **72**:103–115 (1994).
13 Van Hove LWA, Van Kooten O, Van Wijk KJ, Adema EH, and Pieters GA, Physiological effects of long term exposure to low concentrations of SO_2 and NH_3 on poplar leaves. *Physiol Plant* **82**:32–40 (1991).
14 Farquhar GD, Wetselaar R and Weir B, On the gaseous exchange of ammonia between leaves and the environment: Determination of the ammonia compensation point. *Plant Physiol* **66**:710–714 (1980).
15 Faller N, Schwefeldioxid, Schwefelwasserstoff, nitrose Gase und Ammoniak als ausschliebliche S-bzw. N-Quellen der höheren Pflanzen. *Zeitschrift für Pflanzenernährung und Bodenkunde* **131**:120–130 (1972).
16 Castro A, Stulen I, Posthumus FS and De Kok LJ, Changes in growth and nutrient uptake in *Brassica oleracea* exposed to atmospheric ammonia. *Ann Bot* **97**:121–131 (2006).
17 Hao XY, Chang C, Janzen HH, Clayton G and Hill BR, Sorption of atmospheric ammonia by soil and perennial grass downwind from two large cattle feedlots. *J Environ Qual* **35**:1960–1965 (2006).
18 Harper LA, Denmead OT and Sharpe RR, Identifying sources and sinks of scalars in a corn canopy with inverse Lagrangian dispersion analysis II. Ammonia. *Agric For Meteorol* **104**:75–83 (2000).
19 Dueck TA, Effect of ammonia and sulphur dioxide on the survival and growth of *Calluna vulgaris* (L.) Hull seedlings. *Functional Ecology* **4**:109–116 (1990).
20 Chen XL, Li SQ, Ren XL and Li SX, Effect of atmospheric NH_3 and hydroponic solution nitrogen levels on chlorophyll fluorescence of corn genotypes with different nitrogen use efficiencies. *Actaecologica Sinica* **128**:1026–1033 (2008).
21 Van Hove LWA, Bossen ME, Mensink MGJ and Van Kooten O, Physiological effects of a long term exposure to low concentrations of NH_3, NO_2 and SO_2 on Douglas fir (*Pseudotsuga menziesii*). *Physiol Plant* **82**:32–40 (1992).
22 Tisdale SL, Nelson WL, Beaton JD and Havlin JL, *Soil Fertility and Fertilizers*, 5th edition. Macmillan Publishing Company, New York (1993).
23 Pérez-Soba M and Van der Eerden LJM, Nitrogen uptake in needles of Scots pine (*Pinus sylvestris* L.) when exposed to gaseous ammonia and ammonium fertilizer in the soil. *Plant Soil* **153**:231–242 (1993).
24 Clement JMAM, Loorbach J, Meijer J and Van Hasselt PR, The impact of atmospheric ammonia and temperature on growth and nitrogen metabolism in winter wheat. *Plant Physiol Biochem* **34**:159–164 (1997).
25 Nemitz E, Sutton MA, Gut A, San Jose R, Husted S and Schjoerring JK, Sources and sinks of ammonia within an oilseed rape canopy. *Agric For Meteorol* **105**:385–404 (2000).
26 Wilson EJ, Foliar uptake and release of inorganic nitrogen compounds in *Pinus sylvestris* (L.) and *Picea abies* (L.) Karst. *New Phytol* **120**:407–416 (1992).
27 Lockyer DR and Whitehead DC, The uptake of gaseous ammonia by the leaves of Italian ryegrass. *J Exp Bot* **37**:919–927 (1986).

