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Available online: 15 Feb 2012

To cite this article: Xiaoli Chen, Pute Wu, Xining Zhao & Shiqing Li (2012): Effects of atmospheric ammonia enrichment and nitrogen status on the growth of maize, Soil Science and Plant Nutrition, 58:1, 32-40

To link to this article: http://dx.doi.org/10.1080/00380768.2011.654349

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Effects of atmospheric ammonia enrichment and nitrogen status on the growth of maize

Xiaoli CHEN1,2, Pute WU2, Xining ZHAO2 and Shiqing LI1

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Abstract
Enhanced atmospheric ammonia (NH3) concentration could impact growth of crops. The effect of atmospheric NH3 in combination with nitrogen-deprived and -sufficient supply (ND and NS, respectively) on growth of maize plants (Zea mays L.) was investigated. Plants were exposed to 0 and 1000 nL L−1 NH3 for 50 days in open-top chambers (OTCs). At different growing stages after injecting NH3, elevated NH3 significantly increased plant height, leaf area per plant and shoot biomass (total biomass) in the nitrogen-deprived plants in 2006 and 2007, but the corresponding values in the nitrogen-sufficient plants remained the same or slightly decreased. The values for net photosynthesis rate (Pn) and relative content of chlorophyll (SPAD value) increased with NH3 enhancement both in the presence and absence of nitrogen in nutrient solution, but the increment of Pn and SPAD value in nitrogen-deprived plants was two times higher than that of the nitrogen-sufficient plants in each year. The shoot: root ratio (S/R) for maize first decreased and then rose during the growing season. At 50 days after injecting NH3, the S/R in the ND-1000 plants was significantly higher than that in the ND-0 plants in both years. Under these conditions, increased growth was observed, and it was concluded that the effect of atmospheric NH3 on maize could be mainly due to the shoot growth response.

Key words: atmospheric ammonia, N status, shoot/root ratio, hydroponics, OTCs, maize.

1. INTRODUCTION
Atmospheric ammonia (NH3) 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. 1994). Plants absorb NH3 from the air with the above-ground plant parts or via the roots when deposited in the soil. The existence of an NH3 compensation point in plant tissues was first demonstrated by Farquhar et al. (1980). The NH3 compensation point is defined as the NH3 concentration in the air within the substomatal cavities at which no net NH3 exchange with the ambient environment takes place. Plant stomata are a channel of gas exchange between a leaf and the atmosphere. The direction of the gas exchange is controlled by the difference in partial pressure (or mixing ratio or air concentration) between the stomatal cavities and the atmosphere. Therefore, it is quite natural that a markedly high concentration of atmospheric ammonia easily results in plant absorption of ammonia, which is theoretically similar to the dry deposition of ammonia onto the plant. Of course, plant emission of ammonia also occurs when the concentration of atmospheric ammonia is lower than that inside the stomata.

The effects of NH3 on plants may be positive and negative: at lower and more natural NH3 concentrations, the extra nitrogen (N) input results in a stimulation of photosynthesis and a higher biomass production (Van der Eerden and Pérez-Soba 1992; Van Hove et al. 2003). These positive effects often coincide with changes
in shoot:root ratio and nutrient imbalance (Van Dijk et al. 1990; Fangmeier et al. 1994). Exposure of plants to high atmospheric NH₃ concentrations may negatively affect growth and result in direct toxic effects (Van der Eerden 1982). However, mostly these high concentrations are a result of accidents upon the production, storage and transport of ammonia and therefore will only seldom occur (Pérez-Soba 1995). Maize (Zea mays L.) is a major provision crop in the north of China, and is frequently subjected to the impacts of increased atmospheric NH₃ emission, particularly in fertilizer-enriched fields (Li et al. 2004). As a C₄ plant, maize has a large reduction potential in its mesophyll cells due to the special “flower ring” structure, which is formed by the bundle sheath and the outer mesophyll cells adjacent to it. Plants can utilize atmospheric NH₃ as a nutrient to improve plant growth if the mole fraction of NH₃ in the atmosphere is greater than the mole fraction of gaseous NH₃ in the substomatal cavity. Much of our current knowledge of the effects of NH₃ on higher plants is predominantly derived from studies conducted in Europe. It was found that foliar uptake of NH₃ is a passive diffusion process, independent of the metabolism of the plant (Van Hove et al. 1987).

Atmospheric NH₃ exposure may result in impacts on growth of plants (Chen et al. 2008; Li et al. 2009). Different studies relating to effects of enhanced atmospheric NH₃ 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 status in medium. Wilson (1992) suggested that plants growing on low-N soil take up more leaf-derived nitrogen than those fertilized with higher nitrogen. Clement et al. (1997) exposed winter wheat (Triticum aestivum L. cv. Urban) to 1000 and 2000 nL L⁻¹ NH₃ and found reductions in N uptake rate of 8.0% and 13.8%, respectively. Castro et al. (2006) concluded from experiments with Brassica oleracea exposed to 4000 and 8000 nL L⁻¹ NH₃ that in N-sufficient plants N uptake at both concentrations was reduced by 50% and 66%, respectively.

However, the vast majority of studies have reported on the effects of atmospheric NH₃ on the growth of plants in forests and grasslands. Hence, there is little information available in the literature about responses of crops in agricultural fields. In this paper, we present results from a potting experiment using open-top chambers (OTCs) that examined the plant growth and biomass production in maize treated with elevated NH₃ concentrations and N solution medium. We hypothesized that the enhanced atmospheric ammonia had a fertilizing effect on maize even at a high concentration of 1000 nL L⁻¹. Our objectives were (1) to determine whether elevated NH₃ in combination with N status affected net photosynthesis rate ($P_n$) and relative chlorophyll content (SPAD) in maize; and (2) to investigate the influence of elevated NH₃ in combination with N status on changes in shoot:root (S/R) ratio, calculated by measurement of shoots and roots.

2. MATERIALS AND METHODS

2.1. NH₃ control system

Six OTCs were used in this study (Fig. 1). Three OTCs were maintained at an ambient NH₃ concentration of about 0 nL L⁻¹ and the other three at an elevated concentration of about 1000 nL L⁻¹. The chambers were initially designed for exposing plants to elevated carbon dioxide (CO₂) concentrations under close to natural conditions, with a square 3.0 m × 3.0 m base and 2.4-m tall perpendicular glass walls topped by glass
quadrilaterals inclined towards the centre in an iron frame (total volume ca. 21.6 m³). 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% NH₃. During the period from seedling emergence to the end of the experiment, pressurized NH₃ diluted with N₂ (1000 µL L⁻¹) was injected into the incoming air stream and adjusted to the desired concentration by AMS electronic mass flow controllers. The air exchange rate was 40 L min⁻¹ and a ventilator continuously mixed the air inside the OTCs. NH₃ concentrations were verified during the experimental period by leading the air stream at a known flow rate into a 1 mM EDTA solution for 2 h. Aliquots were taken from the initial volume and NH₃ was measured colorimetrically at 410 nm (Starcol SC-60-S, R&R Mechatronics, Hoorn, The Netherlands) with Nessler’s reagent A (Merck, Germany) mixed 1:1 with 9 N NaOH (v/v) (Castro et al. 2006). Air temperature did not differ significantly (one-way ANOVA: F = 0.46, P = 0.63) among the six chambers (26.7 ± 4.6°C in three NH₃ chambers versus. 25.9 ± 5.4°C in three control chambers) throughout the hydroponics experiment, and relative humidity was in the range of 40–50%. A detailed description of the NH₃ control system is provided in Zhang et al. (2010).

### 2.2. Plant materials

Seeds of maize were rinsed with a 10% H₂O₂ 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 imbibed for 12 h and then sown in porcelain trays for 15 min. After rinsing in distilled water, seeds were covered 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 conditions of 25/18°C average day/night temperature, 60–70% relative humidity (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 7222 cm³ (inner diameter 20 cm, height 23 cm) were used to contain 7.2 L of nutrient solution for plant growth. NH₃ fumigation and N treatments were initially preceded on the third day after the seedlings were transferred into OTCs at their three-leaf stage. A completely randomized design was used with a total of four treatments: two NH₃ concentrations (0 and 1000 nL L⁻¹), and two N levels [nitrogen-deprived (ND) and nitrogen-sufficient (NS)]. The four treatment combinations are ND-0, ND-1000, NS-0 and NS-1000, respectively. The pots with three replicates of NH₃ concentration treatment were placed in 6 OTCs, respectively. Twenty-four pots in each chamber covered ten replicates of maize and two N status treatments in solution medium. Each treatment was exchanged in the different chambers at certain intervals (10 d) to reduce the test error caused by the chamber difference. Complete nutrient solution containing all essential minerals for plant growth (Hoagland and Arnon 1938) was supplied for the present solution culture experiment, and all solutions were made up with distilled water. The nutrient solution was added to an emulsion, which was made of higher-pH alcohol to restrain NH₃ change between the two phases of gas and liquid, then the pH of the nutrient solution was adjusted to 6.2 (±0.1). The N-deprived and -sufficient status in the medium were achieved using 1/9 and 1/3 strength of the complete solution, i.e., 5.00 and 1.67 m mol L⁻¹ N, 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 plant fixation with sponge and adhesive tape, and the space between plants and holes was sealed with wax. The solution was aerated without NH₃ for 4 h a day to assure normal growth.

### 2.3. Collection and analysis of plant samples

On August 5 in 2006 and 2007, 8 pots were randomly selected from the 24 pots in each of the OTCs of different NH₃ in combination with N treatments, and leaves were dissected and used to measure the leaf area of maize exposed to ambient NH₃ and elevated NH₃ for 30 d. On August 20 in 2006 and 2007, another 8 pots were randomly selected from the remaining 16 pots in each of the OTCs for two NH₃ levels in combination with N treatments to measure plant leaf area of maize exposed to ambient NH₃ and elevated NH₃ for 45 days. Finally, the last 8 pots were used to measure leaf area of maize exposed to ambient NH₃ and elevated NH₃ for 50 days on September 5 in 2006 and 2007. Leaf area per plant was measured using a digital area meter (model CI-202 CID, Camas, WA, USA). Plant height was then determined on each plant following leaf area determination and measured above the functional leaves. After the determination of leaf area and plant height, the roots of maize were separated from the shoot and both were dried at 60–70°C for 3 d. At this point, shoot and root dry weights were taken and used to calculate biomass production and S/R ratio. Leaf photosynthetic rates and the relative content of chlorophyll (SPAD value) were measured 20 d after injection of NH₃, with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, Nebraska, USA) and a chlorophyll meter.
At intervals of 15 d throughout the growing season, newly fully expanded leaves (the second leaf from the top) were selected for the measurement of leaf photosynthetic rate and SPAD values.

2.4. Statistical analysis

The treatments consisted of all combinations of two levels of NH3 for air concentration and two levels of N for water culture medium. Treatments were assigned to chambers in a completely randomized 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 analyzed as a 2 × 2 factorial using analysis of variance techniques (SAS Proc GLM, SAS Systems for Windows, Ver. 9.1, SAS Institute, Cary, NC). Comparisons between the control and the other treatments were made using estimate statements in one-way analysis of variance tests, and treatments means were compared using the least significant difference (LSD).

3. RESULTS AND DISCUSSION

3.1. Plant height

The interaction between NH3 concentration × N level had a significant effect on plant height of maize ($P < 0.05$) (Table 1). Plant height of maize in different treatments is listed in Table 2. On the whole, in the growth period of maize, the values of plant height for N-deprived plants significantly increased ($P < 0.05$) under elevated NH3, and the values for N-sufficient plants decreased ($P > 0.05$) under elevated NH3 in either experimental year. This is in accord with the results of our experiment on winter wheat in atmospheric NH3 and N status treatment (Li et al. 2009), which show that at the different stages of crop growth, high NH3 concentrations increased plant height grown in ND soil and decreased plant height grown in NS soil compared with ambient NH3. At the different stages after injecting NH3, the mean plant height of ND-1000 treatments in 2006 and 2007 increased by 28.3% and 26.8%, respectively, compared with those of ND-0 treatments in 2006 and 2007, whereas those of NS-1000 treatments in 2006 and 2007 are 4.4% and 3.7% lower than those of NS-0 treatments in 2006 and 2007, respectively. This result suggests that elevated NH3 concentration is beneficial for height increase of plants grown in N-deprived nutrient solution. However, exposure of ND plants to 1000 nL L-1 still resulted in shorter plant heights than was seen in NS plants.

The direct effects of elevated NH3 on individual plant species have been well documented (Van der Eerden and Pérez-Soba 1992). For example, elevated NH3 generally results in increased rates of growth (Krupa 2003). Castro et al. (2006) reported that Brassica oleracea plants grown in elevated NH3 had a significantly higher relative growth rate (RGR) than plants grown in ambient NH3. In this study, significantly higher plant heights were observed in ND plants under elevated NH3 compared with ambient NH3 in successive years, which indicates enriched atmospheric NH3 has a direct “fertilizer” effect on plant growth. However, we also found that elevated NH3 resulted in a slight decrease in plant height of NS plants in both 2006 and 2007 ($P > 0.05$). The results show that the effect of NH3 level on plant height differed from the N status of the plant, which is in accordance with many researchers’ studies (Wilson 1992; Pearson and Stewart 1993). They revealed that plants growing

### Table 1

<table>
<thead>
<tr>
<th>Source</th>
<th>$P_n$</th>
<th>SPAD value</th>
<th>Plant height</th>
<th>Leaf area</th>
<th>Biomass (S)</th>
<th>Biomass (R)</th>
<th>S/R</th>
<th>Total biomass</th>
</tr>
</thead>
<tbody>
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<td>NH3</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>*ns</td>
<td>*</td>
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<tr>
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<td>**</td>
<td>**</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Year</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>NH3 × N</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>*ns</td>
<td>ns</td>
</tr>
<tr>
<td>NH3 × Year</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>N × Year</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>NH3 × N × Year</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Significant treatment effects and interactions $P < 0.05$.

**Significant treatment effects and interactions $P < 0.01$.

***Significant treatment effects and interactions $P < 0.001$.

S, shoot biomass; R, root biomass; S/R, shoot:root ratio.

ns, not statistically significant $P > 0.05$. 

(SPAD-502, Japan), respectively. At intervals of 15 d throughout the growing season, newly fully expanded leaves (the second leaf from the top) were selected for the measurement of leaf photosynthetic rate and SPAD values.
with low N status take up more leaf-derived N than those fertilized with higher amounts of N.

### 3.2. Leaf area

During the 50-d NH₃ injection period, the effects of NH₃ concentration (P < 0.05), N level (P < 0.01), and the interaction between NH₃ concentration × N levels (P < 0.05) significantly influenced the plant leaf area (Table 1). Significantly higher plant leaf area of ND-1000 treatments was found in 2006 and 2007 compared with ND-0 treatments (P < 0.05). This was similar to the results observed with plant height. As shown in Table 2, the values of leaf area were on the rise in the growth period of maize. At the big bell mouth stage of crop growth (after 50 d NH₃ injection), the value of leaf area slightly decreased under elevated NH₃ compared with ambient NH₃ (P < 0.05), while in the NS solution, leaf area slightly decreased under elevated NH₃ compared with ambient NH₃ (P > 0.05). The results suggest that the rate of NH₃ uptake by the leaves seems most relevant to the nutritional status of the plant. Increasing the nutrient solution in ND conditions resulted in an inhibition of plant leaf area under elevated levels of NH₃.

### 3.3. Net photosynthesis and SPAD value

Main treatment effects and their interactions were statistically significant for seasonal midday net photosynthesis rate (Pₚ) (Table 1). As Table 3 shows, the values of Pₚ for both ND and NS maize exposed to elevated levels of NH₃ appeared to significantly increase (P < 0.05) during different crop-growing seasons in each experimental year. In 2006, mean Pₚ was 6.3% and 17.9% greater in the NS-1000 and ND-1000 treatments, respectively, than in the NS-0 and ND-0 treatments, respectively. In 2007, mean Pₚ was 5.9% and 16.5% greater in the NS-1000 and ND-1000 treatments, respectively, than in the NS-0 and ND-0 treatments, respectively. The results indicate that elevated NH₃ concentration can, to some extent, improve plant photosynthetic capacity, and the enhancement amplitude depends on the nutrient status of the plants. Conclusions by Van der Eerden and Pérez-Soba (1992) showed that at intermediate to high ambient NH₃ concentrations, NH₃ may stimulate photosynthesis, most likely by providing a source of N for synthesis of ribulose 1,5-bisphosphate carboxylase-oxygenase, catalyzing the addition of carbon dioxide (CO₂) to 1, 5-bisphosphate in the Calvin cycle.

### Table 2  Plant height and leaf area per plant of maize (Zea mays L.) sampled on different days after injecting (DAI) under ammonia (NH₃) concentrations in combination with nitrogen (N) status in 2006 and 2007

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>NS-0</td>
<td>48.2</td>
<td>89.8</td>
</tr>
<tr>
<td></td>
<td>NS-1000</td>
<td>43.8</td>
<td>87.1</td>
</tr>
<tr>
<td></td>
<td>ND-0</td>
<td>27.4</td>
<td>53.3</td>
</tr>
<tr>
<td></td>
<td>ND-1000</td>
<td>36.7</td>
<td>70.3</td>
</tr>
<tr>
<td>2007</td>
<td>NS-0</td>
<td>43.3</td>
<td>85.5</td>
</tr>
<tr>
<td></td>
<td>NS-1000</td>
<td>41.0</td>
<td>83.0</td>
</tr>
<tr>
<td></td>
<td>ND-0</td>
<td>29.2</td>
<td>52.4</td>
</tr>
<tr>
<td></td>
<td>ND-1000</td>
<td>38.6</td>
<td>71.8</td>
</tr>
</tbody>
</table>

1Plants were exposed for 50 d. ND, nitrogen-deprived plants; NS, nitrogen-sufficient plants; 0, ambient NH₃; 1000, ambient plus 1000 nL nL⁻¹. NS-0, NS-1000, ND-0 and ND-1000 are four experimental treatment combinations. Data represent the mean, with four measurements per treatment with one plant in each pot. Within a column, means indicated by different lowercase letters are significantly different in elevated NH₃ (1000 nL nL⁻¹) (LSD test, P < 0.05; df = 1, 4); means indicated by different uppercase letters are significantly different at the same NH₃ concentration (LSD test, P < 0.05, df = 1, 4).
The NH₃ concentration (P<0.05) and N level (P<0.01) had a significant effect on SPAD value (value of relative content of chlorophyll) (P<0.05) (Table 1). Similar to Pn, high NH₃ concentrations significantly increased the SPAD values of maize both in the presence and absence of N in the nutrient solution during the crop growing seasons in two years. In 2006, the average SPAD values for NS-1000 and ND-1000 treatments increased by 7.4% and 14.1%, respectively, compared with those of NS-0 and ND-0 treatments after 50 d of NH₃ exposure to the plants. In 2007, the average SPAD values of NS-1000 and ND-1000 treatments increased by 7.4% and 14.8% higher than those for NS-0 and ND-0 treatments, respectively (Table 3). These results suggest that changes in SPAD values were consistent with changes in crop photosynthetic capacity. This is in agreement with what Clement et al. (1997) reported on winter wheat. They found NH₃ exposure increased photosynthetic capacity and chlorophyll a contents of plants.

From a large number of studies, it appears that atmospheric NH₃ enrichment usually enhances photosynthesis. Van der Eerden et al. (1990) reported that exposure of *Pinus sylvestris* to 336 nL L⁻¹ NH₃ resulted in increased photosynthesis. Van Hove et al. (1989) assumed that higher demand for carbon skeletons resulting from NH₃ assimilation was responsible for increased CO₂ fixation and increased stomatal conductance to be regulated by internal CO₂ concentration, whereas NH₃ itself had no direct influence on stomatal conductance. Thus, NH₃ uptake may cause an autocatalytic increase of additional NH₃ flux into the leaves by inducing stomatal opening via the internal CO₂ level, as long as photon flux density is sufficient for equivalent photosynthesis (Van der Eerden and Pérez-Soba 1992). In our experiment, elevated NH₃ increased Pn both in the presence and absence of N in the nutrient solution during the two-year experiment (Tables 1 and 3). We also found that the increased Pn was relevant to N status in grown plants. We found that the increment of Pn in ND plants exposed to high levels of atmospheric NH₃ was two times higher than that of the NS plants in both years (Table 3). 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, as previously suggested by Glass and Siddiqi (1995). However, there is some evidence that direct physiological responses of plants to atmospheric NH₃, whether the outcome of status or foliar uptake, are not easily distinguished. This is because the exact mechanism for the stimulation of photosynthetic capacity is not clear, and because of the complications that altered nutritional status may have on these responses. Irrespective of the cause, this increase is likely to be significant with regard to the provision of carbon skeletons for the ready assimilation of the pollutant NH₃ (Pearson and Stewart 1993). In addition, the determination of the relative content of chlorophyll, and SPAD value, indicated that leaf SPAD value increased with the increase of atmospheric NH₃ concentration. This effect can be attributed to the N increase. Van Dijk et al. (1990) found, in three conifer species watered with NH₃ solution, that although magnesium (Mg) and calcium (Ca) levels in the leaves decreased markedly, the pigment content of chlorophyll (chl a and chl b) increased when compared with controls, which may be associated with an enhancement of photosynthesis across a range of photon flux densities and CO₂ concentrations (Eamus and Fowler 1990). However, the exact mechanism for the stimulation of photosynthetic capacity is not clear. Thus, the effect of elevated NH₃ on plant photosynthesis and chlorophyll content merits further study.

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**Table 3** Net photosynthesis (Pn) and the relative content of chlorophyll (SPAD value) of corn (maize) sampled on different days after injecting (DAI) under ammonia (NH₃) concentrations in combination with N status in 2006 and 2007.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatments</th>
<th>Aug.5(20)</th>
<th>Aug.20(35)</th>
<th>Sept.5(50)</th>
<th>Mean</th>
<th>Aug.5(20)</th>
<th>Aug.20(35)</th>
<th>Sept.5(50)</th>
<th>Mean</th>
</tr>
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<tbody>
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<td>NS-0</td>
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<td>24.2</td>
<td>25.3</td>
<td>23.7A</td>
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<td>44.1</td>
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<td>21.0</td>
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<td>24.7</td>
<td>28.8</td>
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<td>43.5</td>
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<td>NS-0</td>
<td>20.8</td>
<td>24.8</td>
<td>25.5</td>
<td>23.7bA</td>
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<td>26.8</td>
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<td>20.3</td>
<td>23.5</td>
<td>20.6bB</td>
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<td>39.9</td>
<td>41.2</td>
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<td>24.0A</td>
<td>44.3</td>
<td>45.8</td>
<td>47.7</td>
<td>45.9A</td>
</tr>
</tbody>
</table>

1Plants were exposed for 50 d. ND, nitrogen-deprived plants; NS, nitrogen-sufficient plants; 0, ambient NH₃; 1000, ambient plus 1000 nL L⁻¹. NS-0, NS-1000, ND-0 and ND-1000 are four experimental treatment combinations. Data represent the mean, with four measurements per treatment with one plant in each pot. Within a column, means indicated by different lowercase letters are significantly different in elevated NH₃ (1000 nL L⁻¹) compared with ambient NH₃ (0 nL L⁻¹) (LSD test, P<0.05, df = 1, 4); means indicated by different uppercase letters are significantly different at the same NH₃ concentration (LSD test, P<0.05, df = 1, 4).
3.4. Biomass accumulation and allocation

NH$_3$ concentration ($P < 0.01$), N level ($P < 0.01$) and interaction between NH$_3$ concentration $\times$ N level ($P < 0.05$) significantly affected shoot dry weight and shoot:root ratio of maize. Significant effects of N level ($P < 0.01$) and the interaction between N $\times$ Year ($P < 0.05$) were observed in plant root dry weight. However, significant effects were only observed in total biomass production of maize ($P < 0.05$) (Table 1).

The effect of NH$_3$ on biomass production depended on the atmospheric concentration, and differed between NS and ND nutrient solutions (Table 4). Significantly higher shoot biomass was found in exposure of ND plants to 1000 nL L$^{-1}$ NH$_3$ in 2006 and 2007 compared to those found in exposure of ND plants to ambient NH$_3$. Root biomass in each treatment showed no difference after injecting NH$_3$ gas for 20 d, 35 d and 50 d in both years, indicating that the effect of NH$_3$ application on biomass accumulation can be seen only from shoot biomass. Changes in total biomass production were observed similar to those in shoot biomass at three different stages in successive years. Total biomass in NS treatments did not decrease or decreased only slightly after injecting NH$_3$ gas for 20 d, 35 d and 50 d in 2006.

### Table 4 Biomass accumulation of corn sampled by different days after injecting (DAI) under ammonia (NH$_3$) concentrations in combination with N status in 2006 and 2007$^*$

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatments</th>
<th>Aug. 5(20)</th>
<th>Aug. 20(35)</th>
<th>Sept. 5(50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Shoots</td>
<td>Total</td>
<td>Roots</td>
</tr>
<tr>
<td>2006</td>
<td>NS-0</td>
<td>0.20A</td>
<td>0.64aA</td>
<td>0.84A</td>
</tr>
<tr>
<td></td>
<td>NS-1000</td>
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<td>0.59aA</td>
<td>0.78aA</td>
</tr>
<tr>
<td></td>
<td>ND-0</td>
<td>0.16A</td>
<td>0.36bB</td>
<td>0.52bB</td>
</tr>
<tr>
<td></td>
<td>ND-1000</td>
<td>0.18A</td>
<td>0.48bB</td>
<td>0.66aB</td>
</tr>
<tr>
<td>2007</td>
<td>NS-0</td>
<td>0.21A</td>
<td>0.65aA</td>
<td>0.86aA</td>
</tr>
<tr>
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<td>0.61aA</td>
<td>0.81aA</td>
</tr>
<tr>
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<td>ND-0</td>
<td>0.17bB</td>
<td>0.34bB</td>
<td>0.51bB</td>
</tr>
<tr>
<td></td>
<td>ND-1000</td>
<td>0.19A</td>
<td>0.47bB</td>
<td>0.66aB</td>
</tr>
</tbody>
</table>

$^*$Plants were exposed for 50 d. ND, nitrogen-deprived plants; NS, nitrogen-sufficient plants; 0, ambient NH$_3$; 1000, ambient plus 1000 nL L$^{-1}$. NS-0, NS-1000, ND-0 and ND-1000 are four experimental treatment combinations. Shoot biomass, root biomass and total biomass production are calculated by the dry weight of plant per pot (g d wt). Data represent the mean, with four measurements per treatment with one plant in each pot. Within a column, means indicated by different lowercase letters are significantly different in elevated NH$_3$ (1000 nL L$^{-1}$) compared with ambient NH$_3$ (0 nL L$^{-1}$) (LSD test, $P < 0.05$, df = 1, 4); means indicated by different uppercase letters are significantly different at the same NH$_3$ concentration (LSD test, $P < 0.05$, df = 1, 4).

Figure 2 The impact of ammonia (NH$_3$) exposure on shoot:root ratio (S/R) in both 2006 (a) and 2007 (b). ND, nitrogen-deprived plants; NS, nitrogen-sufficient plants; 0, ambient NH$_3$; 1000, ambient plus 1000 nL L$^{-1}$. NS-0, NS-1000, ND-0 and ND-1000 are four experimental treatment combinations. S/R calculated on a dry weight basis. Aug.5, injecting NH$_3$ for 20 d; Aug.20, injecting NH$_3$ for 35 d; Sept.5, injecting NH$_3$ for 50 d. Data represent the mean of four pots measurements with four plants from three replicate chambers per treatment ($\pm$ s.d.). Different lowercase letters are significantly different in elevated NH$_3$ (1000 nL L$^{-1}$) compared with ambient NH$_3$ (0 nL L$^{-1}$) (LSD test, $P < 0.05$, df = 1, 4); means indicated by different uppercase letters are significantly different at the same NH$_3$ concentration (LSD test, $P < 0.05$, df = 1, 4).
and 2007 under elevated NH₃ compared with ambient NH₃.

Shoot:root ratio (S/R) of maize in different treatments is shown in Fig. 2. On the whole, in the growth period of maize, the S/R first declined and then rose. At 50 d after injection of NH₃, the value of S/R in four treatments was maximal, indicating that elevated NH₃ concentration benefits for the increase of shoot biomass at that stage, and also the utilization for light energy, were at their best states, whereas at 35 d after injecting NH₃, the value of S/R in four treatments was at a minimum, indicating that this stage is mainly beneficial to the growth of roots and leads to rapid improvement of root biomass. Meanwhile, S/R in NS treatments decreased remarkably with elevated NH₃ concentration at 20 d after injecting NH₃ in 2006 and 2007, indicating high atmospheric NH₃ is not favorable to shoot growth under NS nutrient solution, while S/R in ND-1000 treatments were significantly higher than in ND-0 at the growth stage. After injecting NH₃ gas for 35 d, there was no difference in S/R in both NS-1000 and ND-1000 compared with both NS-0 and ND-0 in either experimental year, which indicates that NH₃ enhancement and N status stimulate the development of the root system. At 50 d after injecting NH₃, S/R in ND-1000 treatments significantly increased compared to that in ND-0 in both years. Nevertheless, there was no significant difference between NS-1000 and NS-0 treatments. This result suggests that NH₃ application is mainly beneficial to the growth of shoot and leaf under N absence.

As we know, one of the most obvious plant responses following NH₃ assimilation is enhanced growth, which has been observed in many experiments, as long as the NH₃ concentrations were not toxic. For example, at lower and more natural NH₃ concentrations, the extra N input results in a stimulation of photosynthesis and in a higher biomass production (Van Hove et al. 1992; Pérez-Soba et al. 1994). These positive effects often coincide with changes in shoot/root ratio and nutrient imbalance (Fangmeier et al. 1994). Our previous experiments (Chen et al. 2008) with maize exposed to 0 and 1000 nL L⁻¹ NH₃ and the present results (Table 4) revealed that higher NH₃ concentration can stimulate maize growth. At 1000 nL L⁻¹ NH₃, shoot biomass production was significantly increased in the absence of N in the nutrient solution, while shoot biomass appeared to decrease in the presence of N in the nutrient solution. This indicates that the present results with maize do support our hypothesis that high N status may restrain NH₃ uptake by the leaves. However, this result is not in agreement with what Clement et al. (1997) reported on winter wheat. They found that the growth of winter wheat was not enhanced or only slightly enhanced upon exposure to NH₃, despite the potential of these plants to take up extra N via the leaves. One reason for the relatively small effect of NH₃ on growth may be a simultaneous reduction of root N uptake in NH₃-exposed plants, which could compensate for the foliar absorbed N. Also, a significant increase in the S/R ratio at 1000 nL L⁻¹ NH₃, both in the presence and absence of N in the nutrient solution after injecting NH₃ for 20 d, was found, which is in agreement with many other studies (Castro et al. 2005, 2006). The increased growth is observed after injecting NH₃ mainly due to the shoot growth response. Root growth increases only slightly or at least less than does shoot growth, leading to higher shoot:root ratios. However, the present experiment showed that total biomass accumulation was significantly increased at elevated NH₃ in the ND treatments compared with ambient NH₃. This is not in accordance with the observation that atmospheric NH₃ has no influence on the total biomass production (Clement et al. 1997), indicating that negative effects of excess N on crops are highly unlikely in the ND conditions.

In addition, exposure of plants to very high concentrations of atmospheric NH₃ often leads to direct toxic effects and growth reduction, especially of the roots (Castro et al. 2006). In our experiment, even though the NH₃ concentrations used were much higher than generally found in polluted areas (Krupa 2003), maize apparently can use an NH₃ concentration of 1000 nL L⁻¹ as a nutrient (Table 2), which indicates that this plant species was tolerant to high atmospheric NH₃ concentrations, even under conditions of high N level. Similar results were obtained with winter wheat exposed to NH₃ in OTCs (Clement et al. 1997). Nevertheless, an exact comparison between the present data and data in the literature is difficult because of different atmospheric NH₃ concentrations and exposure times and differences in growth conditions, which may have resulted in differences in plant relative growth rate. However, the present study did not yield information on N contents of shoots. Further study would increase greatly in value if the shoot N values were obtained in future experiments.

Acknowledgements

This work was jointly supported by the National Natural Science Foundation of China (No. 30571116 and No. 30670326), Ministry of Science and Technology of China (973’ project: Grant No. 2009CB118604), the Department of Agriculture (No. 201103003), and the Special Foundation of National Science & Technology Supporting Plan (2011BAD29B09).
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