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A new urease-inhibiting formulation decreases ammonia volatilization and improves maize nitrogen utilization in North China Plain

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Overuse of urea, low nitrogen (N) utilization, and large N losses are common in maize production in North China Plain (NCP). To solve these problems, we conducted two field experiments at Shangzhuang and Quzhou in NCP to test the ability of a newly developed urease inhibitor product Limus® to decrease NH₃ volatilization from urea applied to maize. Grain yield, apparent N recovery efficiency (RE_N) and N balance when using urea applied with or without Limus were also measured over two maize growing seasons. Cumulative NH₃ loss in the two weeks following urea application without Limus ranged from 9–108 kg N ha⁻¹, while Limus addition significantly decreased NH₃ loss by a mean of 84%. Urea with Limus did not significantly increase maize yields ($P < 0.05$) compared with urea alone. However, a significant 11–17% improvement in RE_N with Limus was observed at QZ. The use of urea-N plus Limus would permit a reduction in N applications of 55–60% compared to farmers' practice and/or further 20% N saving compared with optimized urea-N rate (150 kg N ha⁻¹, based on N requirement by target yield of 7.5 t ha⁻¹), and would achieve the same maize yields but with significantly decreased NH₃ loss and increased N utilization.

Ammonia (NH₃) is one of the key reactive N (Nr) components and the major alkaline gas in the atmosphere. NH₃ directly and/or indirectly contributes to the eutrophication of aquatic ecosystems, soil acidification when nitrified and leached, and the decline in plant biodiversity through its deposition; it also damages human health through secondary aerosol or particle formation (e.g., PM_{2.5})^{1–3}. In China, the total NH₃ emission was estimated to be 9.8–20.4 Tg per year in the 2000s, 33–46% of which was derived from N fertilizer application^{4,5}. Another study⁶ showed that the NH₃ loss was up to 3.7 Tg in the North China Plain (NCP) in 2004, of which N fertilizer contributed 54% (and the rest was mainly from animal manures).

Urea accounts for around 60% of total N fertilizer consumption in China, but it has a large potential for NH₃ loss, amounting to more than 20% in arable soils with a pH > 7 (e.g. those in the NCP)^{7–9}. Strategies to decrease NH₃ loss from urea-N application to croplands have included the incorporation of surface applied N, deep placement of N fertilizer, and irrigation immediately following N topdressing^{10,11}. However, on smallholder farms, these conservation practices are frequently not used due to labour shortages and delayed irrigation, so NH₃ loss is expected to be high. These mitigation practices are even more difficult to apply if the N is applied as a top-dressing or as split applications at selected crop growth stages.

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Site	Treatment	1 st N applied (V0 or V3 stage)	2 nd N applied (V12 stage)	Total N input
SZ	N ₀	0	0	0
	N _{con}	150	150	300
	N _{opt}	60	90	150
	N _{opt/L}	60	90	150
	N _{opt/L-1}	150	0	150
	N _{80%opt/L-1}	120	0	120
QZ	N ₀	0	0	0
	N _{con}	135	135	270
	N _{opt}	75	75	150
	N _{opt/L}	75	75	150
	N _{80%opt/L}	60	60	120
	N _{80%opt/L-1}	120	0	120

Table 1. Experimental treatments at Shangzhuang (SZ) and Quzhou (QZ) (unit: kg N ha⁻¹).

In addition, because of the long-term pressure for food security in China, farmers tend to apply excessive amounts of N fertilizer as an “insurance” to meet crop needs. In the NCP, N recovery efficiency (RE_N) from urea applied according to farmers’ practice averaged only 15% across 148 on-farm maize experiments¹² and 10–19% in a two-year maize based cropping experiment¹³, suggesting a large opportunity for reducing N losses and improving RE_N in current urea-N management practices without sacrificing maize yields¹⁴.

There is, therefore, a strong demand for options to decrease NH₃ losses while achieving higher RE_N and crop yields. Application of a urease inhibitor could contribute to achieving these objectives, as it should delay urea hydrolysis, decrease NH₃ losses and improve N utilization¹⁵. A new urease inhibitor product Limus[®], as a mixture of 75% N-(n-butyl) thiophosphoric triamide (NBPT) and 25% N-(n-propyl) thiophosphoric triamide (NPPT), was recently developed by BASF SE (a German Company). A laboratory study proved that the mixture of NBPT and NPPT (e.g. 75% vs. 25% or 50% vs. 50%) could achieve better effects in decreasing NH₃ loss than single urease inhibitor (NBPT or NPPT)¹⁶. Limus product (here referring to plain urea amended with Limus) can be broadcasted on soil surface without extra irrigation or soil incorporation. Recently our previous study demonstrated the positive effects of Limus on decreasing NH₃ loss in winter wheat at three locations and improving yield and N utilization with 55% less farmer’s N practice under once application in wheat at one of three locations in north and northwest China¹⁷. However, the effects of Limus on maize, the largest crop in NCP, were not evaluated under field trials. Considering completely different meteorological (e.g. warm and humid in maize vs. cold and dry in wheat) and soil (e.g. different urease activities) conditions, we hypothesize that the effects of Limus on urease inhibition and NH₃ loss mitigation may be lower in maize season than in wheat season. This is because the warm and humid condition will accelerate urea’s hydrolysis and the degradation of Limus.

To systematically evaluate the role of Limus in reducing NH₃ volatilization and improving RE_N and grain yields in maize season, we conducted two typical field experiments over two years at Shangzhuang (SZ) and Quzhou (QZ). We wanted to (1) test our hypothesis (e.g. if Limus shows lower role in mitigating NH₃ loss in maize than in wheat) and (2) develop a practical recommendation to minimize NH₃ loss and achieve high yields and N utilization in maize in NCP.

Results

Cumulative NH₃ losses. After each N application over the two-year trial at each site, NH₃ volatilization from urea alone at SZ ranged from 9 to 31 kg N ha⁻¹ (accounting for 4–38% of N application) compared to 0 to 2 kg N ha⁻¹ (accounting for 0–2% of N application) from urea amended with Limus, while at QZ NH₃ loss ranged from 18 to 59 kg N ha⁻¹ (accounting for 18–61% of N application) for urea alone compared to 1 to 17 kg N ha⁻¹ (accounting for 1–28% of N application) from urea with Limus (Table 2). Compared with N_{con}, N_{opt} significantly ($P < 0.05$) decreased total NH₃ loss by up to 2–9 kg N ha⁻¹ at SZ and 13–23 kg N ha⁻¹ at QZ, while urea amended with Limus significantly decreased NH₃ loss by 70–100% at the V3, and 63–100% at the V12 growth stage compared to N_{opt} at both sites. In contrast, no significant differences in NH₃ volatilization were observed between the various Limus treatments (Table 1) at SZ in both years and at QZ in the first year. A significant reduction of total NH₃ loss by 14 kg N ha⁻¹ ($P < 0.05$) was observed in N_{80%opt/L-1} compared to N_{opt/L} and N_{80%opt/L} at QZ in the second year.

At SZ, total cumulative NH₃ loss varied from 0 to 37 kg N ha⁻¹ (accounting for 0–19% of urea-N) under the different fertilizer treatments, while NH₃ loss significantly decreased to almost zero kg N ha⁻¹ (78–100% reduction, $P < 0.05$) when Limus was added. At QZ, Limus addition reduced total NH₃ loss by 38–55 kg N ha⁻¹ ($P < 0.05$), corresponding to a 65–90% decrease in NH₃ loss (Table 2). The greatest decrease in total NH₃ losses due to Limus addition occurred in the second year at QZ. At SZ, even though only 5–11 kg N ha⁻¹ NH₃ was emitted at V12 in the urea-only treatments, urea amended with Limus decreased NH₃ volatilization by 5–7 kg N ha⁻¹ ($P < 0.05$), corresponding to a 78–100% reduction of NH₃ loss.

Time courses of NH₃ fluxes. The dynamics of NH₃ fluxes and weather conditions (precipitation, air temperature, relative humidity and wind speed at two heights, etc.) in the two study years at SZ and QZ are shown

Site	Treatment	1 st Year			2 nd Year		
		V0/V3	V12	Total loss	V3	V12	Total loss
SZ	N _{con}	0	11c	11c	31c	6c	37c
	N _{opt}	0	9b	9b	23b	5b	28b
	N _{opt/L}	0	2a	2a	0a	0a	0a
	N _{opt/L-1}	0	n.d.	0a	1a	n.d.	1a
	N _{80%opt/L-1}	0	n.d.	0a	1a	n.d.	1a
QZ	N _{con}	24c	31c	55c	50c	59c	109d
	N _{opt}	18b	24b	42b	40b	46b	86c
	N _{opt/L}	3a	1a	4a	12a	17a	29b
	N _{80%opt/L}	3a	1a	4a	13a	17a	30b
	N _{80%opt/L-1}	5a	n.d.	5a	17a	n.d.	17a

Table 2. Cumulative NH₃ losses (unit: kg N ha⁻¹) following urea (\pm Limus) application to the different treatments during the two years of maize production at Shangzhuang (SZ) (2011 and 2012) and Quzhou (QZ) (2012 and 2013). Note that values without the same letters within the same column at each site are significantly different ($P < 0.05$, Duncan's test). n.d. means not detectable.

in Figs 1 and 2, respectively. Precipitation events occurred frequently, while the relative humidity ranged from 35–90% (mostly above 55%), and the air temperature mainly around 22–33 °C during the different periods of NH₃ volatilization at SZ and QZ (Figs 1a'–d' and 2a'–d'). The daily NH₃ emission rates in the two N fertilization events (at V0 or V3 and V12 growth stages) varied between the two study years at SZ (Fig. 1a–d); no NH₃ volatilization was detected at V0 in June 2011 (Fig. 1a). At V12 in August 2011 (Fig. 1b), the daily volatilization losses were higher in N_{con} and N_{opt}, in which volatilization peaked at 360 and 300 g N ha⁻¹ h⁻¹, respectively, on the first day after surface application of urea. In contrast, urea with Limus (N_{opt/L}) lost a daily maximum of only about 30 g N ha⁻¹ h⁻¹ on the first day after surface application. The NH₃ fluxes decreased to 0–50 g N ha⁻¹ h⁻¹ on all of the treatments two days later.

Compared with the high NH₃ fluxes at SZ after N fertilization at V12 in 2011, maximum volatilization rates were observed with urea alone at V3 in 2012. The highest NH₃ fluxes from N_{con} and N_{opt} at SZ occurred from the 3rd to the 10th day after fertilization, while much lower peak fluxes (0–30 g N ha⁻¹ h⁻¹) from the Limus treatments (N_{opt/L}, N_{opt/L-1}, N_{80%opt/L-1}) were measured on the 4th and 10th days after fertilization at V3 in 2012 (Fig. 1c).

At QZ, the daily NH₃ volatilization rates (Fig. 2a–d) were approx. 3 times higher than those at SZ (Fig. 1a–d) over the two-years. Fluxes at QZ were higher in the N_{con} and N_{opt} treatments, peaking at 400–800 and 200–700 g N ha⁻¹ h⁻¹, respectively, during the first 3 days after urea application. Thereafter, they quickly decreased to 0–200 g N ha⁻¹ h⁻¹ for both N_{con} and N_{opt} (Fig. 2a–d). In contrast, NH₃ fluxes in the urea with Limus treatments (N_{opt/L}, N_{80%opt/L}, N_{80%opt/L-1}) were only 0–150 g N ha⁻¹ h⁻¹ after application. Generally, similar maximum NH₃ fluxes (600–800 g N ha⁻¹ h⁻¹) at QZ were measured at each fertilization event in spite of slightly different patterns of flux. Following N fertilization at V3 and V12, NH₃ volatilization was only detected for 5–7 days during the first year (Fig. 2a–b), while the volatilization period lasted longer (up to 12 days) in the second year (Fig. 2b–c).

Grain yield and N recovery efficiency. Lower grain dry matter (DM) yields (2.9–6.3 t DM ha⁻¹ with no N) were found at SZ compared with those at QZ (4.5–7.5 t DM ha⁻¹ with no N) (Fig. 3). At SZ, the grain yields ranged from 5.5 to 7.7 t DM ha⁻¹ during the first and second years under different N application treatments, while the grain yields ranged from 9.0 to 10.1 t DM ha⁻¹ d at QZ site. Totally, N fertilizer application significantly ($p < 0.05$) improved maize grain yield by 93–135% (1st year) and 21–33% (2nd year) but no statistically significant differences ($P > 0.05$) were observed between N treatments. Even the most efficient (in terms of RE_N) treatment N_{80%opt/L} obtained maize yields similar to the other N treatments at both sites.

The RE_N (N recovery efficiency, an indicator of N use efficiency) was between 30% (N_{con}) and 52% (N_{opt} & N_{opt/L-1}) in the first year, and 19% (N_{con}) to 39% (N_{80%opt/L-1}) in the second year at SZ (Fig. 3). In contrast, the RE_N was consistently higher (ranging from 1 to 26%) at QZ than at SZ across all N treatments during both years on average. The RE_N was lower in N_{con} than in the N_{opt} treatments because of the greater amount of N applied to N_{con} at both sites. No significant differences between optimized N fertilization (\pm Limus) were found at SZ, but the use of Limus (N_{opt/L}) at QZ led to an average RE_N increase by 17% and 11% when compared to N_{opt} in the 1st and 2nd years, respectively. Also RE_N in the N_{80%opt/L} treatment was 23% ($P < 0.05$) higher than in N_{opt} in the first year at QZ but, on average across the two years and two sites, applying N_{80%opt/L-1} did not result in significant differences in RE_N (ranging from 39 to 60%) compared with N_{opt} (ranging from 31 to 53%).

Nitrogen balance. Nitrogen balances are shown in Table 3. The “other N loss” in Table 3 comprises all N loss pathways other than NH₃ volatilization (e.g., nitrification/denitrification, N leaching) as well as possible errors. Relatively low mineral N (N_{min}) in the 0–1 m soil profiles before sowing was observed at both SZ (68 kg N ha⁻¹) and QZ (35 kg N ha⁻¹) in the first year, while relatively high N_{min} before sowing was measured on each plot at both SZ (62 to 220 kg N ha⁻¹) and QZ (59 to 120 kg N ha⁻¹) in the second year (Table 3). Apparent N mineralization based on an N balance in N₀ was 33 kg N ha⁻¹ at SZ, and 57 kg N ha⁻¹ at QZ during two years on average.

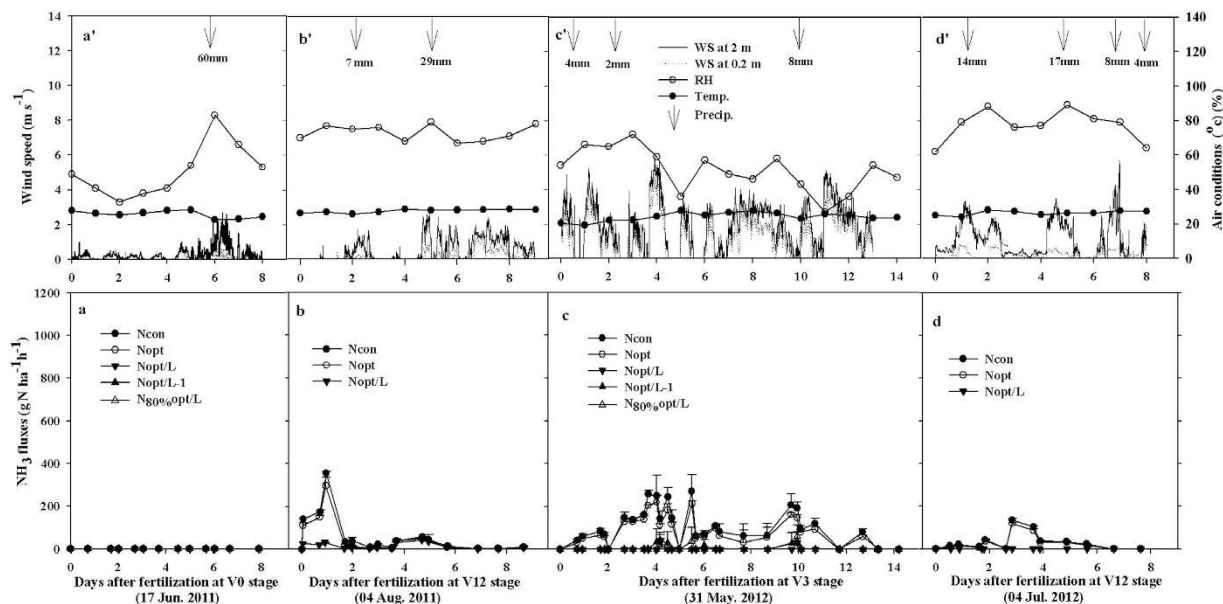


Figure 1. NH₃ fluxes measured by a calibrated Dräger-Tube Method (DTM) following urea (\pm Limus) application at V0 or V3 (seeding or three-leaf extension) and V12 (12-leaf extension) growth stages of summer maize in 2011 and 2012 at Shangzhuang (SZ, Fig. 1a–d) and corresponding time courses of precipitation (Precip., mm), wind speed (WS, 2.0 m and 0.2 m heights), air temperature (Temp., °C) and relative humidity (HM, %) (Fig. 1a'–d'). Bars denote standard deviations of NH₃ fluxes on four replicate plots.

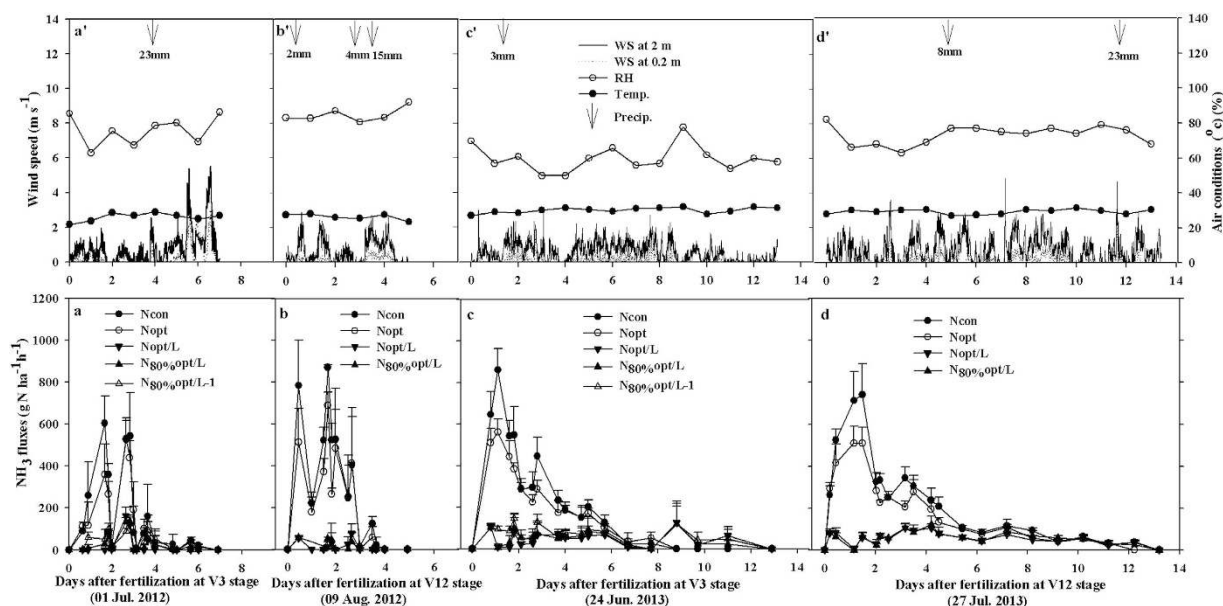


Figure 2. NH₃ fluxes measured by a calibrated Dräger-Tube Method (DTM) following urea (\pm Limus) application at V3 (three-leaf extension) and V12 (12-leaf extension) growth stages of summer maize in 2012 and 2013 at Quzhou (QZ, Fig. 2a–d) and corresponding time courses of precipitation (Prec., mm), wind speed (WS, 2.0 m and 0.2 m heights), air temperature (Temp., °C) and relative humidity (RH, %) (Fig. 2a'–d'). Bars denote standard deviations of NH₃ fluxes on four replicate plots.

At SZ, the residual soil N_{\min} at harvest in N_{con} was 212 and 337 kg N ha⁻¹ during the first and second years, respectively. These values were significantly higher than those in the optimized urea \pm Limus treatments (72–162 kg N ha⁻¹) during both years. The apparent N loss based on an N balance in N_{con} at SZ was 28 and 83 kg N ha⁻¹ during the first and second years, but only 16–44 kg N ha⁻¹ in the optimized urea \pm Limus treatments (Table 3). At QZ, the highest residual N_{\min} was measured in N_{con} (averaging 107 kg N ha⁻¹), while the amounts were about 67% less in the N_{opt} treatment (averaging 34 kg N ha⁻¹) and 56% lower in the urea with Limus ($N_{\text{opt/L}}$, $N_{\text{opt/L-1}}$, $N_{80\%opt/L-1}$) treatments (averaging 47 kg N ha⁻¹). Likewise, the highest mean apparent N loss of 118 kg N ha⁻¹ occurred in N_{con} , followed by 75 kg N ha⁻¹ in N_{opt} , and 40 kg N ha⁻¹ in $N_{\text{opt/L}}$, $N_{\text{opt/L-1}}$, and $N_{80\%opt/L-1}$. As a

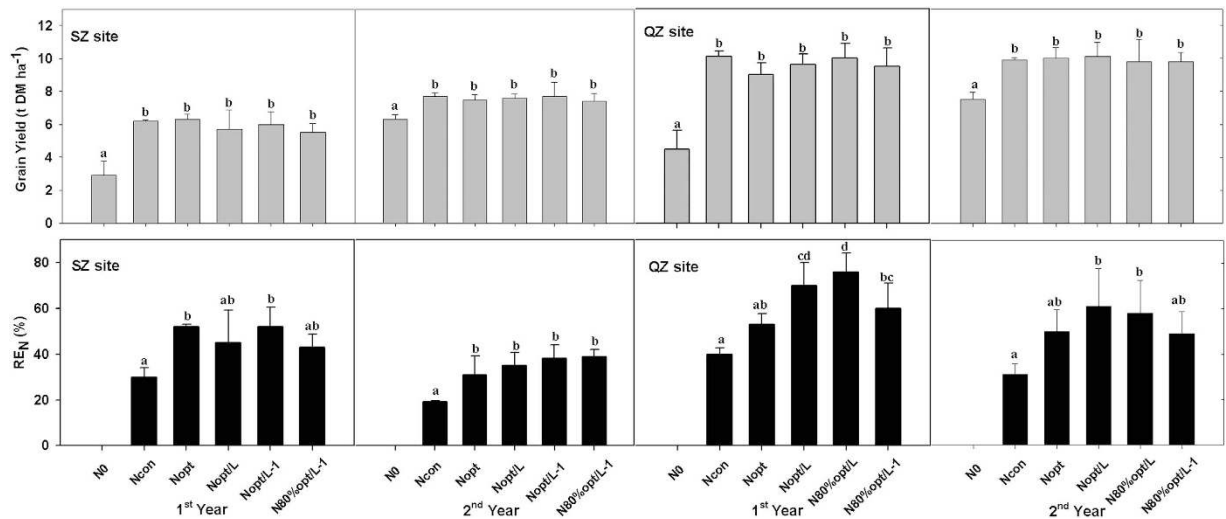


Figure 3. Maize grain yield and apparent nitrogen recovery efficiency (RE_N indicates the percentage of fertilizer N recovered in aboveground plant biomass) in the two years at Shangzhuang (SZ) and Quzhou (QZ). Different letters above the bars indicate the significance under $P < 0.05$.

Year	Item	N_0	N_{con}	N_{opt}	SZ site $N_{opt/L}$	$N_{opt/L-1}$	$N_{80\%opt/L-1}$	N_0	N_{con}	N_{opt}	QZ site $N_{opt/L}$	$N_{80\%opt/L}$	$N_{80\%opt/L-1}$
1 st Year	N input	81	381	231	231	231	201	85	355	235	235	205	205
	Initial soil N_{min}	68	68	68	68	68	68	35	35	35	35	35	35
	Applied N	0	300	150	150	150	120	0	270	150	150	120	120
	App. N mineralization	13	13	13	13	13	13	50	50	50	50	50	50
	N output (N uptake)	51	141	129	118	128	103	64	173	144	169	155	136
	N surplus	30	240	102	113	103	98	21	182	91	66	50	69
	Residual soil N_{min}	30	212	72	110	86	84	21	105	37	48	39	40
	Apparent N loss	0	28	30	3	17	14	0	77	54	18	11	29
	NH_3 volatilization	0	10	9	3	0	0	0	55	42	4	4	5
	Other N loss	0	18	21	0	17	14	0	22	12	14	7	24
2 nd Year	N input	114	572	311	332	321	280	123	454	306	312	274	276
	Initial soil N_{min}	62	220	109	130	119	108	59	120	92	98	90	92
	Applied N	0	300	150	150	150	120	0	270	150	150	120	120
	App. N mineralization	52	52	52	52	52	52	64	64	64	64	64	64
	N output (N uptake)	96	152	142	148	153	142	102	185	177	193	171	161
	N surplus	18	420	169	184	168	138	21	269	129	119	103	115
	Residual soil N_{min}	18	337	110	162	122	88	21	110	32	49	45	59
	Apparent N loss	0	83	59	22	46	50	0	159	97	70	58	56
	NH_3 volatilization	0	37	28	0	1	1	0	108	85	30	31	17
	Other N loss	0	46	31	22	45	49	0	51	12	40	27	39

Table 3. Nitrogen balances in the two maize seasons at SZ and QZ (unit: $kg N ha^{-1}$). Initial soil N_{min} and residual soil N_{min} were the mineral N content within the top 1 meter of soil before planting and after harvest, respectively. N surplus equals N input minus crop N uptake.

whole, both the highest residual soil N_{min} and apparent N losses were in N_{con} plots at both sites. Relatively low apparent N loss was observed for treatments with Limus compared to N_{opt} . Moreover, the proportions of residual soil N_{min} to apparent N losses differed at SZ and QZ: residual soil N_{min} accounted for 80% of the N surplus (76 to 85%) at SZ but only 49% (40 to 58%) at QZ. Compared with the 1st year, the N surplus under farmers' practice (N_{con}) increased by 87 (at QZ) and 180 (at SZ) $kg N ha^{-1}$, while only by 45 (at QZ) and 69 (at SZ) $kg N ha^{-1}$ with optimized N fertilization (N_{opt} , $N_{opt/L}$) in the 2nd year.

Discussion

Our research confirmed that conventional urea application to maize caused large NH_3 losses (average 10–36% of urea-N applied) in the NCP. Similar research by Su *et al.*⁹ found NH_3 losses of up to 26–33% of surface-applied urea at the V3 and V10 growth stages of maize production in the NCP. Optimized urea amended with Limus

significantly decreased NH_3 losses by a mean of 84% and improved RE_N in some cases, consistent with our previous results in winter wheat (e.g. a mean of 83% in NH_3 loss mitigation)¹⁷. Our results deny our prior hypothesis that Limus may have lower role in mitigating NH_3 loss and improving RE_N . We expect that the trade-offs between meteorological conditions (warm and humid in maize vs. cold and dry in wheat) and NH_3 volatilization processes (high flux with short volatilization period in maize vs. low flux with long volatilization period in wheat) could explain why Limus effects on maize (the present study) are comparable to that on wheat¹⁷. In addition, the inhibition period of up to 14 days allowed sufficient time for rain to leach the urea into the subsoil and so prevent further NH_3 losses (Figs 1 and 2).

The dynamics of NH_3 fluxes were mostly determined by the weather (e.g., precipitation, air temperature and wind speed) after N application (Figs 1 and 2). At SZ, for example, NH_3 loss was only detected during the V3 growth stage in 2012, when initial environmental conditions (2–4 mm light precipitation and 60–78% relative humidity) were favorable for NH_3 volatilization. In contrast, the low relative humidity (35–50%) and soil moisture content, together with a heavy rainfall (60 mm) on the 6th day after N top dressing during the V0 growth stage in 2011 were unfavorable for NH_3 volatilization. The magnitude of NH_3 losses was largely influenced by small temporal differences in the weather and initial soil moisture content^{18,19}. Some farmers in China broadcast N fertilizer after precipitation. This would further increase NH_3 volatilization risk because high air humidity or limited rainfall provide adequate moisture for urea hydrolysis and cause the desorption of ammoniacal N from the soil^{20,21}. In contrast, heavy rainfall effectively mitigates NH_3 losses by leaching unhydrolyzed urea into the soil profile where it will be retained^{11,22}. This was clearly demonstrated during the two maize growing seasons at QZ (Fig. 2a–d). Here moist initial soil conditions favoured rapid urea hydrolysis and promoted the initial NH_3 loss. However, the high NH_3 fluxes then rapidly decreased and stopped after precipitation (15–23 mm). In the NCP, we find that NH_3 volatilization will not be inhibited unless the daily precipitation is ≥ 7 mm (Figs 1a'–d' and 2a'–d').

Additionally, the NH_3 flux from urea at QZ was about 3 times higher than that at SZ over the two seasons (Figs 1a–d, 2a–d). This can partly be explained by the much higher urease activity in the QZ soil ($13.0 \mu\text{g N g}^{-1}$ soil h^{-1} or $29.3 \text{ kg N ha}^{-1} \text{ h}^{-1}$) than the SZ soil ($4.2 \mu\text{g N g}^{-1}$ soil h^{-1} or $9.5 \text{ kg N ha}^{-1} \text{ h}^{-1}$). High soil urease activity was reported to have a close positive relationship with the timing of urea hydrolysis as well as NH_3 loss^{23,24}. Nevertheless at SZ, NH_3 loss still amounted to 31% of the urea-N applied (75 kg N ha^{-1}) during the V3 growth stage in 2012 (Table 2). This confirms that urea hydrolysis-induced NH_3 volatilization is mostly dependent upon meteorological (e.g. amount of precipitation and wind speed) and soil (e.g. soil humidity and soil temperature) conditions^{15,18}. In the current study, we found that the NH_3 loss rates in maize seasons (2012/13) were nearly 20% higher than those in the previous winter wheat season (2011/12) at QZ with same amount of urea¹⁷. One factor that possibly influenced the different losses was air-soil temperature since the higher temperature in the summer maize growing season may have increased microbial activity and hydrolysis of urea fertilizers, also more NH_3 in gaseous phase and less in solution phase at higher temperature^{15,23}.

Considering the high potential for NH_3 loss in the NCP, with most soils at $\text{pH} > 7.5$, we expected that Limus will be very effective in decreasing NH_3 emissions in this region. Our two-year experimental data confirm this role of Limus at both SZ and QZ sites in NCP.

Some previous reports have shown that the use of a urease inhibitor could increase the yields of crops^{25,26}, while others did not show any significant yield increases^{27–29}. A meta-analysis conducted by Abalos *et al.*³⁰ found that urease inhibitors had a positive effect on crop yield (10%) and N use efficiency (12%), but their effectiveness was dependent on environmental and management factors. Our study clearly demonstrates a significant effect of Limus on mitigating NH_3 volatilization but no significant effect on yield. During our first study year (2011 at SZ and 2012 at QZ), the initial soil N_{min} contents were low ($35\text{--}68 \text{ kg N ha}^{-1}$) because of previous N depletion (Table 3). However, grain yields were maintained under treatments of urea with Limus ($N_{\text{opt/L-1}}$ & $N_{80\% \text{opt/L-1}}$) applied only once at V0 or V3 (Fig. 3). High NH_3 losses of $42\text{--}55 \text{ kg N ha}^{-1}$ (accounting for 20–28% of total N applied) were measured at QZ during the first maize season (2012) in the absence of Limus but, unexpectedly, maize grain yield was not improved by using Limus despite initial soil N_{min} contents being less than half of those at SZ. However, the RE_N of urea with Limus ($N_{\text{opt/L}}$ & $N_{80\% \text{opt/L}}$) significantly increased by about 20% and 33% compared to N_{opt} (53%) and N_{con} (40%), respectively, and there was an increase in maize N uptake. During the 2nd study year, the sum of inputs to soil mineral N (including initial soil N_{min} , N fertilizer, plus contributions from N mineralization) increased to a mean of 363 kg N ha^{-1} at SZ and 324 kg N ha^{-1} at QZ, respectively (Table 3). Therefore at both sites, despite the NH_3 loss of $28\text{--}37 \text{ kg N ha}^{-1}$ at SZ and $86\text{--}109 \text{ kg N ha}^{-1}$ at QZ, there was still more than sufficient soil N_{min} available to meet crop requirements without the use of Limus. However, residual mineral N contents were approx. 30 kg N ha^{-1} higher (ranging from 11 to 52 kg N ha^{-1}) in treatments with Limus ($N_{\text{opt/L}}$), compared to N_{opt} treatments (Table 3), showing the N conserving effect of Limus and so the need to apply less N fertilizer to the following crop.

Research has shown that continually increasing the amount of N applied does not produce a sustained increase in crop productivity due to diminishing returns³¹. In our experiments, compared with the optimized urea-N treatments, grain yield was not significantly increased by the larger amounts that farmers applied (N_{con}), while the RE_N of N_{con} was significantly reduced to 30% at SZ and 40% at QZ in the 1st year and 19% at SZ and 31% at QZ in the 2nd year. Previous observations indicated that RE_N in the NCP has decreased to $< 20\%$ under current farming N practice¹², because of excessive N fertilizer use by farmers, which is substantially less than the 30–35% achieved in the 1980s in China¹⁰. As a consequence, the various N loss pathways including N leaching, NH_3 volatilization, and N_2O emission^{5,13} cause environmental pollution, such as enhanced N deposition³, pollution haze³², increased greenhouse gas emissions³³, and soil acidification³⁴. These problems have become increasingly serious in rapidly developing nations and their consequences are important on a global scale^{1,35}. In addition, the excessive application of N fertilizer has been shown to lead to the accumulation of nitrate N in the soils of the NCP¹². We found that the residual nitrate N retained in the 0–1 m soil profile after harvest ranged from 203 kg N ha^{-1} to 310 kg N ha^{-1} at SZ after the 2-year study. After harvesting maize, Ju *et al.*³⁶ reported nitrate N to be 275 kg N ha^{-1} in the top

0.9 m soil layer and 213 kg N ha⁻¹ in the 0.9–1.8 m soil layer in Shandong province in the NCP, which was a potential threat to shallow groundwater quality through nitrate N leaching. Over-application of urea-N is common in Chinese intensively managed croplands due to the low economic penalties associated with yield losses and the relatively low price of urea fertilizer³⁷. The optimized urea treatment with or without Limus maintained yields and achieved higher RE_N values (51% with urea alone and 62% with urea with Limus at QZ, and 41% and 42% at SZ, respectively). This and the much smaller applications of N fertilizer (by about 48% compared to farmer's practice) led to less N_r losses and a smaller residual soil N_{min}. A further 20% reduction in optimized N rate by using urea plus Limus (N_{80%opt/L-1}: 120 kg N ha⁻¹, applied at V0 or V3) shows that Limus can maintain maize yields at a mean of approx. 6.5 t DM ha⁻¹ at SZ and 9.6 t DM ha⁻¹ at QZ while increasing the RE_N to an average of 41% at SZ and 55% at QZ, and at the same time saving labour. The once application practice of urea with Limus (e.g. N_{80%opt/L-1}) should be consistent to Chinese smallholder farmers' requirement: fertilization as simple as possible.

Although these practices represent a large step forward, increasing rather than merely maintaining crop yields remains a fundamental challenge. New research should quantify the economics (especially the environmental benefit of NH₃ reduction) of using a urease inhibitor (e.g. Limus) across a range of soils, management, and climate variables.

Methods

Study sites. The North China Plain (NCP) is the main maize (and wheat) production region in China, accounting for 39% of total national maize production¹³. The climate conditions are sub-humid continental. Mean annual rainfall is 500–700 mm, with ~70% of precipitation occurring during the maize growing season. The field experiments were conducted for two consecutive years at two typical sites in the NCP: (1) Shangzhuang (SZ, 40°05' N and 116°12' E) in Beijing, during the 2011 and 2012 maize seasons; (2) Quzhou (QZ, 36°58' N and 115°12' E) in Hebei, during the 2012 and 2013 maize seasons. The soil types of SZ and QZ are fluvo-aquic soil and alluvial soil, respectively. Both soils are of calcareous loamy and silty texture and representative of the NCP. The initial soil pH at both (1:2.5 soil water ratio) was 7.9–8.0 and total N content 0.6–0.7 g kg⁻¹. Urease activity was 4.2 μg N g⁻¹ soil h⁻¹ and 13.0 μg N g⁻¹ soil h⁻¹ before seeding in the SZ and QZ soils, respectively. And soil organic matter content was 9.7 and 12.0 g kg⁻¹ in the SZ and QZ soils.

Application of fertilizers and crop management practices. Before planting, phosphorus (calcium superphosphate, 60 kg P₂O₅ ha⁻¹) and potassium (potassium sulphate, 60 kg K₂O ha⁻¹) fertilizers were applied to the plots, according to local mechanical tillage practices. Table 1 shows the detailed information on the N and Limus treatments at the sites, and there were six N treatments at each of the two maize experiments. All of the urea and urea plus Limus were surface applied in a randomized block design to four replicate plots of 30–40 m². Five of the six treatments were the same at both sites: N₀, no N fertilizer; N_{con}, conventional urea (270–300 kg N ha⁻¹); N_{opt}, optimized urea (150 kg N ha⁻¹); N_{opt/L}, optimized urea amended with Limus (150 kg N ha⁻¹), applied at two doses differently (SZ) or equally (QZ) at V0 (seeding, only at SZ in 2011) or V3 (three-expanded leaves) and V12 (twelve-expanded leaves) growth stages for N_{con}, N_{opt} and N_{opt/L}; N_{80%opt/L-1}, 80% of optimized urea amended with Limus (120 kg N ha⁻¹) applied only once at V0 or V3 stage. The additional treatment was N_{opt/L-1} at SZ, which was optimized urea amended with Limus (150 kg N ha⁻¹) applied only once at V0 or V3, or at QZ, N_{80%opt/L} which was optimized urea amended with Limus (120 kg N ha⁻¹) applied half at V3 and half at V12. The amount of optimized urea N was based on previous research on N fertilizer recommendations^{38,39}. V3 and V12 were two key stages of maize for N topdressing. The maize variety *Xianyu 335* was used at SZ at sowing densities of 60,000 and 70,000 seeds ha⁻¹ in the first and second years, respectively. The maize variety at QZ was *Zhengdan 958* sown at 90,000 seeds ha⁻¹, based on local practice. Maize crop management (e.g. applications of pesticide and herbicide) was conducted according to traditional local practices at each site. Before the experiment, the winter wheat and summer maize rotation had been planted for at least one year without fertilizer inputs. No irrigation water was supplied for maize production at each site.

NH₃ loss measurements. A calibrated Dräger-Tube Method (DTM) was adopted to measure NH₃ loss, which is a dynamic chamber technique adjusted to actual meteorological conditions (e.g., temperature, wind speed) by an empirical calibration equation⁸. The calibrated DTM has been shown to be well-suited for NH₃ measurements in multi-plot trials, and has been widely used around the world^{40–42}. Briefly, four chambers (total surface area approx. 400 cm²) were gently pressed about 2 cm into the soil, and connected to a Teflon tube, a Dräger NH₃ indicator tube, and a hand pump to detect the NH₃ concentration. The primary NH₃ flux was converted into the ambient NH₃ flux using a calibration equation that included outside temperature and wind speed (at 0.2 m and 2 m height). The NH₃ indicator tubes and pump were supplied by Drägerwerk AG, Lübeck, Germany. More details of the calibrated DTM method are presented in^{40,43}, where the comparison of the DTM with NH₃ volatilization observed from micrometeorological measurements showed very good agreement, especially for surface applied and evenly distributed N fertilizers.

Sampling and laboratory procedures. The maize plants were harvested manually and aboveground crop residues, except stubble, were removed from each plot at maturity during the two maize cropping seasons. The plant samples (including grains, cobs and straws) from six maize plants per plot were oven-dried at 65 °C to a constant weight. For determination of above-ground N uptake, plant samples were digested by H₂SO₄-H₂O₂ solution and N concentrations in grain and straw analyzed by the micro-Kjeldahl method.

Soils were sampled with an auger to 1 m before planting and after harvest, taking five cores per plot in 20-cm increments (i.e. 0–20, 20–40, 40–60, 60–80 and 80–100 cm). The fresh soil samples were extracted with a 1:10 ratio of soil to 0.01 mol L⁻¹ CaCl₂ and analyzed for NO₃⁻-N and NH₄⁺-N by an AA3 continuous flow analyzer (Bran + Luebbe GmbH, Norderstedt, Germany).

Calculations and Statistical analysis. The RE_N was calculated using the following equation: $RE_N = (\text{crop N uptake in applied N plot} - \text{crop N uptake in } N_0 \text{ plot}) / \text{applied fertilizer N} \times 100$ and indicates the percentage of fertilizer N recovered in aboveground plant biomass⁴⁴.

Other components of N balances (e.g., Apparent N mineralization, N surplus, N loss) were estimated using the following equations^{37,45,46}:

$$\text{Apparent (App.) N mineralization} = \text{crop N uptake in } N_0 \text{ plot}_{(\text{at harvest})} + \text{residual soil } N_{\min} \text{ in } N_0(\text{at harvest}) - \text{initial soil } N_{\min} \text{ in } N_0 \text{ plot.}$$

$$\text{N surplus} = \text{Initial soil } N_{\min} + \text{N fertilizer applied} + \text{apparent N mineralization} - \text{crop N uptake}_{(\text{at harvest})}$$

$$\text{Apparent N loss} = \text{N surplus} - \text{residual soil } N_{\min(\text{at harvest})}$$

$$\text{Other N loss} = \text{Apparent N loss} - \text{NH}_3 \text{ volatilization.}$$

where the initial soil N_{\min} and residual soil N_{\min} were the mineral N content within the top 1 m of soil before planting and after harvest, respectively.

One-way analysis of variance was performed on NH_3 loss, grain yield, RE_N and other related parameters using the SPASS 17.0 statistical software. Significant differences among means were identified using Duncan's test at $P < 0.05$.

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Author Contributions

X.J.L., G.P., W.Z. and A.H.W. designed the experiments. Q.Q.L. and X.Q.C. performed the experiments. Q.Q.L. and X.J.L. analyzed data and wrote the paper. X.J.L., M.R., X.P.C., K.G. and F.S.Z. discussed and contributed to interpretation of the manuscript.

Additional Information

Competing Interests: Dr Qianqian Li's work has partly been funded by BASF SE. About the trial design, Prof. Xuejun Liu consulted for Drs. Gregor Pasda, Wolfram Zerulla and Alexander H. Wissemeyer who work in BASF SE as staff. Dr Marco Roelcke, Profs. Keith Goulding, Xinping Chen and Fusuo Zhang declare no potential conflict of interest.

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