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Effect of combining urea fertilizer with P and K fertilizers on the efficacy of urease inhibitors under different storage conditions

Zhipeng Sha[1] · **Tiantian Lv**[1] · **Maarten Staal**[2] · **Xin Ma**[1] · **Zhang Wen**[1] · **Qianqian Li**[1] · **Gregor Pasda**[2] ·
Tom Misselbrook[3] · **Xuejun Liu**[1]

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Abstract

Purpose Urease inhibitors provide a simple solution to mitigate ammonia loss from fertilized soil. Consumption of bulk blend fertilizers and compound fertilizers were increased in recent year and its enhanced efficiency and stabilized techniques were urgently required. However, it is essentially unknown if and how the efficacy of urease inhibitors is influenced by the inclusion of phosphorus (P) and potassium (K) fertilizers with urea.

Materials and methods A laboratory study was therefore conducted to assess the impact of P and K (bulk blend scenario: combing urea with di-ammonium phosphate (DAP); compound fertilizer scenario: nitrogen (N)-P-K proportion as 16-16-16 (CN16) or 32-0-6 (CN32)) and additionally the impact of fertilizer storage duration and temperature on the efficacy of two different urease inhibitors NBPT and Limus® in reducing ammonia volatilization following application to soil.

Results and discussion Both urease inhibitors significantly reduced ammonia loss from urea regardless of storage temperature and time. However, mixed storage of urea and DAP with urease inhibitors significantly decreased the efficacy of NBPT and Limus® in reducing ammonia loss. Ammonia loss increased exponentially with DAP addition rate and with storage time of the mixture. Storage at a higher temperature (30 °C compared with 20 °C) also reduced the efficacy of the inhibitors. Adding magnesium sulfate ($MgSO_4$) to urea plus Limus® significantly mitigated the negative effect of DAP mixed storage on the efficacy of Limus® regardless of storage temperature and time. The urease inhibitors did not significantly reduce ammonia loss from CN16, but were effective for reducing ammonia loss from CN32.

Conclusions The efficacy of urease inhibitors was compromised by P fertilizer. Urease inhibitor inclusion in the production of CN32, urea, and its blends (DAP + $MgSO_4$) are recommended as an effective means of reducing the environmental cost causing by intensive agricultural production.

Keywords Ammonia volatilization · Compound fertilizer · Phosphorus fertilizer · Potassium fertilizer · Urease inhibitors

1 Introduction

Since the development of the Haber-Bosch process to synthesize ammonia at an industrial scale in 1908, widespread use of synthetic nitrogen (N) fertilizers has greatly boosted global agricultural production (Erisman et al. 2008). However, increased N fertilizer use has been associated with increased N losses to the environment. Ammonia volatilization (AV) is a major N loss pathway (Jiang et al. 2017), with emissions to the atmosphere of approximately 10–12 Tg/year arising from global fertilizer application (accounting for 29–41% of total agricultural ammonia emission) (Beusen et al. 2008). With increased anthropogenic activity, global ammonia emissions may increase 2-fold from 2008 (65 Tg N) to 2100 (132 Tg N) with associated detrimental effects (Sutton et al. 2013; Fowler

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✉ Xuejun Liu
liu310@cau.edu.cn

[1] College of Resources & Environmental Sciences, Key Laboratory of Plant-Soil Interactions of MOE, China Agricultural University, Beijing 100193, China

[2] BASF SE, Agricultural Center Limburgerhof, Speyerer Strasse 2, 67117 Limburgerhof, Germany

[3] Rothamsted Research, North Wyke, Devon EX20 2SB, UK

et al. 2015). These include eutrophication, soil acidification, and loss of biodiversity through dry/wet deposition of ammonia to terrestrial or aquatic ecosystems (Bergstrom and Jansson 2006; Clark and Tilman 2008; Liu et al. 2013; Zhu et al. 2016), also the formation in the atmosphere of secondary inorganic aerosols which contribute to fine particulate matter (diameter < 2.5 μm, $PM_{2.5}$) (Griffith et al. 2015; Wang et al. 2016). High concentrations of $PM_{2.5}$ can influence visibility and human health (Kampa and Castanas 2008; Pozzer et al. 2017). Many studies have demonstrated the benefits of ammonia emission reduction in reducing the concentration of $PM_{2.5}$ (Tsimpidi et al. 2007; de Meij et al. 2009; Wang et al. 2011). Pozzer et al. (2017) estimated that a 50% reduction in ammonia emissions could reduce the mortality attributable to air pollution by ~ 0.25 million people per year worldwide.

Ammonia mitigation strategies in crop production systems include fertilizer application method (e.g., soil placement), N source (substitution of a higher emitting fertilizer type with a lower one), and use of enhanced efficiency fertilizer products (Abalos et al. 2014; Pan et al. 2016; Tingyu et al. 2018). Urea fertilizer has a high ammonia loss potential; surface applied urea could lose more than 40% of total N input by volatilization (Misselbrook et al. 2006). However, urea is the major N fertilizer used in many regions of the world due to its high N concentration and comparatively lower cost (Elaine 1994; Zhang et al. 2011). Urease inhibitors (UIs), like N-(n-butyl) thiophosphoric triamide (NBPT) (phosphoramide derivatives), could easily bound with the active sites of urease enzymes that include two Ni atoms and a carbamate group, thereby retarding urease activity and urea hydrolysis, can be very effective at reducing ammonia emissions following urea applications to land (Amtul et al. 2002). Of these, one of the most widely studied regarding efficacy of ammonia reduction, N use efficiency, and crop yield improvement is NBPT (Pan et al. 2016; Silva et al. 2017; Tingyu et al. 2018). Other novel urease inhibitors, such as Limus® (a combination of NBPT and N-(n-propyl) thiophosphoric triamide (NPPT)), have also been shown to be effective in reducing ammonia emissions and improving production over multiple field trials (Li et al. 2015; Schraml et al. 2016; Li et al. 2017). The variation in the efficacy of urease inhibitors may be a result of many factors, and studies to date have focused largely on soil properties and meteorological conditions (Carmona et al. 1990; Watson et al. 1994). UI storage stability is an important indicator for assessing product efficacy, and previous study suggested stability of NBPT in the fertilizer product was very dependent on storage temperature and duration (Watson et al. 2008). Soares and Cantarella (2012) demonstrated NBPT content dramatically decreased and the ability to reduce AV was compromised after 6-month storage at 35 °C. As described by Pasda et al. (2016), the novel Limus® formulation shows improved efficacy and longer active ingredient (a.i.) storage stability on urea compared to NBPT.

Consumption of N-P-K fertilizers has dramatically increased in many regions of the world. Bulk blended or compound fertilizers are a common type of N-P-K fertilizer (Kumar et al. 2014; Hasler et al. 2015). Integrated enhanced efficiency N-P-K fertilizers may have great opportunity to reduce reactive N loss and increase crop yield (Zhou et al. 2017). However, little is known as to how the efficacy of urease inhibitors may be affected by combining urea with P and K fertilizers when applied to the soil.

To address this current knowledge gap and provide information pertinent to product formulation of compound fertilizer (inclusion UI), storage, and use, a laboratory study was conducted to assess the impact on urease inhibitor efficacy at mitigating ammonia emission following fertilizer application to soil of the following: (i) mixed storage of urea (with or without urease inhibitor) with phosphorus fertilizer (di-ammonium phosphate (DAP)) in different ratios; (ii) compound N-P-K fertilizer with or without urease inhibitor; (iii) duration and temperature of fertilizer storage prior to use.

2 Materials and methods

2.1 Fertilizer preparation

Two urease inhibitors were used in the study: Limus® (containing 25% mixture of NBPT and NPPT, supplied by BASF, Germany) and NBPT (Agrotain Ultra®, containing 26.2% NBPT, manufactured by Koch Agronomic Services, USA). Urea granules and urea-based compound fertilizer granules (wetblend 16-16-16 (CN16) and wetblend 32-0-6 (CN32) in which the N:P:K ratios were 16:16:16 and 32:0:6 respectively) were prepared for urease inhibitor amendment. Urea granules were separately coated with each urease inhibitor at the same addition dose of 0.056% (wt) active ingredient. Compound fertilizer granules were treated with 0.050% (wt) of each urease inhibitor active ingredient separately. DAP were mixed with urea granules (with or without urease inhibitors) at a range of addition rates from 0 to 50% (Table S1, Electronic Supplementary Material—ESM). Extra urea granules treated with Limus® were amended with magnesium sulfate ($MgSO_4$) at an addition rate of 5% by weight of urea or the urea/DAP mixtures.

Fifty grams of each fertilizer or fertilizer mixture (according to Table S1—ESM) was put into a 100-mL plastic bottle. The bottles were closed and shaken thoroughly by hand and stored in climate chambers at either 20 or 30 °C. After 0, 0.5, 1, 2, 3, 4, 5, and 6 months of storage, bottles were removed for assessment of ammonia emissions by laboratory incubation from soils.

2.2 Soil preparation and ammonia volatilization measurement

Arable topsoil (0–20 cm), classification fluvo-aquic, was sampled from the Shangzhuang experimental station of the China Agricultural University in Beijing, China (40° 08′ N, 116° 12′ E elevation 51 m). The sampled soil was air-dried and ground to pass a 2-mm sieve as preparation for the laboratory study. Some of the major soil properties and methods of determination are given in Table S2 (ESM). Three incubation jars were prepared for each fertilizer treatment. One hundred twenty grams of air-dried soil was packed into a 500-mL incubation jar (diameter 8 cm, height 10 cm). Deionized water was added to each jar to achieve a soil water content of 50% water holding capacity, the top of jar sealed with plastic film through which nine needle holes were evenly distributed. The jars were pre-incubated at 25 °C for 48 h, following which jars were weighed and water added to replace loss during pre-incubation.

Dräger tubes were employed to assess the AV from the urea (with or without urease inhibitor) for each fertilizer treatment. The Dräger tube measurement system comprised an incubation jar with a modified lid supporting a Dräger tube (Ammonia 20/a-D) (Fig. S2—ESM). In the middle of the lid was a hole, inset with a rubber plug sealed around with glue. A hole was drilled through the rubber plug of a size to perfectly match the Dräger tube (ensuring no gas escape). The measuring principle of the Dräger tube is based on the color reaction of ammonia with bromophenol blue and acid. During soil incubation, ammonia volatilized and diffused from the soil surface results in volumetric concentration changes of ammonia. Each Dräger tube can determine ammonia concentration changes in the range 20 to 1500 ppm. The Dräger tubes are calibrated, so cumulative ammonia concentration change over time can be easily determined from the progressive color change in the tube.

For the urea/DAP mixtures, urea was firstly separated from the DAP and 200 mg of urea granules was distributed evenly over the pre-incubated soil surface. For the compound fertilizers, 400 mg of the CN16 and 800 mg of the CN32 were weighed out and distributed evenly over the pre-incubated soil surface. Following fertilizer application, the jar was immediately closed with a lid through which a Dräger tube (Ammoniac 20/a-D) was inserted (ensuring that the Dräger tube fitted tightly, so no ammonia gas could escape). Jars were incubated at 25 °C under dark conditions. The amount of ammonia volatilized within each jar was indicated by progressive color change on the Dräger tubes. Dräger tube readings were taken for each jar every 12 h until the 1500 ppm level was reached during the 14-day incubation period.

2.3 Statistics and analysis

The cumulative ammonia loss from un-amended urea or compound fertilizers were expected to reach the maximum load of one Dräger tube in less time than those from inhibitor-amended fertilizers. Therefore, comparisons of ammonia loss among different fertilizer treatments were made at the time the first Dräger tube reached maximum load. Cumulative ammonia losses from different fertilizer treatments were compared using a one-way analysis of variance (ANOVA) and post hoc multiple comparisons were conducted by LSD test at a significance level of 0.05. Statistical analyses were carried out using SPSS version 21.0. It is, however, hard to illustrate product efficiency over such a short time. Therefore, instead of using the time needed for untreated urea to reach the Dräger tube maximum load as “end time,” we selected the time at which the first urease inhibitor experiment reached the Dräger tube maximum load as “end time.” To better understand the influence of increasing DAP addition rate on AV, an allometric model was adopted (using Origin 9.0), with model parameters sensitive to storage duration.

3 Results

3.1 The efficacy of different urease inhibitors in reducing ammonia volatilization

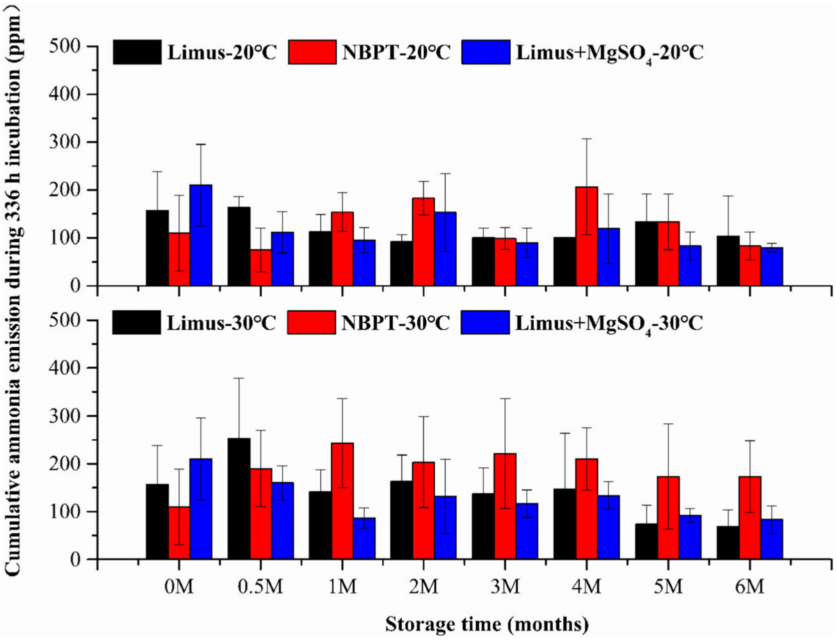
Both assessed urease inhibitors significantly decreased cumulative ammonia loss from soils compared to untreated urea during 336-h incubation ($P < 0.01$). Storage temperature significantly impacted ($P < 0.05$) on ammonia loss (Table S3—ESM). The efficacy of Limus® was not affected by storage duration at either storage temperature (Fig. 1, Fig. S3—ESM). Cumulative ammonia loss from urea amended with NBPT stored at 30 °C was 31.6% greater than from that stored at 20 °C (Fig. 1, Fig. S3—ESM). At 30 °C, ammonia loss was 25.2% and 33.6% lower from urea amended with Limus® and Limus® plus $MgSO_4$ treatments, respectively, than from NBPT (Fig. S3—ESM).

3.2 Impact of mixed storage of urea and DAP on the efficacy of different urease inhibitors in reducing ammonia volatilization

3.2.1 Ammonia volatilization from urea and from urea plus urease inhibitors

Without any urease inhibitor, urea rapidly hydrolyzed under the favorable environmental conditions of the incubation, with maximum load on the Dräger tube reached after about 84 h. Mixed storage of urea with DAP did not affect ammonia volatilization (AV) from urea under any DAP addition rate

Fig. 1 Cumulative ammonia volatilization from urea amended with different urease inhibitors during 336-h incubation following storage at two temperatures (20 vs 30 °C) and 0, 0.5, 1, 2, 3, 4, 5, or 6 months duration. Error bars indicate standard deviation of each mean ($n = 3$)



(Table S4—ESM). There was no significant effect of storage temperature on ammonia loss from urea (Table S4—ESM) and while storage duration was significant, there was no apparent pattern to the effect (Fig. S4—ESM).

Addition of the assessed urease inhibitors significantly reduced AV compared to using urea alone ($P < 0.01$) (Fig. 2). However, the efficacy of Limus® in the urea/DAP mixtures over the 84-h incubation declined with increasing addition rate of DAP and extending the storage duration (Fig. 2; Table S5—ESM). The fertilizer storage temperature also had a significant effect, with greater ammonia loss from the urea amended with Limus® stored at 30 °C than that at 20 °C. A similar response to DAP addition rate and storage treatment was observed for urea amended with NBPT (Fig. 2, Table S5—ESM). The efficacy of Limus® plus $MgSO_4$ during the 84-h incubation was not influenced by DAP mixed storage regardless of addition rate, storage temperature, or duration (Fig. 2, Table S5—ESM).

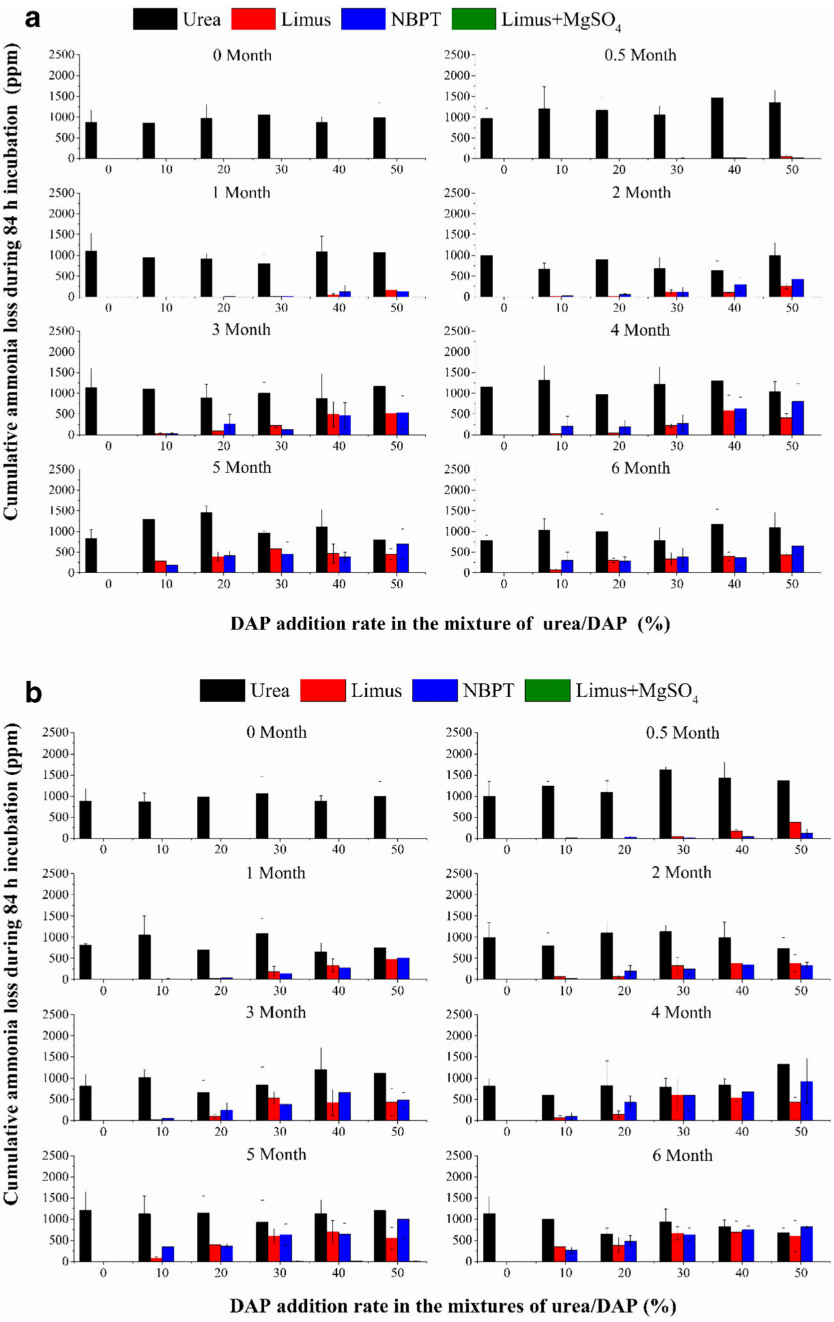
3.2.2 Effect of storage mixed with DAP on the efficacy of Limus®

As mentioned above, urea Limus® was very effective in reducing AV from urea. However, its efficacy was reduced by mixed storage with DAP (Fig. 3). DAP addition rate, storage temperature, and duration significantly impacted on the efficacy of Limus®, and there were also significant interactions between DAP addition rate and storage temperature, and DAP addition rate and storage duration. Good fits were obtained for the allometric model of AV as a function of DAP addition rate across all storage temperature and duration combinations (Table 1) and it was evident that the value of parameter b declined with increased storage duration. Cumulative AV over the 108-h incubation from fertilizer stored at 20 °C up until 4 months increased exponentially with DAP addition rate (Fig. 3). For longer storage duration, AV tended to plateau at the higher DAP addition rates (30–50%), at which rates the Limus® was giving almost no reduction compared with the mixture without inhibitor over the 108-h incubation period. This occurred where the parameter b was < 1, and this may be defined as a threshold point. Using this, the threshold at 30 °C storage was reached at a shorter storage duration than at 20 °C (3 months compared with 5 months, respectively), indicating that the effect of mixed storage with DAP on reducing the efficacy of Limus® was greater at the higher storage temperature. At 30 °C, there was no significant difference in cumulative AV during the 84-h incubation from urea and urea plus Limus® at urea/DAP mixtures of over 30% after 5 and 6 months storage (Fig. 3, Table S6—ESM).

3.2.3 Effect of storage mixed with DAP on the efficacy of NBPT

Cumulative AV of urea plus NBPT mixed storage treatments were similar to those observed for urea plus Limus® (Fig. 4). NBPT was also effective at reducing AV when not mixed with DAP during storage. DAP addition rate, storage temperature, and duration significantly affected the efficacy of NBPT, and there was significant interaction between DAP addition rate and storage duration (Table S6—ESM). From fitting the allometric model (Table 2), at 20 °C storage, the DAP addition rate had a similar effect as observed with the urea/DAP plus Limus® treatments, with the threshold point at the same storage duration of 5 months. Similarly, storage temperature had a

Fig. 2 Comparison of ammonia volatilization from urea and urea amended with urease inhibitors during 84-h incubation after 0, 0.5, 1, 2, 3, 4, 5, or 6 months storage. **a** The mixtures stored at 20 °C. **b** The mixtures stored at 30 °C. AV after 84-h incubation was zero for all Limus + $MgSO_4$ experiments. Error bars indicate standard deviation of each mean (*n* = 3)



significant impact, with the threshold at 30 °C being reached after 2 months storage.

3.2.4 Effect of storage mixed with DAP on the efficacy of Limus® plus $MgSO_4$

Adding $MgSO_4$ to urea plus Limus® significantly reduced AV during 336-h incubation (Fig. 5). DAP addition rate, storage temperature, and duration all had significant effects on the efficacy of Limus® + $MgSO_4$, and there was a significant interaction between storage temperature and duration (Table S6—ESM). No good fit was obtained with the allometric model for any of the different mixed storage treatments. The efficacy of Limus® + $MgSO_4$ tended to decline with increasing DAP addition rate, and, unlike other urease inhibitors, reductions in cumulative AV tended to be greater following storage at 30 °C compared with 20 °C.

3.3 Effect of storage temperature and duration on the efficacy of urease inhibitors in reducing ammonia volatilization from compound fertilizer

The ammonia loss rate from CN16 was slower than from urea. For storage at 20 °C, the inhibitors Limus®, NBPT, or

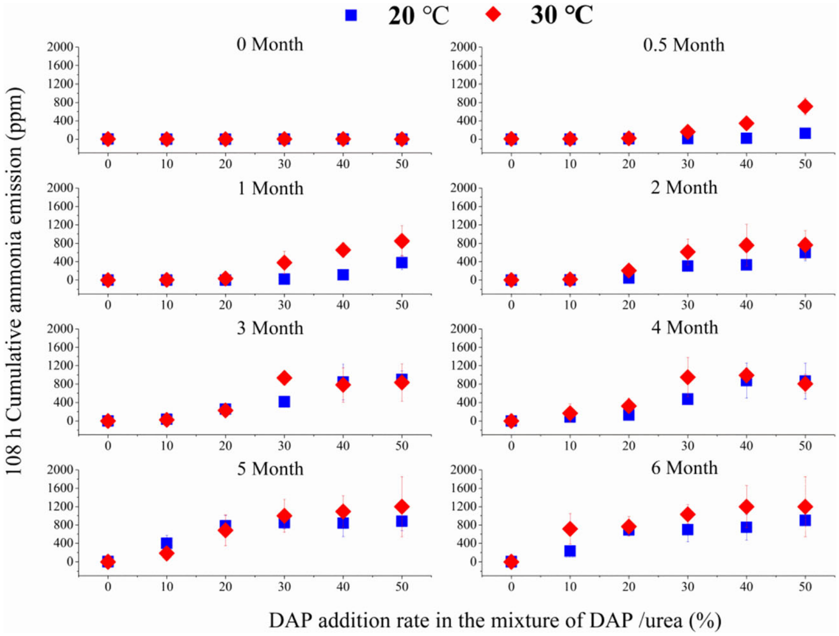


Fig. 3 Ammonia volatilization during 108-h incubation from urea amended with Limus stored together with di-ammonium phosphate (DAP) following storage at two temperatures (20 vs 30 °C) and 0, 0.5, 1, 2, 3, 4, 5, or 6 months duration. Error bars indicate standard deviation of each mean ($n = 3$)

Limus® + $MgSO_4$ did not show significant reduction in ammonia emission during the 204-h incubation (Fig. 6, Fig. S5—ESM). At 30 °C storage however, Limus® + $MgSO_4$ reduced ammonia loss by 22% compared to CN16. The storage duration significantly affected the efficacy of all urease inhibitors (Table S7—ESM).

Table 1 Fitting function and parameters of the allometric model using cumulative AV during 108-h incubation from urea plus Limus following different storage treatments

Time (month)	Parameters		R^2	P	Function
	a	*b*			
Storage in 20 °C					
0	–	–	–	–	–
0.5	0.01	7.77	0.97	< 0.01	$y = 0.01x^{7.77}$
1	0.07	5.37	0.99	< 0.01	$y = 0.07x^{5.37}$
2	26.21	1.94	0.92	< 0.01	$y = 26.21x^{1.94}$
3	97.25	1.43	0.93	< 0.01	$y = 97.25x^{1.43}$
4	95.01	1.44	0.87	< 0.01	$y = 95.01x^{1.44}$
5	514.89	0.37	0.70	< 0.01	$y = 514.89x^{0.37}$
6	569.16	0.28	1.00	< 0.01	$y = 569.16x^{0.28}$
Storage in 30 °C					
0	–	–	–	–	–
0.5	4.45	3.16	0.99	< 0.01	$y = 4.45x^{3.16}$
1	39.87	1.93	0.94	< 0.01	$y = 39.87x^{1.93}$
2	131.56	1.17	0.83	< 0.01	$y = 131.56x^{1.17}$
3	191.49	0.99	0.61	0.01	$y = 191.49x^{0.99}$
4	282.25	0.79	0.58	< 0.01	$y = 282.25x^{0.79}$
5	358.28	0.79	0.88	< 0.01	$y = 358.28x^{0.79}$
6	666.19	0.38	0.88	< 0.01	$y = 666.19x^{0.38}$

Limus®, NBPT, and Limus® + $MgSO_4$ significantly decreased AV when included with CN32 compared with the unamended compound during 108-h incubation for all storage temperature × duration combinations (Fig. 7, Fig. S6—ESM). Storage temperature and duration had a significant effect on the efficacy of all urease inhibitors (Table S7—ESM). There was no clear effect of storage duration.

4 Discussion

4.1 Effect of storage temperature and duration on the efficacy of urease inhibitors

Results confirmed that the urease inhibitors NBPT and Limus® could reduce ammonia loss from soil surface applied urea, which is consistent with previous studies under either laboratory or field conditions (Li et al. 2015; Schraml et al. 2016; Li et al. 2017). These urease inhibitors are classified as phosphoramide derivatives and share a similar urease inhibition mechanism in which the active sites of urease enzymes that include two Ni atoms and a carbamate group are bound with these compounds (Amtul et al. 2002). The inhibition ability of urease inhibitors are known to be influenced by soil properties and ambient conditions. Previous studies have shown that high ambient temperature can decrease the stability of NBPT and decrease the inhibitor efficacy in reducing ammonia emissions (Carmona et al. 1990). In our study, we focused on the influence of temperature during storage (and duration) on the stability of the urease inhibitors, rather than incubation environment. A similar effect was observed, i.e., that NBPT and Limus® stored at high temperature had a lower

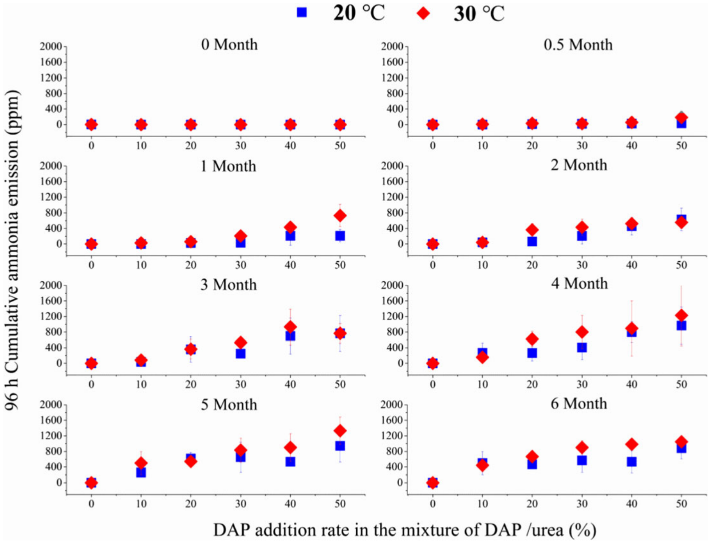


Fig. 4 Ammonia volatilization during 96-h incubation from urea amended with NBPT stored together with di-ammonium phosphate (DAP) following storage at two temperatures (20 vs 30 °C) and 0, 0.5, 1, 2, 3, 4, 5, or 6 months duration. Error bars indicate standard deviation of each mean ($n = 3$)

efficacy in reducing ammonia emission from urea. A study from Soares and Cantarella (2012) showed a much stronger decrease in NBPT efficacy after storage of NBPT-treated urea for more than 1 month at 35 °C or more than 3 months at 25 °C. The difference in results can be explained by the use of different test methods and soil types. The Dräger tube test used in this study is a simplified and less sensitive version of the volatilization test used by Soares and Cantarella (2012). On the other hand, according to the manufacturer, the use of these tubes is for only 2 to 8 h, but in reality, we used these tubes for one to several days testing which may cause uncertainties. Li (2014) demonstrated that increasing soil temperature from 15 to 25 °C and from 25 to 35 °C resulted in 3-fold increases in ammonia emission from Limus®-treated urea. In contrast, the higher storage temperature appeared to increase the efficacy of Limus® + $MgSO_4$. However, there are no relevant research regarding Limus® plus $MgSO_4$ and it is unclear what the mechanism for this increase in Limus® stability at high storage temperature when coupled with $MgSO_4$ might be.

Table 2 Fitting function and parameters of the allometric model using cumulative AV during 96-h incubation from urea plus NBPT following different storage treatments

Time (month)	Parameters		R^2	P	Function
	a	b			
Storage in 20 °C					
0	–	–	–	–	–
0.5	1.16	5.30	0.92	< 0.01	$y = 1.16x^{5.30}$
1	2.13	7.69	0.79	< 0.01	$y = 0.07x^{5.37}$
2	25.57	2.06	0.98	< 0.01	$y = 25.57x^{2.06}$
3	95.17	1.32	0.81	< 0.01	$y = 95.17x^{1.32}$
4	131.52	1.24	0.88	< 0.01	$y = 131.52x^{1.24}$
5	324.59	0.60	0.60	< 0.01	$y = 324.59x^{0.60}$
6	406.81	0.36	0.33	< 0.01	$y = 406.816x^{0.36}$
Storage in 30 °C					
0	–	–	–	–	–
0.5	0.15	4.41	0.92	< 0.01	$y = 0.15x^{4.41}$
1	14.15	2.46	0.99	< 0.01	$y = 39.87x^{1.93}$
2	161.32	0.83	0.81	< 0.01	$y = 161.32x^{0.83}$
3	184.70	0.98	0.78	< 0.01	$y = 184.70x^{0.98}$
4	272.37	0.93	0.92	< 0.01	$y = 272.37x^{0.93}$
5	373.12	0.73	0.84	< 0.01	$y = 373.12x^{0.73}$
6	473.37	0.52	0.96	< 0.01	$y = 473.37x^{0.52}$

4.2 Effect of P and K fertilizers on the efficacy of urease inhibitors

Influencing factors on NBPT degradation in soil or chemical buffer media have been comprehensively studied (Douglass and Hendrickson 1991; Hendrickson and Douglass 1993; Engel et al. 2015). pH is considered as an important factor (Watson et al. 1994; San Francisco et al. 2011; Suter et al. 2011; Engel et al. 2013; Engel et al. 2015; Zheng et al. 2018). The half-life of NBPT in acidic soil was shown to increase 49-fold from acidic (pH 5.5) to alkaline (pH 8.2) soil conditions (Engel et al. 2015). In our study, there was a significantly negative impact on the efficacy of the urease inhibitors when stored as a mixture with DAP. This may have been as a result of phosphoric acid from DAP creating relatively acidic storage conditions and accelerating the degradation of NBPT and Limus® (Engel et al. 2015). Another explanation could be the effect of phosphorolysis of NBPT and Limus®, caused by phosphates present in the storage accelerating degradation (Garrett and Grisham 2005). Higher storage temperature may accelerate these degradation processes. Compared to NBPT, Limus® had greater stability to any negative impacts of mixed storage with DAP. This is likely due to the

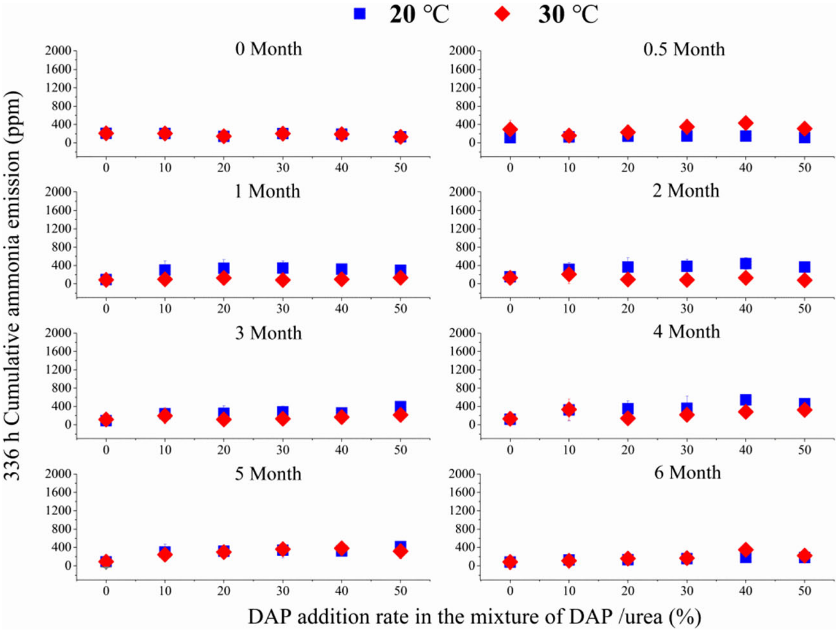


Fig. 5 Ammonia volatilization during 336-h incubation from urea plus Limus amended with $MgSO_4$ following storage at two temperatures (20 vs 30 °C) and 0, 0.5, 1, 2, 3, 4, 5, and 6 months duration. Error bars indicate standard deviation of each mean ($n = 3$)

differences in chemical structures, which could cause differences in the charge of the P atom. Engel et al. (2015) reported that the larger positive charge of P creates more polarity in the P–N bond, which makes the urease inhibitor molecule more susceptible to nucleophilic attack from other substances and cleavage of the P–N bond. Limus® is a combination of NBPT and NPPT (3:1 mixed ratio), potentially making it more stable under diverse soil conditions and more effective in controlling ammonia emission from urea. Previous studies comparing NBPT and Limus® have shown that, at relatively low addition rates (0.0025% and 0.01%), Limus® performed better at mitigating ammonia loss compared to NBPT (Li 2014). Our study has shown that treatment of Limus® with $MgSO_4$ can significantly mitigate the negative effects of mixed storage with DAP on the efficacy of Limus®. The chemical reaction between DAP and $MgSO_4$ may create more neutral ambient conditions. The reaction product magnesium ammonium phosphate may also decrease the effect of phosphorolysis by limiting the availability of phosphate. In this study, we failed to test the efficacy of NBPT plus $MgSO_4$, but believe that they would show similar effects to Limus® due to the analogous inhibition mechanism.

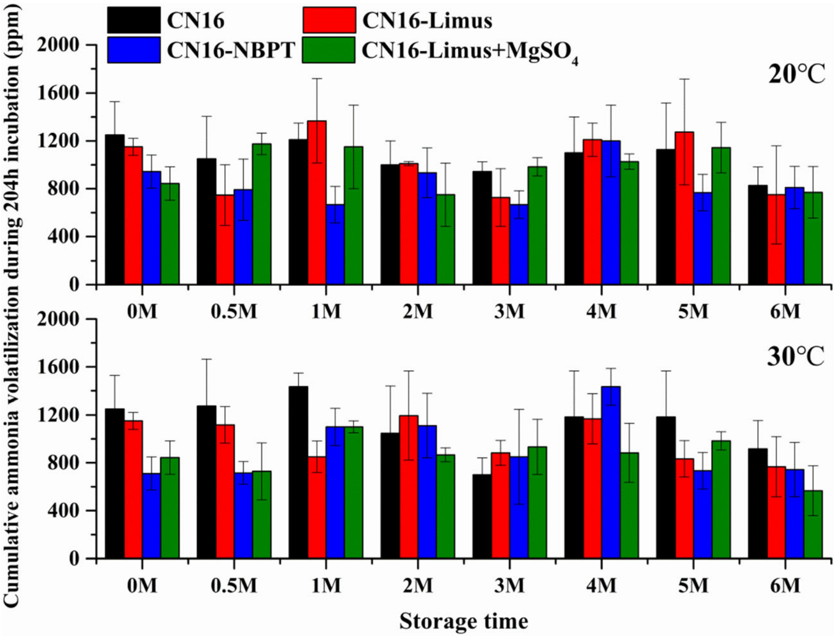


Fig. 6 Ammonia volatilization during 204-h incubation from wetblend 16-16-16 (CN16) amended with different urease inhibitors following storage at two temperatures (20 vs 30 °C) and 0, 0.5, 1, 2, 3, 4, 5, and 6 months duration. Error bars indicate standard deviation of each mean ($n = 3$)

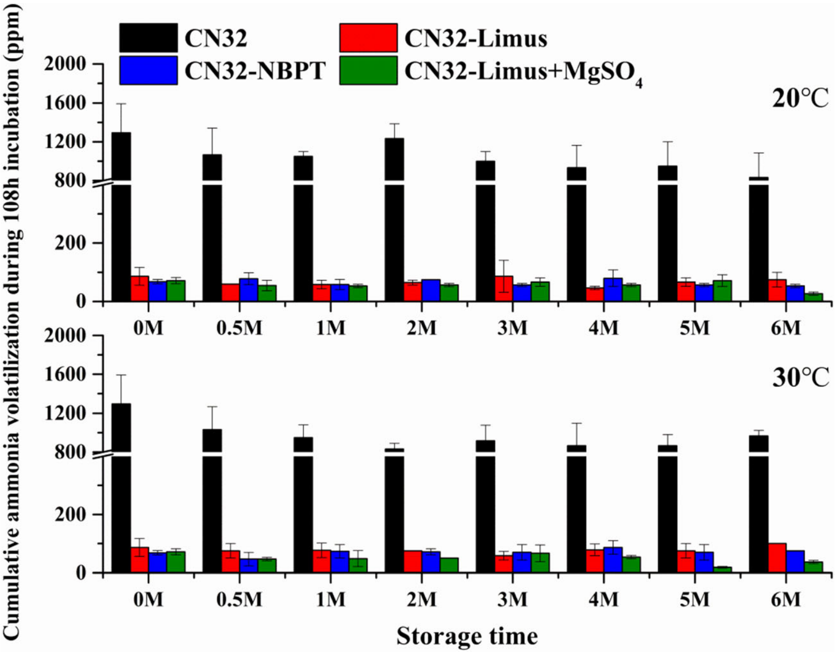


Fig. 7 Ammonia volatilization during 108-h incubation from wetblend 32-0-6 (CN32) amended with different urease inhibitors following storage at two temperatures (20 vs 30 °C) and 0, 0.5, 1, 2, 3, 4, 5, or 6 months duration. Error bars indicate standard deviation of each mean (n = 3)

Inclusion of the urease inhibitors in N-P-K compound fertilizer granules showed different results regarding their efficacy when compared to use with urea alone or following mixed storage with DAP (following which only the urea was applied to soil). The presence of phosphate through the whole soil incubation period may be the reason for this observed lack of effectiveness (Engel et al. 2015). When incorporated in the N-K compound fertilizer, the urease inhibitors were very effective in reducing ammonia loss, confirming the presence of the phosphorus in the N-P-K compound as the influencing factor.

4.3 Implications for fertilizer production and application in the field

Blends between enhanced efficiency fertilizers and regular fertilizers could delay nutrient dispersal and offset the high cost of enhanced efficiency fertilizers (Chen et al. 2008). Higher crop yields have also been noted from the use of blended products compared to single use of enhanced efficiency fertilizers or regular fertilizers (Farmaha and Sims 2013; Ye et al. 2013; Zhou et al. 2017). This study found that bulk blending of urease inhibitor (NBPT and Limus®) treated urea with DAP was not a suitable way to produce fertilizer or to apply in the field as the resulting chemical reaction decreased the efficacy of the urease inhibitors. However, $MgSO_4$ amendment may be a potential solution to this problem. This could result in a product which reduces undesired gaseous N loss (Schraml et al. 2016; Li et al. 2017), and simple application of multiple nutrients, in proportions adjusted to meet crop production requirements (Miserque and Pirard 2004; Hasler et al. 2015).

Unlike the bulk blending fertilizer scenario, direct inclusion of urease inhibitors in N-P-K compound fertilizer granules (urea-N base) did not show ammonia reduction potential. However, the rate of ammonia loss from CN16 was much slower than from urea or CN32, which may be related to the physical structure and the presence of phosphoric acid (Fan and Mackenzie 1993). In this situation, urease inhibitor amendment is not necessary. Improving the fertilizer application method, such as deep placement, could also give effective ammonia mitigation potential (Huang et al. 2017). On the other hand, urease inhibitor inclusion in the production of CN32 is recommended as an effective means of reducing the environmental cost causing by intensive agricultural production.

5 Conclusions

Two urease inhibitors (NBPT and Limus®) were shown to significantly reduce ammonia loss from urea fertilizer application across a number of pre-application storage temperature and duration combinations. However, mixed storage of urease inhibitor-amended urea with DAP significantly decreased the efficacy of the inhibitors in reducing ammonia loss. Ammonia loss increased exponentially with increasing DAP addition rate and with storage duration, and storage at a high temperature also negatively influenced inhibitor efficacy. Adding $MgSO_4$ to urea plus Limus® significantly mitigated the effect of mixed storage with DAP across all storage temperature and duration combinations. Urease inhibitors did not significantly reduce ammonia loss when incorporated in a compound N-P-K fertilizer.

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