

Dynamics of ammonia volatilization from NBPT-treated urea in tropical acid soils

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ABSTRACT: The urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) reduces NH₃ losses from urea (UR) surface-applied to soils, but its efficacy may be lower in acidic soils. The period when urease inhibition occurs efficaciously may change with soil pH. This needs to be clarified in tropical soils which are commonly acidic. This study evaluated the effectiveness of NBPT-treated urea to delay and reduce ammonia volatilization in two soils at three pH levels. Two experiments were conducted under laboratory conditions in soils with different textures (sandy-clay and clay). The treatments consisted of three soil pH levels and two N sources (UR and UR + NBPT), with five replicates. The soil pH values were adjusted and reached values of 4.5, 5.6, and 6.4 in the sandy-clay, and 4.5, 5.4, and 6.1 in the clay soil. Ammonia volatilization was measured using glass chambers (1.5 L). In the sandy-clay soil, NH₃ losses were 40-47 % of the UR-N. In the clay soil, losses were 26-32 %. The addition of NBPT to UR reduced the NH₃ volatilization by 18-53 %; the inhibitor decreased the N losses under all soil pH conditions but was significantly less efficient in acidic soils (pH 4.5). The lower efficiency of the inhibitor under acidic conditions was more evident in the first few days: 50 % of the total NH₃ losses occurred in less than four days in soils with pH 4.5, but in 8-11 days in soils with pH above 5.4. The rapid loss in efficiency in more acidic soils is a drawback. Using NBPT in severely acidic soils showed a relatively small advantage over untreated UR as the inhibitor did not provide extra time for fertilizer incorporation and further reduction of NH₃ losses.

Keywords: enhanced-efficiency fertilizers, urease inhibitor, soil pH, buffer capacity

Introduction

Urea, which represented 50 % to 60 % of N fertilizer consumption in the world in 2019, is the soluble N source most likely to drive future N fertilizer expansion capacity, corresponding to 75 % of the projected N increment for 2020-2024 (IFA, 2020). High N losses through NH₃ volatilization can occur when UR is surface-applied to soils, as it reduces fertilizer use efficiency (Cantarella et al., 2018), resulting in environmental pollution, soil acidification, and water eutrophication. Ammonia also generates particulate matter in the atmosphere and, when deposited elsewhere, causes nitrous oxide emission which depletes the ozone layer and is a potent greenhouse gas (Behera et al., 2013; Ferm, 1998).

Meta-analysis studies reported mean NH₃ losses of 15 to 35 % of the N applied as UR (Pan et al., 2016; Silva et al., 2017). However, depending on climatic conditions, soil properties, and agricultural practices, N losses can be higher and reach 40-60 % of the N fertilizer (Trivelin et al., 2002; Cantarella et al., 2008, 2018). Urease inhibitors have been used to reduce the NH₃ losses from UR applied to soils. NBPT (N-(n-butyl) thiophosphoric triamide) is currently the most widely used inhibitor globally (Cantarella et al., 2018; Modolo et al., 2018; Klimczyk et al., 2021). Adding NBPT to UR reduces NH₃ volatilization by approximately 60 % and can subsequently increase crop yields (Trenkel, 2010; Linqvist et al., 2013; Abalos et al., 2014; Silva et al., 2017).

Soil properties affect NH₃ volatilization from UR and influence the efficacy of NBPT. Soil pH and

buffer capacity are soil properties best related to NH₃ volatilization (Watson et al., 1994). Increasing total exchange capacity and soil pH caused a decrease in NH₃ losses from UR + NBPT (Sunderlage and Cook, 2018). In addition, acidic soils can accelerate NBPT degradation (Hendrickson and Douglass, 1993; Engel et al., 2015). This is a matter of concern in tropical soils, which typically are highly weathered and predominantly acidic (Lopes and Guilherme, 2016). Therefore, the implication of soil acidity on the performance of NBPT-treated urea should be investigated.

The magnitude of NH₃ losses also depends on the time it takes for UR to hydrolyze and diffuse into the soil after UR + NBPT is surface-applied, which may be faster in sandy than in clay soils (Cantarella et al., 2018). Understanding the dynamics of these reactions under different soil pH values and textures may help devise strategies to reduce losses. This study aimed to evaluate, under controlled conditions, the efficiency of NBPT in delaying and reducing NH₃ volatilization from UR in two soils with contrasting textures at three soil pHs.

Materials and Methods

Soil samples were collected from the 0-20 cm layer of two acidic soils with different textures in the experimental farm at the Instituto Agronômico (IAC) in Campinas, São Paulo, Brazil. The soils are representative of the leading agricultural soils in Brazil and are classified as Typic Hapludox (sandy-clay soil) and Rhodic Hapludox (clay soil) (Soil Survey Staff, 1999). Approximately 1 m² of the 0-20 cm soil layer was dug, sieved (0-4 mm), and

homogenized in each area. The soil samples were air-dried and stored in a greenhouse for approximately ten days at room temperature. Next, the soils were sieved (0.2 mm) and analyzed for chemical (Raij et al., 2001) and physical properties (Camargo et al., 1986) and urease activity (Tabatabai, 1982) (Table 1).

The original pH values (CaCl_2) of the soil samples were 4.2 and 4.4 (Table 1). Reagent grade calcium and magnesium carbonates were added to increase soil pH to three levels: 4.5, 5.5, and 6.5, within the range of soil pH values depending on the crop under cultivation (Quaggio, 2000). Calcium and magnesium carbonates were mixed in the proportion of 2:1 (mass). The rates of liming material were calculated following the formula relating to soil base saturation and pH (Quaggio, 2000). Three samples of each soil (15 L) were incubated with the carbonates in plastic bags with small holes for gas exchange. The soils were maintained at 60 % water retention capacity for 21 days. Next, soil samples were air-dried, sieved at 2 mm, and the pH was measured in 10 mmol L^{-1} CaCl_2 solution (soil:solution ratio 1:2.5). The pH values reached 4.5, 5.6, and 6.4 in the sand-clay soil and 4.5, 5.4, and 6.1 in the clay soil.

Two NH_3 volatilization experiments were set up separately in a laboratory while the temperature was maintained at 25 ± 3 °C. The study with the clay soil was performed in Sept-Oct and the sandy-clay soil in Jan-Feb. The experimental design for both studies was a 2×3 factorial with two N sources (UR and NBPT-treated UR) and three pH levels, with five replicates in a randomized design. In addition, unfertilized control plots with soils of intermediate pH values (pH 5.6 and 5.4, respectively) and an empty chamber to control atmospheric contamination were included.

Prilled urea (1-2 mm in diameter and containing 45.6 % of N) was treated with an NBPT solution (20 % of active ingredient) at 530 mg NBPT kg^{-1} one day before it was used in the experiments. The NBPT solution was weighed, added to 1 kg of urea in plastic bags, and thoroughly homogenized for 5 min. The solid fertilizers were surface-applied to the soil contained in volatilization chambers at a rate of 30 g N m^{-2} (200 mg kg^{-1}), equivalent to approximately 100 kg ha^{-1} when fertilizers are applied in bands in the field.

Volatilized ammonia was measured in chambers according to Soares et al. (2012). The chambers consisted of hermetic 1.5 L cylindrical glass vessels. Two opposite lateral holes 0.5 cm in diameter were perforated in the

glass vessels 4 cm below the lid and fit with 6 cm glass tubes to allow air in and out of the chambers. Dried soil samples (1,200 g) were placed in the chambers so that the soil surface was ~ 2.5 cm below the air flowing tubing. One week before the fertilizer application, the soil was moistened to reach 60 % water retention capacity (0.25 g g^{-1} in the sandy clay and 0.30 g g^{-1} in the clay soil). This was necessary to restore the soil's microbial and enzymatic activities.

Air flowing from a compressor was bubbled through an H_2SO_4 solution (0.05 mol L^{-1}) and then through deionized water contained in 15 L glass flasks to remove traces of NH_3 from the air and keep the air humidified. The air was carried through PVC tubes to each volatilization chamber and allowed to flow over the soil inside the chambers at a rate of 2.0 L min^{-1} to carry the volatilized NH_3 , which is collected in flasks containing 150 mL of a trapping solution [boric acid solution (20 g L^{-1}) containing pH indicators (44 mL L^{-1} methyl red and 66 mL L^{-1} bromocresol green)]. The ammonia was determined by potentiometric titration (Cantarella and Trivelin, 2001). The flasks with the trapping solution were replaced daily until NH_3 volatilization was negligible (0.1 % of N applied), which occurred 21 and 23 days after the fertilizer application for the sandy-clay and the clay soils, respectively.

At the end of the volatilization experiment, the 0-2 cm soil layer was removed, homogenized, and frozen at -20 °C for further analysis. The N content in the ammonium and nitrate forms was extracted with a 2 mol L^{-1} KCl solution and determined by steam distillation (Cantarella and Trivelin, 2001). The soil pH was determined in a 10 mmol L^{-1} CaCl_2 solution (soil:solution ratio of 1:2.5).

The soil urease activity after UR application was evaluated in a separate experiment. Dried soil samples (50 g of the same soils used in the volatilization experiments) were transferred to 200 mL glass vials. The UR was applied at 300 mg N kg^{-1} as a solution (1.5 g N L^{-1}) to avoid NH_3 losses. Next, the soil was moistened at 60 % of the water retention capacity, and the vials were placed in a laboratory incubator at 25 °C and sealed with a plastic film with small holes to allow for gas exchange. Urease activity was evaluated soon after UR application ($t = 0$) and after incubation for 1, 2, and 4 days. Urease activity was determined by the remaining UR method (Tabatabai, 1982). Urea-N was extracted with a 2 mol L^{-1} KCl solution containing phenylmercuric acetate

Table 1 – Chemical, physical and biological soil properties¹.

| Soil | pH CaCl_2 | OM | P | K | Ca | Mg | H+Al | CEC | Clay | Silt | Sand | UA |
|------------|-----------------------|--------------------|---------------------|-------------------------|----|----|------|--------------------|------|------|------------------------------------|----|
| | | g dm^{-3} | mg dm^{-3} | mmol c dm^{-3} | | | | g kg^{-1} | | | mg $\text{kg}^{-1} \text{ h}^{-1}$ | |
| Sandy-clay | 4.2 | 24 | 3 | 1.5 | 5 | 5 | 52 | 64 | 359 | 80 | 561 | 20 |
| Clay | 4.4 | 55 | 8 | 3.2 | 14 | 7 | 58 | 82 | 525 | 84 | 391 | 23 |

¹pH $\text{CaCl}_2 = \text{CaCl}_2$ 10 mmol L^{-1} ; OM = Oxi-Red.; P, K, Ca, Mg = extracted with ion-change resin; H + Al = buffer solution at pH 7.0; CEC = cation-exchange capacity; Soil texture = densimeter method. UA = urease activity: urea-N hydrolyzed.

and determined by the diacetyl monoxime method (Mulvaney and Bremner, 1979).

The statistical analyses were conducted separately for each experiment. The data were checked for normal distribution of residuals, submitted to analysis of variance, and one- or two-way pairwise differences of means compared by Tukey ($p < 0.05$) using the SISVAR software package (Ferreira, 2011). The following sigmoid function was fitted to data of cumulative ammonia losses and time according to Soares et al. (2012), using the SigmaPlot software (version 12.5):

$$f = \frac{a}{1 + e^{-\left(\frac{t-t_0}{b}\right)}} \quad (1)$$

where f is the cumulative NH_3 loss (in percentage of applied N), t the time (in days after application), e a constant ("Euler's number"), and a , t_0 , and b the parameters of the equation; a is also the maximum NH_3 loss (in percentage of N applied) and t_0 the time (in days after application) in which 50 % of the losses occur.

Results

In the sandy-clay soil, the UR treatment had a higher peak of NH_3 volatilization at soil pH 4.5 than at 5.6 and 6.4, occurring on the second and third day, respectively (Figure 1A). The treatment with UR+NBPT resulted in a reduced peak of NH_3 volatilization which varied at different soil pH values. Under the more acidic condition, pH 4.5, the volatilization peak occurred on the third day after fertilizer application. In contrast, at pH 5.6 and 6.4, the NH_3 volatilization peak was delayed until the eighth day and was lower than at pH 4.5 (Figure 1A). The UR treatments had cumulative NH_3 losses of 44, 40, and 47 % and UR + NBPT of 36, 19, and 22 % of N applied, at soil pH of 4.5, 5.6, and 6.4, respectively (Figure 1B).

A similar pattern of daily NH_3 volatilization according to soil pH was observed in the clay soil. The peak of NH_3 loss in the treatment with UR was higher and occurred earlier than with UR+NBPT. In addition, in the UR+NBPT treatment, NH_3 volatilization peaked on the third day at soil pH 4.5; however, at soil pH 5.4 and 6.1, the peak was delayed until the ninth day after fertilization (Figure 1C). The total NH_3 volatilized was lower in the clay soil than in the sandy-clay soil. UR had NH_3 losses of 26, 26, and 32 %, and UR+NBPT losses of 21, 18, and 19 % of N applied at soil pH of 4.5, 5.6, and 6.1, respectively (Figure 1D).

In general, increasing the soil pH resulted in increased NH_3 losses from UR. Cumulative NH_3 volatilized from UR applied in sandy-clay soil was higher at soil pH 6.4 than pH 5.6 for all periods but was not different from pH 4.5 at five days and 20 + days (Figure 2A-C). Total NH_3 losses from UR in the clay soil were higher at pH 6.1 than at pH 5.4 and 4.5 at ten days and 20 + days but were not different from pH 4.5 at five days (Figure 2D-F).

The addition of NBPT to UR reduced NH_3 losses in both soils at all soil pH levels examined (Figure 2A-F). However, NBPT was less efficient at soil pH 4.5, and NH_3 losses were higher than in soils with pH > 5.4. Five days after fertilization, NH_3 volatilization from untreated UR was already close to the maximum observed. During this period, NBPT reduced NH_3 losses from UR by > 95 % in both soils at higher soil pH (> 5.4) but decreased only by 30 and 25 % when soil pH was 4.5 in the sandy-clay and clay soils, respectively (Figure 2A and D). At ten days in the sandy-clay soil, UR+NBPT reduced NH_3 volatilized by 78 and 68 % at soil pH 6.4 and 5.6, respectively, but the reduction was 20 % at soil pH 4.5 (Figure 2B); in the clay soil, the reduction at ten days was 60 and 52 % at pH 6.1 and 5.4, respectively, but was 20 % at pH 4.5 (Figure 2E).

At 20 + days, the reduction of NH_3 losses caused by NBPT in the sandy-clay soil at pH 4.5 was only 18 %, compared to those of untreated UR, whereas the losses were reduced by more than 50 % at soil pH 5.6 and 6.4 (Figure 2C). No differences ($p < 0.05$) were observed in total NH_3 volatilized from UR+NBPT in the three soil pH conditions in the clay soil. Compared with UR, NBPT reduced NH_3 volatilized by 19 % at soil pH 4.5 and 31 and 41 % at soil pH 5.4 and 6.1, respectively (Figure 2E). The effectiveness of NBPT in delaying NH_3 losses was affected under acidic conditions (pH 4.5) compared with higher pH in both soils (Table 2). In the sandy-clay soil, 50 % of total NH_3 loss occurred after 9 and 11 days (t_0 in Table 2) at pH 5.6 and 6.4, respectively, but the time was shortened to four days at pH 4.5. In the clay soil, the time when 50 % of total NH_3 occurred was shortened from nine days (pH 5.4 and 6.1) to four days at pH 4.5 (t_0 in Table 2).

Table 2 – Sigmoid equation parameters and regression coefficients (R^2) of cumulative NH_3 losses from urea (UR) treated with urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) applied to sandy-clay and clay soils with different values of pH¹.

| Treatments | Parameters | | | R^2 |
|------------------------|------------|------|-------|--------|
| | a | b | t_0 | |
| Sandy-clay soil | | | | |
| UR pH 4.5 | 41.26 | 0.50 | 1.90 | 0.9172 |
| UR pH 5.6 | 38.93 | 0.88 | 2.82 | 0.9866 |
| UR pH 6.4 | 46.08 | 0.89 | 2.83 | 0.9887 |
| UR + NBPT pH 4.5 | 34.20 | 1.11 | 3.79 | 0.9717 |
| UR + NBPT pH 5.6 | 18.33 | 1.86 | 8.84 | 0.9964 |
| UR + NBPT pH 6.4 | 21.55 | 2.43 | 10.60 | 0.9976 |
| Clay soil | | | | |
| UR pH 4.5 | 24.84 | 1.17 | 3.12 | 0.9451 |
| UR pH 5.4 | 25.11 | 1.45 | 4.63 | 0.9768 |
| UR pH 6.1 | 31.19 | 1.30 | 3.97 | 0.9772 |
| UR + NBPT pH 4.5 | 20.28 | 1.32 | 3.90 | 0.9518 |
| UR + NBPT pH 5.4 | 16.89 | 1.92 | 9.03 | 0.9931 |
| UR + NBPT pH 6.1 | 18.60 | 1.88 | 9.23 | 0.9954 |

¹Parameters = a is the total NH_3 loss, in % of N applied; t_0 is the time that 50 % of the losses occurs in days.

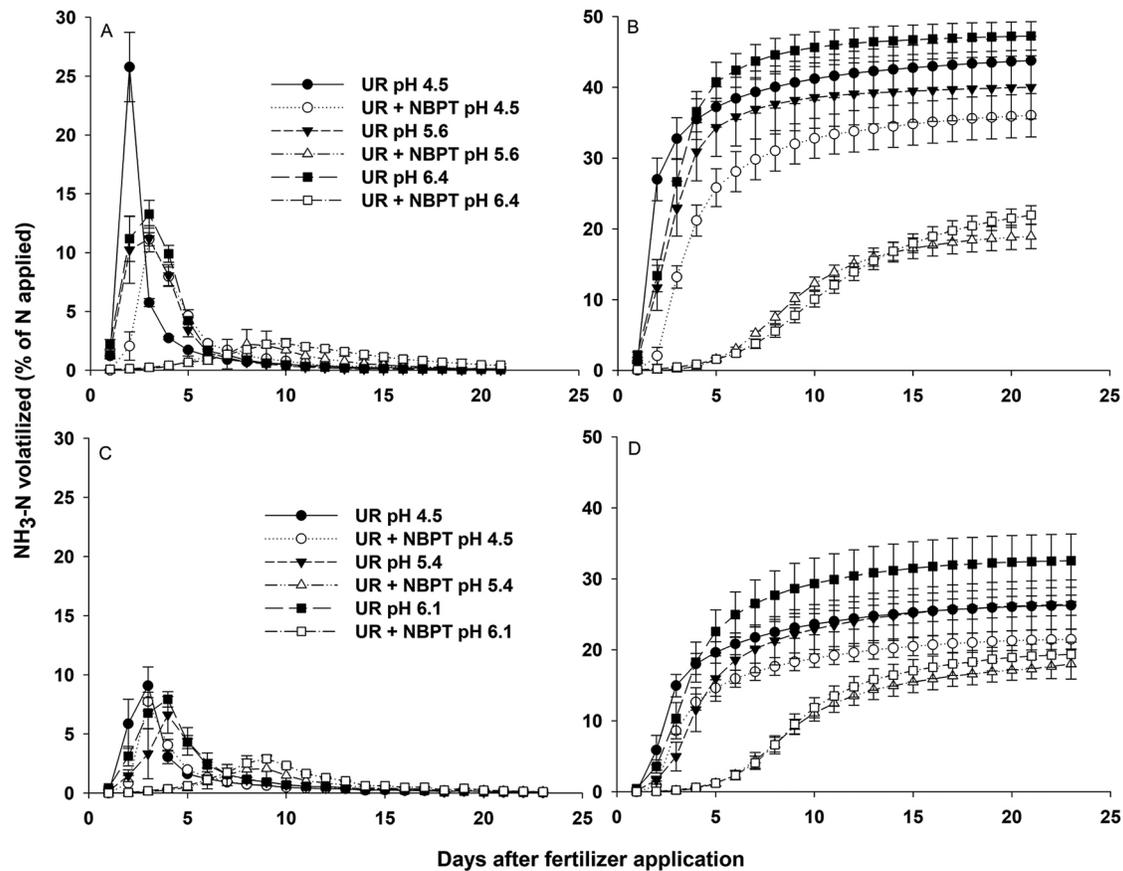


Figure 1 – Daily (A, C) and cumulative (B, D) NH_3 volatilization from urea (UR) treated with urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) applied in sandy-clay (A, B) and in clay soil (C, D) with different pH values. Mean \pm Standard Deviation.

Table 3 – Urease activity in sandy-clay and clay soils with different pH values in the first four days after urea (UR) application¹.

| Treatments | Urease activity | | | |
|-----------------|-------------------------------------|---------------|---------------|----------------|
| | 0 day | 1 day | 2 days | 4 days |
| | mg kg ⁻¹ h ⁻¹ | | | |
| Sandy-clay soil | | | | |
| UR pH 4.5 | 22 \pm 9 ns | 52 \pm 9 a | 44 \pm 6 a | 23 \pm 8 ns |
| UR pH 5.6 | 28 \pm 2 ns | 22 \pm 1 b | 32 \pm 8 ab | 14 \pm 2 ns |
| UR pH 6.4 | 32 \pm 5 ns | 19 \pm 2 b | 22 \pm 3 b | 22 \pm 4 ns |
| Clay soil | | | | |
| UR pH 4.5 | 19 \pm 6 b | 23 \pm ns | 19 \pm 7 ns | 18 \pm 2 ns |
| UR pH 5.4 | 28 \pm 5 ab | 29 \pm 1 ns | 23 \pm 2 ns | 19 \pm 12 ns |
| UR pH 6.1 | 35 \pm 3 a | 33 \pm 7 ns | 26 \pm 6 ns | 20 \pm 8 ns |

¹Urea-N hydrolyzed. Means with the same letter in the column did not differ ($p < 0.05$), ns = not significant. Mean \pm SD.

Urease activity was higher at soil pH 6.1 than at pH 4.5 in the clay soil at time zero; in the sandy-clay soil, no differences were found (Table 3). One and two days after UR application, different responses were found depending on the soil texture; higher urease activity was observed at pH 4.5 than at pH 5.6 and 6.4 in the sandy-clay soil. However, in the clay soil, no differences were

observed (Table 3). Four days after UR application no differences were observed in urease activity according to soil pH (Table 3).

The soil NH_4^+ -N concentrations in the 0-2 cm layer at the end of the NH_3 volatilization experiment were higher in the UR+NBPT than in the UR treatments in both soils at the higher pH values. Nevertheless, they did not differ at pH 4.5 (Table 4). The NO_3^- concentrations were not different for UR and UR+NBPT treatments. Low soil pH resulted in higher NH_4^+ -N and lower NO_3^- -N concentrations than treatments with higher soil pH (Table 4).

Discussion

NH_3 losses from the surface application of UR were of higher magnitude in the coarse soil than in the fine texture soil corroborating other findings in the literature (Sunderlage and Cook, 2018). The clay soil has a higher cation-exchange capacity and total acidity and can retain more ammonium and reduces NH_3 losses compared with coarse soils (Watson et al., 1994; Sunderlage and Cook, 2018).

The effectiveness of NBPT in reducing NH_3 losses from UR was lower in the clay soil than in the sandy-

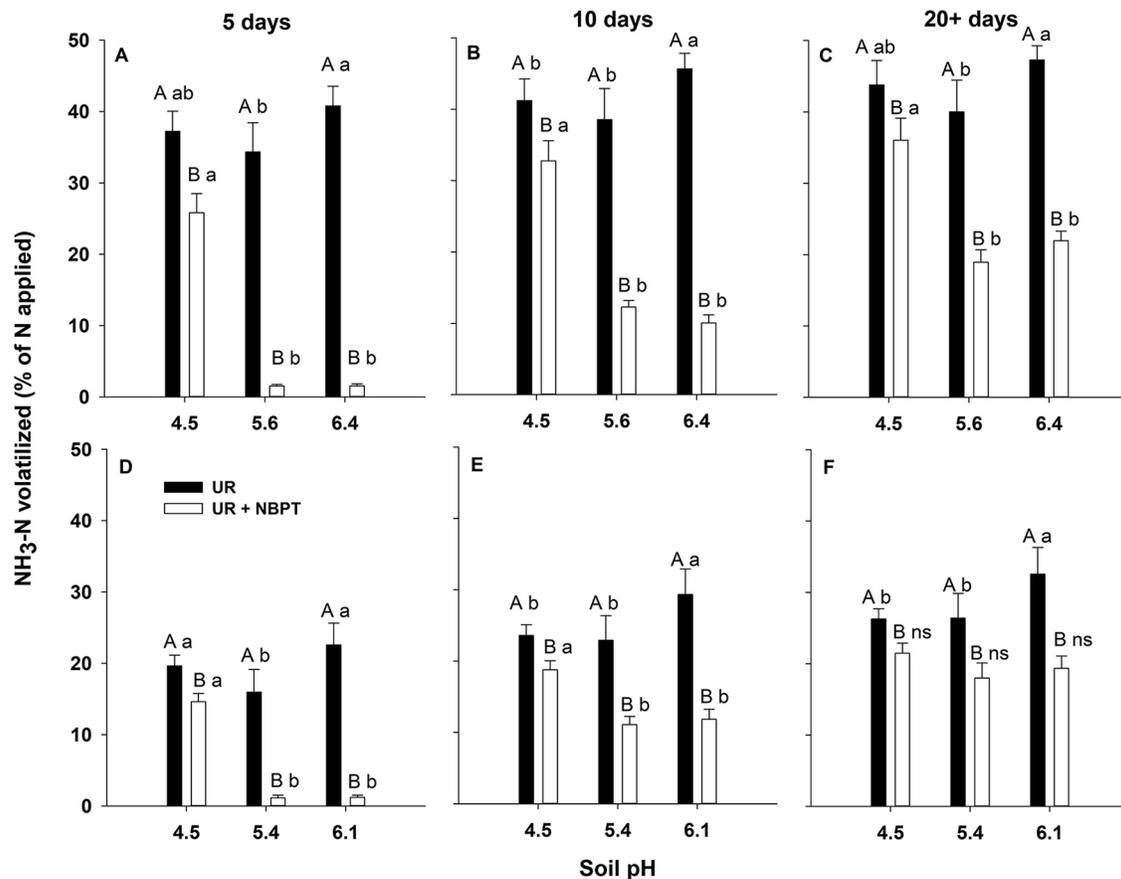


Figure 2 – Partial (5, 10 days) and total NH₃ (20+ days) volatilized from urea (UR) treated with urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) applied in sandy-clay (A-C) and in clay soil (D-F) with different pH values. Error bars represent standard deviations. Uppercase letters above bars compare N sources at each pH level, and lowercase letters compare pH levels in each N source; ns: not significant ($p < 0.05$).

clay. The degradation of NBPT combined with UR diffusion in soils was relevant to this result. In clay soil, NBPT-treated UR may move slower than in sandy-clay soil (Christianson et al., 1993). This, combined with a probably faster degradation of NBPT due to higher OM content (> 2-fold) in the clay soil (Watson et al., 1994), resulted in a lower effectiveness of the inhibitor to reduce NH₃ losses from UR compared with sandy-clay soil.

UR hydrolysis causes a localized increase in soil pH as the reaction consumes H⁺. As NH₃ losses were high in all UR treatments, it is likely that the soil pH had increased in the 0-2 cm soil layer soon after UR application. However, after 20+ days, the soil pH values had declined to 5.0-6.0 (Table 4) mainly due to nitrification (Cantarella et al., 2018). The nitrification process was probably lower under more acidic conditions (pH 4.5) than under higher soil pH (> 5.4), resulting in higher NH₄⁺-N and lower NO₃⁻-N contents. Increasing soil pH changes microbial diversity, increases the abundance of ammonia-oxidizing bacteria, and reduces archaea, resulting in increased nitrification rates (Nicol et al., 2008).

In a study on several soils, increasing soil pH increased NH₃ volatilization after UR application (Watson

et al., 1994). However, the initial soil pH is not always determinant of NH₃ losses (Sunderlage and Cook, 2018). In the present study, NH₃ volatilized in the sandy-clay soil at pH 4.5 did not differ from that at pH 5.6 and 6.4, reaching 47 % of the N applied. The urease activity has a pH optimum which can vary according to soil characteristics (Venkatesan et al., 2007). In the present study, the urease activity after UR application was higher at pH 4.5 than soil pH 5.6 and 6.4 in the sandy-clay soil, like that found by Venkatesan et al. (2007), which showed higher activity at lower pH conditions in certain soils. Therefore, the higher urease activity and low buffer capacity of the sandy-clay soil probably caused high NH₃ volatilization from UR at pH 4.5, which was no different from soil pH 5.6 and 6.4.

The urease inhibitor NBPT added to UR partially lost its effectiveness in reducing NH₃ volatilization in both soils when applied under acidic conditions (pH 4.5) compared with pH > 5.4. After 20 days, NBPT decreased losses by 52-53 % at higher soil pH conditions but only by 18 % at pH 4.5 in the sandy-clay soil. The corresponding loss reductions in the clay soil were 31-41 % and 19 %, respectively. In the clay soil, the effect of NBPT was

Table 4 – Soil pH, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ content in the 0-2 cm soil layer after 20+ days of the NH_3 volatilization losses from urea (UR) treated with urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) applied to sandy-clay and clay soils with different values of pH¹.

| Treatments | pH (CaCl ₂) | mg kg^{-1} | |
|------------------|----------------------------|--------------------------|--------------------------|
| | | $\text{NH}_4^+\text{-N}$ | $\text{NO}_3^-\text{-N}$ |
| Sandy-clay soil | | | |
| UR pH 4.5 | 5.0 ± 0.1 | 284 ± 21 abc | 193 ± 36 b |
| UR pH 5.6 | 5.2 ± 0.3 | 185 ± 49 de | 370 ± 80 a |
| UR pH 6.4 | 5.5 ± 0.2 | 125 ± 34 e | 348 ± 38 a |
| UR + NBPT pH 4.5 | 5.4 ± 0.2 | 357 ± 57 a | 168 ± 47 b |
| UR + NBPT pH 5.6 | 5.4 ± 0.1 | 305 ± 80 ab | 310 ± 18 a |
| UR + NBPT pH 6.4 | 5.9 ± 0.4 | 206 ± 58 cde | 391 ± 71 a |
| Clay soil | | | |
| UR pH 4.5 | 5.3 ± 0.2 | 453 ± 66 a | 128 ± 7 c |
| UR pH 5.4 | 5.5 ± 0.1 | 289 ± 9 b | 203 ± 12 b |
| UR pH 6.1 | 5.7 ± 0.2 | 158 ± 23 c | 286 ± 31 a |
| UR + NBPT pH 4.5 | 5.2 ± 0.1 | 449 ± 44 a | 129 ± 4 c |
| UR + NBPT pH 5.4 | 5.8 ± 0.2 | 405 ± 74 a | 192 ± 30 b |
| UR + NBPT pH 6.1 | 6.0 ± 0.2 | 270 ± 44 b | 282 ± 23 a |

¹Means with the same letter in the column did not differ ($p < 0.05$). Mean ± SD. N content from soil control was subtracted = 9 mg kg^{-1} of $\text{NH}_4^+\text{-N}$ and 26 mg kg^{-1} of $\text{NO}_3^-\text{-N}$ in the sandy-clay soil; 9 mg kg^{-1} of $\text{NH}_4^+\text{-N}$ 56 mg kg^{-1} of $\text{NO}_3^-\text{-N}$ in the clay soil. In control plots (soil pH 5.4 and 5.6) soil pH was 5.2 for both soils.

little affected by soil pH at the end of the experiment. However, soon after fertilization, the low soil (pH 4.5) strongly influenced the inhibitor's action; for example, at five days, NBPT reduced NH_3 loss from UR by > 95 % in both soils at pH > 5.4, but under acidic conditions (pH 4.5), the NBPT declined by only 20-30 %. The fast NH_3 loss in UR+NBPT treatments in acid soils not only reduces the effectiveness of the urease inhibitor but also decreases the time span for management options to incorporate UR into the soil such as irrigation.

Studies of different soils have reported a negative correlation between soil pH and cumulative NH_3 volatilization from UR+NBPT, suggesting a lower inhibitor efficiency in soils at low pH (Watson et al., 1994; San Francisco et al., 2011; Sunderlage and Cook, 2018). The benefits of NBPT in reducing NH_3 volatilization can be partially explained by the extra time that the inhibitor provides for UR to diffuse into the soil before UR is hydrolyzed (Dawar et al., 2011; Cantarella et al., 2018). The rate of UR diffusion into the soil combined with the lower stability of NBPT in acidic soils (Engel et al., 2015; Sunderlage and Cook, 2018) helps to explain our results.

In temperate soils, the protection of NBPT in UR lasted 2-3 weeks in acidic soils (pH 5.5-6.4) and more than seven weeks in alkaline soils (pH 6.5-8.4) (Engel et al., 2011). Our laboratory conditions that mimic the relatively high temperature of tropical soils resulted in faster reactions (< one week) than those observed by Engel et al. (2011) (temperate climate and field conditions). To our knowledge, this is the first study to

evaluate the effectiveness of NBPT according to soil pH exclusively in tropical soils, with detailed observations of the NH_3 volatilization dynamics, showing how acidic soils reduce the ability of NBPT to delay and decrease the NH_3 losses from UR.

The differences between temperate and tropical conditions can change the stability, longevity, and efficacy of NBPT (Cantarella et al., 2018). The degradation of NBPT after nine months of storage was much less at 4 °C (66 % recovered) than at 25 °C (19 % recovered) (Watson et al., 2008). In two regions in Brazil, NBPT-treated urea preserved part of its inhibitor effectiveness for up to nine months of storage under mild temperature exposure. However, the inhibitory effectiveness decreased under hotter conditions (Cantarella et al., 2018). When applied to soils, NBPT degrades rapidly, depending on soil properties. In tropical soils at high temperatures, NBPT degradation can start 2-4 days after application (Soares et al., 2012), but under temperate conditions, it can take 10-15 days (Watson et al., 2008). Therefore, understanding how climate, management, and soil conditions affect NBPT effectiveness can help to optimize the use of the inhibitor in reducing NH_3 losses from urea.

In our study, the detailed measurements of NH_3 losses in soils with different textures and acidic conditions allowed us to determine not only the effect of soil pH on the efficacy of NBPT to reduce losses but, more importantly, to establish the period in which NBPT remains effective at different soil pH values. This information has practical implications for fertilizer management. The period that NBPT effectively reduced NH_3 losses was shortened at low soil pH compared with higher pH (Table 2). It took 8-11 days for 50 % of total NH_3 loss to occur for soil with pH values above 5.4 (t_0 in Table 2), but the time was reduced to four days at lower pH, a period similar to untreated UR; that is to say, NBPT reduced losses for a much shorter time in acidic soils. Probably, the lower efficacy of NBPT occurred because its analogue NBPTO degrades faster at low soil pH (Engel et al., 2015), resulting in faster UR hydrolysis (Fan et al., 2018; Lasisi et al., 2020) and lower effectiveness in reducing NH_3 losses in acidic soils (Suter et al., 2011; Mira et al., 2017).

In the present study, the inhibitor reduced NH_3 losses from UR in all situations but was significantly less effective at low soil pH (4.5), regardless of soil texture. Soils with pH < 5.0 are common in tropical regions (Lopes and Guilherme, 2016), in which the inhibitor NBPT can result in lower efficiency in reducing NH_3 losses from UR. One consequence of the high NH_3 losses of NBPT-treated UR in acidic soils in the first four days is that NBPT appears to give only a small advantage over untreated UR if strategies such as fertilizer incorporation by rain, irrigation, or mechanical means are used to reduce NH_3 losses (Fontoura and Bayer, 2010; Viero et al., 2015). This finding has practical implications, which, to our knowledge, had not been raised in previous studies.

Better fertilizer formulations may be necessary if more substantial effectiveness of the inhibitor in acidic soils is to be achieved. New products are already available in the market to boost NBPT performance, such as its combination with n-(n-propyl) thiophosphoric triamide (NPPT) and duromide (Cassim et al., 2021; Klimczyk et al., 2021), as well as other urease inhibitors such as N-(2-nitrophenyl) phosphoric triamide (2-NPT) (Cantarella et al., 2018; Klimczyk et al., 2021) or controlled release fertilizer (Mariano et al., 2019). Despite promising results with these combinations/new products in reducing NH_3 losses from UR application (Schraml et al., 2016; Krol et al., 2020; Cassim et al., 2021), more studies are necessary to evaluate them in a comparison with NBPT alone, especially in situations that affect the inhibitor, such as acidic soils.

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Authors' Contributions

Conceptualization: Cantarella, H. **Data curation:** Soares, J.R. **Formal analysis:** Soares, J.R. **Funding acquisition:** Cantarella, H. **Investigation:** Soares, J.R.; Cantarella, H. **Methodology:** Cantarella, H. **Project administration:** Soares, J.R.; Cantarella, H. **Resources:** Cantarella, H. **Supervision:** Cantarella, H. **Writing-original draft:** Soares, J.R. **Writing-review & editing:** Soares, J.R.; Cantarella, H.

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