



## An inhibitor of urease activity effectively reduces ammonia emissions from soil treated with urea under Mediterranean conditions

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Received 25 July 2007; received in revised form 1 February 2008; accepted 4 February 2008

### Abstract

Urea is an important source of ammonia (NH<sub>3</sub>) emissions to the atmosphere from agricultural soils. Abatement strategies are necessary in order to achieve NH<sub>3</sub> emission targets by reducing those emissions. In this context, a field experiment was carried out on a sunflower crop in spring 2006 with the aim of evaluating the effect of the *N*-(*n*-butyl) thiophosphoric triamide (NBPT) in the mitigation of volatilized NH<sub>3</sub> from a urea-fertilised soil. Ammonia emission was quantified, using the integrated horizontal flux (IHF) method, following application of urea with and without the urease inhibitor NBPT. Urea and a mixture of urea and NBPT (0.14%, w/w) were surface-applied at a rate of 170 kg N ha<sup>-1</sup> to circular plots (diameter 40 m). The soil was irrigated with 10 mm of water just after the application of urea to dissolve and incorporate it into the upper layer of soil. Over the duration of the measurement period (36 days) three peaks of NH<sub>3</sub> were observed. The first peak was associated with hydrolysis of urea after irrigation and the others with the increase of ammonia in soil solution after changes in atmospheric variables such as wind speed and rainfall. The total NH<sub>3</sub> emission during the whole experiment was 17.3 ± 0.5 kg NH<sub>3</sub>-N ha<sup>-1</sup> in the case of urea treated soils and 10.0 ± 2.2 kg NH<sub>3</sub>-N ha<sup>-1</sup> where NBPT was included with the urea (10.1 and 5.9%, respectively, of the applied urea-N). The lower NH<sub>3</sub> emissions from plots fertilised with urea + NBPT, compared with urea alone, were associated with a reduction in urease activity during the first 9 days after inhibitor application. This reduction in enzymatic activity promoted a decrease in the exchangeable NH<sub>4</sub><sup>+</sup> pool.

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**Keywords:** Ammonia volatilization; Urease; NBPT; Urea; Mediterranean climate

### 1. Introduction

Urea (U) is the predominant source of inorganic N fertiliser used in agriculture throughout the world, accounting for 50% of the total world fertiliser N consumption. However, the efficiency of urea is decreased by losses of N

as ammonia gas (NH<sub>3</sub>) after its hydrolysis in soil by reaction with the enzyme urease (Harrison and Webb, 2001). As well as the economical impact for farmers (Van der Stelt et al., 2005), NH<sub>3</sub> lost to the atmosphere from applied urea will subsequently be deposited to land or water causing eutrophication and acidification of natural ecosystems on a regional scale (Sommer and Hutchings, 2001). Therefore, the United Nations have included NH<sub>3</sub> in the Convention on Long-Range Transboundary Air Pollution, and the EU Commission has set a target to the emission of NH<sub>3</sub> from European countries (EEA, 1999). To get a reduction for NH<sub>3</sub> emissions in these countries, abatement strategies in

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agriculture are necessary (Webb et al., 2005). Some of these measures are based on incorporating the fertiliser into the soil, favouring the rapid adsorption of ammonium ( $\text{NH}_4^+$ ) from solution to soil colloid (Sommer et al., 2004).

The use of urease inhibitors is also a possible way to reduce  $\text{NH}_3$  emissions to the atmosphere after urea fertiliser application, especially when urea is surface-applied (Wang et al., 1995; Grant et al., 1996; Grant and Bailey, 1999). These compounds can retard the hydrolysis of urea by inhibiting the urease enzyme in the soil (Gill et al., 1997). Slowing urea hydrolysis would allow more time for urea to diffuse onto the soil from the application point and would hence reduce the concentration of  $\text{NH}_4^+$  present in the soil solution and the potential for  $\text{NH}_3$  volatilization (Grant et al., 1996). Among the various types of urease inhibitors which have been identified and tested, *N*-(*n*-butyl) thiophosphoric triamide (NBPT) has been found significantly effective at relatively low concentrations under laboratory conditions (Carmona et al., 1990; Gill et al., 1997). This inhibitor forms stable complexes with urease and is among the most efficient inhibitors of this enzyme after the conversion to its oxidised form (NBPTO) in soil (McCarty et al., 1989; Manunza et al., 1999). As urease is an enzyme present in a wide variety of fungi and bacterial species of soil, the efficiency of NBPT to decrease urease activity may depend on its diffusion from the application point and the concentration of inhibitor maintained in microsites. Under field conditions, the inhibitor is normally applied by including it in granules of urea, which may limit the proportion of microsites where the enzyme is inhibited. However, information on the effect of NBPT on urease activity and its possible influence in the N volatilization and exchangeable N pool is very limited, especially under field conditions.

Quantification of  $\text{NH}_3$  emissions is essential as a first step for the compliance with international protocols on atmospheric pollution. To date, most of the  $\text{NH}_3$  measurements under field conditions have been carried out in West Europe and USA. From these studies, losses of the urea–N applied ranged from 0 to 50% (Sommer et al., 2004), mainly depending on climatic and soil conditions, agronomic practices and management of fertiliser application (Malhi et al., 2001). Additionally, much of this variability has been ascribed to the various techniques employed in measuring ammonia emission (Fillery et al., 1984). There are a number of methodologies which can be used to determine  $\text{NH}_3$  fluxes between fertilised areas and the atmosphere. Micrometeorological methods, such as the mass-balance integrated horizontal flux (IHF) (Misselbrook et al., 2005), are preferred since they are sensitive to changes in the environmental conditions, do not disturb the natural environment where the measurements are being performed and they provide a measure of the average flux density of a large area (Leuning et al., 1985; Ferguson et al., 1988). Additionally, cumulative emission can be calculated with this method, based on continuous or semi-continuous measurements over an entire period.

There are relatively few data relating to Mediterranean areas (none using micrometeorological measurement techniques), where fertilisation is often carried out under high soil temperature. These conditions favour  $\text{NH}_3$  volatilization (Carmona et al., 1990), which increases rapidly during a short period of time after fertiliser application. These conditions also favour nitrification, which, in contrast, would limit volatilization as the soluble and exchangeable N pools are rapidly diminished (Sánchez-Martín et al., 2007). Additionally, it has been shown that the effectiveness of NBPT on reducing  $\text{NH}_3$  emissions is decreased under warm temperatures (Carmona et al., 1990). However, we hypothesise that, even under warm temperatures and favourable conditions for nitrification, the use of the urease inhibitor NBPT would significantly reduce  $\text{NH}_3$  emissions from urea-fertilised soils. With the aim of evaluating the effect of the NBPT in the mitigation of  $\text{NH}_3$  volatilization from urea-fertilised soil, a field experiment in an arable soil was carried out for an irrigated sunflower crop. As atmospheric conditions may affect  $\text{NH}_3$  emission rates, the IHF micrometeorological method was used.

## 2. Materials and methods

### 2.1. Experimental location

The study was carried out in a Calcaric Fluvisol at “La Poveda” field station in Madrid ( $40^\circ 18' 14''\text{N}$ ;  $3^\circ 25' 57''\text{W}$ ) on a sunflower crop in spring 2006. Some relevant characteristics (0–20 cm) were: total organic matter, 1.4%;  $\text{pH}_{\text{H}_2\text{O}}$ , 8.1; bulk density,  $1.47 \text{ Mg m}^{-3}$ ;  $\text{CaCO}_3$ , 3.4%; field capacity, 20.2% (w/w); porosity, 46%; sand, 37%; silt, 50% and clay, 13%. The dominant clay mineral was vermiculite. The average annual temperature and rainfall (over the last 10 years) in this area were  $13.5^\circ\text{C}$  and 460 mm, respectively.

### 2.2. Experimental procedure

Four circular plots ( $r = 20 \text{ m}$ ) were selected in the experimental area on 6th May 2006. There were no wind-disturbing elements, e.g., trees, houses or other protruding landscape elements; within 150 m. Sunflower (cv. San Jose) was sown at a rate of  $4 \text{ kg ha}^{-1}$  on 10th May. Sunflower is becoming increasingly important as a source of oil both for food and biodiesel in Mediterranean areas. The seeds were mechanically and firmly pressed into the soil at 3 cm depth in lateral rows, spaced 80 cm apart. Fertilisers were applied by hand on 24th May at a rate of  $170 \text{ kg N ha}^{-1}$ . This rate is towards the upper end of the range applied by farmers in this area under irrigation conditions ( $120\text{--}200 \text{ kg N ha}^{-1}$ ), chosen deliberately to increase the probability of detecting a treatment effect. Fertiliser treatments were: urea, used in a granular form, and urea mixed within the urease activity inhibitor NBPT (U + NBPT), also in a granular form. The

proportion of inhibitor in the mixture was 0.14% of the dry weight of urea, and it was prepared by Fertiberia S.A. (Huelva, Spain) 10 days prior to application. Plots were irrigated with 10 mm of water immediately after application of the two treatments with the aim of dissolving and incorporating the fertiliser granules into the first layer of soil. A mobile sprinkler irrigation system was used to apply the water homogeneously.

Wind speed, wind direction, rainfall as well as atmospheric and soil temperature data were collected by means of a weather station and data logger (Davis Instruments, California, EEUU) located in the experimental field. The station was equipped with three anemometers at heights 0.30, 0.80 and 1.25 m. Soil temperature was measured using a temperature probe inserted 10 cm into the soil. All data were monitored every 10 min.

### 2.3. Ammonia measurement

The ammonia measurement technique was the micrometeorological mass-balance integrated horizontal flux technique. This method equates the vertical flux of  $\text{NH}_3$  from a treated area of limited upwind extent with the net integrated horizontal flux at a known downwind distance (Misselbrook et al., 2005). In this study, one central mast, equipped with five passive flux samplers (PFS) at heights of 0.25, 0.65, 1.2, 2.05 and 3.05 m above ground, was placed in the centre of each plot. Two further masts, equipped with three PFS at heights of 0.25, 1.25 and 3.05 m were placed upwind of the treated area. Samplers were prepared as described by Leuning et al. (1985), coated internally with oxalic acid. Passive flux samplers were removed and replaced on the masts every 24 h during the 1st week, every 48 h during the 2nd week, and every 3 days in the following weeks. At the end of each sampling period and immediately following exposure, the PFS were carried to the laboratory in order to extract the samples of ammonium oxalate. The PFS were leached with 40 ml of deionised water and the solution was analysed for  $\text{NH}_4^+\text{-N}$  by spectrophotometry using the nitroprussiate method (Searle, 1984).

Net horizontal flux of  $\text{NH}_3\text{-N}$  ( $F$ ,  $\mu\text{g m}^{-2} \text{s}^{-1}$ ) was obtained by mean of the following equation (Leuning et al., 1985; Misselbrook et al., 2005):

$$F = \frac{1}{x} \left[ \int_0^z (\overline{uc})_{\text{dw}} dz - \int_0^z (\overline{uc})_{\text{uw}} dz \right]$$

The mean horizontal flux,  $\overline{uc}$  ( $\mu\text{g m}^{-2} \text{s}^{-1}$ ), at each height was calculated from

$$\overline{uc} = \frac{M}{A}$$

where  $M$  is the mass of  $\text{NH}_3\text{-N}$  collected ( $\mu\text{g}$ ) in the sampler during sampling period and  $A$  the effective cross-sectional area of the sampler ( $\text{m}^2$ ) as determined in wind tunnel calibrations. The mean fetch length ( $x$ ) equated to the radius

of the treated circle (20 m). As samplers were continuously replaced during the experiment, the IHF method allowed us to calculate cumulative  $\text{NH}_3$  emission over an entire period as the sum of  $\text{NH}_3$  volatilized at each measurement interval. Flux rates were converted to  $\text{g ha}^{-1} \text{h}^{-1}$  for reporting.

As there were high ambient temperatures during the whole experiment (maximum temperature was  $29.8^\circ\text{C}$  and average temperature was  $26.7^\circ\text{C}$ ), the effect of high temperature on the volatilization of oxalic acid and ammonium oxalate from the inner part of the PFS was tested in a laboratory experiment. Thirty grams of both ammonium oxalate and oxalic acid were placed into an oven at a temperature of  $80^\circ\text{C}$  for 8 h. From these experiments, no significant evidence of volatilization was observed.

### 2.4. Analysis of soil

Soil samples (0–10 cm) were collected several times over the duration of the experiment in order to analyse changes in soil moisture, soil nitrate ( $\text{NO}_3^-\text{-N}$ ) and ammonium ( $\text{NH}_4^+\text{-N}$ ) concentration as well as urease activity. Both soil  $\text{NO}_3^-\text{-N}$  and  $\text{NH}_4^+\text{-N}$  were extracted by using 8 g of fresh soil with 60 ml of distilled water and 50 ml 1 M KCl, respectively. Soil  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  were determined colorimetrically using a Technicon AAII Auto-analyser (Technicon Hispania, Spain). Urease activity was determined by following Nannipieri's method (Nannipieri et al., 1980), involving the incubation of soil with buffered urea solution, the extraction of  $\text{NH}_4^+$  with 1N KCl and colorimetric  $\text{NH}_4^+$  determination.

## 3. Results and discussion

### 3.1. Environmental conditions

Average wind speed at a height of 1.25 m ranged between 0.7 and  $2.4 \text{ m s}^{-1}$  during the measurement period (Fig. 1a). Soil temperature ranged between 20 and  $30^\circ\text{C}$  (Fig. 1b). Two rainfall events of 5 and 7 mm were recorded on 9th and 15th June, respectively (Fig. 1c). At the beginning of the experiment, following irrigation, soil moisture was 14.6% (w/w) (Fig. 1d). This decreased to 8.5% (w/w) by the 5th day of the experiment (29th May). The two rainfall events produced a significant, but short-lived increase in soil moisture (by 12.6, and 14.0% respectively) (Fig. 1d).

### 3.2. Urease activity and mineral N

There was a significant treatment effect on urease activity in the initial days following fertiliser application. Urease activity remained almost constant until the 15th day ( $8 \mu\text{mol NH}_3 \text{ g}^{-1} \text{h}^{-1}$ ) for U, but declining sharply on the 4th day after application ( $<1 \mu\text{mol NH}_3 \text{ g}^{-1} \text{h}^{-1}$ ) for U + NBPT. This important decrease in urease activity was indicative of a high percentage of NBPT diffusing to the soil

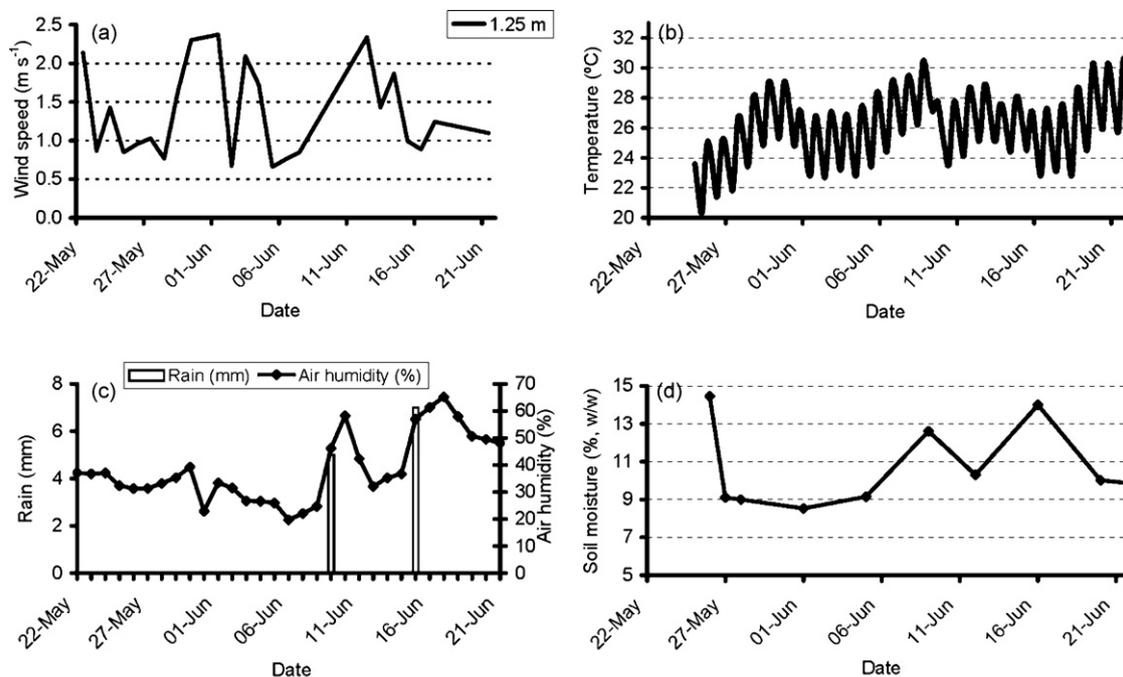


Fig. 1. Wind speed ( $\text{m s}^{-1}$ ) (a), daily mean soil temperature ( $^{\circ}\text{C}$ ) (b), rainfall (mm) and air humidity (%) (c) and soil moisture (w/w) (d) during the experimental period in the upper soil layer (0–10 cm).

microsites and an important concentration of the active form, NBPTO, in the upper part of soil. It is probable that the addition of water, through irrigation, on the day after fertiliser application facilitated the diffusion of the inhibitor. Inhibition of urease activity was maintained until 9 days after application of NBPT (Fig. 2).

The inclusion rate of NBPT in the fertiliser (0.14%, w/w) was sufficient to inhibit urease activity. Carmona et al. (1990) and Watson et al. (1994), using the amount of volatilized  $\text{NH}_3$  as criteria for evaluating the inhibitor efficiency, considered this concentration sufficient for NBPT in temperate climates. A direct comparison of this study with other experiments has not been possible because, to our knowledge, there have been no studies evaluating the effect of NBPT directly on urease activity under field conditions.

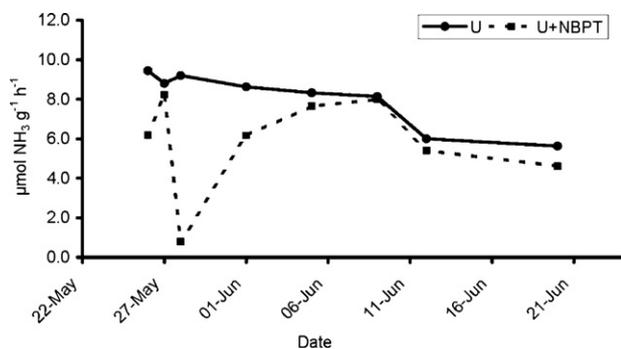


Fig. 2. Urease activity ( $\mu\text{mol NH}_3 \text{ g}^{-1} \text{ h}^{-1}$ ) in the 0–10 cm soil layer during the measurement period. Standard errors were lower than  $0.3 \mu\text{mol NH}_3 \text{ g}^{-1} \text{ h}^{-1}$  in all cases.

As a result of the irrigation, urea was rapidly dissolved and incorporated into the 0–10 cm upper soil layer. Under these warm experimental conditions (average temperature  $26.7^{\circ}\text{C}$ ), hydrolysis of urea occurred rapidly and, therefore, the highest  $\text{NH}_4^+$  concentration ( $19.7 \text{ mg NH}_4^+\text{-N kg}^{-1}$ ) was observed on the 2nd day after fertiliser application (Fig. 3a). The NBPT delayed, but did not eliminate, the hydrolysis of urea, with the highest  $\text{NH}_4^+$  concentration ( $10.1 \text{ mg NH}_4^+\text{-N kg}^{-1}$ ) observed on the 3rd day for this treatment. Thereafter, the  $\text{NH}_4^+\text{-N}$  concentration declined slowly to 0.8 and  $1.9 \text{ mg NH}_4^+\text{-N kg}^{-1}$ , respectively for U and U + NBPT 27 days after fertiliser application. This was probably because soil conditions favoured nitrification. This suggestion is supported by the rapid evolution of  $\text{NO}_3^-\text{-N}$  in soil, with  $\text{NO}_3^-\text{-N}$  content in the upper layer of soil increasing in both treatments during the 1st week after the beginning of the experiment (Fig. 3b).

The observed decrease of  $\text{NH}_4^+$  may also have partly been caused due to its fixation in the clay interlayers. Vermiculite, a 2:1 expanding clay mineral, was the dominant form of clay in this soil and would have favoured the rapid interlayer fixation of  $\text{NH}_4^+$  (Brady and Weil, 2002). The fixation could also have been promoted in the period when soil was drying, because the reduction of the interlayer space of the vermiculite during drying periods favours fixation (Gouveia and Eudoxie, 2007). A fixation of N of  $>35\%$  of the N applied in soil with 2:1 expanding clays is in accordance with several authors (Scherer and Mengel, 1986; Elmaci et al., 2002).

The presence of NBPT had a positive effect in the maintenance of  $\text{NO}_3^-\text{-N}$  in soil as a higher  $\text{NO}_3^-\text{-N}$

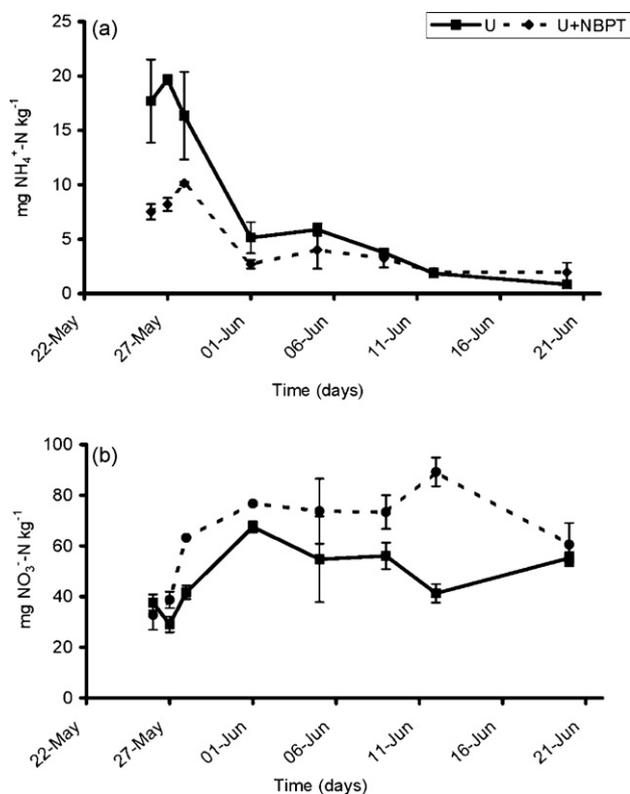


Fig. 3.  $\text{NH}_4^+\text{-N}$  (a) and  $\text{NO}_3^-\text{-N}$  (b) concentrations in the 0–10 cm soil layer during the measuring period. Vertical lines indicate standard errors.

concentration was observed for U + NBPT than for U over the first 3 weeks. Consequently, higher maximum values of  $\text{NO}_3^-\text{-N}$  were observed for U + NBPT ( $89.2 \text{ mg NO}_3^-\text{-N kg}^{-1}$ ) than for U ( $67.5 \text{ mg NO}_3^-\text{-N kg}^{-1}$ ) (Fig. 3b). Possibly the slow hydrolysis of urea in the period during which the urease activity was partly inhibited, produced a slower release of  $\text{NH}_4^+$  to the soil solution, reducing the  $\text{NH}_4^+$  fixation (Nommik, 1981; Kissel et al., 2004; Gouveia and Eudoxie, 2007) and  $\text{NH}_3$  volatilization resulting in more  $\text{NO}_3^-$  formation than in the case of urea only treated soils.

### 3.3. Ammonia emission from urea treated plots

Irrigation after fertiliser application is considered an abatement strategy to mitigate  $\text{NH}_3$  volatilization (Sommer et al., 2004). Our results support this hypothesis as the highest volatilization rate for treatment U in the days following irrigation (4th day) was  $36 \text{ g N ha}^{-1} \text{ h}^{-1}$  (Fig. 4a), which was lower than the result noticed by other authors such as Sainz et al. (1999) who measured a maximum of  $335 \text{ g N ha}^{-1} \text{ h}^{-1}$  in a Typic Argiudoll treated with urea at a rate of  $140 \text{ kg N ha}^{-1}$ . After irrigation, high temperatures (maximum  $29.8 \text{ }^\circ\text{C}$ ) promoted water evaporation and, under such conditions, more  $\text{NH}_4^+$  was probably fixed by vermiculite (Brady and Weil, 2002). The high nitrification rate of this soil also contributed to decrease the  $\text{NH}_4^+$  present in the soil solution and, thereafter,  $\text{NH}_3$  volatilization rates dropped to less than  $10 \text{ g N ha}^{-1} \text{ h}^{-1}$  (Fig. 4a). Ammonia

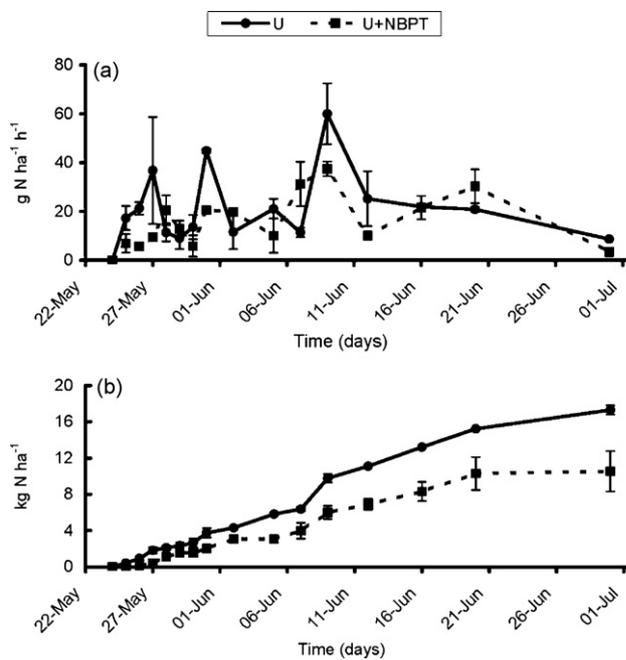


Fig. 4. Ammonia emission rates (a) and cumulative emission (b) after application of urea (U) with or without urease inhibitor (NBPT). Immediately after fertiliser application, crop was irrigated at a rate of 10 mm. Vertical lines indicate standard errors.

volatilization peaked again after changes in atmospheric conditions. The second peak ( $44.8 \text{ g N ha}^{-1} \text{ h}^{-1}$ ), occurred on the 1st of June, could have been associated to an increase in wind speed (average  $2.5 \text{ m s}^{-1}$ ) (Fig. 1a). The source of this  $\text{NH}_3$  was mainly the exchangeable  $\text{NH}_4^+$  present in soil at this day ( $6\text{--}7 \text{ mg N kg}^{-1}$ ), which was in equilibrium with soil solution. A higher wind speed than in previous days, increased the pressure gradient of  $\text{NH}_3$  between soil and atmosphere enhancing the  $\text{NH}_3$  volatilization (Ferguson et al., 1988; Cabrera et al., 2001). Finally, ammonia emission peaked again ( $60 \text{ g N ha}^{-1} \text{ h}^{-1}$ ) on 9th June in response to both high wind speeds ( $2.5 \text{ m s}^{-1}$ ) and rainfall (5 mm) (Fig. 4a).

The total  $\text{NH}_3$  emission over the measuring period (36 days) was  $17.3 \pm 0.5 \text{ kg NH}_3\text{-N ha}^{-1}$ , representing 10.1% of the total N applied as urea. This value was lower than those found by other authors (Grant et al., 1996; Sainz et al., 1999; Kissel et al., 2004; Pacholski et al., 2006) for this fertiliser type. For example, Grant et al. (1996) reported cumulative losses of 38% of applied N in a fine sandy loam soil (Orthic Chernozem). The default emission factor given in the CORINAIR Emission Inventory Guidebook for a region with spring temperatures  $>13 \text{ }^\circ\text{C}$  is 20% of the applied N (CORINAIR, 2006). This reference value is normally used for arable soils under Mediterranean climate as there are very few results of  $\text{NH}_3$  losses measured under field conditions (Rana and Mastroianni, 1998), particularly using micrometeorological methods. However, our results demonstrate that a lower  $\text{NH}_3$  emission from urea treated soils is possible.

### 3.4. Effect of NBPT on NH<sub>3</sub> volatilization

The use of the urease inhibitor NBPT has reduced NH<sub>3</sub> volatilization. The inhibitor was effective in maintaining a lower exchangeable NH<sub>4</sub><sup>+</sup> over the first 9 days (Fig. 3a); during which NH<sub>3</sub> emissions were lower than in those soils where only urea was applied. In this way, the first peak of NH<sub>3</sub> (20.5 g N ha<sup>-1</sup> h<sup>-1</sup>) produced 3–4 days after fertiliser application was reduced by 45% due to the presence of the NBPT (Fig. 4a). However, there was not a total efficacy of the NBPT on retarding hydrolysis of urea and, therefore, reducing NH<sub>3</sub> emissions. The distribution of the inhibitor in the soil and the probability of forming the urease–NBPTO complex must be considered as two of the main limiting factors on the effectiveness of the NBPT as a way of abating NH<sub>3</sub> emissions (Christianson et al., 1990). Ammonia fluxes were also lower for U + NBPT for subsequent peak emission rates (Fig. 4a) with reductions of 54% (1st June) and 36% (9th June). The effect of the inhibitor on volatilized NH<sub>3</sub> was a consequence of its influence on soil N pools (soluble, exchangeable and fixed NH<sub>4</sub><sup>+</sup>–N), occurring as a result of the reduction of urease activity during the first days after application. The higher exchangeable NH<sub>4</sub><sup>+</sup> content maintained until the 1st of June in the U treated soils explained higher volatilization fluxes, promoted by increasing wind speed. However, by the 9th June there were no differences in the exchangeable NH<sub>4</sub><sup>+</sup> content of the soil for U and U + NBPT, and differences in NH<sub>3</sub> volatilization may be associated with differences in the fixed NH<sub>4</sub><sup>+</sup>–N. Probably, the proportion of fixed NH<sub>4</sub><sup>+</sup>–N was higher at this date for U, because a higher release of NH<sub>4</sub><sup>+</sup> to the soil solution from hydrolysis of urea during the first days temporarily increased the NH<sub>4</sub><sup>+</sup> fixation (Nommik, 1981; Gouveia and Eudoxie, 2007). Although this N pool was not quantified, the unaccounted for N was higher for U (47% of N applied) than for U + NBPT (34%) on 9th June. If a micrometeorological method had not been used, important differences in NH<sub>3</sub> emissions between treatments would probably not have been detected, as the most important differences occurred under atmospheric conditions characterised by a high wind speed and rainfall.

Ammonia emissions from the urea U + NBPT treated plots were 10.0 ± 2.2 kg NH<sub>3</sub>–N ha<sup>-1</sup>, 42% lower than the plots where the inhibitor was not applied. These results are similar to the observed by Grant et al. (1996), who noticed, in an Orthic Chernozem, a reduction of 36% in the emitted NH<sub>3</sub>. Other authors (Carmona et al., 1990; Christianson et al., 1990; Antisari et al., 1996) observed, under laboratory conditions, reductions of 51, 39 and 22%, respectively, after fertilising with a mixture of urea and NBPT (0.1%).

In the case of Mediterranean countries, such as Spain, a substantial reduction in NH<sub>3</sub> emissions must be achieved in order to comply with the emissions targets established through the UNECE Gothenburg Treaty (UNECE, 1999). Since the majority of those NH<sub>3</sub> emissions come from the agriculture sector, the results of this study suggest that the

use of urease inhibitors such as the NBPT could be considered as a good abatement strategy towards achieving those emission reduction requirements.

## 4. Conclusions

This study has demonstrated that urease inhibitors, such as NBPT, represent a mitigation measure of NH<sub>3</sub> volatilization from arable soils fertilised with urea. Besides, the incorporation of urea into the soil by irrigation has been an effective way for reducing NH<sub>3</sub> losses under high temperature conditions. Volatilization losses from urea-fertilised soil, measured with the mass-balance method (IHF), were lower than under other climatic conditions despite the high temperatures during the measuring period, mainly because the effect of management practices, environmental and soil characteristics. The use of the NBPT reduced urease activity for 9 days. This resulted in a reduction in cumulative NH<sub>3</sub> emission of 42% through the slower release of NH<sub>4</sub><sup>+</sup> to the soil solution from the hydrolysis of urea, which also promoted a lower exchangeable N pool. More studies under semiarid conditions are necessary to confirm these results and to study the influence of urease inhibitors on NH<sub>3</sub> volatilization, from both non-irrigated and irrigated crops fertilised with urea.

## Acknowledgements

The authors are grateful to the Autonomous Community of Madrid and Fertiberia S.A. for financing this research and proving us with the fertilisers and the inhibitor. It is a pleasure to acknowledge Pilar López, Ana Ros, Pedro Hernaiz and Susana Fernández for their assistance on both laboratory and field work. We also want to thank Mark Theobald for his helpful comments in the preparation of this article.

## References

- Antisari, L.V., Marzadori, C., Gioacchini, P., Ricci, S., Gessa, C., 1996. Effects of the urease inhibitor N-(n-butyl) phosphorothioic triamide in low concentrations on ammonia volatilization and evolution of mineral nitrogen. *Biol. Fertil. Soils* 22, 196–201.
- Brady, N.C., Weil, R.R., 2002. *Nature and Properties of Soil*. Prentice Hall, New Jersey.
- Cabrera, M.L., Kissel, D.E., Davis, R.C., Qafoku, N.P., Segars, W.I., 2001. Design and ammonia-recovery evaluation of a wind speed-sensitive chamber system. *Soil Sci. Am. J.* 65, 1302–1306.
- Carmona, G., Christianson, C.B., Byrnes, B.H., 1990. Temperature and low concentration effects of the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) on ammonia volatilisation from urea. *Soil Biol. Biochem.* 22, 933–937.
- Christianson, C.B., Byrnes, B.H., Carmona, G., 1990. A comparison of the sulfur and oxygen analogs of phosphoric triamide urease inhibitors in reducing urea hydrolysis and ammonia volatilization. *Fertil. Res.* 26, 21–27.

- CORINAIR, 2006. <http://reports.eea.europa.eu/EMEP/CORINAIR4/en/B1010vs4.0.pdf>.
- EEA, 1999. Environment in the European Union at the Turn of the Century. Environmental Assessment Report No. 2. Copenhagen, European Environment Agency.
- Elmaci, O., Secer, M., Erdemir, O., Iqbal, N., 2002. Ammonium fixation properties of some arable soils from the Aegean region of Turkey. *Eur. J. Agron* 17, 199–208.
- Ferguson, R.B., McInnes, K.J., Kissel, D.E., Kanemasu, E.T., 1988. A comparison of methods of estimating ammonia volatilization in the field. *Fertil. Res.* 15, 55–69.
- Fillery, I.R.P., Simpson, J.R., De Datta, S.K., 1984. Influence of field environment and fertilizer management on ammonia loss from flooded rice. *Soil Sci. Soc. Am. J.* 48, 914–920.
- Gill, J.S., Bijay-Singh, Khind, C.S., Yadvinder-Singh, 1997. Efficiency of N-(n-butyl) thiophosphoric triamide in retarding hydrolysis of urea and ammonia volatilization losses in a flooded sandy loam soil amended with organic materials. *Nutr. Cycl. Agroecosyst.* 53, 203–207.
- Gouveia, G.A., Eudoxie, G.D., 2007. Distribution of fertiliser N among fixed ammonium fractions as affected by moisture and fertiliser source and rate. *Fertil. Soils* 44, 9–18.
- Grant, C.A., Bailey, L.D., 1999. Effect of seed-placed urea fertilizer and N-(n-butyl) thiophosphoric triamide (NBPT) on emergence and grain yield of barley. *Can. J. Plant Sci.* 79, 491–496.
- Grant, C.A., Jia, S., Brown, K.R., Bailey, L.D., 1996. Volatile Losses of NH<sub>3</sub> from surface-applied urea and urea ammonium nitrate with and without the urease inhibitors NBPT or ammonium thiosulphate. *Can. J. Soil Sci.* 417–419.
- Harrison, R., Webb, J., 2001. A review of the effect of N fertilizer type on gaseous emissions. *Adv. Agr.* 73, 65–108.
- Kissel, D.E., Cabrera, M.L., Vaio, N., Craig, J.A., Rema, J.A., Morris, L.A., 2004. Rainfall timing and ammonia loss from urea in a loblolly pine plantation. *Soil Sci. Soc. Am. J.* 68, 1744–1750.
- Leuning, R., Freney, J.R., Denmead, O.T., Simpson, J.R., 1985. A sampler for measuring atmospheric ammonia flux. *Atmos. Environ.* 19, 1117–1124.
- Malhi, S.S., Grant, C.A., Johnston, A.M., Gill, K.S., 2001. Nitrogen fertilization management for no-till cereal production in the Canadian Great Plains: a review. *Soil Till. Res.* 60, 101–122.
- Manunza, B., Deiana, S., Pintore, M., Gessa, C., 1999. The binding mechanism of urea, hydroxamic acid and N-(N-butyl)-phosphoric triamide to the urease active site. A comparative molecular dynamics study. *Soil Biol. Biochem.* 31, 789–796.
- McCarty, G.W., Bremner, J.M., Chai, H.S., 1989. effect of N-(n-butyl) thiophosphoric triamide on hydrolysis of urea by plant, microbial, and soil urease. *Biol. Fertil. Soils* 8, 123–127.
- Misselbrook, T.H., Nicholson, F.A., Chambers, B.J., Johnson, R.A., 2005. Measuring ammonia emissions from land applied manure: an intercomparison of commonly used samplers and techniques. *Environ. Pollut.* 135, 389–397.
- Nannipieri, P., Ceccanti, B., Cervelli, S., Matarese, E., 1980. Extraction of phosphatase, urease, proteases, organic carbon, and nitrogen from soil. *Soil Sci. Soc. Am. J.* 44, 1011–1016.
- Nommik, H., 1981. Fixation and biological availability of ammonium in soil clay minerals. *Ecol. Bull.* 33, 273–279.
- Pacholski, A., Cai, G., Nieder, R., Richter, J., Fan, X., Zhu, Z., Roelcke, M., 2006. Calibration of a simple method for determining ammonia volatilization in the field-comparative measurements in Henan Province, China. *Nutr. Cycl. Agroecosyst.* 74, 259–273.
- Rana, G., Mastroilli, M., 1998. Ammonia emissions from fields treated with greenmanure in a Mediterranean climate. *Agr. Forest Meteorol.* 90, 265–274.
- Sainz, H., Echeverría, H., Studdert, G., Andrade, F., 1999. No-till maize nitrogen uptake and yield: effect of urease inhibitor and application time. *Agron J.* 91, 950–955.
- Sánchez-Martín, L., Vallejo, A., Dick, J., Skiba, U.M., 2007. The influence of soluble carbon and fertilizer nitrogen on nitric oxide and nitrous oxide emissions from two contrasting agricultural soils. *Soil Biol. Biochem.* 40, 142–151.
- Scherer, H.W., Mengel, K., 1986. Importance of soil type on the release of nonexchangeable NH<sub>4</sub><sup>+</sup> and availability of fertilizer NH<sub>4</sub><sup>+</sup> and fertilizer NO<sub>3</sub><sup>-</sup>. *Fertil. Res.* 8, 249–258.
- Searle, P.L., 1984. The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen—a review. *Analyst* 109, 549–568.
- Sommer, S.G., Hutchings, N.J., 2001. Ammonia emissions from field applied manure and its reduction. *Eur. J. Agron* 15, 1–15.
- Sommer, S.G., Schjoerring, J.K., Denmead, O.T., 2004. Ammonia emission from mineral fertilizers and fertilized crops. *Adv. Agr.* 82, 557–622.
- UNECE, 1999. Protocol to The 1979 Convention on Long-Range Transboundary Air Pollution to Abate Acidification, Eutrophication and Ground-Level Ozone. United Nations Economic Commission for Europe, Geneva.
- Van der Stelt, B., Temminghoff, E.J.M., Riemsdijk, W.H., 2005. Measurement on ion speciation in animal slurries using the Donnan membrane technique. *Anal. Chim.* 552, 135–140.
- Wang, X.B., Xin, J.F., Grant, C.A., Bailey, L.D., 1995. Effects of placement of urea with a urease inhibitor on seedling emergence, N uptake and dry matter yield of wheat. *Can. J. Soil Sci.* 75, 449–452.
- Watson, C.J., Miller, H., Poland, P., Kilpatrick, D.J., Allen, M.D.B., Garrett, M.K., Christianson, C.B., 1994. Soil properties and the ability of the urease inhibitor to reduce ammonia volatilization from surface-applied urea. *Soil Biol. Biochem.* 26, 1165–1171.
- Webb, J., Menzi, H., Pain, B.F., Misselbrook, T.H., Dammgén, U., Hendriks, H., Döhler, H., 2005. Managing ammonia emissions from livestock production in Europe. *Environ. Pollut.* 135, 399–406.