Original Research Article

Use of urease and nitrification inhibitors to reduce gaseous nitrogen emissions from fertilizers containing ammonium nitrate and urea

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A R T I C L E   I N F O

A B S T R A C T

Nitrogen (N) fertilizers increase agricultural yields, but also lead to the release of the greenhouse gases nitrous oxide (N_2O) and ammonia (NH_3). This not only reduces the efficiency of N use, but also results in climate change and loss of biodiversity. The use of nitrification inhibitors may improve the efficiency of N use and reduce the emission of greenhouse gases. We tested three inhibitors (NZONE MAX, Piadin and *N*-(*n*-butyl) thiophosphoric triamide (NBPT)) added to two common N fertilizers (urea and urea ammonium nitrate (UAN)) and determined emissions of CO_2, N_2O and NH_3 to evaluate the effectiveness of these three inhibitors and to improve our understanding of the soil nitrogen cycle. NBPT effectively reduced NH_3 volatilization by 50% (from 3.0 g NH_3-N m^{-2} in urea alone to 1.4 g NH_3-N m^{-2} in urea + NBPT). Piadin decreased N_2O emissions (from 0.98 g N_2O-N m^{-2} in urea alone to 0.15 g N_2O-N m^{-2} in urea + Piadin and from 0.81 g N_2O-N m^{-2} in UAN alone to 0.39 g N_2O-N m^{-2} in UAN + Piadin) by inhibiting the conversion of NH_4^+ to NO_3^-. However, although Piadin was found to be an effective nitrification inhibitor, the risk of higher NH_3 emissions (from 3.0 g NH_3-N m^{-2} in urea alone to 4.5 g NH_3-N m^{-2} in urea + Piadin) with the addition of Piadin cannot be neglected in environmental and economical evaluations.

1. Introduction

Large-scale inputs of nitrogen (N) fertilizers in agriculture have increased crop yields worldwide, allowing global agricultural production to keep pace with the rapidly growing population (Burney et al., 2010). The global use of N fertilizers is unlikely to decrease while the world's population continues to increase (Bakken and Frostegard, 2017; van Beek et al., 2010). The most widely used synthetic N fertilizers are urea and urea-containing N fertilizers. Urea accounts for about 56% of the global production of N fertilizers (Bremner, 2007; International Fertilizer Industry Association, 2013; Suter et al., 2016). Urea is a solid fertilizer with a high N content (46%). It can be stored and applied to crops easily and it can be added to the soil in combination with other N fertilizers. A common urea-containing fertilizer is urea ammonium nitrate (UAN), which is a liquid N fertilizer consisting of 50% urea and 50% ammonium nitrate and ranging from 28% to 32% N by weight.

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The efficiency of N use is often low (Sun et al., 2015), and typically <50% of the applied N fertilizer can be used by a corn crop owning to environmental and management constraints (Drury et al., 2017). About 25% of the urea applied to the soil surface is converted to ammonia (NH_3) and volatilized to the atmosphere (FAOSTAT, 2015); also, the rate of NH_3 volatilization may be even higher at warm temperatures and under moist soil conditions (Camberato, 2017; Tasca et al., 2011). Such large losses of N not only constitute an economic loss for farmers, but are also an important source of greenhouse gases. NH_3 is known to cause acidification and eutrophication of both soils and surface waters, and may also have an indirect impact on Earth's climate owning to its short lifetime in the atmosphere and its relationships with other climate-relevant gases, such as N_2O (Pietzner et al., 2017). It is estimated that about 1%−2% of volatilized NH_3 is later on converted into N_2O (Wulf et al., 2002). The effect of the emission of N_2O on the atmosphere might be one of the most serious environmental consequences of N fertilizer losses (Bakken and Frostegard, 2017), as it contributes to both global warming and the depletion of the ozone layer (Erisman et al., 2007; Ravishankara et al., 2009). About 70% of N_2O and 90% of NH_3 emissions are caused by agricultural activities (Boyer et al., 2002; Zaman and Blennerhassett, 2010). Therefore, improvement in the efficiency of N use is not only a question for policymakers aiming to meet the demands of the United Nations Framework Convention on Climate Change (the Kyoto Protocol) to estimate anthropogenic greenhouse gas emissions (UNFCCC, 1997), but may also increase profits for farmers.

To increase the efficiency of N use, in addition to good agricultural practices (e.g. the correct application techniques, good timing and soil testing to determine the amounts of fertilizer required, which may be constrained by physical conditions), the use of N stabilizers and nitrification inhibitors may potentially delay detrimental processes such as the volatilization of NH_3, the leaching of nitrate (NO_3^-) and the reduction of N_2O emissions. A number of chemical products have been developed to delay the transformation of N, and these can be added to urea and UAN. These slow-release products are classified as (1) urease inhibitors or (2) nitrification inhibitors (Franzen, 2017):

(1) Urease inhibitors. When urea is applied to the soil, it rapidly hydrolyzes to ammonium carbonate. Ammonium carbonate is unstable and breaks down to NH_3 and CO_2. The NH_3 is either absorbed by the soil or volatilizes. The hydrolysis reaction is determined by the urease enzyme, and urease inhibitors block this enzyme to prevent the conversion of urea to NH_3 for a period of 1−2 weeks, allowing time for the incorporation of urea into the soil by rainfall or other means. Many reports have shown that *N*-(*n*-butyl) thiophosphoric triamide (NBPT) can effectively prevent the loss of NH_3 (Drury et al., 2017; Liu et al., 2017; Mira et al., 2017; Silva et al., 2017; Tian et al., 2015).

(2) Nitrification inhibitors. The enzymatic activity of NH_3 oxidizing bacteria is strongly affected by nitrification inhibitors (Ruser and Schulz, 2015). With the addition of nitrification inhibitors to urea, the conversion of ammonium ions (NH_4^+) to NO_3^- is delayed, possibly also limiting N_2O emissions from soil denitrification. Dicyandiamide (DCD) (Di et al., 2014; Guo et al., 2014; Liu et al., 2017; Zaman et al., 2013) and 3,4-dimethylpyrazol-phosphate (DMPP) (Liu et al., 2015; Rose et al., 2018; Shi et al., 2017) are the most researched compounds and are effective in reducing N_2O emissions. In Germany, however, Vizura (containing DMPP) and Piadin (1H-1, 2, 4-triazole and 3-methylpyrazole) are more often used as nitrification inhibitors, and Piadin has also been shown to be effective in reducing N_2O emissions (Pietzner et al., 2017; Wolf et al., 2014; Wu et al., 2017).

To find new, effective chemical ingredients, novel fertilizer additives should also tested—for example, NZONE MAX (also called a penetrant/nitrogen management aid), which has only been mentioned in a few informal reports. NZONE MAX contains 27.5% alkylarylpolyoxyethylene glycol, 7.25% calcium aminoethylpiperazine and 6.5% calcium heteropolysaccharides. NZONE MAX is an ammonium stabilizer intended to open the exchange sites on the soil colloid and improve the attachment of NH_4^+ to soil colloids. Therefore the loss of N by volatilization, leaching and denitrification can be reduced.

Although there has been a wealth of studies on urease (e.g. NBPT) and nitrification (e.g. DMPP and DCD) inhibitors, new compounds still require research. The effectiveness of inhibitors in reducing NH_3 and N_2O emissions in different types of soil and in different climates is variable. As a result of the complex interactions between N_2O and NH_3 emissions, the mitigation of one gas flux may enhance the emission of another; so, apart from losses by leaching and runoff, both N_2O and NH_3 fluxes need to be considered in environmental evaluations (Ferm et al., 2006; Webb et al., 2010). Therefore, more experimental data about the emissions of NH_3 and N_2O when using new inhibitors are needed. To improve our knowledge of the environmental impact of different inhibitors, we conducted a pot experiment using urea and UAN as N fertilizers, and using NBPT, Piadin and NZONE MAX as N additives, and measured their effects on greenhouse gas emissions. We used analyses of CO_2, N_2O and NH_3 emissions to evaluate the effectiveness of these three chemical additives in improving the efficiency of N use and their environmental impact. Our hypotheses were: (1) the urease inhibitor NBPT can effectively reduce NH_3 emissions; (2) the nitrification inhibitor Piadin can effectively reduce N_2O emissions; and (3) NZONE MAX will decrease NH_3 volatilization and N_2O emissions when used as an additive.

2. Materials and methods

2.1. Soil properties and sample preparation

A loamy loess soil was collected from Reinshof agricultural research station, University of Goettingen, Lower Saxony, Germany (51°29′50.3″N 9°55′59.9″E, 155m asl). The annual mean temperature and mean annual precipitation were 8.5 °C

Table 1
Soil properties (0−25 cm depth) of the soil used in the pot experiments, cited from Roemer et al. (2015).

Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	Bulk density (g cm^{-3})	pH (CaCl$_2$)
16	61	23	2.0	1.30	7−7.2

and 650 mm, respectively. The soil was classified as Luvisol (IUSS, 2015) and the texture of the topsoil (0−25 cm) is described in Table 1 (Römer et al., 2015). It had previously been used for a three-year field rotation consisting of winter barley (*Hordeum vulgare*) (2013−2014), winter oilseed rape (*Brassica napus*) (2014−2015) and winter wheat (*Triticum aestivum*) (2015−2016). The soil was collected on 4th April 2016 and stored in a container for three months before incubation. Before use, the soil was passed through a 2-mm sieve. The soil taken from the field had a moisture content of 30% water-filled pore space (WFPS), which was adjusted to a WFPS of 55% (equivalent to a 60% water holding capacity) at the start of the experiment. White rectangular polypropylene buckets with dimensions of 0.39 m (length) × 0.29 m (width) x 0.27 m (height) and an air-tight lid were used as the incubation system. The soil column therein was 16.5 cm high and consisted of three layers of soil adjusted to a soil bulk density of 1.30 g cm^{-3}. There was a 10-cm headspace above the soil surface when the air-tight lid was closed. The soil was pre-incubated in the buckets at 25 °C for 5 days before the addition of fertilizers. All experiments were conducted under the same controlled environmental conditions.

2.2. Experimental treatments

The experiment consisted of eight treatments (including CK, U, U + NZ, U + P, U + NBPT, UAN, UAN + NZ, UAN + P, described in Table 2) and four replicates. The total amount of N applied to each pot, except the control treatment, was 12 g N m^{-2} (corresponding to 120 kg N ha^{-1}). The calculated amount of fertilizer added to each pot was only 2.066 g of urea or 2.64 ml of UAN and therefore the required amount of inhibitors was very small. The inhibitors were bought in liquid form and diluted according to the manufacturer's recommendations. The fertilizers and diluted inhibitors for each pot were dissolved in 7.5 ml of water and the required volume of liquid was applied evenly to the soil surface using a pipette.

2.3. Gas flux measurements

2.3.1. Measurement of CO$_2$ and N$_2$O emissions

Trace gas concentrations of gas samples were analyzed after manual gas sampling from each closed chamber. Lids on the top of the buckets were sealed and samples were taken via silicon stoppers therein. Samples were taken using 60-ml syringes and then 30 mL of gas was transferred into evacuated 12-ml Exetainer vials (Labco, Lampeter, UK). Samples were taken at 0, 20 and 40 min after the chambers had been sealed and measurements were taken each day during the first week, then every two or three days for a period of one month. Gas samples were analyzed on a BRUKER SCION™ 456 gas chromatograph (BRUKER, Bremen, Germany) equipped with electron capture detection for analysis of N$_2$O, a flame ionization detector for CH$_4$ and a thermal conductivity detector for CO$_2$ analysis. Flux rates were calculated with linear or non-linear regression of the gas concentration with time (Parkin et al., 2012; Wang et al., 2013). Cumulative emissions were calculated by linear interpolation.

2.3.2. Measurement of NH$_3$ emissions

NH$_3$ emissions were determined by the Dräger tube method (Pacholski et al., 2006) using an X-act 5000 automatic tube pump (Dräger, Kiel, Germany). Four gas collection cylinders were inserted into the soil surface within each bucket and emitted gases were extracted through the tube pump and flushed through NH$_3$ color indicator-equipped NH$_3$ absorber tubes (Dräger Safety, Lübeck, Germany). The measured concentrations were converted from ppm into absolute values (kg N ha^{-1}) and the NH$_3$ fluxes were calculated as reported by Pacholski et al. (2006). Measurements were taken each day during the first week, then every two or three days for a period of one month.

2.4. Additional parameters

On the first day of the experiment, the soil moisture was adjusted to a WFPS of 55% and fertilizer was added. This corresponds to typical spring time moisture conditions when soils tolerate management measures such as fertilizer spreading by

Table 2
Total mineral N (g N m^{-2}) additions and added inhibitors in different treatments.

	CK	U	U + NZ	U + P	U + NBPT	UAN	UAN + NZ	UAN + P
NO$_3^-$-N	0	0	0	0	3	3	3	3
NH$_4^+$-N	0	12	12	12	9	9	9	9
Added Inhibitors	0	0	NZONE MAX	Piadin	NBPT	0	NZONE MAX	Piadin

CK: control without fertilization, U: urea, NZ: NZONE MAX, P: Piadin, NBPT: N-(n-butyl) thiophosphoric triamide, UAN: urea ammonium nitrate.

agricultural machinery. The moisture decreased to a WFPS of 51% on day 5. Then, simulating a rainfall event, it was adjusted to a WFPS of 80% to stimulate high N_2O emission rates under oxygen depleted soil conditions. By the end of the experiment WFPS had decreased to 60%.

Soil samples were taken before application of fertilizers and at the end of the experiment (30 days later) to determine the soil moisture content and the concentration of mineral N (NO_3^-, NH_4^+). 50 g soil samples were dispersed in 250 ml of 0.0125 mol L^{-1} $CaCl_2$ solution, shaken for 1 h and filtered for later analysis with a San++ continuous flow analyzer (Skalar Analytical, Breda, The Netherlands).

2.5. Calculations and statistical analysis

Emission rates are expressed as arithmetic means $\pm$ the standard error of the mean of four replicates. Least significance difference tests were used to check significant pairwise differences among the treatments. Statistical analyses were performed using Statistica 11 (Dell, Round Rock, TX, USA), with $p < 0.05$ as the criterion for a statistical significance.

3. Results

3.1. CO_2 emissions

The time course of the CO_2 emissions showed that all added fertilizers induced a significant increase in respiration before the simulated rainfall/irrigation (Fig. 1A and B). Before irrigation (<55% WFPS), all fertilized treatments had almost the same CO_2 emission rates, and only on day 2 and 3 did they differed from the control treatment. After irrigation to a WFPS of 80%, the CO_2 emissions were much lower, suggesting that the simulated irrigation affected the microbial activity (Fig. 1A and B). The soil respiration rate began to increase again after a few days, and the differences between treatments were more distinct. In the urea series, a reduction in CO_2 emissions only occurred after addition of the nitrification inhibitor Piadin. The addition of NZONE MAX and NBPT did not decrease the emission of CO_2. In the UAN series, neither the addition of Piadin nor NZONE MAX reduced CO_2 emissions. In fact, even slightly higher emission rates were observed (Fig. 1A and B).

The treatment with urea plus Piadin (U + P) resulted in significantly lower cumulative CO_2 emissions (Fig. 2). They were 38% lower than the treatment without Piadin. The other inhibitors did not lead to significant reductions in cumulative CO_2 emissions compared with the N fertilizer treatments without an inhibitor.

3.2. N_2O emissions

N_2O emissions were low in all treatments from the onset of fertilizer treatment to day 5. Upon irrigation the WFPS reached 80% at day 5, N_2O fluxes increased strongly and the emissions from treatments U + P and UAN + P rose to significantly higher levels than those of the other treatments (Fig. 3A and B). Fig. 3 (A and B) shows a remarkable reduction in N_2O emissions in treatments U + P and UAN + P after day 5. Cumulative emissions of N_2O from soil treated with urea alone amounted to 0.98 g N_2O-N m^{-2}, whereas N_2O emission from U + P was only 0.15 g N_2O-N m^{-2}; therefore, the use of Piadin reduced N_2O emissions by >80% (Fig. 4). In the UAN series, the emissions from the UAN + P (0.39 g N_2O-N m^{-2}) treatment was about 48% of that from UAN alone (0.81 g N_2O-N m^{-2}). The cumulative N_2O emissions from U + NBPT (0.67 g N_2O-N m^{-2}) was 31% lower than from the treatment with urea alone (0.98 g N_2O-N m^{-2}) (Fig. 4), although it was not significant at $p < 0.05$. The addition of NZONE MAX did not show any reduction in N_2O emissions in either fertilizer series. The emission rate was higher with urea + NZONE MAX (U + NZ) than with urea alone (Fig. 4).
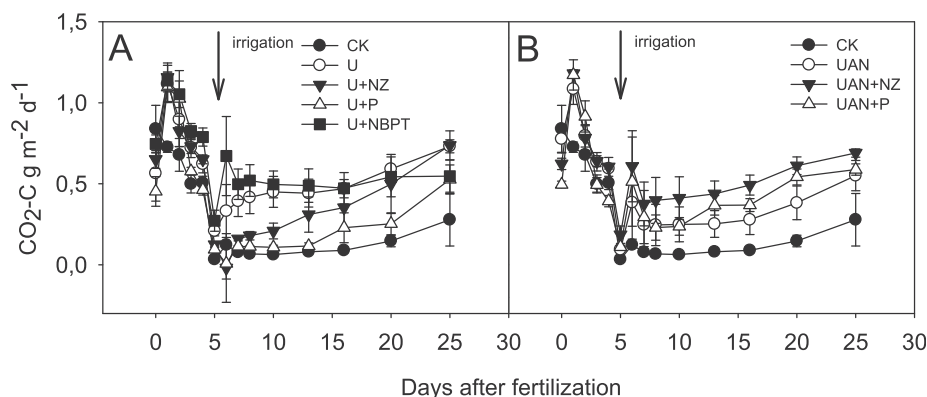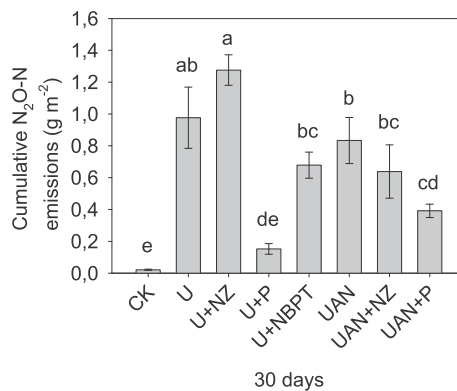

Fig. 1. Time course of CO_2 emissions of different fertilizer treatments. **A**, urea series; **B**, UAN series. Error bars correspond to ± 1 SE ($n = 4$). CK: control without fertilization, U: urea, NZ: NZONE MAX, P: Piadin, NBPT: N-(n-butyl) thiophosphoric triamide, UAN: urea ammonium nitrate.
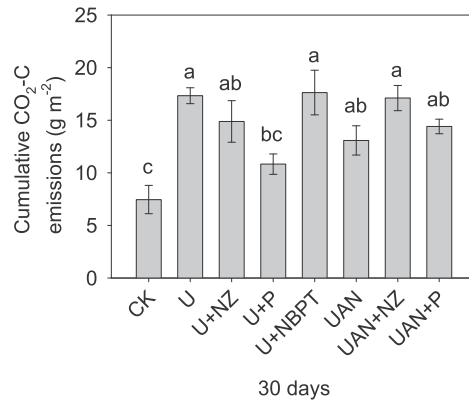
Fig. 2. Cumulative CO_2 emissions of different fertilizer treatments. Error bars correspond to ± 1 SE ($n = 4$). Treatments labeled with the same letters did not show statistically differences at the 0.05 probability level. CK: control without fertilization, U: urea, NZ: NZONE MAX, P: Piadin, NBPT: N-(n-butyl) thiophosphoric triamide, UAN: urea ammonium nitrate.
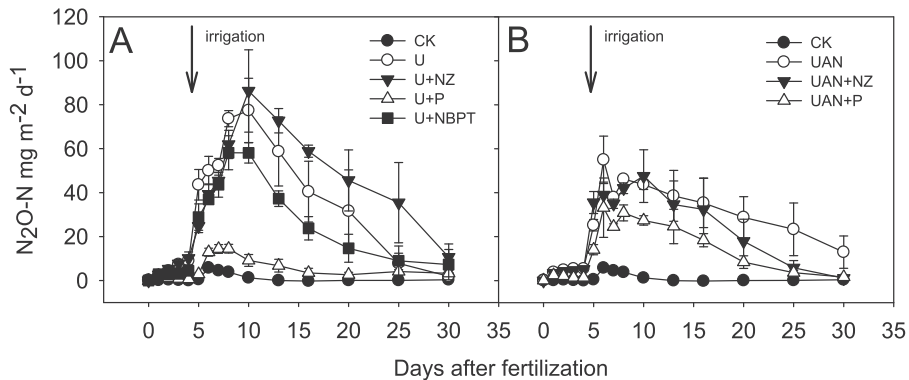
Fig. 3. Time course of N_2O emissions of different fertilizer treatments. **A**, urea series; **B**, UAN series. Error bars correspond to ± 1 SE ($n = 4$). CK: control without fertilization, U: urea, NZ: NZONE MAX, P: Piadin, NBPT: N-(n-butyl) thiophosphoric triamide, UAN: urea ammonium nitrate.

Fig. 4. Cumulative N_2O emissions of different fertilizer treatments. Error bars correspond to ± 1 SE ($n = 4$). Treatments labeled with the same letters did not show statistically significant differences at the 0.05 probability level. CK: control without fertilization, U: urea, NZ: NZONE MAX, P: Piadin, NBPT: N-(n-butyl) thio-phosphoric triamide, UAN: urea ammonium nitrate.
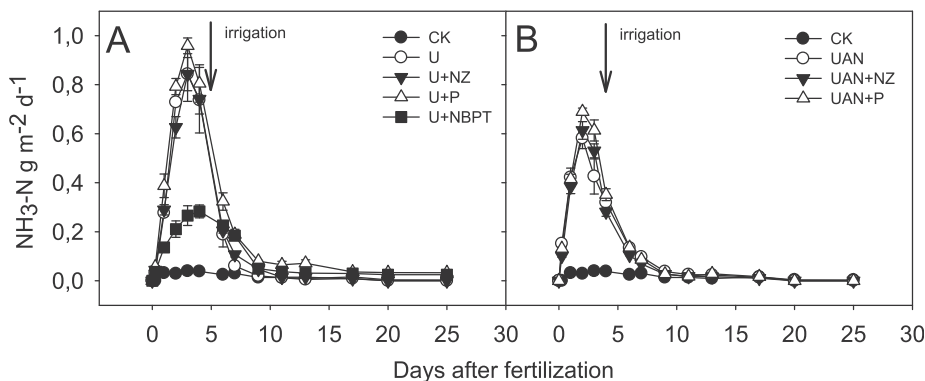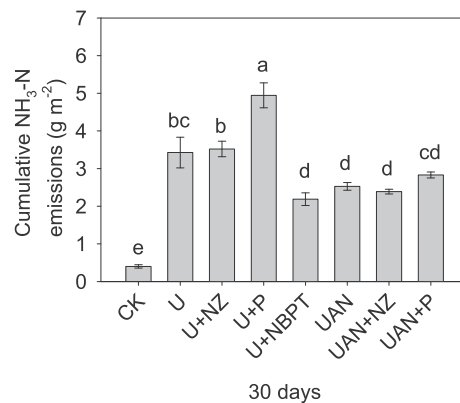
3.3. NH$_3$ emissions

Fig. 5 shows that all treatments resulted in a sharp increase in NH$_3$ emissions after addition of fertilizers. In the urea series, the emissions after the urea alone, U + NZ and U + P treatments showed similar time courses and reached a peak on the third day (Fig. 5A and B). By contrast, the emissions in treatment U + NBPT were much lower, with the peak value on day four. The increase persisted for three days longer than in the other treatments. The peak emission after the U + NBPT treatment was only 0.27 g NH$_3$-N m^{-2} d^{-1} on day 4, compared with 0.84, 0.84 and 0.96 g NH$_3$-N m^{-2} d^{-1} at day 3 for the urea alone, U + NZ and U + P treatments (Fig. 5A and B). The time courses of the emissions were similar for the three treatments in the UAN series, with peak values at day 3. The peak emissions in the UAN, UAN + NZ and UAN + P treatments were 0.58, 0.61 and 0.69 g NH$_3$-N m^{-2} d^{-1}, respectively. In contrast to CO$_2$ and N$_2$O fluxes there was no response to the simulated irrigation on day 5 in any treatment.

Cumulative emissions of NH$_3$ from the soil surfaces of the experimental pots in the urea treatment amounted to 3.4 g NH$_3$-N m^{-2} in 30 days (Fig. 6), minus the emission of 0.4 g NH$_3$-N m^{-2} from the control treatment, which was considered as the background emission from the original soil N pool. The emission related to the application of urea alone was therefore about 3 g NH$_3$-N m^{-2}. In relation to 12 g NH$_3$-N m^{-2} fertilization, the rate of ammonium volatilization was thus 25% of the applied urea-N. With addition of the urease inhibitor (U + NBPT), the emission was reduced to 1.7 g NH$_3$-N m^{-2} (the cumulative emission minus the background emission). Therefore, after the treatment with U + NBPT, the cumulative NH$_3$ emissions were reduced by ca. 50% relative to urea alone.

NH$_3$ emissions from the Piadin + fertilizer treatment were higher than for urea and UAN alone (Fig. 6). In the urea series, the cumulative emission from the U + P treatment was 4.95 g NH$_3$-N m^{-2}, i.e. 44% more than after treatment with urea alone (3.42 g NH$_3$-N m^{-2}). In the UAN series, the cumulative emission of NH$_3$ after treatment with UAN + P (2.83 g NH$_3$-N m^{-2}) was 12% higher than after treatment with UAN alone (2.53 g NH$_3$-N m^{-2}).

Fig. 5. Time course of NH$_3$ emissions of different fertilizer treatments. **A**, urea series; **B**, UAN series. Error bars correspond to ±1 SE ($n = 4$). CK: control without fertilization, U: urea, NZ: NZONE MAX, P: Piadin, NBPT: N-(n-butyl) thiophosphoric triamide, UAN: urea ammonium nitrate.

Fig. 6. Cumulative NH$_3$ emissions of different fertilizer treatments. Error bars correspond to ±1 SE ($n = 4$). Treatments labeled with the same letters did not show statistically significant differences at the 0.05 probability level. CK: control without fertilization, U: urea, NZ: NZONE MAX, P: Piadin, NBPT: N-(n-butyl) thiophosphoric triamide, UAN: urea ammonium nitrate.

3.4. NO_3^--N and NH_4^+-N remaining in the soil after 30 days

The mineral N in the soil samples was determined before the addition of the fertilizers and the concentrations of NO_3^--N and NH_4^+-N were 6.80 and 0.23 g N m^{-2}, respectively (Fig. 7A and B). Thirty days after the addition of 12 g N m^{-2} to all treatments, the remaining soil NO_3^--N ranged from 7.7 g N m^{-2} (U + P) to 14.6 g N m^{-2} (U + NZ) and 2.8 g N m^{-2} in the control treatment (Fig. 7A). The soils treated with U + P showed a lower but not significant NO_3^--N content than those treated with urea alone. The NH_4^+-N remaining after treatment with U + P (1.6 g NH_3-N m^{-2}) was significantly higher than that remaining after the other treatments (<0.5 g NH_3-N m^{-2}) (Fig. 7B).

4. Discussion

4.1. CO_2 emissions

The additional emission of CO_2 from the soils treated with urea fertilizer was a result of two processes: the hydrolysis of urea and induced heterotrophic microbial activity. During hydrolysis of urea, urea is cleaved into NH_3 ($2 \times NH_3$) and carbon dioxide (CO_2) and this goes along with a net increase in the soil pH. In this experiment, the treatment with UAN alone (13.1 g CO_2-C m^{-2}, Fig. 1) resulted in CO_2 emissions that were 25% lower than the treatment with urea alone (17.3 g CO_2-C m^{-2}) ($p < 0.05$). As N in UAN consists of only 50% urea-N that can be hydrolyzed, this figure indicates that, in both treatments, the hydrolysis of urea made a considerable contribution to the volume of CO_2 emitted.

The other source of CO_2 is respiration resulting from the activity of heterotrophic microorganisms, such as the NH_3-oxidizing bacteria population (Kowles, 2018). All treatments showed a surge in the emission of CO_2 after 24–72 h. The soil moisture content was low (55% WFPS) during this time period and the temperature remained constant at 25 °C. Irrigation to a WFPS of 80% on day 5 caused a dramatic decrease in the emission of CO_2, after which the emission of CO_2 increased slowly, with a simultaneous decrease in the WFPS. Therefore it seems that at 55% WFPS conditions were more favorable for microbial respiration than 80% WFPS conditions. The observed decrease in CO_2 emissions after treatment with urea and a nitrification inhibitor has been reported previously (Florio et al., 2016; Maienza et al., 2014; Weiske et al., 2001). The decreased CO_2 emissions after irrigation were mainly from i) disturbed microbial activity and ii) the slower diffusion rate of CO_2 out of the soil with a higher water content.

4.2. N_2O emissions

N_2O emissions were relatively low in all treatments during the first four days of the experiment, before irrigation at day 5. However, the emissions increased rapidly to a high level after irrigation, suggesting that the increase in the soil moisture content (WFPS) from 50% to 55% between days 0 and 5–80% at day 6 was the key driver of N_2O emissions (Cardenas et al.,
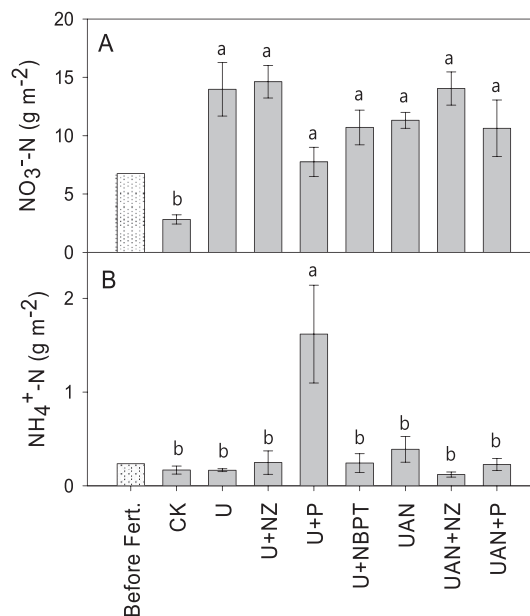
Fig. 7. Nitrate and ammonium present in the soil samples before the application of fertilizer and after 30 days of application for the different treatments. Error bars correspond to ±1 SE ($n = 4$). Treatments labeled with the same letters did not show statistically significant differences at the 0.05 probability level. CK: control without fertilization, U: urea, NZ: NZONE MAX, P: Piadin, NBPT: N-(n-butyl) thiophosphoric triamide, UAN: urea ammonium nitrate.

2017; Yu et al., 2018; Zaman et al., 2013). It is widely accepted that soil moisture has an important impact on N_2O emissions and that a WFPS of 60% is the threshold between aerobic and anaerobic soil conditions (Menéndez et al., 2012). Soil moisture below a WFPS of 60% is unfavorable for the emission of N_2O. Low N_2O emission rates have been observed previously in similar studies reported by Menéndez et al. (2012) and Volpi et al. (2017).

Only a few earlier studies (Pietzner et al., 2017; Wolf et al., 2014; Wu et al., 2017) have evaluated 1H-1,2,4-triazole and 3-methylpyrazole (Piadin) as a nitrification inhibitor. However, the results of these studies were similar to our findings, confirming that Piadin can significantly reduce N_2O emissions. Research has also been carried out on other nitrification inhibitors (e.g. DMPP, DCD and Nitrapyrin), demonstrating their effectiveness in reducing N_2O emissions. As nitrification inhibitors aim to suppress, reduce or delay the oxidation of NH_4^+ to NO_3^- in soils, our observations of reduced N_2O fluxes in the treatments with nitrification inhibitors were probably related to variations in the availability of the substrate (NO_3^-) for denitrification. They may also have been influenced by different contributions from the two major N_2O-forming processes of nitrification and denitrification (Zaman and Nguyen, 2012). In a number of studies (Guo et al., 2014; Yu et al., 2018; Zaman et al., 2013; Zaman and Nguyen, 2012) the time courses of soil NH_4^+ and NO_3^- concentrations after application of fertilizers have shown that treatment with nitrification inhibitors (DMPP or DCD) result in higher NH_4^+ and lower NO_3^- concentrations.

Cumulative emissions of N_2O were high in all treatments in this study, except for the U + P and UAN + P treatments. This suggests that the chosen incubation environment did favor denitrification, probably as a result of the high soil moisture content (80% WFPS), high incubation temperature (25 °C) and high soil NO_3^- content. The N_2O emissions in studies under similar conditions were predominantly from denitrification (Grave et al., 2018; Menéndez et al., 2012; Senbayram et al., 2012; Luo et al., 2008), most likely as a result of limited nitrification due to the low availability of oxygen (Tian et al., 2015). The lowest N_2O emissions in our study were observed in the treatments with the lowest NO_3^- concentrations in the soil (with Piadin treatment), which is seen as further evidence of this assumption.

4.3. NH_3 emissions

The release of large amounts of NH_3 after the application of urea is a serious agricultural problem (Engel et al., 2017; Li et al., 2015; Pacholski et al., 2018; Schraml et al., 2016; Sun et al., 2015; Tian et al., 2015). In this study, the U + NBPT treatment reduced NH_3 fluxes by about 50%, which is in agreement with previously published work (Connell et al., 2011; Drury et al., 2017; Mira et al., 2017; Suter et al., 2013). The meta-analysis of Silva et al. (2017) showed that urea + NBPT reduced 52% losses of NH_3. The trend of reduction was observed in soils over all classes of soil pH, organic carbon content and rate of N addition. Moreover, the addition of NBPT to urea has also been suggested to be effective in increasing crop yields (Drury et al., 2017; Silva et al., 2017).

As UAN is composed of urea and ammonium nitrate in a ratio of 1:1, the volatilization losses of NH_3 from the group of UAN treatments should theoretically be lower than those from the soils treated with the different urea fertilizers. This was confirmed by our results. Although we did not include a UAN + NBPT solution in this study, a number of other studies (Goos, 2012; Grant, 2013; Rajkovich et al., 2017) have shown that the addition of NBPT to UAN can significantly reduce NH_3 losses relative to the application of UAN alone.

By contrast, nitrification inhibitors tend to induce increased NH_3 emissions because NH_4^+ is available for extended periods of time. The addition of Piadin to both groups of N fertilizers increased the cumulative NH_3 emissions by 44% and 12%, respectively, relative to urea or UAN alone. This increase in NH_3 emissions agrees with earlier reports showing that nitrification inhibitor treatments increased NH_3 emissions from 3% to 65% (Fan et al., 2018; Ferm et al., 2006; Lam et al., 2018, 2017; Pan et al., 2016; Qiao et al., 2015; Webb et al., 2010). However, Piadin performed well in reducing N_2O emissions owing to lower NO_3^--N concentrations in the soil. Therefore, the benefit of nitrification inhibitors in reducing N_2O emissions has to be judged against the higher risk of NH_3 volatilization, or additional strategies need to be implemented to reduce NH_3 volatilization.

4.4. Soil NO_3^--N and NH_4^+-N

The remaining mineral N was determined at the end of experiment. It was expected that large amounts of total mineral N (NO_3^--N + NH_4^+-N) would remain in the soil due to the absence of plants utilizing N and the shallow depth of the experiment in the soil layer (16 cm). In addition, N leaching was impeded as a result of the use of water-tight incubation vessels. Consequently, all the treatments (urea alone, U + NZ, U + NBPT, UAN and UAN + NZ) showed residual mineral N of >10 g N m^{-2}. As the total amount of mineral N at the start of the experiment was 19 g N m^{-2} (12 g N m^{-2} fertilizer N and 7 g N m^{-2} initial soil mineral N), more than half of the original amount of N remained in the treated soils. The range of N losses in our experiment was similar to previously reported experiments carried out under similar conditions (Wu et al., 2017; Zaman and Nguyen, 2012). Some of the applied N not recovered as inorganic N was probably taken up by soil microbes and would have been part of the soil organic N pool.

Nitrification inhibitors such as Piadin inhibit the oxidation of NH_4^+ to NO_3^-. In our study, the residual soil NH_4^+-N in U + P treatment was 1.6 g N m^{-2}, whereas in all other treatments it was <0.5 g N m^{-2}. The soil NO_3^- concentration was still low at the end of the incubation period of 30 days, accounting for only 7.7 g NO_3^--N m^{-2}, which was the lowest of all treatments. In the pot experiments of Goos and Johnson (1999) and Sassman (2014), conducted at 25 °C for tests of application rates of 15 g NH_4^--N m^{-2}, the half-life of soil NH_4^+ after the application of urea alone and UAN alone was 2–3 weeks. This is consistent with

our study, in which the conversion of NH_4^+ to NO_3^- was almost complete 30 days after the addition of fertilizer. This process is always clearly delayed in the presence of a nitrification inhibitor (Wu et al., 2017; Yu et al., 2018; Zaman et al., 2013).

4.5. Evaluation of the novel fertilizer additive NZONE MAX

We included the product NZONE MAX because this novel compound has been reported to be a powerful additive, improving the efficiency of N fertilizers by improving the attachment of NH_4^+ to soil colloids and preventing their volatilization. However, we found that NZONE MAX was ineffective in reducing both NH_3 and N_2O emissions with our soil. Goos (2012) and Harrel (2012) reported similar results. Our study clearly confirms that the addition of NZONE MAX to major types of N fertilizer had no effect on the reduction of N losses by volatilization and denitrification and, based on final soil N concentration in our incubation experiment, there was no indication of potential effects on NO_3^- leaching. The impact of that mechanism would likely be dependent on soil texture. A soil with moderate to high clay content and/or organic matter would probably already have sufficient CEC and readily retain ammonium. We refer that product may be more likely to have an impact on emissions in a low CEC soil. Future studies should test if NZONE MAX increases the ammonium sorption capacity (Venterea et al., 2015).

5. Conclusion

This laboratory study shows that NBPT is an effective urease inhibitor and reduces NH_3 volatilization and probably also N_2O emissions. The nitrification inhibitor Piadin was also found to be effective in reducing N_2O emissions. However, the potential of increasing NH_3 volatilization with the use of Piadin or similar nitrification inhibitors should not be neglected. In our study, the novel additive NZONE MAX was found to be unsuitable for reducing greenhouse gas emissions and improving the efficiency of fertilizer use. However, future studies should test this novel additive on soils with a lower clay content or organic matter that limits NH_4^+ attachment on soil colloids. Future studies also need to focus on improving management methods, or on new chemical or biochemical additives.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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