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Turnover of Urea in a Soil from the North China Plain as Affected by the Urease Inhibitor NBPT and Wheat Straw

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Abstract: In the North China Plain (NCP), urea is applied to predominantly high-pH soils and high gaseous losses of N are observed in the forms of ammonia (NH_3) and nitrous oxide (N_2O). The production of N_2O and N_2 is further stimulated by the addition of fresh organic materials. The urease inhibitor (UI) *N*-(*n*-butyl) thiophosphoric triamide (NBPT) restricts the loss of N by reducing the rate of urea hydrolysis and limiting the substrates for NH_3 and N_2O emission. We investigated the turnover of N—as well as the emissions of NH_3 , N_2O and CO_2 —as affected by the addition of wheat straw and urea +NBPT in an incubation experiment using soil from a farmer's field, typical for the NCP. Added wheat straw prolonged the persistence of NH_4^+ after the application of both urea and urea +NBPT, while reducing the formation of nitrate. Wheat straw may therefore either act as a stimulant of hydrolysis or as an inhibitor of nitrification. Urea increased soil respiration and the emission of N_2O , possibly acting as a primer for microbial activity as described in earlier studies. In combination with the application of organic C sources, this effect of urea may be a main driver of gaseous N loss.

Keywords: nitrogen; mineralization; *N*-(*n*-butyl) thiophosphoric triamide; N_2O emission; ammonia volatilization

1. Introduction

Since the late 1980s, the loss of nitrogen (N) to the environment has steadily increased in China [1], where the fertilization behavior of farmers and their attitude towards the overuse of chemical fertilizers has the biggest impact on the loss of reactive N [2–4]. The most common synthetic N source in China, the world's largest producer and consumer of synthetic fertilizer, is urea. Under normal soil conditions, the urease enzyme catalyzes the hydrolysis of urea to ammonium (NH_4) and carbonate within a few days [5]. In soil solution, there is a pH-dependent balance between ammonia (NH_3), which is dominant in alkaline environments, and ammonium (NH_4^+). Only the latter is stable in the soil and dominates in acidic environments. Additionally, the formation of hydroxide ions (OH^-), when ammonia is hydrogenated, may lead to a further increase in pH and further increased ammonia volatilization [6].

It has been estimated that 95% of NH_3 emissions from crop production in China are in the form of ammonia from urea and ammonium bicarbonate, the second most important N fertilizer in China [7]. A recent meta-analysis has shown that N loss as NH_3 from urea ranges between 1.7 and 48% for East Asia and 0.9 and 64% globally [8].

A proven chemical agent for the inhibition of the urease enzyme is the chemical compound *N*-(*n*-butyl) thiophosphoric triamide (NBPT), which reduces the loss of ammonia and thereby increases

the efficiency of urea-based fertilizers for a variety of crops [6,9–11], even though the effectiveness of the inhibitor with regard to yield increases must be regarded in light of the application rate [12].

While the urease inhibitor NBPT may potentially reduce the loss of N_2O from agricultural soils by reducing the available substrate for denitrification [13], the results for the efficiency of NBPT in reducing N_2O emissions are inconsistent [14,15].

Besides the risk of increased ammonia losses, N-fertilization with urea provides the substrate for nitrous oxide (N_2O)-producing microorganisms in soils after the hydrolysis and subsequent nitrification of ammonium. N_2O is a potent climate-relevant trace gas that accounts for approximately 7.9% of the anthropogenic greenhouse effect [16] and contributes to stratospheric ozone depletion [17]. Although some further processes are discussed as a source of N_2O in soils, there is no doubt that nitrification and denitrification contribute substantially to N_2O production in soils [18–20]. Nitrification is the autotrophic oxidation of ammonium in aerobic soil compartments, whereas nitrate is reduced to gaseous nitrogen oxides and N_2 during heterotrophic denitrification in compartments with low oxygen partial pressure. It has been described that both nitrification and denitrification processes significantly contribute to the emission of N_2O at lower soil moistures, while processes of denitrification are predominant when high soil moisture leads to anaerobic conditions [21,22].

Further, denitrification relies on organic carbon as an electron donor. It has been shown that, especially, the incorporation of fresh organic materials stimulates N_2O production by favoring the anaerobic conditions for denitrification due to oxygen consumption during the respiration of the easily available carbon [23,24]. Especially, the combination of NO_3^- and carbon sources such as oat residues resulted in strongly increased N_2O emissions as compared to the control treatments, which received only NO_3^- or only oat residues [25]. The assumption that denitrification was the major N_2O source under such conditions with easily available C and NO_3^- was confirmed by Garcia-Ruiz and Baggs [25] using ^{15}N tracers, even though this assumption is not automatically valid for loess-derived soils of the North China Plain (NCP).

Pfab et al. [26] reported increasing N_2O emissions from a chard field the closer the mineral N fertilization was temporally placed after the incorporation of the preceding green rye, suggesting that a reduction in N_2O emission can be achieved if the temporal availability of C and NO_3^- can be successfully decoupled.

For Chinese soils under sub-humid climate conditions, it has been shown that N_2O is mainly derived from nitrification. The main reason may be the lack of carbon for denitrifiers but also differences in the microbial population, as Northern Chinese soils characteristically have rapid nitrification rates and organic matter turnover, despite low carbon contents [27–30]. In a recent study, it has been shown that high urea application rates may shift the microbial community structure in soils towards rapid nitrifying communities [31].

Traditionally, straw residues are burnt in China to prepare fields for the following crops, even though the burning of straw significantly contributes to the emission of particulates [32,33], and recent studies suggest that the incorporation of straw may increase soil carbon contents [34]. As of yet, there is little information on how the simultaneous application of straw residues and urea influences N_2O emissions, but it has been suggested that N_2O emissions may increase as carbon is available for nitrifying communities [35]. Similarly, the simultaneous application of N and C may increase CO_2 emissions, as the addition of N and external C primes the turnover of soil organic carbon (SOC) [36].

The objective of this article is to give an overview of how the application of the urease inhibitor NBPT affects NH_3 , N_2O and CO_2 emissions after the simultaneous application of wheat residues and urea, using soil taken from a farmer's field in the county of Quzhou, Hebei Province. The soil has a history of over-application of N, as is common in the wheat–maize double cropping system of the NCP [37], and may therefore give valuable insight to the dynamics of gaseous N loss after N fertilizer application in the region.

Our first main hypothesis was that NBPT reduces the rate of urea hydrolysis, resulting in lower concentrations of ammonium-N in incubated soil and subsequently to lower concentrations of nitrate-N. NBPT therefore reduces the volatilization of ammonia-N and the emission of N_2O , as the substrate for denitrification is reduced in concentration.

Our second hypothesis was that added wheat straw leads to lower concentrations of mineral-N in incubated soil but increases biological activity, measurable through CO_2 respiration, and N_2O loss as the decomposition of straw increases denitrifying processes.

We tested our hypotheses in an incubation experiment by observing the turnover of urea in soils amended with urea, wheat straw and NBPT and by measuring the gaseous emissions of NH_3 , N_2O and CO_2 .

2. Materials and Methods

The soil used in this experiment was a homogenized sample taken from the Ap horizon of a farmer's field, typical for the NCP (Quzhou County, Hebei Province, PRC, 115° E, 36° N, 40 m a.s.l.). The field was chosen as its history of N application to a wheat–maize double cropping system of the NCP lay in the median range of 550 kg (N) $ha^{-1} a^{-1}$ [37]. According to the IUSS working group WRB, the soil can be classified as a Cambisol with a silt-loam texture. Soil analysis showed a total C concentration of 1.8%, a total N concentration of 0.1% and 110 mg of Olsen-P $kg\ soil^{-1}$, and the pH (0.01 M $CaCl_2$) was 7.5. Before transport, the soil was air-dried and homogenized after sieving through a 0.5 cm mesh sieve. In order to restore soil activity and avoid a nitrification flush on beginning the experiments, the soil water content was adjusted to 10% (gravimetric) using distilled H_2O and stored in a cold room at 7 °C for a period of two months.

The wheat straw used in this experiment showed a C/N ratio of 70:1. Before transport and analysis, the plant material was oven-dried at 60 °C and homogenized using a rotating disc-mill to allow homogeneous incorporation into the soil.

Two N-fertilizers were used in this experiment, the first being pure urea with an N concentration of 46% and the second being the UTEC® product from Eurochem Agro, a urea-based fertilizer with an N concentration of 46% and 0.1% NBPT as a urease inhibitor (urea + UI).

The two experiments of the research described in this report were designed as 2-factorial experiments (Table 1) and were carried out under the same conditions to ensure the comparability of the results. The amount of wheat straw homogeneously mixed into the soil was calculated to reflect the addition of 5 Mg of straw ha^{-1} in a farmer's field at a depth of 20 cm, corresponding to 1.92 g of wheat straw $kg\ soil^{-1}$. For the fertilized treatments, granules of urea or urea + UI (UTEC) were applied to the soil surface in the incubation glasses. The addition of N was calculated to reflect the application of 250 kg N ha^{-1} . For the determination of N turnover, the calculation was based on soil weight (40 mg urea 75 g $soil^{-1}$); for the determination of gaseous flux, the application of urea was based on the surface area of the incubated soil (250 mg of urea 200 g $soil^{-1}$) (Table 1). Both experiments included 5 repetitions of each treatment to allow appropriate statistical analysis of the results.

Table 1. 2-factorial design of treatments in the 1st (determination of mineralization) and 2nd (determination of gaseous flux) experiments. Wheat residues (ws) were incorporated into the soil, and fertilizer granules without or with urease inhibitor (UI) were applied to the surface of individual repetitions.

Treatment	Residues	Fertilizer
–ws – N	No	No
–ws + urea	No	urea
–ws + urea + UI	No	urea + UI
+ws – N	Yes	No
+ws + urea	Yes	urea
+ws + urea + UI	Yes	urea + UI

Treatments for the determination of N turnover were prepared as described above. For each treatment, repetition and sampling date, an individual screw-top glass was filled with 75 g of soil and brought to a soil water content of 20% (gravimetric) using distilled H₂O. Each glass was closed using plastic paraffin film to avoid evaporation while allowing gaseous exchange. The soil was incubated in a climate chamber at 18 °C for 19 days.

Sampling occurred on Days 0, 1, 3, 7, 11 and 19. For the extraction of mineral N from soil, 200 mL of 0.5 M K₂SO₄ were added to each screw-top glass and shaken at 180 rpm for 60 min. The concentrations of NH₄-N and NO₃-N of the extract were analyzed through micro-flow auto analysis (SEAL QuAAtro). The results were calculated as mg NH₄-N and NO₃-N kg soil⁻¹. The apparent net N mineralization was calculated.

To investigate the effects of NBPT and the addition of plant residues on the flux of CO₂, N₂O and NH₃, soil was incubated in a climate chamber at 18 °C for 16 days. Incubation bottles were closed with silicone plugs provided with an air inlet and outlet. Compressed air was continuously passed over the samples at a rate of 13 mL min⁻¹ for each chamber individually. The air passed through a glass vial for the determination of CO₂ and N₂O flux rates and finally through an acid trap containing 200 mL of 0.2 M H₂SO₄ for the determination of the total NH₃ emitted from the samples. Sampling occurred on Days 2, 4, 6, 8, 10, 12, 14 and 16. For each replicate treatment, 200 g of soil, without or with wheat residues as described in Table 1, were brought to a soil water-content of 20% (optimal, judged on experience) using distilled water (dH₂O) and put into the incubation bottles. For the fertilized treatments, granules of Urea or UTEC were applied to the surface of the soil before the individual glasses were attached to the air-flow system. At sampling, the glass vials were removed, and their contents were analyzed for CO₂ and N₂O using gas chromatography (5890 series II, Hewlett Packard, CA, USA) with an electron capture detector (ECD); the ammonia concentration in the acid-traps was analyzed through micro-flow analysis (SEAL QuAAtro, Southampton, UK).

The gas fluxes of CO₂ and N₂O were calculated according to the following equation:

$$gas\ flux_{CO_2, N_2O} = \frac{k_{CO_2, N_2O} \times ([CO_2, N_2O]_{in} - [CO_2, N_2O]_{out}) \times s}{A} \quad (1)$$

where *gas flux* is the emission of CO₂ in mg (CO₂-C, N₂O-N) m⁻² h⁻¹ and N₂O in µg (CO₂-C, N₂O-N) m⁻² h⁻¹; *k* is the conversion constant for the mass conversion of CO₂-C and N₂O-N; [CO₂, N₂O]_{IN} and [CO₂, N₂O]_{OUT} are the concentrations of CO₂-C and N₂O-N before and after passing over the samples, respectively; *s* is the air flow rate of the system in mL min⁻¹; and *A* is the inner diameter (surface area) of the incubation bottles.

NH₃-N was calculated as the total amount of NH₃-N emitted from the treatments at the point of sampling.

The experimental results were analyzed applying the R environment for statistical computing [38], using a linear, 2-factorial analysis of variance (ANOVA) for each individual sampling date. Differences between treatments were calculated using Tukey's test for Honestly Significant Difference (Tukey-HSD) at a confidence interval of $\alpha = 0.05$.

3. Results

3.1. N Turnover Experiment

There was a significant effect of added fertilizer (urea and urea + UI) on the concentrations of ammonium-N and nitrate-N in the soil (Figure 1). For ammonium-N, this effect was evident up to the 11th day of the experiment, after which no significant differences between treatments were observed.

Comparisons between the two fertilizers showed significantly higher concentrations of ammonium-N following the urea treatment compared to with urea + UI only for the first 7 days of the experiment, showing that UI reduces the appearance of ammonium-N, indicating inhibition of the urease enzyme.

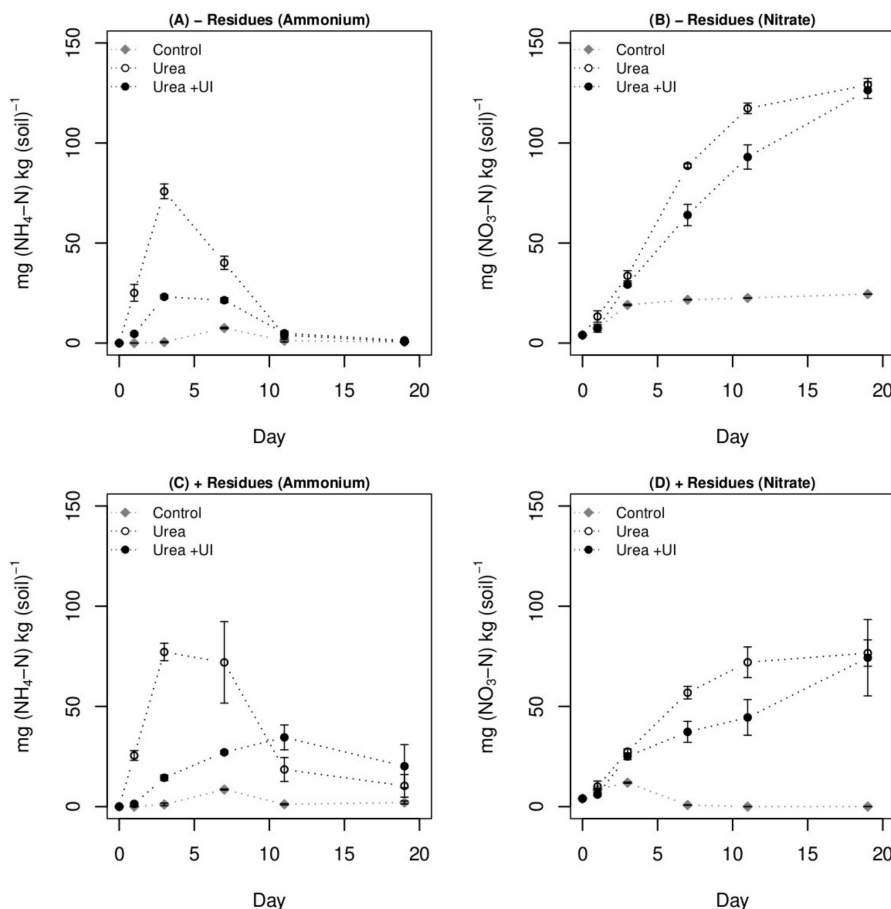


Figure 1. Turnover of ammonium-N (A,C) and nitrate-N (B,D) in alkaline soil of the North China Plain (NCP) after application of urea and urea + UI (*N*-(*n*-butyl) thiophosphoric triamide (NBPT)) without (A,B) and with (C,D) incorporated residues (wheat straw). Error bars indicate the standard errors of the mean.

The soil nitrate-N concentrations under both urea and urea + UI treatments increased over the entire experimental period. The nitrate-N concentrations with the urea treatment were higher compared to those with urea + UI up to the final day of sampling, at which no significant differences between the two fertilizer treatments were determined.

Regarding the main effect of added fertilizer, the concentration of mineral N at the conclusion of the experiment was 141.6 and 141.8 mg N kg soil⁻¹ for urea and urea + UI, respectively (Table 2).

The incorporation of the wheat straw showed a trend towards higher ammonium-N concentrations in the second week of the experiment both after the addition of urea and urea + UI (Figure 1A,B), with a significant main effect on the concentrations of ammonium N in the final two days of sampling (Days 11 and 19). By contrast, the addition of wheat straw led to a 35% lower concentration of nitrate-N, compared to that in soil without added wheat straw (Figure 1C,D).

Urea as the N-source led to peak concentrations of ammonium-N on the third day of the experiment (–wheat straw: 76 mg N kg soil⁻¹; +wheat straw: 77 mg N kg soil⁻¹). For urea + UI, peak concentrations of ammonium-N were observed on Day 3 (–wheat straw: 23 mg kg soil⁻¹) and Day 11 (+wheat straw: 35 mg kg soil⁻¹). These days must be regarded as the points in time where the rate of nitrification exceeded the rate of urea hydrolysis (Figure 1).

Nitrate-N concentrations continuously increased up to the final day of sampling. The observed values of mineral N (ammonium-N + nitrate-N) at the conclusion of the experiment are approximately 50% of those of the added fertilizer-N (Table 2).

Table 2. Apparently net mineralized N ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$), total $\text{CO}_2\text{-C}$ respiration, total $\text{N}_2\text{O-N}$ emission and total $\text{NH}_3\text{-N}$ volatilization at the conclusion of the experiments (2 weeks), showing the main effects of the 2-factorial experimental design (wheat straw x fertilizer) in the two experiments (Exp. 1 and Exp. 2). Letters behind values indicate significant differences between treatments for either the main factors or, where relevant, the interaction between main factors of the experiment.

	Net Mineralized N	$\text{CO}_2\text{-C}$	$\text{N}_2\text{O-N}$	$\text{NH}_3\text{-N}$
Treatment	$\text{mg kg}^{-1} \pm \text{SE}$	$\text{g m}^{-2} \pm \text{SE}$	$\text{mg m}^{-2} \pm \text{SE}$	$\text{g m}^{-2} \pm \text{SE}$
−ws − N	29.6 ± 0.1	3.9 ± 0.3	3.4 ± 0.4 c	0.0 ± 0.0
−ws + urea	170.3 ± 4.7	24.0 ± 1.0	84.6 ± 5.0 b	2.9 ± 0.4
−ws + urea + UI	167.7 ± 5.1	12.4 ± 3.9	14.3 ± 4.1 c	0.1 ± 0.1
+ws − N	-1.2 ± 1.0	33.2 ± 4.6	5.8 ± 0.4 c	0.0 ± 0.0
+ws + urea	112.9 ± 4.5	56.9 ± 3.9	432.8 ± 33.0 a	3.5 ± 0.3
+ws + urea + UI	115.9 ± 9.0	48.6 ± 1.5	29.7 ± 2.0 bc	0.0 ± 0.0
Factor A x B	n.s.	n.s.	***	n.s.
−ws	122.5 ± 17.7 a	13.4 ± 2.5 b	34.1 ± 9.8 b	1.0 ± 0.4
+ws	75.9 ± 14.9 b	46.2 ± 3.2 a	156.1 ± 53.3 a	1.2 ± 0.4
Factor A	***	***	***	n.s.
−N	14.2 ± 5.2 b	18.5 ± 5.4 c	4.6 ± 0.5 c	0.0 ± 0.0 b
urea	141.6 ± 10.0 a	40.4 ± 5.8 a	258.7 ± 60.1 a	3.2 ± 0.3 a
urea + UI	141.8 ± 9.9 a	30.5 ± 6.4 b	22.0 ± 3.4 b	0.1 ± 0.0 b
Factor B	***	***	***	***

Tukey HSD: Significance Codes: $p < 0.001$: “***”; no difference: “n.s”. Net mineralized N: $(\text{NH}_4\text{-N} + \text{NO}_3\text{-N})_{\text{End}} - (\text{NH}_4\text{-N} + \text{NO}_3\text{-N})_{\text{Start}}$.

3.2. Gaseous Flux Experiment

The addition of wheat straw led to a significant ($P < 0.001$) increase in CO_2 respiration as compared to that under the treatments without added wheat straw over the entire course of the experiment. For the treatments without added wheat straw, the CO_2 respiration rates under the fertilized treatments increased throughout the experiment, leading to peak CO_2 respiration rates of $105 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ (urea) and $54 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ (urea + UI) on Day 16. For the treatments with added wheat straw, peak CO_2 respiration was observed on Day 3 of the experiment with 180 and 170 $\text{mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ for urea and urea + UI, respectively.

The control in both instances (with and without wheat straw) showed similar CO_2 dynamics compared to the fertilized treatments at lower concentrations (Figure 2A,B).

With regard only to the main effect of added wheat straw, total CO_2 respiration increased from a mean of 13.4 to $46.2 \text{ g CO}_2\text{-C m}^{-2}$ over the experimental period. Furthermore, there was a significant effect of added fertilizer on CO_2 respiration (control < urea + UI < urea, Table 2), in excess of the possible $5.8 \text{ g CO}_2\text{-C m}^{-2}$ that theoretically may be released from urea at the chosen application rates. There was no significant interaction between the main factors of the experiment (wheat straw x fertilizer).

The incorporation of wheat straw increased the emission of N_2O in soil treated with urea and urea + UI. The observed effect of both fertilizers was evident from Day 3 onward and remained elevated compared to the control throughout the experiment. In the second week of sampling, a significant interaction between the main effect of added wheat straw and fertilizer became evident. This interaction was evident only for the treatments fertilized with urea, not urea + UI.

In all the fertilized treatments, the highest N_2O emission rates were observed on the final day of sampling, and flux rates increased with the addition of wheat straw. With urea as the fertilizer, the N_2O flux rates were 515 and 3390 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$; with urea + UI, the flux rates were 76 and 113 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ without and with added wheat straw, respectively (Figure 2C,D).

There was a significant interaction effect between the factors of wheat straw and fertilizer on the emission of N_2O , which is reflected in the calculated total emission of N_2O for the duration of the

experiment. For soil fertilized with urea, the total emission of N_2O at 432.8 mg m^{-2} with added wheat straw was five times higher compared to that from soil without added wheat straw at 84.6 mg m^{-2} . By contrast, N_2O emission from soil fertilized with urea + UI remained at a low level, with total values of 14.3 mg m^{-2} without added wheat straw and 29.7 mg m^{-2} with added wheat straw (Table 2).

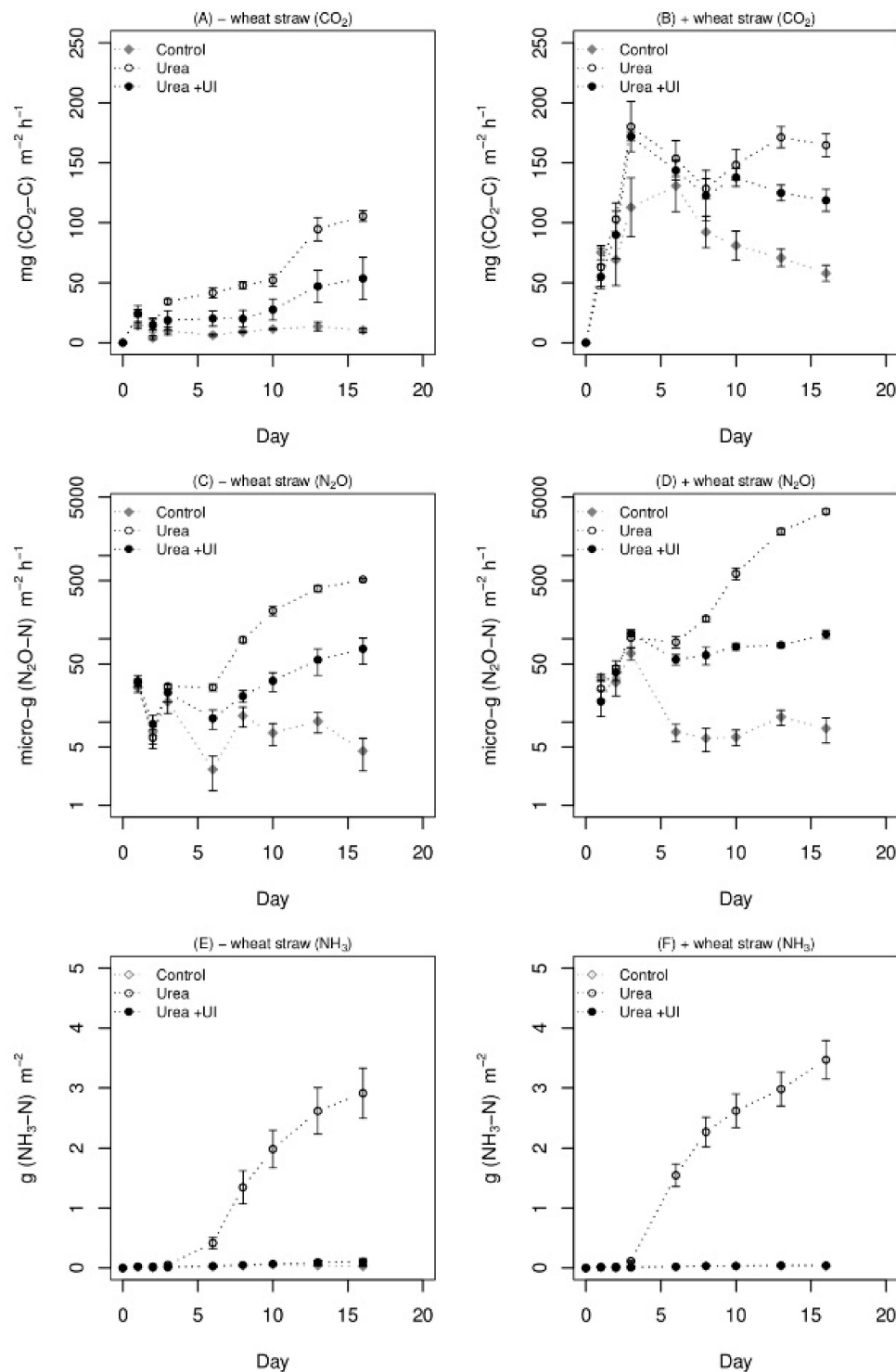


Figure 2. Flux rates of $\text{CO}_2\text{-C}$ (A,B) and $\text{N}_2\text{O-N}$ (C,D, logarithmic scale) and total loss of $\text{NH}_3\text{-N}$ (E,F) from alkaline soil of the NCP after application of urea and urea + UI (NBPT) without (left) and with (right) incorporated wheat straw.

There was a response of ammonia volatilization only from treatments with urea fertilization. For both the control and urea + UI, only trace amounts of ammonia were determined in the sulfuric acid traps used in this experiment.

The peak volatilization rates of ammonia from soil fertilized with urea were observed on the eighth day of the experiment for soil without added wheat straw ($19.3 \text{ mg NH}_3\text{-N m}^{-2} \text{ h}^{-1}$) and on the sixth day for soil with added wheat straw ($19.2 \text{ mg NH}_3\text{-N m}^{-2} \text{ h}^{-1}$), after which the volatilization rates decreased until the conclusion of the experiment (Figure 2E,F).

The total cumulative ammonia losses over the experimental period (16 days) from soil fertilized with urea amounted to 2.9 and 3.5 g N m^{-2} for soil without and with added wheat straw, which corresponds to a loss of 12 and 14% of applied urea-N, respectively (Table 2).

4. Discussion

For treatments without added fertilizer, the N dynamics in the soil used for these experiments are consistent with previous research on the subject [39,40]. The non-amended soil showed a total apparent mineralization of about 21 mg N kg soil⁻¹ over a 3-week period, which translates to about 20 kg (N) ha⁻¹, taking a depth of 10 cm as the basis for calculation. By contrast, soil amended with wheat straw led to a net immobilization of 2 mg N kg soil⁻¹. Similar results have been described by Mundra et al. [41], Nieder and Richter [42], and Mary et al. [39], who state that net immobilization takes place over a short-term period when organic material with a C/N ratio greater than 25:1 is incorporated into soil.

As expected, the addition of wheat straw led to a strong increase in CO₂ respiration, i.e., microbial activity, over the first week of the experiment, before it gradually declined in the second week of the experiment. Similarly, without additional N, N₂O flux rates were elevated only for the first few days of observation, before decreasing to levels similar to those observed in pure soil. These observations may indicate that microbial activity was limited by N as a substrate for microbial activity, as described by Zumft et al. [43].

The observations of the turnover of urea, especially the concentrations of ammonium-N, indicate that the hydrolysis of urea as catalyzed by the urease enzyme reached its maximum within 3 days after application and that nitrification was induced quickly. These dynamics of N were expected after urea application and are consistent with previous research on the subject [5,44].

The addition of wheat straw in this experiment led to a prolonged presence of ammonium-N in the soil and reduced concentrations of nitrate-N throughout the experiment. It is therefore possible that the addition of wheat straw either stimulated the activity of the urease enzyme or reduced the rate of nitrification, thereby acting as a nitrification inhibitor. This specific result in part contradicts observations by Marsh et al. [5], who stated that nitrification after urea application is induced quickly under a wide range of conditions, as urea acts as both a C and N source for ammonium-oxidizing bacteria. Concerning total mineral-N, the addition of wheat straw led to lower mineral N concentrations, which may be explained through an increase in the microbial-N fraction [39] during the decomposition of organic material [42].

As has been previously reported, the application of urea to neutral-to-alkaline soils may lead to a significant loss of N in the form of ammonia [27,45–49]. In our experiments, the loss of N through ammonia volatilization after the application of urea amounted to 2.9 g NH₃-N m^{-2} without added wheat straw, and 3.5 g NH₃-N m^{-2} with added wheat straw, which is 12 and 14% of the applied urea-N, respectively. These rates of volatilization are lower compared to those in research conducted by McKinnes et al. [45], Zhang et al. [27] and Soares et al. [10] but are similar to the results obtained by Sanz-Cobena et al. [13]. While indicating a strong effect of NBPT on the reduced volatilization of ammonia, higher volatilization may occur when higher air-exchange rates are chosen during experimental set-up. The addition of urea significantly increased CO₂ respiration rates compared to the unfertilized treatments without and with added wheat straw. Taking the application rate of urea as a basis for calculation, only 5.8 g CO₂-C m^{-2} can be explained by the hydrolysis of urea; CO₂-emission

above these values must be explained through background soil respiration (no added wheat straw) or the turnover of added organic material (wheat straw). These values indicate that there is a priming effect of urea on the activity of microbial organisms and, thereby, CO₂-respiration, as described by Kuzyakov et al. [50] and Qiu et al. [36]. In this case, urea would act as both a N and C source for microbial organisms, leading to an increase in soil activity and the turnover of added organic material.

A similar priming effect must also be responsible for the increase in N₂O flux rates observed in this experiment. The addition of urea increased the emission rates of N₂O-N by a factor of 10 compared to those in soil without added fertilizer and without added wheat straw, and by a factor of 100 when wheat straw was incorporated. Generally, N₂O emission is limited either by the absence of N as a substrate [51] or by C as a limiting factor for microbial activity [26]. Especially for soils of the NCP, N₂O emissions are mainly derived from nitrification processes [27–30]. In the case of the research presented here, the addition of urea may act as both a C and N source [5], having a priming effect on microbial activity, thereby leading to the interactive effect of added urea and wheat straw on N₂O emission.

Three principal points must be made when comparing urea + UI to urea. Firstly, the use of UI does effectively limit the rate of hydrolysis as catalyzed through the urease enzyme. However, while the appearance of ammonium-N is reduced in incubated soil and the subsequent appearance of nitrate-N is slightly reduced, the activity of the urease enzyme seems sufficient to provide ammonium-N as a substrate for nitrification.

Secondly, UI almost completely prevented the loss of N as ammonia in these experiments. The volatilization of NH₃-N was undiscernibly higher compared to that in the unfertilized control over a period of 2 weeks. While the efficiency of NBPT concerning the volatilization of ammonia has previously been described [6,9,10,52], this near complete restriction of ammonia volatilization is surprising and should be confirmed using more sensitive methods of analysis.

Finally, UI strongly reduced the loss of N as N₂O after the application of urea, which supports the suggestion of Ding et al. [53], who described that the loss of N₂O may be reduced by the urease inhibitor NBPT.

In addition to the results for NH₃ volatilization and N₂O emission, the results further show that CO₂ respiration is reduced through UI. In the context of N₂O-emission, this result specifically may lead to either of two conclusions: UI may either simply act to restrict microbial activity in general, or the principal mechanisms of the urease inhibitor may prevent the priming effect of urea on soil microbial activity, as the C fraction of urea is not available as a substrate for microbial growth [5,50]. Further research on the subject, specifically on the effect of the urease inhibitor NBPT on the C and N turnover in soils, is therefore necessary and should use marker-based studies to observe the development of mineral and organic C and N fractions in soils.

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