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Urease inhibitors for mitigation of ammonia losses resulting from fertilization of granulated urea

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List of Abbreviations

2-NPT	N-(2-nitrophenyl) phosphoric triamide
AL	arable land
CAN	calcium ammonium nitrate
DM	dry matter
Corg	organic carbon content
GL	grassland
IPAT	N-(Isopropoxycarbonyl) phosphoric acid triamide
NBPT	N-(n-butyl) thiophosphoric triamide
Ntotal	content in total nitrogen
NUE	nitrogen use efficiency
UI	urease inhibitor
w/w	weight by weight

Zusammenfassung

Harnstoff ist mit einem Marktanteil von 58% der weltweit bedeutendste mineralische Stickstoffdünger. Allerdings ist es auch einer der Dünger mit dem höchsten Potenzial für gasförmige Stickstoffverluste in Form von Ammoniak (NH_3). Aufgrund dessen negativer Auswirkungen auf die Umwelt und auf die menschliche Gesundheit ist NH_3 ein bedeutsames Gas, dessen Reduktion ein gesamtgesellschaftliches Ziel ist.

Das grundsätzliche Potenzial für NH_3-Verluste nach der oberflächigen Ausbringung von Harnstoff wird durch die Bodeneigenschaften und die Nutzung des Standorts beeinflusst. Der Verlauf sowie das Ausmaß der NH_3-Verluste ist allerdings stark von den tatsächlichen klimatischen Bedingungen bestimmt. In der Literatur werden NH_3-Emissionen nach oberflächiger Ausbringung von Harnstoff von bis zu 44% des ausgebrachten Stickstoffs in Winterweizen, 58% in Grünland und 38% auf brachem Boden beschrieben. Für die nationale Emissionsberichterstattung werden nach Ausbringung von granuliertem Harnstoff in der temperierten Klimazone NH_3-Emissionen von 15.5% (pH des Bodens < 7.0) oder 16.4% (pH des Bodens > 7.0) angenommen. Diese Verluste können durch eine unverzügliche Einarbeitung des Düngers vermieden werden. Wenn eine Einarbeitung nicht möglich ist, kann die Zugabe eines Ureaseinhibitors (UI) die NH_3-Verluste reduzieren oder sogar verhindern.

Über 7 Jahre hinweg wurde in 24 Messreihen im Feld und Gewächshaus in Getreide, im Grünland oder auf brachem Boden das Ausmaß der NH_3-Emissionen nach oberflächiger Ausbringung von Harnstoff erfasst, sowie das Potenzial der zwei neu entwickelten Ureaseinhibitoren IPAT und 2-NPT zur Reduzierung dieser NH_3-Verluste untersucht und mit dem etablierten UI NBPT verglichen.

In Feldversuchen sowie in Großwannenversuchen im Gewächshaus wurden die NH_3-Emissionen nach oberflächiger Ausbringung von granuliertem Harnstoff mit einem dynamischen Kammersystem direkt und kontinuierlich erfasst. In Gefäßversuchen wurden

die NH_3-Verluste indirekt über die Ausnutzung des Düngerstickstoffs bestimmt. Die Messungen im Feld erfolgten auf brachem Boden, in Winterweizen sowie auf Grünland. Im Gewächshaus wurden Untersuchungen auf brachem Boden, in Hafer, in Sommerweizen sowie auf Grünland durchgeführt.

Nur in Messungen auf brachem Boden erreichten die NH_3-Verluste mit bis zu 16% des gedüngten N den von der nationalen Emissionsberichterstattung angenommenen Emissionswert von 15.5%. Allen anderen Versuchen war gemeinsam, dass die Verluste an NH_3 nach oberflächiger Ausbringung von Harnstoff deutlich niedriger waren als in der Literatur berichtet. In einer Mehrzahl der Versuche hatte die Zugabe der UI NBPT, IPAT und 2-NPT einen signifikanten Einfluss auf den Verlauf der NH_3-Emissionen. Der UI NBPT bestätigte seine gute Wirkung in unseren Feldversuchen (2002 - 2004) und reduzierte die NH_3-Verluste um durchschnittlich 33%. Der UI IPAT zeigte in diesen Versuchen mit durchschnittlich 23% Emissionsminderung ebenfalls eine gute Wirkung, erreichte allerdings trotz einer gegenüber NBPT erhöhten Wirkstoffkonzentration keine verbesserte Wirkung. Der im Verlauf des Projektes entwickelte UI 2-NPT zeigte hingegen bereits in einer niedrigen Konzentration eine sehr gute Wirkung von durchschnittlich 63% Emissionsminderung, und wurde unter anderem basierend auf diesen Versuchen als erster Ureaseinhibitor in die deutsche Düngemittelverordnung aufgenommen.

Abstract

Urea is the most important fertilizer worldwide with a world market share of 58% in mineral nitrogen fertilizer consumption. However, it is also the mineral N fertilizer with one of the highest potential for ammonia (NH_3) losses. Due to NH_3's negative impact on the environment as well as on human health, society aims for a reduction of NH_3 emissions.

A site's soil characteristics and land use define its potential for NH_3 losses, while climatic conditions following the application of urea determine their actual extent. Following the surface application of granulated urea, high NH_3 losses of up to 44% in winter wheat, 58% in grassland and 38% from bare soil have been reported. In a temperate climate, the European emission inventory guidebook estimates a loss of 15.5% (soil pH < 7.0) or 16.4% (soil pH > 7.0) of the applied urea as NH_3. For mitigating NH_3 losses from urea, the best option would be to immediately incorporate the fertilizer. If incorporation is not possible, the addition of a urease inhibitor (UI) represents a potent alternative in reducing or mitigating NH_3 emissions.

Over 7 years, 24 measurement periods were conducted in the field and the greenhouse, in winter wheat, in grassland and on bare soil, to evaluate the extent of NH_3 losses following the application of urea and to determine the mitigating effect of two newly developed UIs, IPAT and 2-NPT, in comparison to the established UI NBPT.

In field and tray experiments, NH_3 emissions were directly and continuously measured using a dynamic chamber system. In pot experiments, fertilizer N losses as NH_3 were detected indirectly by determination of fertilizer N use efficiency. Field experiments were done on bare soil, in winter wheat and on grassland. Greenhouse experiments were carried out on bare soil, in oat, in summer wheat and on grassland.

Only on bare soil, NH_3 emissions of up to 16% of applied N corresponded to the emission factor of 15.5% stated by the European emission inventory guidebook. In

all other experiments, low NH_3 emissions were detected from broadcasted urea when compared to literature. In most measurement periods, addition of the UIs IPAT, 2-NPT and NBPT had a significant effect on the course of NH_3 emissions. The UI NBPT confirmed its good reduction potential in field experiments (2002 - 2004) and reduced NH_3 emissions on average by 33%. IPAT showed a similarly good reducing potential of on average 23%. However, despite its higher concentration of active substance, it did not attain a similarly good result as NBPT. 2-NPT, which had been developed in the course of this project, showed a good reduction potential of on average 63% even in a low concentration. Based on these experiments and other results, the UI 2-NPT was the first urease inhibitor to be registered in the German fertilizer regulation.

Chapter 1

Introduction

1.1 Urea as a mineral fertilizer

In the Haber Bosch process, ammonia (NH_3) is produced using hydrogen from natural gas and nitrogen from air. Together with carbon dioxide (CO_2), NH_3 is further processed to urea (chemically expressed as carbamide, $CO(NH_2)_2$) under temperatures of 400 °C to 500 °C and pressure of 150 bar to 350 bar (Boswell et al., 1985).

$$N_2 + 3\,H_2 \longrightarrow 2\,NH_3$$

$$CO_2 + 2\,NH_3 \longrightarrow CO(NH_2)_2 + H_2O$$

Compared to other mineral nitrogen fertilizers, urea has the advantages of a low cost per unit of N due to lower production costs, a high N content of 46%, availability in most markets, low corrosion, compatibility with most fertilizers, high foliar uptake, prompt availablility to plants, and a good water solubility of $1\,\mathrm{kg\,l^{-1}}$ at 20 °C (Finck, 1992; Silva et al., 2017).

Due to these advantages, urea is the most important fertilizer worldwide with a world market share of 58% in mineral nitrogen fertilizer consumption (IFA, 2017).

1.2 Ammonia emissions from urea

In the presence of water, urea is quickly hydrolyzed to ammonium carbonate which, in turn, decomposes to CO_2 and ammonium (NH_4^+).

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} 2\,NH_3 + CO_2$$

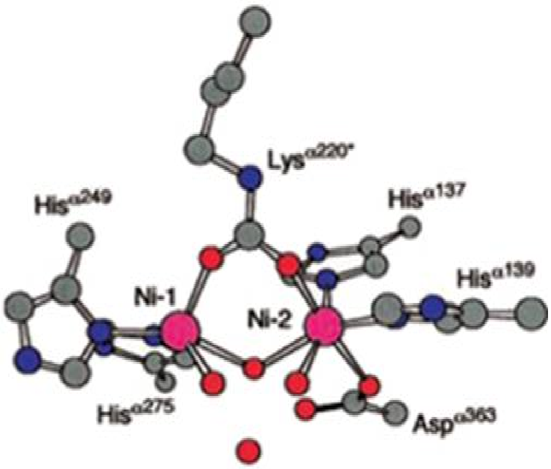

Figure 1.1: Active site of a urease enzyme (Universita di Bologna - FaBIT, 2013).

The reaction is catalyzed by the ubiquitous enzyme urease (Figure 1.1; Bremner and Mulvaney, 1978; Zaman and Blennerhassett, 2010), which is produced by microbes and plants (Kot et al., 2008). Total soil urease activity consists of intracellular as well as extracellular urease, where the extracellular fraction has been released from dead and decaying organisms (Pettit et al., 1976). Also according to Pettit et al. (1976), soil urease is partly resistant to proteolysis, gamma irradiation as well as storage and geological preservation. Conrad (1940) attributes this high resistance to its adsorption to organic soil constituents, which have the ability to stabilize the urease.

The hydrolysis of urea takes place within 1 to 2 days (Nannipieri et al., 1980) and results in an elevated pH surrounding the fertilizer granule. High emissions of ammonia into the atmosphere are a result of this increase in pH, together with a switch in the NH_4^+/NH_3-equilibrium tipping towards a higher NH_3 concentration in the soil solution (Figure 1.2; Harrison and Webb, 2001; Sommer et al., 2004).

$$NH^+_{4(aq)} \rightleftharpoons NH_{3(aq)} + H^+_{(aq)}$$

$$NH_{3(aq)} \rightleftharpoons NH_{3(gas)}$$

Since the strong increase in pH is locally concentrated around the granule (Overrein and Moe, 1967), these losses can occur even when urea is applied onto acidic soils (Soares et al., 2012). The accumulation of NH_4^+ and thus the increase in pH around the granule is considered to be the main reason for urea's importance concerning NH_3 emission (Haynes, 1986; He et al., 1999).

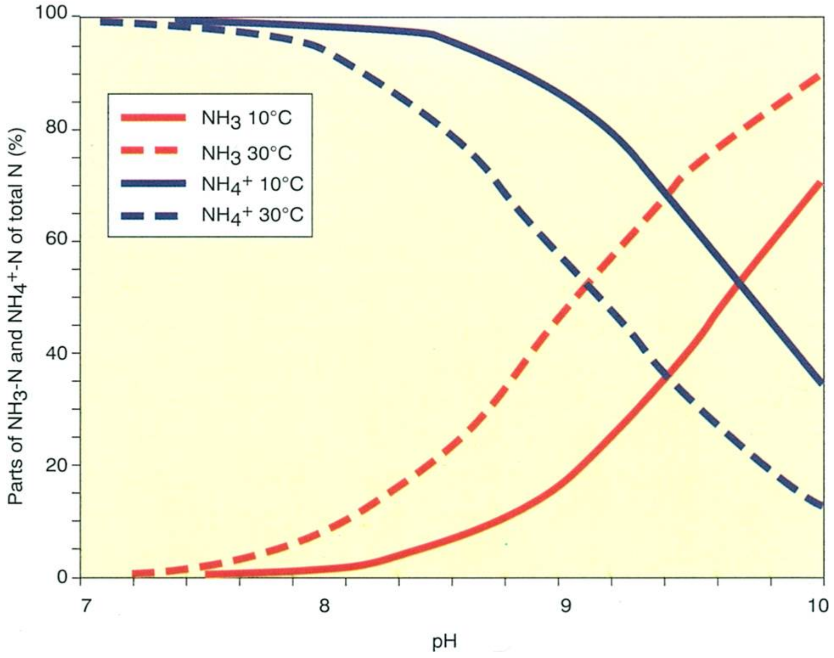

Figure 1.2: Relative concentrations of NH_4^+ and NH_3 in water at different pH and at 10 °C and 30 °C (ECETOC).

1.3 Soil parameters and climatic conditions influencing ammonia emissions

Influence of climatic conditions. Besides the pH, the NH_4^+/NH_3-equilibrium in the soil water points out the importance of temperature and soil water for the extent of fertilizer N losses as NH_3 following the application of urea (Figure 1.2).

Between these two factors, water has the predominant impact on the extent, length and course of NH_3 emissions following application of urea (van der Weerden and Jarvis, 1997). Water is relevant at two points following application of urea:

1. Soil water status at the time of urea application is crucial for the course of dissolution of the fertilizer granules as well as for the following hydrolysis of urea. In general, if the soil surface is dry during urea application, no NH_3 losses occur (Ernst and Massey, 1960; Herbst et al., 2005; Misselbrook et al., 2004; Sommer et al., 2004). However, if an initially moist soil surface is followed by several days of slow drying with no or low rainfall events, then large total NH_3 losses are to be expected (Engel et al., 2011; Ernst and Massey, 1960; Hargrove, 1988).

2. Precipitation during the phase of NH_3 emissions influences the course of losses. Two possible effects of rainfall on NH_3 emissions can be observed (Bouwmeester et al., 1985; Forrestal et al., 2016; Hargrove, 1988; Ma et al., 2010):
    - Emissions are terminated by rainfall as it transports urea and ammoniacal N into deeper soil layers.
    - Rainfall enhances losses by adding water to the top soil for further dissolution of urea granules and hydrolysis of urea.

Black et al. (1987) stated that the sooner the precipitation event occurred after fertilization, the higher was its emission terminating or reducing effect. The amount of rainfall needed to mitigate NH_3 losses depends on soil characteristics and the timing of the rain relative to urea hydrolysis, but for most soils 20 mm of rain terminate NH_3 volatilization (Black et al., 1987; Hargrove, 1988; Misselbrook et al., 2004; Sommer et al., 2004).

If soil water content is not limiting, temperature directly influences the magnitude of NH_3 loss in several ways (Cai et al., 2002; Ernst and Massey, 1960; Hargrove, 1988; Sherlock et al., 1995; Watson, 2000):

1. It induces a faster urea hydrolysis rate which results in a higher soil pH and greater ammoniacal N concentration near the soil surface.
2. Temperature influences the NH_4^+/NH_3-equilibrium (Figure 1.2) and the equilibrium between NH_3 in solution and NH_3 as gas.

Therefore, with higher soil temperatures more N gets lost as NH_3 from applied urea (Carmona et al., 1990). Misselbrook et al. (2004) postulated an increase of NH_3 emissions by a factor of 2 with every increase in temperature by 5 °C.

Influence of soil parameters. Although the actual magnitude of NH_3 losses is determined by climatic conditions, soil parameters determine the potential for NH_3 emissions (Hargrove, 1988). Relevant soil properties influencing NH_3 volatilization are—among others—soil pH, soil texture, content in organic carbon, organic residues and urease activity.

In general, the higher the initial soil pH is, the higher NH_3 losses will result from application of urea (Bronson et al., 1989; He et al., 1999; Sommer et al., 1996; Watson et al., 1990a; Watson et al., 1994b). In the course of urea hydrolysis and N transformation in soil, NH_3 emissions and nitrification of NH_4^+ to NO_3^- end in an acidifying effect of urea of 36 val H^+ per kg urea (Soares et al., 2012), which corresponds to a consumption of 1 kg lime. This reduction in pH takes place on all soils, but on alkaline soils it results in a pH that is still high enough for severe losses. This is one reason why NH_3 losses from alkaline soils are higher.

Generally, urease activity of almost all agricultural soils is adequate for rapid hydrolysis of urea (Zantua and Bremner, 1976). However, the higher the site's urease activity is, the more rapidly urea hydrolysis takes place, resulting in a higher concentration of ammoniacal N at the soil surface (Hargrove, 1988). Urease activity of a soil is determined by several soil and climatic factors like temperature, total N content, soil texture and soil organic matter (Hargrove, 1988; Reynolds et al., 1985; Zantua et al., 1977). In grassland, urease activity and consequently urea hydrolysis rate is mostly determined by the grass sward (Reynolds et al., 1985), in which the herbage material, both standing and as organic residues, increases NH_3 losses in several ways (Hargrove, 1988; McGarity and Hoult, 1971; Mira et al., 2017):

1. It forms a physical barrier between the N source and the soil, thus blocking or delaying the contact between soil and fertilizer, and as a result slowing diffusion of N into the soil.

2. It prevents rapid drying of the soil surface, thereby maintaining higher loss rates for a longer period of time.

3. It enhances urease activity far above that of bare soil and consequently favors NH_3 volatilization.

Although urease activity and initial soil pH have an impact on extent of NH_3 losses following application of urea, their effect may be less important in predicting NH_3 volatilization than soil properties that influence the retention of NH_4-N (Reynolds and Wolf, 1987). Among others, a high content in soil organic matter is reported to absorb urea and thus reduce NH_3 loss potential (Rao and Ahmad, 1984). The same effect was observed for soils with a high content in clay, which is reported to reduce NH_3 emissions, as clay minerals have a high potential to bind the NH_4^+ resulting from hydrolysis of urea (Reynolds and Wolf, 1987; San Francisco et al., 2011; Watson et al., 1994b). High sand contents however favor NH_3 losses (Reynolds and Wolf, 1987; San Francisco et al., 2011).

1.4 Environmental and health impact of ammonia

Once in the atmosphere, NH_3 is either directly deposited in the vicinity of the source, or reacts with acids to form ammonium aerosols like $(NH_4)_2SO_4$ or NH_4HSO_4 (Galloway et al., 2003). These can be transported over long distances before they are deposited (Hertel et al., 2011). When deposited on vegetation surfaces, ammoniacal N forms cause soil acidification, eutrophication and increased leaching on a regional scale (van Breemen et al., 1982). The impact is substantial, especially when they are deposited in natural and semi-natural ecosystems, where the nitrogen can cause an ecological shift in species diversity (Bouwman and van Vuuren; van Breemen et al., 1982). Already in the late 1990s, the deposition of ammoniacal N forms into semi-natural ecosystems substantially exceeded their critical load in 7 to 18 % of the global area (Bouwman and van Vuuren). Furthermore, input of nitrogen into ecosystems always results in increased nitrous oxide (N_2O) emissions, giving NH_3 an indirect global warming potential of 2.98 CO_2 equivalent emissions over 100 years (IPCC, 2006; IPCC).

Besides its environmental impact, NH_3 also poses a threat to the human health. As the only base in the atmosphere, NH_3 plays an important role in neutralizing the existing acids. The resulting ammonium salts (NH_4NO_3, $(NH_4)_2SO_4$) can further evolve to secondary particulates. 40% of the secondary particulates in the atmosphere consist of ammonium salts (LUBW). Depending on the size (PM10, PM2.5, $<$ PM2.5), these particulate matters can penetrate the human body beyond the lungs up to the blood circuit, where they cause irritations of the mucous membranes, asthma attacks, bronchitis, and even changes in the autonomic nervous system and heart attacks (Stokstad, 2014).

1.5 Extent of ammonia losses from urea

In laboratory and greenhouse experiments, NH_3 losses following the application of urea have been reported to range from 0.4% to up to 63% of applied urea (Carmona et al., 1990; Frame, 2017; Reynolds and Wolf, 1987; San Francisco et al., 2011; Soares et al., 2012; Sommer et al., 1996; Watson et al., 1990a; Watson et al., 1994b; Watson et al., 2008).

In field experiments, NH_3 losses of 0.1% to up to 38% have been detected when urea was applied to bare arable soil (Ma et al., 2010; Pacholski et al., 2006).

Losses following the application of urea to a standing crop have been reported to range between 1% and 44% of the applied fertilizer N (Cai et al., 2002; Chadwick et al.; Engel et al., 2011; Gioacchini et al., 2002; Nastri et al., 2000; Pacholski et al., 2006; Pan et al., 2016; Schjoerring and Mattsson, 2001; Turner et al., 2012). On average, emissions peak 4.8 days after urea application and last for 10 days (Silva et al., 2017).

On grassland, NH_3 losses following urea application vary between 6% and 58% of applied N and occur within a period of 3 to 7 days, with the maximum rate being recorded on days 1 to 3 following fertilization (Black et al., 1985; Black et al., 1987; Chadwick et al.; Dawar et al., 2011; Forrestal et al., 2016; Sanz-Cobena et al., 2011; Sherlock et al., 1995; van der Weerden and Jarvis, 1997; Watson et al., 1994a).

For the calculation of the German national emission inventory, the European emission inventory guidebook (EEA, b) indicates NH_3 emissions following the application of urea to be situated between 155 (soil pH < 7.0) and 164 (soil pH > 7.0) g $NH_3\,kg^{-1}$ N applied. However, for Germany there are not many field studies to corroborate these emission factors (EEA, a).

1.6 Possibilities for mitigation of ammonia losses

Immediate incorporation of the applied fertilizer into the soil is known to decrease NH_3 losses distinctly (Cai et al., 2002; Ernst and Massey, 1960; Rochette et al., 2013; Sommer et al., 2004). However, incorporation is not always feasible. Especially when nitrogen is applied to grassland, mechanical incorporation into the soil usually disturbs the grass sward and is therefore not economically viable.

In these cases, the most promising alternative would be an active substance to block the enzyme urease. Watson (2000) explained that the main working principle of such a urease inhibitor (UI) is to slow down the hydrolysis of urea, providing more time for the urea to diffuse away from the application spot, or for rain or irrigation water to dilute urea and the NH_4^+ concentration at the soil surface and increase its dispersion into the soil. A urease inhibitor reversibly blocks the active center of the enzyme urease.

This delay in hydrolysis (Medina and Radel, 1988) caused by the UI also comes with a reduced increase in pH around the urea granule and, consequently, significantly lower NH_3 emissions (Watson et al., 1994).

Throughout the last decades, many chemical compounds have been tested for their ability of blocking urease and showed greater or lesser effects on mitigation of ammonia emissions (Medina and Radel, 1988; Watson, 2000). The most widely used and well-studied urease inhibitor is N-(n-butyl) thiophosphoric triamide (NBPT; Agrotain International L.L.C., St. Louis, USA), with a reported mitigation efficiency of 25% to up to 89% in laboratory and greenhouse experiments (Frame, 2017; San Francisco et al., 2011; Soares et al., 2012; Watson et al., 1994b; Watson et al., 2008), 42% to up to 100% on arable land (Bronson et al., 1989; Chadwick et al.; Engel et al., 2011; Gioacchini et al., 2002; Nastri et al., 2000; Pan et al., 2016; Sanz-Cobena et al., 2008) and of 45% to up to 79% on grassland (Dawar et al., 2011; Forrestal et al., 2016). Besides its generally reducing effect, addition of NBPT delays NH_3 emissions following application of urea by several days (Mira et al., 2017; Silva et al., 2017). However, NBPT is not the active form and has to be converted into its oxygen analogue first (Manunza et al., 1999; McCarty et al., 1989), which can delay its efficacy by minutes, hours or even days (Saggar et al., 2013; Sanz-Cobena et al., 2011). Furthermore, degradation of NBPT is promoted by higher temperatures (Cantarella et al., 2005; Cantarella et al., 2008; Carmona et al., 1990; Mira et al., 2017; Soares et al., 2012) and on extremely acidic soils (Engel et al., 2015; Frame, 2017; Mira et al., 2017; San Francisco et al., 2011), and NBPT shows a reduced effect under cold temperatures (Engel et al., 2011) as well as in the presence of organic residues (Carmona et al., 1990; Saggar et al., 2013).

1.7 Objectives of this work

From 2001 to 2006, in two approved joint projects financed by the German Federal Ministry of Education and Research (BMBF), Stickstoffwerke Piesteritz GmbH together with Humboldt Universität zu Berlin, Martin-Luther-Universität Halle-Wittenberg and Technische Universität München (Support code 0330202) developed and tested new urease inhibitors on their efficiency to reduce NH_3 emissions following the application of granulated urea (Niclas and Schuster; Niclas). Among others, N-(Isopropoxycarbonyl) phosphoric acid triamide (IPAT; SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt-

Wittenberg, Germany) and N-(2-nitrophenyl) phosphoric triamide (2-NPT; SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt-Wittenberg, Germany) were developed. Being the two most promising UIs, they were first evaluated in multi-year field experiments in winter wheat (2002 - 2005) and in greenhouse experiments (2004 - 2005).

From these, 2-NPT turned out to be the most promising UI. Besides its high efficiency in NH_3 loss reduction, it is very stable during storage (Hucke et al., 2010). Therefore, the potential of 2-NPT to reduce NH_3 emissions following the application of urea was evaluated in field experiments on grassland in 2007 and 2008.

Chapter 2

Material and Methods

2.1 Measurement of ammonia emissions

A dynamic chamber system was used for the NH_3 measurements (Khalil et al., 2009; Schraml et al., 2016). These 'open top'-chambers (area $0.125\,m^2$, height 0.40 m, volume $0.05\,m^3$; Figure 2.1) had a stainless steel ring on the bottom that was pushed into the soil by about 0.03 m. The chamber's body consisted of acrylic glass. Heating wires were wrapped around the chamber to prevent surface condensation, which would cause adsorption of emitted NH_3.

There were two measuring stations in operation, each equipped with six chambers. Ventilators were used to collect ambient air 2 m above ground level and to blow it tangentially into the chambers through 12 m of Teflon tubes (fluorinated ethylene propylene (FEP); inner diameter: 18 mm; Wolf-Technik, Stuttgart, Germany) at an average volumetric flow rate of $42.3\,l\,min^{-1}$ (41.2 to $43.7\,l\,min^{-1}$; inlet velocity: 2.70 to $2.86\,m\,s^{-1}$). The air took up the emitted gaseous N and spiraled upwards to the narrow top of the chamber, where the sample collection system was installed (Figure 2.1). A wind protection cover permitted inside air to flow out while simultaneously inhibiting the entry of external air. The sample collection system continuously drew a sample out of the air stream (wind velocity: $0.005\,m\,s^{-1}$) at a constant flow rate of $4\,l\,min^{-1}$ through 9 m of insulated (Foamed Polyurethane, PUR) and heated (50 °C) Teflon tubing (FEP; inner diameter: 8 mm; Wolf-Technik, Stuttgart, Germany). Before entering the measurement device, an upstream distribution system consisting of magnetic valves ensured that the air samples of the six chambers per measurement system were pro-

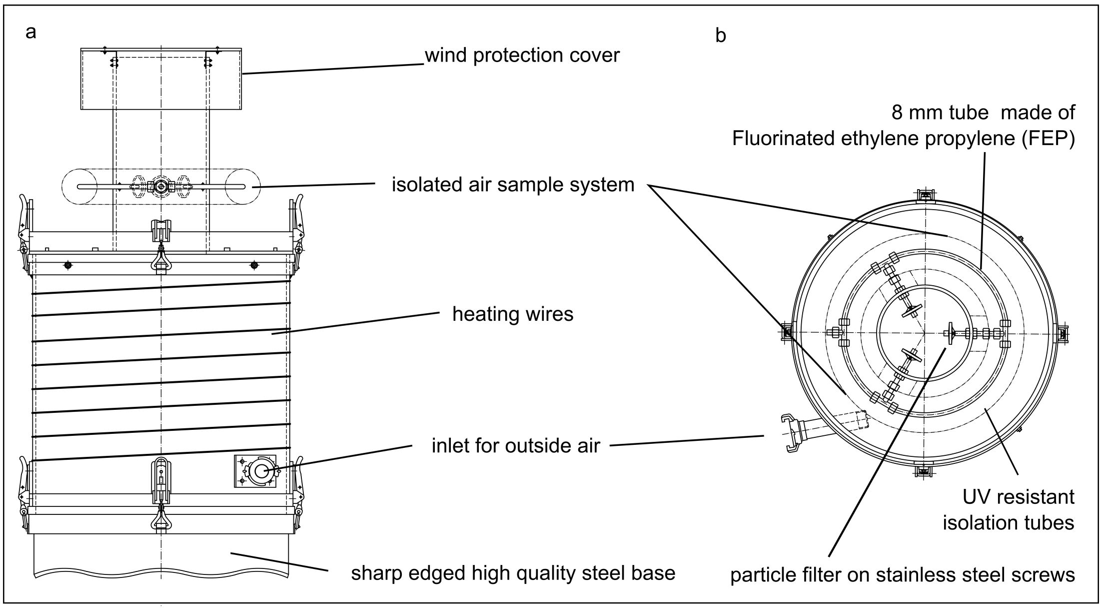

Figure 2.1: Scheme of the dynamic chamber (a: side view; b: top view). ©Thomas Kammerloher, Bavarian State Research Center, Institute of Agricultural Engineering and Animal Husbandry, Freising, Germany, 2013

cessed one at a time, and only the air of the actually sampled chamber was sucked into the converters and the detection unit (Figure 2.2).

As NH_3 has a high affinity to react with all surface materials, the measuring system was flushed with the air from the sampled chamber first, before NH_3 content was detected. In field experiments on arable land and in tray experiments in the greenhouse, the flushing time was 15 min. Chambers were analyzed in turn throughout the day, so that each chamber was measured once within 1.5 h. During experiments in the greenhouse, we realized that a flushing time of 15 min was not sufficient in some cases. Especially when a chamber containing a low NH_3 concentration in the air was measured right after one with a high concentration, the actual NH_3 level could not be reached within 15 min, and NH_3 emissions were overestimated for this chamber. As a consequence, we replaced the control system for the magnetic valves and programmed it to enable a flexible setting of the flushing time separately for each chamber. Consequently, in field experiments in grassland, sampling time for a chamber varied between 15 and 30 min, depending on the expected NH_3 concentration in the sampled air and on the expected difference in the NH_3 concentration to the sample in the preceding chamber.

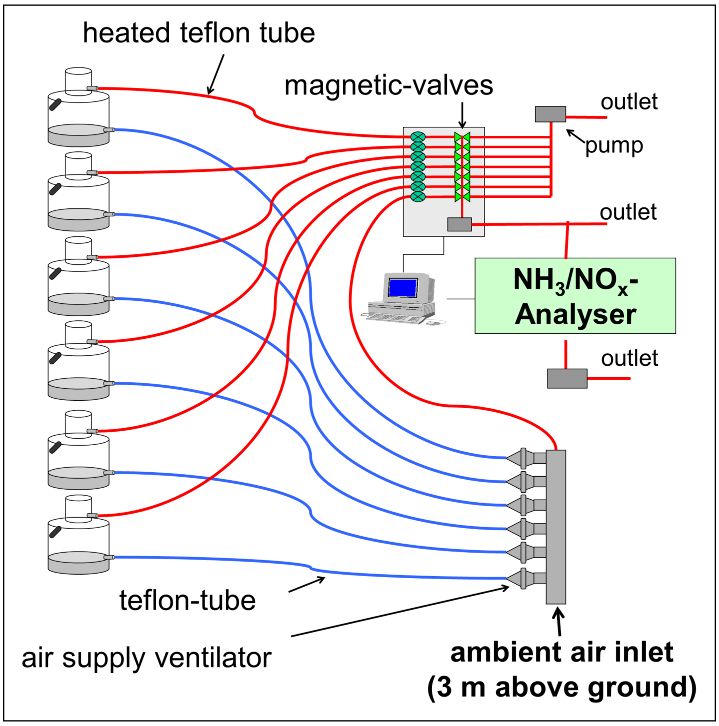

Figure 2.2: Simplified sketch of the NH_3 measurement system (Weber et al., 2001).

The measurement device for the detection of nitric oxide (NO) content in the air (CLD 700 AL, EcoPhysics, Gürnten, Switzerland) was based on the chemiluminescent gas phase reaction of NO with ozone (O_3) detected in a photomultiplier. For this purpose, all NOx and NH_3 had to be converted into NO first. For nitrogen dioxide (NO_2), this conversion was performed by a molybdenum (Mo) thermal converter ($3\,NO_2 + Mo \longrightarrow 3\,NO + MoO_3$) at 375 °C. Ammonia was converted into NO by the addition of O_3 in a stainless steel converter at a temperature of 600 °C. The two-chamber principle of the measuring device divided the sampled air into two streams. One stream passed only through the Mo converter ([NOx]=[NO] + [NO_2]), the other passed through both, first the steel converter and then the Mo-converter ([NOxamines]=[NOx] + [NH_3]). The concentration of NO within these two streams was measured in the photomultiplier. Thereafter, the ammonia content was calculated by [NH_3]=[NOxamines] − [NOx]. Samples were collected and analyzed throughout the day, establishing the progression of NH_3 emissions for every applied fertilizer treatment.

For every measurement day, the daily amount of NH_3 emissions (g NH_3-N ha^{-1} d^{-1}) was calculated for each chamber by:

$$F(NH_3) = \rho_N \ \Delta c \ Q \ A^{-1}$$

where $F(NH_3)$ is the NH_3-flux ($ng\ N\,m^{-2}\,s^{-1}$), ρ_N the standard density of the nitrogen in NH_3 ($ng\ N\,l^{-1}$), Δc the concentration difference between sample and ambient air ($ppbv = nl\,l^{-1}$), Q the volume flow rate ($l\,s^{-1}$) and A the chamber area ($0.125\,m^2$). These daily emissions from each chamber were used for statistical evaluation.

2.2 Experimental setup

Two different categories of experiments were carried out: field experiments and experiments under controlled conditions in the greenhouse.

Urea and urea with NBPT were provided in granular form by SKW Stickstoffwerke Piesteritz GmbH (Lutherstadt-Wittenberg, Germany). Application of the urease inhibitors IPAT and 2-NPT to the urea granule was carried out by SKW Stickstoffwerke Piesteritz GmbH and did not influence the granules' size of approximately 3 mm in diameter. CAN was obtained from the local distributor.

2.2.1 Field experiments

Design of experiments on arable land

Field experiments to determine NH_3 emissions from urea applied to winter wheat as well as to bare soil, and to evaluate the effectiveness of the urease inhibitors NBPT, IPAT and 2-NPT, were conducted at the experimental station Dürnast in Southern Germany (48°24′ N, 11°41′ E; 485 m a.s.l; Chair of Plant Nutrition, Technical University of Munich, Freising, Germany). Over four consecutive years 2002 to 2005, a total of 17 measurement campaigns were carried out, 14 of which took place in winter wheat and 3 on bare soil. Winter wheat was chosen because it is the primary crop in Germany, and because N application is carried out several times in the vegetation period from early March to mid-June. Measurements on bare soil were done to evaluate the NH_3 loss potential of urea without a possible influence of a standing crop.

The experimental site is characterized by a temperate climate with a mean annual temperature of 7.8 °C and an average annual precipitation of 821 mm (14.2 °C and 557 mm from March to September, respectively). The soil of this site is a Cambisol (IUSS Working Group WRB). In accordance with VDLUFA recommendations (VDLUFA, 1991; VDLUFA, 2007), representative soil samples were collected from the experimental site in a depth of 0-0.3 m. Samples were analyzed for the parameters listed in Table 2.1. Analytical methods used for soil analysis are described in Chapter 2.3.

Table 2.1: Soil characteristics of the site chosen for field experiments on arable land.

soil type	**pH**	**clay**	**silt**	**sand**	**Ntotal**	**Corg**	**urease activity**
	($CaCl_2$)	%	%	%	% DM	% DM	$\mu g\ NH_4^+ - N\ (g\ DM)^{-1}\ h^{-1}$
Cambisol	5.7	24.7	54.9	20.4	0.19	1.43	15.7

Corg, organic carbon content; DM, dry matter; Ntotal, content in total nitrogen

In all years, the measurement plots were 2 × 10 m in size. Per measurement period, 12 plots were installed in a randomized block design for simultaneous comparison of four fertilizer treatments with three replicates each. Each of the 12 chambers was placed on one of these 12 plots for an individual measurement period. Therefore, every plot was only used once per year for measurement of NH_3 emissions. In the course of a measurement period, the chambers were relocated daily onto a yet unused spot within the plots, both to avoid a ‘greenhouse’ effect, and to record the influence of preceding precipitation on the occurrence of NH_3 emissions.

Every year, sowing of winter wheat took place within the two last weeks of October. At the given row distance of 0.13 m for winter wheat, a sowing density of approximately 310 kernels m^{-2} and a field emergence rate of 95%, each ‘open top’-chamber always covered 3 rows with about 27 winter wheat plants, in total. Installation of the dynamic chambers in winter wheat is shown in Figure 2.3 a.

At the beginning of each growing season, all plots received a compound fertilizer as basal fertilization, applying 25 kg $P\,ha^{-1}$, 100 kg $K\,ha^{-1}$, 10 kg $Mg\,ha^{-1}$ and 40 kg $S\,ha^{-1}$.

At the start of their NH_3 measurement period, the experimental plots received 80 kg $N\,ha^{-1}$ as a single dressing. The granulated fertilizer was surface applied using an exact plot fertilizer spreader. The spreader had been customized for us by the Institute of Agricultural Engineering and Animal Husbandry at the Bavarian State Research Center for Agriculture (Freising, Germany). In all measurement periods a treatment consisting of urea was included. In the years 2002 to 2004, the two additional fertilizer treatments were urea + IPAT and urea + NBPT. IPAT was tested in its most effective

Figure 2.3: NH_3 measurement system installed on winter wheat (a) and grassland (b) experimental site Dürnast in the years 2004 (a) and 2008 (b).

concentration of 0.4% w/w. Being a well-studied and established UI, NBPT was evaluated in a concentration of 0.3% w/w, the commercially available concentration at the time. In 2005, urea + NBPT was replaced by the fertilizer treatment containing the newly developed UI 2-NPT. This treatment consisted of urea + 2-NPT in a concentration of 0.06% w/w. Not all UIs tested and evaluated within this project are discussed here.

Directly after fertilization, the NH_3 emission measurement system was installed and started. Ammonia emissions were recorded over a period of 6 to 22 days. Apart from technical reasons, measurement periods were terminated when the next fertilization took place and thus a new experiment started on adjacent plots. For the experiments with winter wheat, measurements were conducted during the growing season from March to June. Additional fertilizer applications were carried out on the bare soil of the already harvested field on July 22 and 29, 2004 as well as on July 13, 2005.

Statistical evaluation. All statistical evaluation was performed in R (R Development Core Team, 2011). NH_3 emission sums (dependent variable) for all fertilizer treatments (independent variable) were compared for each measurement period, by adding up daily NH_3 emission sums over the measurement period for every treatment. As data were normally distributed, sums of NH_3 emission of all three fertilizer treatments were submitted to a POLYANOVA analysis to evaluate possible differences. If treatment effects were detected, data were subjected to a mean value analysis using Fisher's least significant difference (LSD) procedure ($P \leq 0.05$). If only two fertilizer treatments were compared, sums of NH_3 emission of both treatments were submitted to the Mann-Whitney U test procedure ($P \leq 0.05$).

As emissions were low in most of the measurement periods with simultaneously high deviations, the effect of UIs on the course of NH_3 emissions was evaluated as well. For this, differences in the courses of daily NH_3 emission sums from directly adjacent plots were calculated for all fertilizer treatments using the Wilcoxon matched-pairs signed-ranks test ($P \leq 0.05$).

To enlarge the data sample for evaluation, all daily NH_3 emission sums from urea and urea + UI treatments were compared across all experimental years. This was done separately for measurements in winter wheat and on bare soil. As data were not normally distributed, the Mann-Whitney U test procedure ($P \leq 0.05$) was used for comparison of the independent data sets.

Possible correlations between the extent of NH_3 losses and different environmental factors, (e.g. air and soil temperature, air humidity, amount and time of precipitation) before and after fertilization were investigated by calculating the Pearson or Spearman correlation coefficient.

Design of experiments on grassland

In both years 2007 and 2008, field experiments were carried out from late May to late July. They were conducted on two permanent grassland sites in Southern Germany. In 2007, the experimental site was at Veitshof (48°24′ N, 11°41′ E, 446 m a.s.l.). In 2008, experiments were conducted at Dürnast (48°40′ N, 11°69′ E, 485 m a.s.l.) to evaluate the efficiency of the UI 2-NPT on a second site. Both sites consisted of an established sward of perennial ryegrass (*Lolium perenne* L.), which was cut four or five times per year, mainly for silage production.

For both sites, the long-time mean air temperature in the months of May, June and July is 12.4, 15.1 and 17.0 °C, respectively. Average precipitation in these months amounts to 77.5, 97.5 and 108.8 mm, respectively. In 2007, Deutscher Wetterdienst (DWD) provided data on precipitation from a nearby weather station (located 3 km north-west of the experimental site) at a daily resolution. Soil temperature was measured in a depth of 0.05 m at the experimental site (Th2-h, UMS GmbH, Munich, Germany), except during the first measurement period, where the measurement system failed and air temperature also had to be obtained from the DWD at a daily resolution. In 2008, both, air temperature and precipitation were recorded by the DWD at the same weather station used in 2007 (now located 400 m east of the experimental site) and were provided at a resolution of 30 min.

Soils were Endofluvic Chernozem in 2007 and Cambisol in 2008, according to IUSS Working Group WRB. Prior to the experiment, representative soil samples were taken from a depth of 0-0.15 m and analyzed for the parameters listed in Table 2.2. Analytical methods used for soil analysis are described in Chapter 2.3.

In both years, the plots were 2 × 5 m in size. Per measurement period, 12 plots were installed in a random block design for simultaneous comparison of four fertilizer treatments with three replicates. Four measurement periods were carried out in 2007 and three in 2008. A measurement period lasted at least 10 days and was stopped when suitable climatic conditions for the start of the next period were expected. Thus, experimental periods lasted between 10 and 21 days. However, for a better comparison of the course and extent of NH_3 losses, all measurement periods are reported over 10 days in the current work.

All experimental periods were started after a period of precipitation and as soon as a warmer and rainless phase was forecast, which ensured moist conditions at the soil surface and thus a better dissolution of the granules. Warm temperatures enhanced conditions for high NH_3 losses. The only exception was the first measurement period in 2007, where soil conditions at the time of fertilization were dry.

The day before fertilization, the grassland of the plots to be measured was cut down to a stubble height of 0.05 m with a plot grass harvester. The fertilizer was surface-applied using an exact plot fertilizer spreader. Straight after fertilization, the NH_3 emission measurement system was installed and started. Installation of the dynamic chambers on grassland is shown in Figure 2.3 b.

On both experimental sites for grassland, $30\,\mathrm{m}^3$ of cattle slurry ($145\,\mathrm{kg\ Ntotal\,ha}^{-1}$) were applied at the start of vegetation (end of March). In all measurement periods there was a control treatment which received no N (0N), and a treatment consisting of urea. In 2007, the two additional fertilizer treatments were urea + 2-NPT in a concentration of 0.10% w/w and calcium ammonium nitrate (CAN). CAN was chosen because it is the most widely used chemical fertilizer in Germany, where it accounts for 37% of nitrogen fertilizer consumption, followed by urea with 21% (IFA, 2015). Therefore, it is often used as a reference point when comparing fertilizer efficiency. As no NH_3 losses following the application of CAN were detected in 2007, CAN was left out in 2008. In that year, the two additional fertilizer treatments consisted of urea + 2-NPT in two different concentrations of 0.075 and 0.15% w/w. For all experimental periods, mineral fertilizer rate was $80\,\mathrm{kg\ N\,ha}^{-1}$, which is a common dose per cut for intensively used grassland in Germany (Wendland et al.).

Harvesting of the experimental plots was performed using a plot grass harvester to determine biomass yields. Harvest dates were scheduled according to agronomic criteria, and in some cases had to be delayed due to climatic conditions. Therefore, harvesting took place 33 to 52 days after fertilization.

Statistical evaluation. All statistical evaluation was performed in R (R Development Core Team, 2011). For each measurement period, fertilizer treatments (independent variable) were compared according to their NH_3 emissions (dependent variable). Daily NH_3 emissions occurring over the measurement period of 10 days were added up for every fertilizer treatment. As on both sites cattle slurry had been applied prior to the experiment, average NH_3 emissions detected on control plots were subtracted from average NH_3 emissions measured on the fertilized plots to only determine the amount of N lost as NH_3 following the chemical fertilizer application. As data were normally distributed, sums of NH_3 emissions of all four fertilizer treatments were submitted to a POLYANOVA analysis to evaluate possible differences. Subsequently, data were subjected to a mean value analysis using Fishers LSD procedure ($P \leq 0.05$). Grass yields were also subjected to a POLYANOVA.

Regression analyses were performed to investigate possible effects of climatic and soil factors on NH_3 emissions. A multiple regression analysis could not be conducted, since only a few degrees of freedom were left.

2.2.2 Greenhouse experiments

Different forms of experiments were carried out in the greenhouse: Pot experiments and tray experiments. Soils used in these experiments are described in Table 2.3.

Pot experiments

In 2004 and 2005, the possible impact of the soil texture on NH_3 emissions from urea and the reducing potential of the urease inhibitors NBPT, IPAT and 2-NPT on NH_3 emissions was specifically worked upon under controlled conditions in pot experiments.

All pot experiments were carried out in Mitscherlich pots (height: 0.21 m; area: $0.03\,m^2$). Soils to be evaluated were filled in to a bulk density of $0.9\,g\,cm^{-3}$ and watered up to their water holding capacity.

In 2004, soils of the two experimental stations Dürnast and Cunnersdorf, which were part of the joint project and showed a different emission potential for NH_3 losses following the application of urea, were compared (Table 2.3).

Table 2.2: Soil characteristics of the experimental sites chosen for grassland experiments.

site	**year**	**soil type**	**pH**	**clay**	**silt**	**sand**	**Corg**	**urease activity**
			(CaCl$_2$)	%	%	%	% DM	µg $NH_4^+ - N\,(g\ DM)^{-1}\,h^{-1}$
Veitshof	2007	Endofluvic Chernozem	7.2	26.8	45.7	27.5	7.10	279
Dürnast	2008	Cambisol	5.7	28.1	46.6	25.3	10.65	105

Corg, organic carbon content; DM, dry matter

Table 2.3: Soil characteristics of the soils chosen for greenhouse experiments.

site	**land use**	**pH**	**clay**	**silt**	**sand**	**Corg**	**urease activity**
		(CaCl$_2$)	%	%	%	% DM	µg $NH_4^+ - N\,(g\ DM)^{-1}\,h^{-1}$
Cunnersdorf	AL	5.8	12.0	45.0	43.0	0.80	23.9
Dürnast	AL	5.7	24.7	54.9	20.4	1.43	20.2
Mintraching	AL	7.3	53.2	34.6	12.2	4.83	45.3
Scheyern	AL	5.5	11.7	21.5	66.8	1.30	27.0
Schrobenhausen	AL	5.1	4.7	6.8	88.5	0.50	7.2
Westermeier	AL	5.8	17.4	53.6	29.0	1.24	26.4
Westermeier	GL	6.2	17.4	53.6	29.0	1.64	54.3

AL, arable land; Corg, organic carbon content; DM, dry matter; GL, grassland

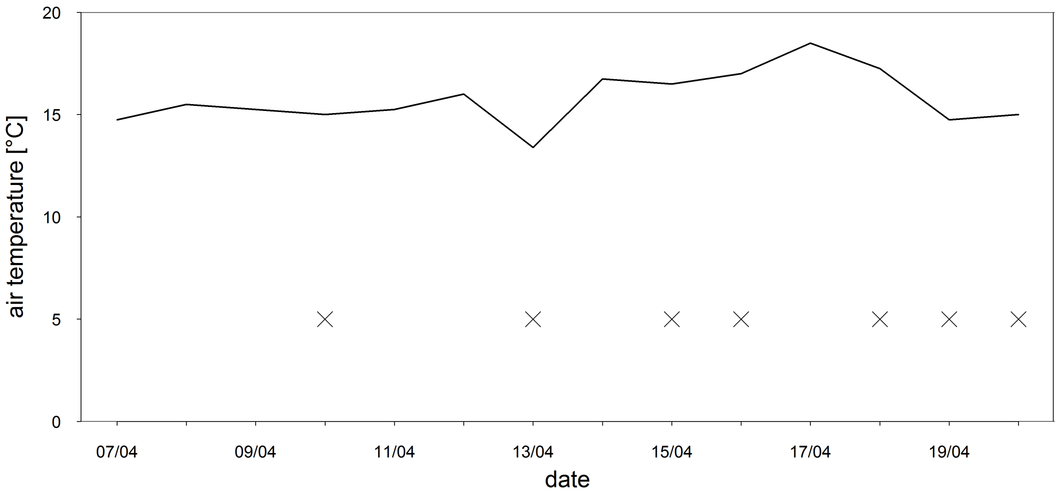

Figure 2.4: Course of air temperature during phase for NH_3 emission losses induced by 6 hours of ventilation per day. Days on which soil surface of all pots were moistened are indicated (x).

In 2005, the two soils Dürnast and Mintraching, differing in pH, soil texture and organic carbon content (Corg), were chosen (Table 2.3).

Fertilizer treatments. Except for the control treatment which received no N, 0.6 g N were applied per pot.

To indirectly determine NH_3 losses, the standard treatment consisted of urea incorporated into the soil to a depth of 5 cm. This should guarantee that no N losses as NH_3 occurred from this treatment. For all further fertilizer treatments, granules were surface applied onto the bare soil. All treatments were carried out in a fourfold replication.

Experimental setup. Fertilizer treatments, duration of the loss phase as well as soils used in pot experiments in 2004 and 2005 are listed in Table 2.4.

In 2004, after fertilizer placement, an NH_3 loss phase of 14 days followed. Temperature was logged during the phase of emissions and soils were moistened when their surface appeared dry (Figure 2.4). Pots were ventilated for 6 hours per day to induce higher NH_3 losses.

In 2005, fertilizer placement was also followed by a loss phase of 13 days for enhanced NH_3 losses. Temperature was not logged during the phase of emission. Low precipita-

Table 2.4: Information about soil and fertilizer treatments used in pot experiments in the years 2004 and 2005.

year	**soil**	**crop**	**fertilizer**	**duration of loss phase** d
2004	Dürnast Cunnersdorf	spring wheat	control (nil N) urea incorporated CAN urea urea + IPAT 0.4% urea + NBPT 0.3%	14
2005	Dürnast Mintraching	oat	control (nil N) urea incorporated urea urea + IPAT 0.4% urea + NBPT 0.3% urea + 2-NPT 0.06%	13

CAN, calcium ammonium nitrate

tion events of 1.7 mm per day, on average, were simulated and pots were ventilated for 6 hours from 09:00 a.m. to 03:00 p.m., every day.

In all experiments, at the end of the period of NH_3 emissions, surface applied fertilizers were manually incorporated to a depth of 5 cm. Plants were sown (2004: spring wheat; 2005: oat) and thinned to a density of 85 plants m^{-2} two weeks later. Pots were installed in a randomized block design. Around BBCH 77 plants were harvested.

Harvest. To determine yield, N uptake and fertilizer N use efficiency, all plants of each pot were cut directly aboveground and collected. All plants were weighed for each pot, then ears were cut and weighed, separately. Ears were manually threshed. For each pot, total straw and total grains were used for gravimetric dry weight determination and chemical analysis, separately. Analytical methods used for plant analysis are described in Chapter 2.3.

Statistical evaluation. Statistical evaluation was performed the same way for all experiments. Dry matter grain yield, N content in grains, N content in the total aboveground plant and N use efficiency (dependent variables) for all fertilizer treatments (independent variable) were compared. As they were normally distributed, data of all fertilizer treatments were submitted to a POLYANOVA analysis to evaluate possible differences. If treatment effects were detected, they were subjected to a mean value analysis using the Tukey test procedure ($P \leq 0.05$).

Tray experiments

Following field experiments in winter wheat (2002 - 2005) and prior to field experiments in grassland (2007 - 2008), tray experiments were carried out under an optimum environment for high rates of NH_3 losses in the greenhouse. Especially the reducing impact of regular precipitation on NH_3 emissions should be factored out while using the same NH_3 measurement technique as in field experiments. In two measurement periods, a possible influence of differences in land use or in soil characteristics (Corg, pH, texture) on NH_3 emissions following application of urea, as well as the mitigation potential of the added urease inhibitor IPAT 0.4% w/w, were investigated.

For both measurement periods, six trays were installed. Trays had a surface of $1.98\,\text{m} \times 0.78\,\text{m}$ and were 0.1 m in depth. Each tray was divided into two plots. On

Figure 2.5: NH_3 measurement system installed on trays in the greenhouse.

each of these plots one chamber was placed. In contrast to measurements in the field, the chambers remained on their placement throughout the measurement period. Figure 2.5 shows the installation of the dynamic chambers on the trays in the greenhouse.

Except for grassland, application of urea was carried out onto bare soil. All treatments were applied in a threefold replication and consisted of urea alone and urea + IPAT 0.4% w/w. In both measurement periods 80 kg N ha^{-1} were fertilized.

Soil water content was adjusted to soil water holding capacity at the time of fertilizer application, to ensure high soil humidity over the whole NH_3 loss phase. The elevated temperature in the greenhouse was expected to be favorable for high NH_3 losses. As chambers were not moved during the measurement period, temperature within the chamber was recorded (Th2-h, UMS GmbH, Munich, Germany) and used for evaluation. Three days before the measurement period, grassland was cut to a stubble height of 5 cm. Immediately following application of urea, chambers were placed on the plots and NH_3 emissions were recorded over a period of 10 days.

Statistical evaluation. All statistical evaluation was performed in R (R Development Core Team, 2011). To detect possible differences in NH_3 emissions, NH_3 sums from urea with or without the UI IPAT 0.4% w/w were compared separately for each soil or land use. Daily NH_3 emission sums occurring over the measurement period were added up. As data were normally distributed, sums of NH_3 emission were submitted to a POLYANOVA analysis to evaluate possible differences. If effects were detected, data were subjected to a mean value analysis using the Tukey test procedure ($P \leq 0.05$).

Furthermore, the effect of IPAT 0.4% w/w on the course of NH_3 emissions was evaluated as well. For this evaluation, differences in the courses of daily NH_3 emission from urea with or without IPAT 0.4% w/w were calculated separately for each soil or land use using the Wilcoxon matched-pairs signed-ranks test ($P \leq 0.05$).

2.3 Laboratory analyses

Soil pH was determined following Schofield et al. (1955) (0.01 M $CaCl_2$). Soil texture was analyzed in compliance with Gee and Bauder (1986), whereby organic matter had to first be removed by the use of hydrogen peroxide (H_2O_2). Organic carbon content (Corg) was calculated as (Corg = Ct − Ccarb), with the total C content (Ct) determined in a C/N-analyzer (ANCA-NT system, Europa Scientific Ltd., Crewe, United Kingdom) and the carbonatic carbon content (Ccarb) measured following the volumetric calcimeter method (Loeppert and Suarez, 1996). Total-nitrogen (Nt) was analyzed using the Dumas procedure (combustion at 1100 °C; VDLUFA, 2016). Urease activity was determined using a 0.01 M $CaCl_2$ solution following the non-buffered method of Kandeler and Gerber (1988). Cation exchange capacity was analyzed following extraction into a buffered 0.1 M $BaCl_2$ solution (pH 8.1).

All plant material was dried at 105 °C, and ground to pass through a 1.5 mm sieve. Total N was determined using a macro N (4.5a macro N, elementar, Hanau, Germany).

Chapter 3

Results

3.1 Field experiments on arable land

3.1.1 Ammonia losses from urea applied to winter wheat

Over the 14 measuring campaigns in winter wheat, cumulative ammonia (NH_3) losses following the application of urea varied between 0.05 and 2.2 kg $NH_3 - N\,ha^{-1}$ (Table 3.1), corresponding to 0.1% up to 2.7% of the applied N. On average, 0.6% of the fertilizer N got lost into the atmosphere as NH_3. Time of urea application within the vegetation period had no influence on the extent of resulting NH_3 losses following the application of urea ($r_{Pearson}$ = -0.11).

In most measurement periods, NH_3 emissions from urea peaked between 2 and 7 days after fertilization. The extent of NH_3 emissions was slightly positively correlated with the average temperature during the phase of emissions ($r_{Spearman}$ = 0.31). Nearly all measurement periods were prematurely terminated by rainfall. When not terminated by rainfall, emissions seemed to last for up to two weeks (Figure 3.2 c).

When urea was applied to winter wheat, the resulting NH_3 emissions were significantly reduced by all urease inhibitors (UI) IPAT (600 measurement days; $P = 0.042$), NBPT (435 days; $P = 0.002$) and 2-NPT (93 days; $P < 0.001$) when the evaluation was done over all measurements. On average, IPAT reduced NH_3 losses by 23% (13 measurement periods), NBPT by 32% (10 periods) and 2-NPT by 53% (2 periods; Table 3.1). Only following fertilizations on April 12, 2002, these differences in NH_3 emission sums from urea and urea with any urease inhibitor were significant (Table 3.1).

Table 3.1: Ammonia emissions (g $NH_3 - N\,ha^{-1}$) occurring following the surface application of urea without or with a UI, NBPT, IPAT or 2-NPT, to winter wheat in the years 2002 to 2005. Sums in NH_3 emission and standard errors are displayed. Letters indicate statistical groups determined by Mann-Whitney U test procedure (2 treatments) or POLYANOVA (> 2 treatments) together with Fisher's LSD procedure ($P \leq 0.05$).

date	**urea**	**urea + NBPT 0.3%**	**urea + IPAT 0.4%**	**urea + 2-NPT 0.06%**
12.04.02 (13*)	$307^a \pm 34.0$	$132^b \pm 59.6$	$111^b \pm 40.6$	
26.04.02 (11*)	$84^a \pm 36.1$	$27^a \pm 21.9$	$70^a \pm 33.4$	
08.05.02 (12*)	$78^a \pm 24.1$	$40^a \pm 9.0$	$45^a \pm 26.0$	
23.05.02 (12*)	$84^a \pm 26.7$	$68^a \pm 26.2$	$66^a \pm 13.5$	
27.03.03 (20*)	$797^a \pm 201.1$	$418^a \pm 149.3$	$703^a \pm 160.1$	
08.05.03 (6*)	$48^a \pm 14.7$	$30^a \pm 15.0$	$41^a \pm 12.7$	
26.05.03 (12*)	$2192^a \pm 407.9$	$1941^a \pm 905.2$	$1600^a \pm 471.1$	
11.06.03 (16*)	$732^a \pm 258.3$	$493^a \pm 7.6$	$538^a \pm 101.3$	
18.03.04 (10*)	$1539^a \pm 480.8$		$394^a \pm 261.9$	
31.03.04 (21*)	$375^a \pm 117.2$		$406^a \pm 95.1$	
04.05.04 (22*)	$112^a \pm 10.5$	$88^a \pm 9.5$	$100^a \pm 14.7$	
27.05.04 (21*)	$121^a \pm 25.8$	$153^a \pm 15.8$	$121^a \pm 23.7$	
02.05.05 (9*)	$57^a \pm 59.0$			$59^a \pm 20.0$
11.05.05 (22*)	$108^a \pm 38.9$		$115^a \pm 86.6$	$-10^a \pm 40.2$

* length of measuring period

2002

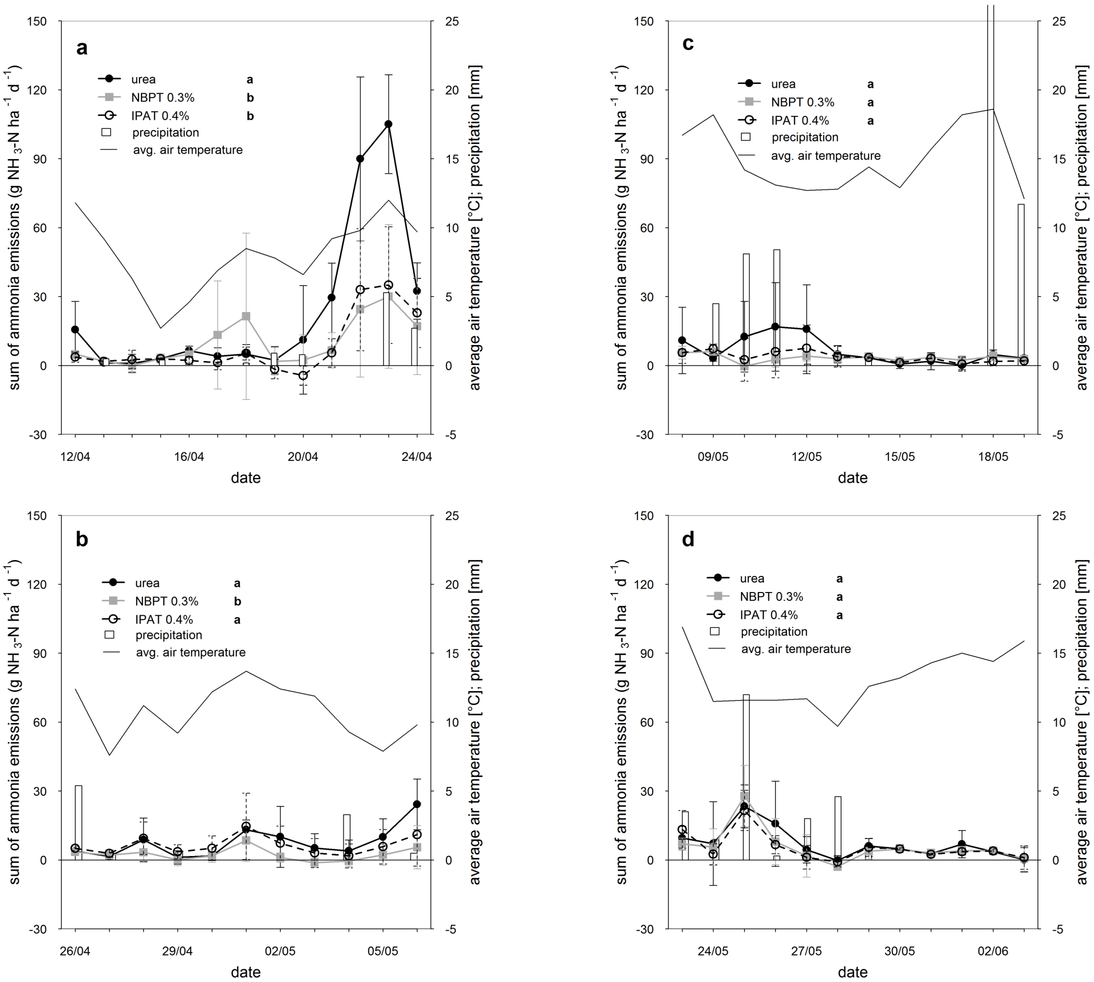

Figure 3.1: Courses of daily NH_3 emissions ($g\ NH_3 - N\,ha^{-1}$) following the surface application of urea without or with a UI, NBPT or IPAT, to winter wheat in the year 2002. The daily average for air temperature and the daily sum of precipitation are indicated. Letters behind the fertilizers indicate significant differences in the course of NH_3 emissions detected using Wilcoxon matched-pairs signed-ranks test ($P \leq 0.05$).

2003

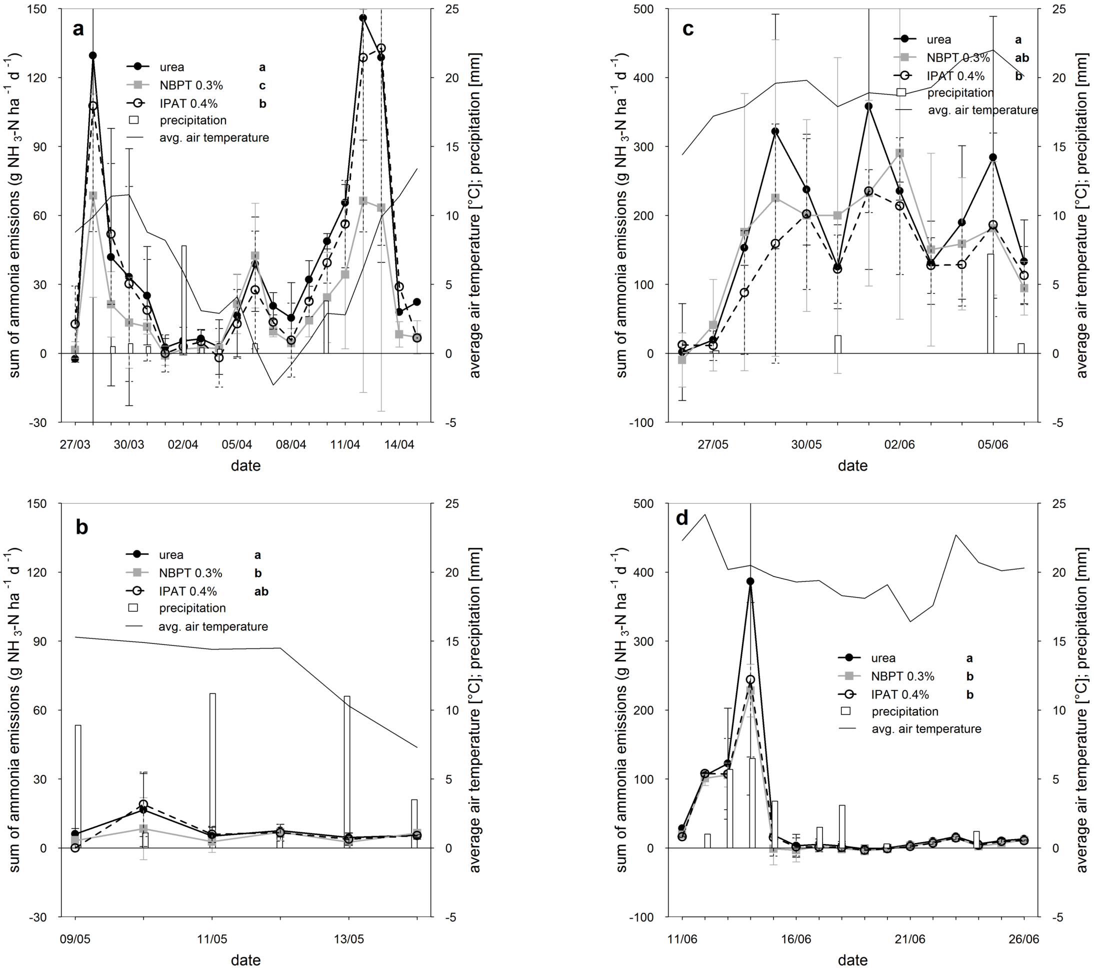

Figure 3.2: Courses of daily NH_3 emissions ($\mathrm{g\ NH_3 - N\,ha^{-1}}$) following the surface application of urea without or with a UI, NBPT or IPAT, to winter wheat in the year 2003. The daily average for air temperature and the daily sum of precipitation are indicated. Letters behind the fertilizers indicate significant differences in the course of NH_3 emissions detected using Wilcoxon matched-pairs signed-ranks test ($P \leq 0.05$).

2004

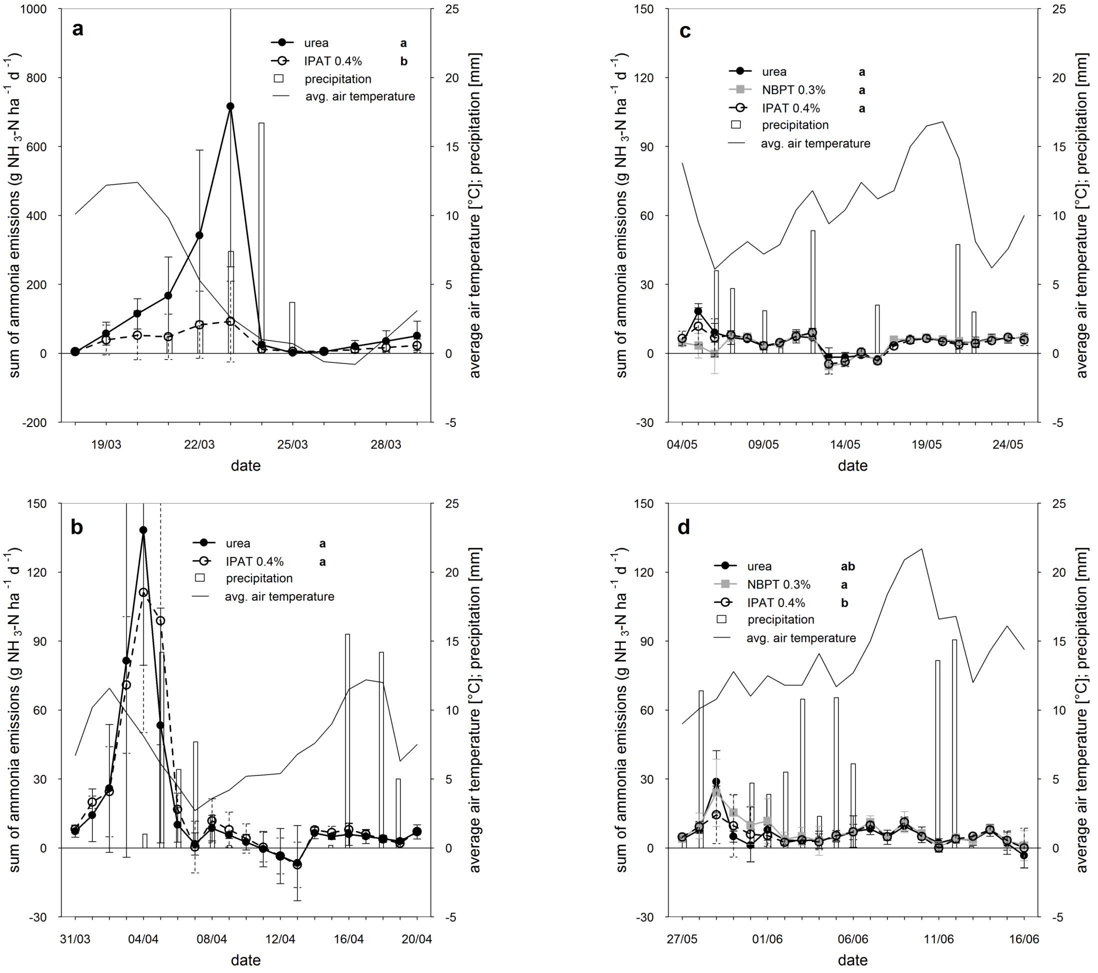

Figure 3.3: Courses of daily NH_3 emissions $(\mathrm{g\ NH_3 - N\,ha^{-1}})$ following the surface application of urea without or with a UI, NBPT or IPAT, to winter wheat in the year 2004. The daily average for air temperature and the daily sum of precipitation are indicated. Letters behind the fertilizers indicate significant differences in the course of NH_3 emissions detected using Wilcoxon matched-pairs signed-ranks test ($P \leq 0.05$).

2005

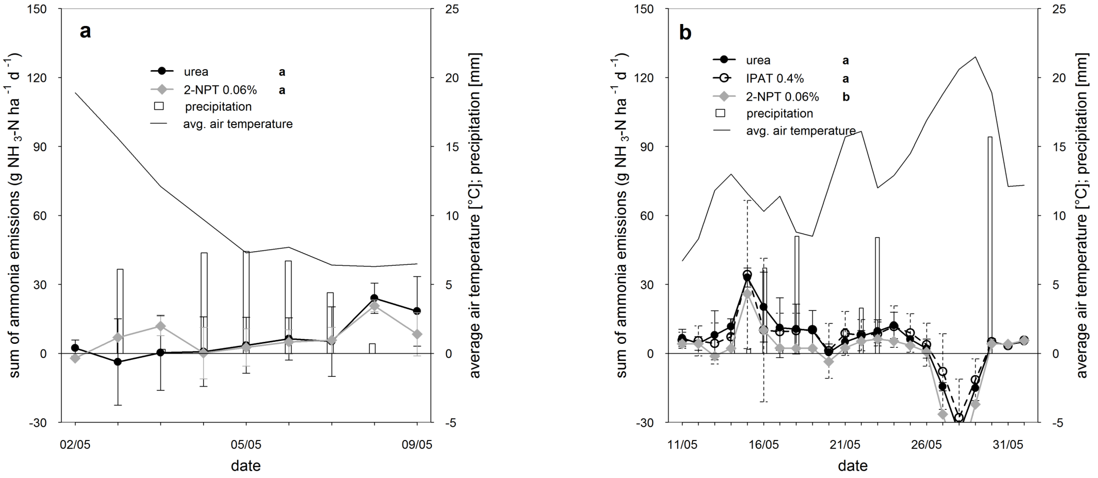

Figure 3.4: Courses of daily NH_3 emissions ($g\ NH_3 - N\,ha^{-1}$) following the surface application of urea without or with a UI, IPAT or 2-NPT, to winter wheat in the year 2005. The daily average for air temperature and the daily sum of precipitation are indicated. Letters behind the fertilizers indicate significant differences in the course of NH_3 emissions detected using Wilcoxon matched-pairs signed-ranks test ($P \leq 0.05$).

3.1.2 Ammonia losses from urea applied onto bare soil

In all three measuring campaigns, application on bare soil led to considerable NH_3 emissions between 2.1 and 13.1 kg $NH_3 - N\,ha^{-1}$, corresponding to 2.6% up to 16.3% of the applied N (Table 3.2).

Under these conditions, a peak in NH_3 emissions from urea could be observed after 2 days (Figure 3.5). Emissions lasted for up to 9 days, when not interrupted by rainfall (Figure 3.5 b).

Evaluated over all measurements, only 2-NPT significantly reduced the losses from urea applied to bare soil, though NH_3 emissions were much higher. When evaluating the single measurement periods, addition of NBPT resulted in an average reduction by 42%, compared to 28% by IPAT and 49% by 2-NPT (Table 3.2). However, only following fertilization on July 13, 2005, these differences in NH_3 emission sums from urea and urea with any urease inhibitor were significant (Table 3.2).

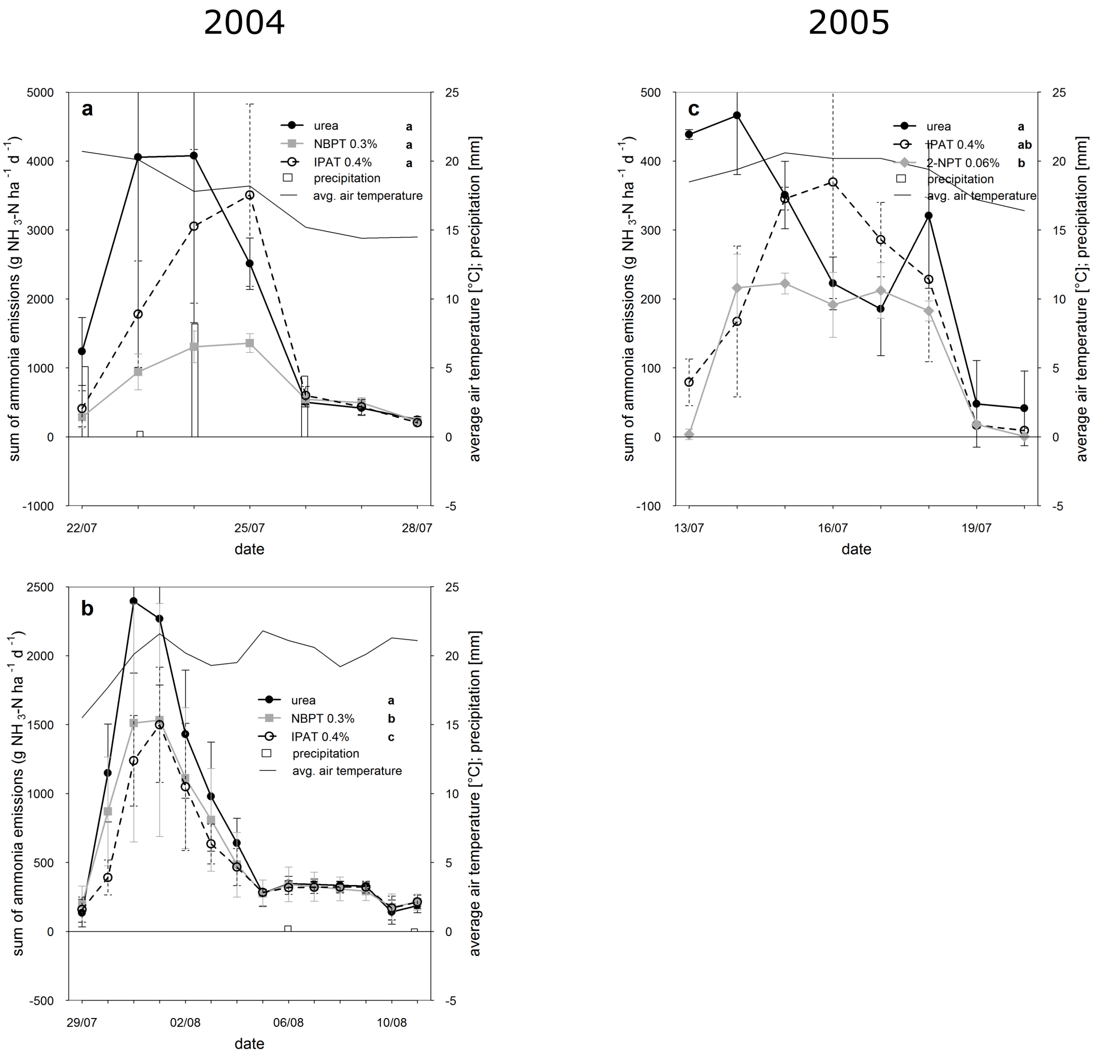

Figure 3.5: Courses of daily NH_3 emissions ($\mathrm{g\ NH_3 - N\,ha^{-1}}$) following the surface application of urea without or with a UI, NBPT, IPAT or 2-NPT, to bare soil in the years 2004 (a and b) and 2005 (c). The daily average for air temperature and the daily sum of precipitation are indicated. Letters behind the fertilizers indicate significant differences in the course of NH_3 emissions detected using Wilcoxon matched-pairs signed-ranks test ($P \leq 0.05$).

Table 3.2: Ammonia emissions (g $NH_3 - N\,ha^{-1}$) occurring following the surface application of urea without or with a UI, NBPT, IPAT or 2-NPT, to bare soil in 2004 and 2005. Sums in NH_3 emission and standard errors are displayed. Letters indicate statistical groups determined by Mann-Whitney U test procedure (2 treatments) or POLYANOVA (>2 treatments) together with Fisher's LSD procedure ($P \leq 0.05$).

date	**urea**	**urea + NBPT 0.3%**	**urea + IPAT 0.4%**	**urea + 2-NPT 0.06%**
22.06.04 (7*)	$13058^a \pm 3328.4$	$5163^a \pm 503.5$	$9996^a \pm 1893.6$	
29.07.04 (14*)	$10952^a \pm 1533.7$	$8454^a \pm 1841.8$	$7392^a \pm 757.3$	
13.07.05 (8*)	$2073^a \pm 164.6$		$1502^b \pm 134.6$	$1048^b \pm 36.7$

* length of measuring period

3.2 Greenhouse experiments

3.2.1 Pot experiments

Pot experiment in 2004. In 2004, the experiment focused on a comparison of soils from the experimental stations Dürnast and Cunnersdorf, differing in texture, organic carbon content and urease activity.

Evaluation of control pots (nil N) showed, that soil Dürnast had a significantly higher yield potential with an 80% higher grain yield compared to soil Cunnersdorf (Table 3.3). Furthermore, N uptake by total aboveground plants was significantly higher in Dürnast control pots, although N content in grains from Cunnersdorf control pots was significantly higher (Table 3.3).

Fertilization led to a significant increase in yield as well as in N uptake. On both soils, the different fertilizer treatments showed a similar effect. The incorporation of urea safeguarded fertilized N and resulted in highest grain yields, N contents, N uptake and as a consequence in the highest fertilizer N use efficiency (NUE) by spring wheat (Figure 3.6). Application of CAN resulted in slightly lower yield, N contents and consequently lower NUE (Figure 3.6). However, except for grain yield on soil Cunnersdorf, these differences were not significant (Table 3.3). On both soils, surface application of urea led to lowest grain yields and N uptake and thus to significantly reduced NUEs (by

Table 3.3: Evaluation of pot experiment in 2004. Two soils were compared with six fertilizer treatments. Averages and standard errors are indicated. Letters indicate statistical groups determined with POLYANOVA together with Tukey test procedure ($P \leq 0.05$).

soil	**treatment**	**DM weight grains** g/pot	**N content grains** %	**N uptake plant** g N/pot
Dürnast	control (nil N)	$13.6^{b} \pm 0.35$	$1.26^{a} \pm 0.026$	$0.24^{b} \pm 0.007$
	urea incorporated	$36.8^{e} \pm 0.32$	$1.71^{e} \pm 0.021$	$0.81^{f} \pm 0.014$
	CAN	$36.2^{de} \pm 0.69$	$1.60^{de} \pm 0.028$	$0.76^{ef} \pm 0.008$
	urea	$33.4^{de} \pm 0.37$	$1.53^{cd} \pm 0.013$	$0.66^{bc} \pm 0.005$
	urea + IPAT 0.4%	$33.9^{de} \pm 0.72$	$1.58^{d} \pm 0.021$	$0.68^{cd} \pm 0.015$
	urea + NBPT 0.3%	$34.5^{de} \pm 0.89$	$1.62^{de} \pm 0.031$	$0.72^{de} \pm 0.009$
Cunnersdorf	control (nil N)	$7.7^{a} \pm 0.55$	$1.40^{b} \pm 0.045$	$0.15^{a} \pm 0.007$
	urea incorporated	$34.2^{de} \pm 1.16$	$1.54^{cd} \pm 0.013$	$0.65^{fg} \pm 0.013$
	CAN	$30.2^{c} \pm 0.34$	$1.59^{de} \pm 0.029$	$0.61^{ef} \pm 0.004$
	urea	$27.3^{c} \pm 0.57$	$1.45^{bc} \pm 0.023$	$0.50^{c} \pm 0.016$
	urea + IPAT 0.4%	$28.1^{c} \pm 0.58$	$1.52^{bcd} \pm 0.021$	$0.53^{cd} \pm 0.005$
	urea + NBPT 0.3%	$29.3^{c} \pm 0.74$	$1.52^{bcd} \pm 0.029$	$0.56^{de} \pm 0.021$

CAN, calcium ammonium nitrate; DM, dry matter

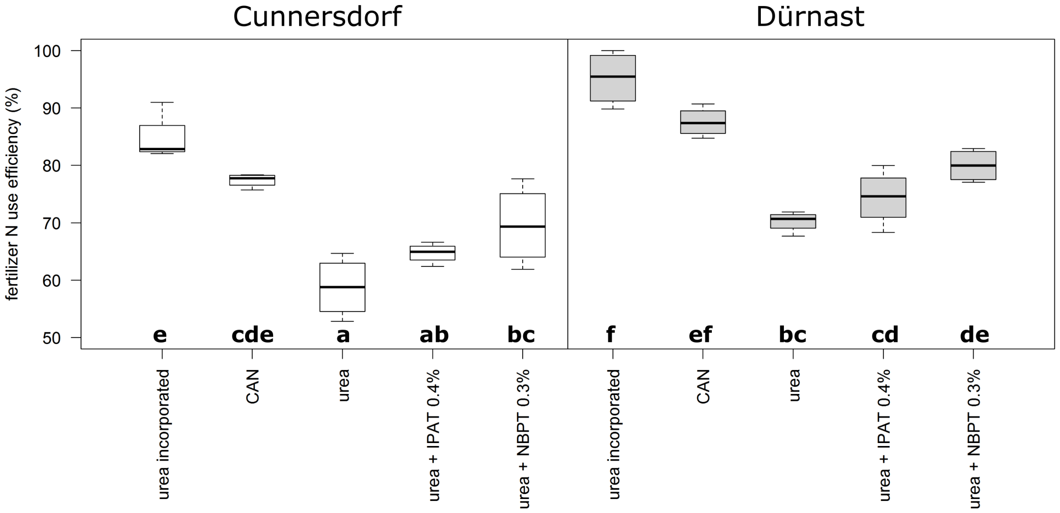

Figure 3.6: Efficiency of fertilizer nitrogen use (%) in pot experiment on two different soils Dürnast and Cunnersdorf with spring wheat in 2004. Letters indicate statistical groups evaluated with POLYANOVA together with Tukey test procedure ($P \leq 0.05$).

31% and 26%) on soil Cunnersdorf and Dürnast, respectively (Figure 3.6). Addition of both urease inhibitors, IPAT and NBPT, led to a higher grain yield and N uptake and thus to an increased NUE. However, only when adding NBPT 0.3% w/w, this effect was significant on both soils.

Pot experiment in 2005. In 2005, the experiment's focus was on comparison of soils differing in organic carbon content (Table 2.3). Besides a higher Corg, soil Mintraching also had a higher pH, a finer soil texture and a higher urease activity. CAN was left out as a fertilizer treatment and was replaced by urea + 2-NPT 0.06% w/w.

Soil Mintraching showed an elevated oat grain yield potential and a higher N uptake in control pots (nil N). However, differences between the two soils were not significant (Table 3.4).

On both soils, fertilization of 0.6 g N per pot as urea with or without any UI significantly increased yield and N uptake.

Incorporation of the urea granules slightly enhanced grain yield as well as N uptake compared to surface applied urea. As a consequence, fertilizer NUE of oat plants was improved by 17% and 12% to 98.8% and 95.4% on soils Dürnast and Mintraching, respectively. This effect was only significant on soil Dürnast. The two soils showed no difference in the extent of NH_3 losses, as NUE of surface applied urea was on the same

Table 3.4: Evaluation of pot experiment in 2005. Two soils were compared with six fertilizer treatments. Averages and standard errors are indicated. Letters indicate statistical groups determined with POLYANOVA together with Tukey test procedure ($P \leq 0.05$)

soil	**treatment**	**DM weight grains**	**N content grains**	**N uptake plant**
		g/pot	%	g N/pot
Dürnast	control (nil N)	$18.1^{a} \pm 0.6$	$1.31^{a} \pm 0.041$	$0.38^{a} \pm 0.014$
	urea incorporated	$42.8^{e} \pm 0.5$	$1.48^{abc} \pm 0.017$	$0.98^{cd} \pm 0.017$
	urea	$40.9^{de} \pm 0.9$	$1.49^{abc} \pm 0.045$	$0.87^{b} \pm 0.019$
	urea + IPAT 0.4%	$39.5^{cde} \pm 0.9$	$1.48^{ab} \pm 0.039$	$0.92^{bc} \pm 0.017$
	urea + NBPT 0.3%	$43.1^{e} \pm 1.4$	$1.39^{a} \pm 0.047$	$0.94^{bcd} \pm 0.023$
	urea + 2-NPT 0.06%	$42.1^{de} \pm 1.4$	$1.46^{a} \pm 0.018$	$1.00^{cd} \pm 0.021$
Mintraching	control (nil N)	$22.4^{a} \pm 0.32$	$1.39^{a} \pm 0.022$	$0.44^{a} \pm 0.009$
	urea incorporated	$35.0^{bc} \pm 1.71$	$1.85^{d} \pm 0.070$	$1.02^{d} \pm 0.017$
	urea	$33.0^{b} \pm 0.88$	$1.80^{d} \pm 0.033$	$0.95^{bcd} \pm 0.015$
	urea + IPAT 0.4%	$32.8^{b} \pm 1.03$	$1.80^{d} \pm 0.029$	$0.96^{cd} \pm 0.021$
	urea + NBPT 0.3%	$35.6^{bc} \pm 0.90$	$1.68^{bcd} \pm 0.017$	$0.94^{bcd} \pm 0.006$
	urea + 2-NPT 0.06%	$37.1^{bcd} \pm 1.34$	$1.68^{cd} \pm 0.066$	$0.97^{cd} \pm 0.023$

DM, dry matter

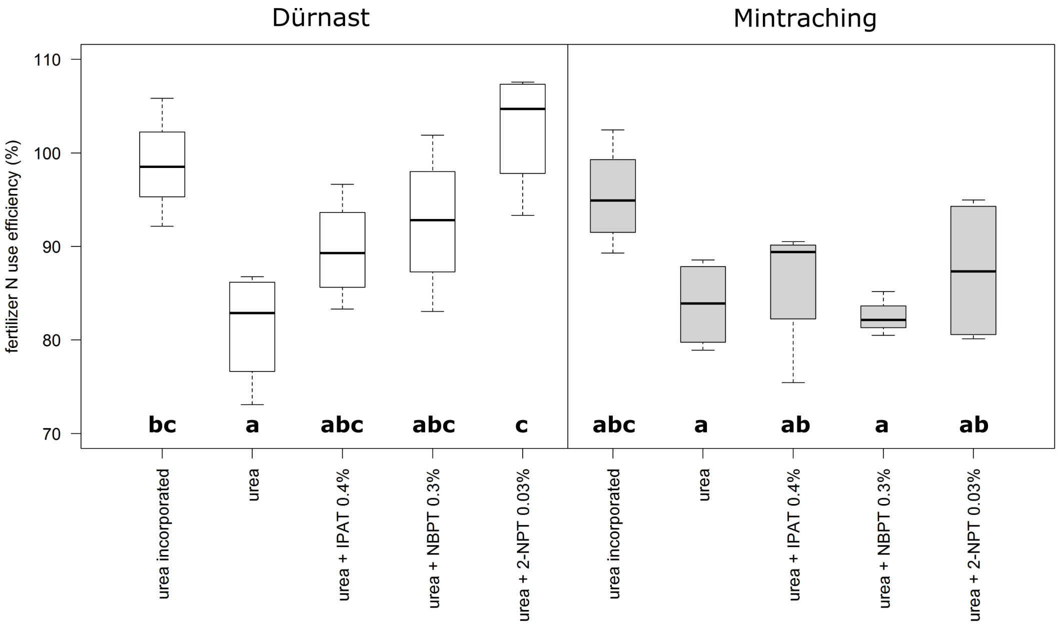

Figure 3.7: Efficiency of fertilizer nitrogen use (%) in pot experiment on two different soils Dürnast and Mintraching with oat in 2005. Letters indicate statistical groups evaluated with POLYANOVA together with Tukey test procedure ($P \leq 0.05$).

level (Figure 3.7). While the UI IPAT 0.4% w/w showed no effect, neither on grain yield nor on N uptake, the addition of NBPT 0.3% w/w and 2-NPT 0.06% w/w led to slightly higher grain yields. At the same time, their addition caused lower N contents in grains on both soils. However, none of these effects were significant (Table 3.4). Only on soil Dürnast, addition of 2-NPT significantly increased N uptake by the total aboveground plant as well as fertilizer N use efficiency (Table 3.7).

3.2.2 Tray experiments

With an average of 23.8 °C (17.6 to 34.3 °C) and 19.2 °C (14.4 to 24.9 °C) during the first and second measurement period, respectively, temperatures within the emission chambers were slightly lower in the second measurement period of tray experiments in the greenhouse.

Tray experiment on sandy soils. In the first measurement period, the two sandy soils Schrobenhausen (SO) and Scheyern (SY), differing in pH, Corg and urease activity, were compared (Table 2.3). Following the surface application of urea, 17.1% and 10.3% of the fertilized N got lost as NH_3 from SY and SO soil, respectively (Table 3.5).

Table 3.5: Ammonia emissions (kg $NH_3 - N\,ha^{-1}$) occurring within 10 days following the surface application of urea and urea + IPAT 0.4% w/w to bare sandy soils Schrobenhausen (SO) and Scheyern (SY). Sums in NH_3 emission and standard errors are displayed. Letters indicate statistical groups determined by POLYANOVA together with Tukey test procedure ($P \leq 0.05$).

soil		**urea**	**urea + IPAT 0.4%**
Schrobenhausen	SO	$8.23^{ab} \pm 1.744$	$3.30^{b} \pm 0.703$
Scheyern	SY	$13.66^{a} \pm 2.062$	$8.27^{ab} \pm 1.286$

Emissions slowly started on the second day after urea application and peaked on the third and fourth day on SY and SO soil, respectively. After 10 days, emissions were nearly terminated (Figure 3.8 a).

Tray experiment on soil under different land use. In the second measurement period, two forms of land use, arable land (AL) and grassland (GL), on the same soil Westermeier were compared (Table 2.3). The manner of surface application of urea differed, as on grassland urea granules were broadcasted onto the grass sward, while on arable land they were applied onto bare soil. Within the 10 days of emission measurement, NH_3 losses of 10.1% and 22.3% of fertilized urea-N were detected on arable land and grassland, respectively (Table 3.6). NH_3 emissions started right after fertilizer application and peaked on the second (GL) and fourth day (AL). After 7 (GL) and 10 (AL) days, emissions had nearly ended (Figure 3.8 b).

Effiency of UI IPAT in tray experiments. On all soils and land uses, addition of the UI IPAT 0.4% w/w reduced NH_3 emissions following the application of urea. Although this reduction was considerable, on soil SY by 40%, on soil SO by 60% and on soil Westermeier AL by 31%, only on soil Westermeier GL the reduction by 45% was significant (Table 3.5; Table 3.6). On soil SY, addition of IPAT delayed NH_3 losses by two days (Figure 3.9 a), but there was no significant difference in the course of emissions. On all other soils or land uses, NH_3 emissions from urea with or without IPAT followed the same course. However, emissions from urea + IPAT continuously proceeded on a clearly lower level. As a result, courses of NH_3 emissions from urea +

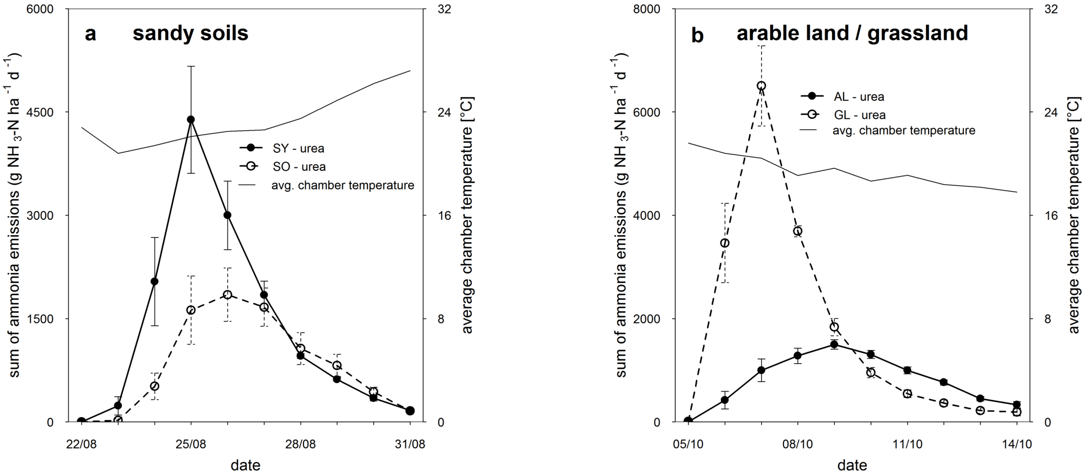

Figure 3.8: Courses of NH_3 emissions ($\mathrm{kg\ NH_3-N\,ha^{-1}\,d^{-1}}$) occurring within 10 days following the surface application of urea onto (a) sandy soils Scheyern (SY) and Schrobenhausen (SO) and onto (b) soil Westermeier under arable land (AL) and grassland (GL) use. Courses of daily average temperatures within the emission chambers are indicated.

Table 3.6: Ammonia emissions ($\mathrm{kg\ NH_3-N\,ha^{-1}}$) occurring within 10 days following the surface application of urea and urea + IPAT 0.4% w/w to soil Westermeier under arable land (AL) and grassland (GL) use. Sums in NH_3 emission and standard errors are displayed. Letters indicate statistical groups determined by POLYANOVA together with Tukey test procedure ($P \leq 0.05$).

soil	**land use**	**urea**	**urea + IPAT 0.4%**
Westermeier	arable land AL	$8.05^b \pm 0.628$	$5.56^b \pm 1.010$
Westermeier	grassland GL	$17.82^a \pm 1.072$	$9.84^b \pm 1.703$

Table 3.7: Ammonia emissions (kg $NH_3 - N\,ha^{-1}$) occurring within 10 days following the surface application of different fertilizer treatments to grassland at the site Veitshof in 2007. Sums in NH_3 emission and standard errors are indicated. Letters denote the arrangement in statistical groups, which was performed by comparison of means according to Fishers LSD procedure ($P \leq 0.05$).

date	**urea**	**urea + 2-NPT 0.10%**	**CAN**	**control (nil N)**
05.06.07	$4.67^a \pm 0.601$	$1.70^b \pm 0.635$	$1.04^b \pm 0.226$	$1.31^b \pm 0.247$
19.06.07	$7.52^a \pm 2.604$	$2.81^{ab} \pm 1.504$	$1.67^b \pm 0.211$	$1.56^b \pm 0.645$
13.07.07	$10.14^a \pm 2.017$	$4.13^b \pm 0.490$	$1.85^b \pm 0.751$	$1.48^b \pm 0.418$
26.07.07	$11.84^a \pm 0.663$	$2.34^b \pm 0.842$	$1.61^b \pm 0.205$	$0.65^b \pm 0.166$

CAN, calcium ammonium nitrate

IPAT were all significantly different from the course of NH_3 emissions from urea alone (Figure 3.9).

3.3 Field experiments on grassland

3.3.1 Ammonia losses from the Veitshof site in 2007

The temperature course and distribution of rainfall events in the months of May to July were typical for an average year. Ammonia losses over 10 days following the application of urea varied between 4.7 and 11.8 kg $NH_3 - N\,ha^{-1}$ (Table 3.7), corresponding to 4.2% up to 14.0% of the applied N. In all experiments, the emissions started immediately following fertilization and peaked on the second day (Figure 3.10).

In the first experimental period, fertilizer was applied to dry soil, which resulted in comparatively low losses of 4.2% of the applied N (Table 3.7), even though the temperatures during the loss phase were similar to the other experiments, with an average of 19.7 °C, and even though the loss phase lasted for about six days longer than during the other 10 day measurement periods (Figure 3.10 a).

In the following experimental periods, fertilizer was applied following periods of precipitation. High moisture contents at the time of fertilization enabled a rapid disso-

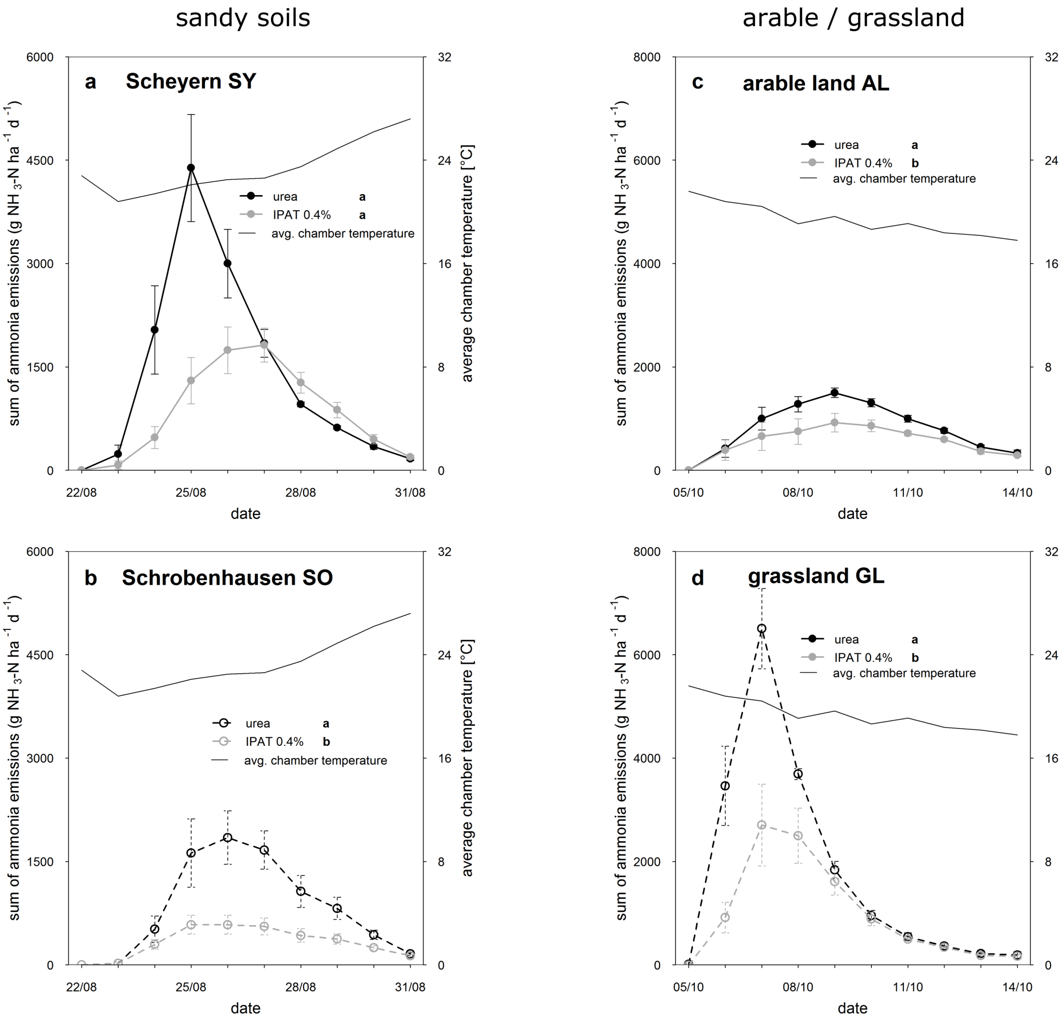

Figure 3.9: Courses of NH_3 emissions ($\mathrm{kg\ NH_3 - N\,ha^{-1}\,d^{-1}}$) occurring within 10 days following the surface application of urea without and with the UI IPAT 0.4% w/w onto sandy soils Scheyern (a) and Schrobenhausen (b) in the first and onto soil Westermeier under arable land (c) and grassland (d) use in the second measurement period. Courses of average daily temperatures within the emission chambers are indicated. Letters behind the treatment names indicate significant differences in the course of NH_3 emissions detected using Wilcoxon matched-pairs signed-ranks test ($P \leq 0.05$).

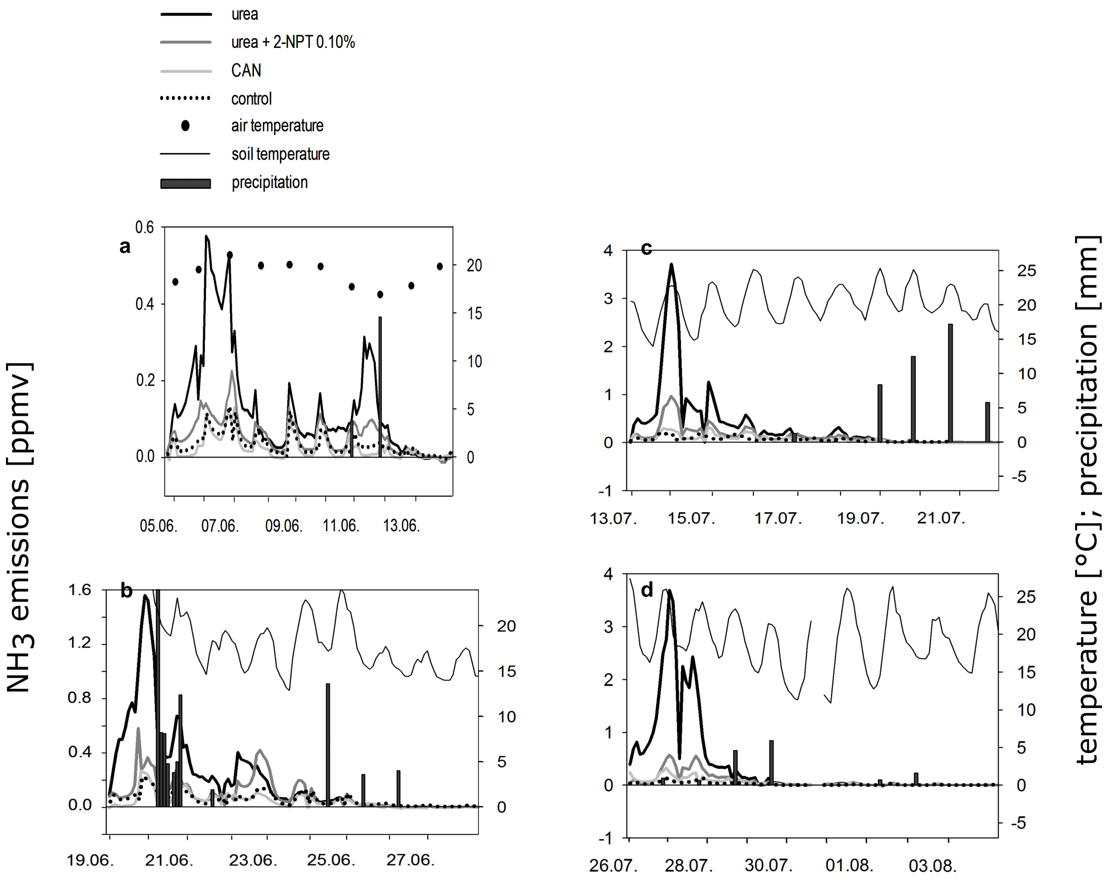

Figure 3.10: Courses of NH_3 emissions (ppmv) within 10 days following application of urea, urea + 2-NPT 0.10% w/w, calcium ammonium nitrate (CAN) and control (nil N) to grassland at the Veitshof experimental site in 2007 during four measurement periods (a-d). Precipitation (a-d), air temperature (a) and course of soil temperature (b-d) are also indicated.

lution of urea granules. In the absence of subsequent precipitation, more than 80% of the NH_3 losses occurred within three days (Figure 3.10 c). Precipitation considerably reduced (Figure 3.10 b) or even terminated (Figure 3.10 d) the NH_3 emissions.

In 2007, highly significant differences ($P \leq 0.001$) and significant differences ($P \leq 0.03$) were detected between fertilizer treatments and application dates, respectively. However, no significant interaction was observed between treatments and application dates ($P \leq 0.06$). In all experiments, the addition of the UI 2-NPT at a concentration of 0.10% w/w resulted in lower courses of NH_3 volatilization. The UI reduced NH_3 emissions by 69% to 88%. Except for the second measurement, this reduction was significant (Table 3.7). Unlike urea, CAN was not significantly different from the control or 2-NPT treatment. NH_3-N losses from CAN varied between 0% and 1.2% of the fertilized N (Table 3.7).

No significant differences in yield were detected between fertilizer treatments, including control. Therefore, yield data is not displayed and results are not further discussed.

3.3.2 Ammonia losses from the Dürnast site in 2008

In 2008, the first two 10 day measurement periods were performed during phases of above-average soil temperatures (by 5 °C and by 3 °C, respectively). Like in 2007, fertilization was performed following a period of precipitation. The last experimental period was characterized by high rainfall of 22.0 mm on the second day of measurement.

Ammonia losses following the application of urea varied between 4.6 and 10.2 $\mathrm{kg\ NH_3 - N\, ha^{-1}}$ and reached a maximum loss of 11.6% of the fertilized N (Table 3.8).

Similar to measurements in 2007, the emissions started immediately following fertilization and peaked on the second day (Figure 3.11). In the absence of rainfall, 80% of the NH_3 losses occurred within three days (Figure 3.11 a). The second and third NH_3 emission period were prematurely terminated due to high rainfall events of 32.5 mm on the fourth day and 22.0 mm on the second day after fertilization, respectively (Figure 3.11 b and c).

In 2008, highly significant differences were detected between treatments ($P \leq 0.001$) and application dates ($P \leq 0.001$), and a significant interaction was observed between treatments and application dates ($P \leq 0.05$). In all measurements, the addition of

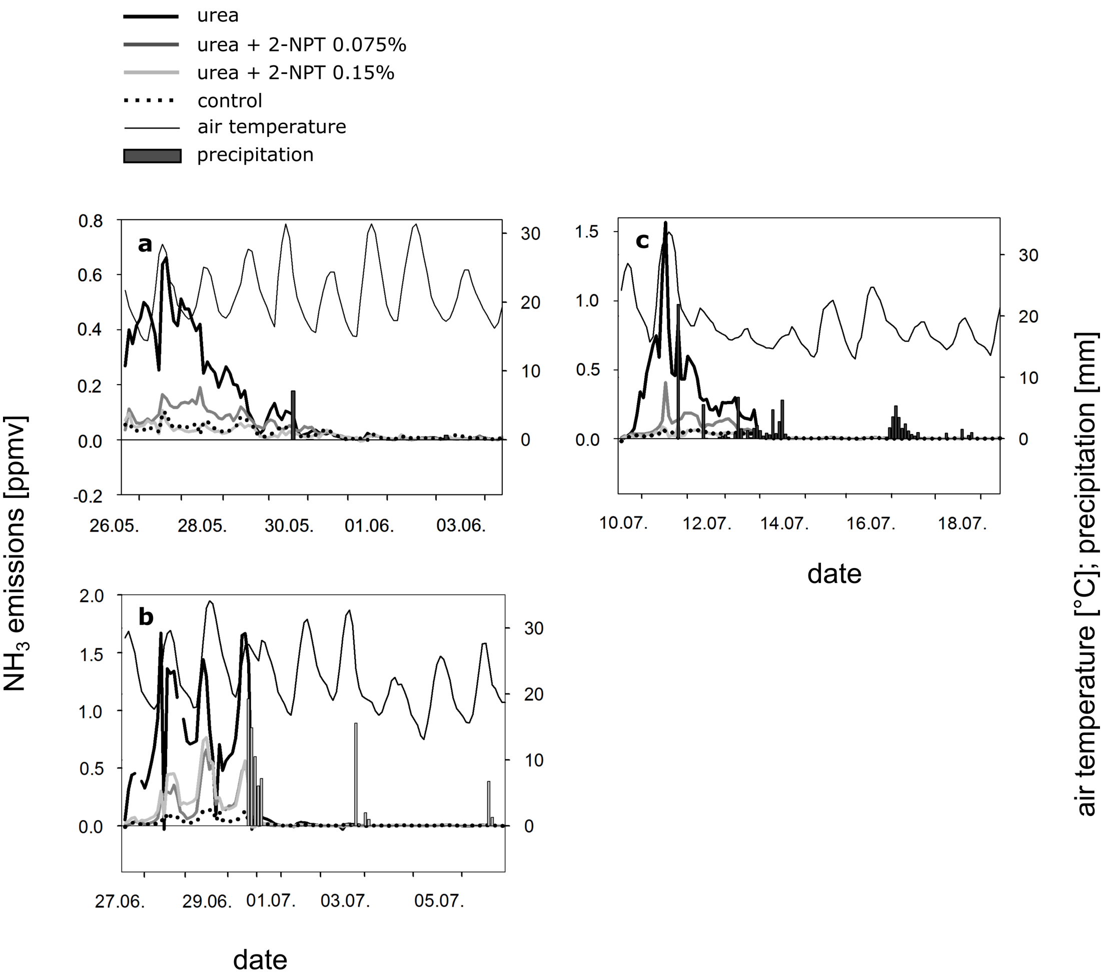

Figure 3.11: Courses of NH_3 emissions (ppmv) within 10 days following application of urea, urea + 2-NPT in concentrations of 0.075% and 0.15% w/w and control (nil N) to grassland at the Dürnast experimental site in 2008 during three measurement periods (a-c). Precipitation and courses of air temperature are also indicated.

Table 3.8: Ammonia emissions (kg $NH_3 - N\,ha^{-1}$) occurring within 10 days following the application of different fertilizer treatments to grassland at the Dürnast experimental site in 2008. Sums in NH_3 emission and standard errors are indicated. Letters denote the arrangement in statistical groups, which was performed by comparison of means according to Fishers LSD procedure ($P \leq 0.05$).

date	**urea**	**urea + 2-NPT 0.15%**	**urea + 2-NPT 0.075%**	**control (nil N)**
26.05.08	$4.69^a \pm 1.832$	$0.70^b \pm 0.463$	$1.41^b \pm 0.455$	$0.73^b \pm 0.425$
27.06.08	$10.15^a \pm 0.957$	$3.63^b \pm 0.689$	$3.26^b \pm 0.145$	$0.84^c \pm 0.244$
10.07.08	$4.59^a \pm 0.637$	$0.47^b \pm 0.221$	$1.13^b \pm 0.771$	$0.47^b \pm 0.345$

2-NPT in both concentrations significantly mitigated NH_3 losses from urea (Table 3.8). These reductions ranged from 70% to 100% and from 74% to 84% for UI concentrations of 0.15 and 0.075% w/w, respectively.

In contrast to measurements conducted in 2007, fertilization significantly increased grass yield. Again, no differences in yield were detected between fertilizer treatments. Therefore, yield data is not displayed and results are not further discussed.

Chapter 4

Discussion

4.1 Field experiments on arable land

On average, 0.6% of the N applied as urea to winter wheat got lost into the atmosphere as NH_3, varying between 0.1 and 2.7% of the applied N. Generally, these emissions were quite low compared to ammonia losses of up to 44% reported in literature (Cai et al., 2002; Chadwick et al.; Engel et al., 2011; Gioacchini et al., 2002; Nastri et al., 2000; Pacholski et al., 2006; Pan et al., 2016; Schjoerring and Mattsson, 2001; Turner et al., 2012) and compared to NH_3 losses of on average 16% assumed for the calculation of the national emissions inventory for Germany (EEA, b).

In all three measuring campaigns, application on bare soil led to considerably higher NH_3 emissions of 2.6% up to 16.3% of the applied N. Under similar environmental conditions, NH_3 emission potential from urea was many times higher than when applied into a standing crop. However, these losses were also less than NH_3 emissions of up to 38% reported in literature (Ma et al., 2010; Pacholski et al., 2006).

Influence of climatic factors. The extent of fertilizer N losses as NH_3 following application of urea depends on a multitude of environmental factors influencing urea hydrolysis rate, like soil moisture, precipitation and temperature (Bouwmeester et al., 1985; Sommer et al., 2004; Watson, 2000). In our work, a slightly positive correlation ($r_{\text{Spearman}} = 0.31$) was only detected between the average air temperature during the emission phase and the extent of NH_3 emissions. When soil water availability was not a limiting factor, higher temperatures enhanced urea hydrolysis, as reported by Sommer et al. (2004), leading to a decreased solubility of NH_3, and consequently an increased diffusion rate (Figure 3.2 c). However, in accordance with observations made by Engel et al. (2011), NH_3 losses also occurred at low temperatures (Figure 3.3 a).

As already stated, nearly all measurement periods were prematurely terminated by rainfall. Two possible effects of rainfall on NH_3 emissions (Bouwmeester et al., 1985; Forrestal et al., 2016), could be confirmed:

1. terminating emissions by transporting urea and ammoniacal N into deeper soil layers (e.g. Figure 3.2 d; Figure 3.3 a and b) and
2. as also observed by Ma et al. (2010), enhancing losses by adding water to the top soil for further dissolution of urea granules and hydrolysis of urea (Figure 3.1 a; Figure 3.2 c).

However, while precipitation of 7.8 mm was insufficient to dissolve and move the urea to a safe depth (Figure 3.2 a, 7^{th} day), precipitation of 5.4 mm on a humid soil surface was sufficient to mitigate NH_3 emissions (Figure 3.1 b, first day).

Mitigation of NH_3 losses by addition of a urease inhibitor. Although only following fertilizations on April 12, 2002 (Table 3.1) and July 13, 2005 (Table 3.2) the differences in NH_3 emission sums from urea and urea with any urease inhibitor were significant, the addition of any UI reduced NH_3 losses over all measurements in winter wheat and on bare soil. The mitigation potential of all UIs was independent from whether urea was applied to winter wheat or onto bare soil, and had a similar reducing effect of 23% and 28% for IPAT, 32% and 42% for NBPT, and 53% and 49% for 2-NPT (Table 3.1; Table 3.2). Similar results for NBPT in reducing NH_3 losses by 42% to up to 100% (Bronson et al., 1989; Chadwick et al.; Engel et al., 2011; Gioacchini et al., 2002; Nastri et al., 2000; Pan et al., 2016; Sanz-Cobena et al., 2008) and 2-NPT by 56% (Gassner, 2014) have also been reported in literature. Generally, the addition of IPAT appeared to reduce NH_3 emissions the better the higher emissions were following the application of urea ($r_{\text{Pearson}} = 0.36$), whereas no such correlation was detected when using NBPT or 2-NPT.

Observations made by Watson et al. (1994b) that the addition of a UI delays the time of the maximum loss rate could also be noticed in some of our measurements (Figure 3.5 a und c). In most experimental periods though, the loss phase of urea with a UI occurred at the same time as for urea alone, just on a smaller scale (Figure 3.1 a;

Figure 3.2 d; Figure 3.3 a; Figure 3.5 b). In many measurements, this difference in the course of NH_3 emissions following fertilization of urea and fertilization of urea with any UI was significant (Figures 3.1 to 3.5). Rainfall which terminated NH_3 emissions from urea had the same effect on urea with any UI (Figure 3.2 d; Figure 3.3 a and b). Both observations confirm Watson (2000), who asserts that the primary benefit of a UI consists in slowing down the hydrolysis of urea, and thus allowing more time for rain to dilute urea and the NH_4^+ concentration at the soil surface, and increase its dispersion into the soil.

Conclusions from field experiments on arable land. On the investigated site in Southern Germany, NH_3 losses from urea were on a much lower level as reported in literature and as used by EEA (b) for the German national emission inventory. This can partly be explained by the soil's generally low loss potential and the site's favorable climatic conditions for a low NH_3 loss potential from urea.

Reduction by IPAT 0.4% w/w was on par with NBPT 0.3% w/w. Thus, addition of a UI allowed to preserve the fertilized nitrogen, preferably as dissolved urea, until the next rainfall event, which relocated it into deeper soil layers and thus prevented emission of ammonia.

4.2 Greenhouse experiments

4.2.1 Pot experiments

The depth to which incorporation is necessary to inhibit all NH_3 losses following the application of urea depends on different soil characteristics (Hargrove, 1988). Rochette et al. (2013) indicated a reduction rate of 12.5% per centimeter, making an incorporation depth of 7 cm the optimum for all soils. In our experiment, a depth in incorporation of 5 cm was sufficient for soils Dürnast and Mintraching to mitigate NH_3 losses, as fertilizer N use efficiencies (NUE) close to 100% (95% to 100%) were attained. However, this depth was not sufficient to mitigate all NH_3 emissions on the sandy soil Cunnersdorf, which is indicated by its lower NUE of 87%. In future pot experiments, an incorporation depth of at least 7 cm should be chosen for urea, especially when evaluating soils characterized by a light texture.

In both experiments, characteristics of all soils as well as climatic conditions in the greenhouse were not expected to negatively influence effectiveness of NBPT, e.g. by very low pH or extreme temperatures (Cantarella et al., 2005; Cantarella et al., 2008; Carmona et al., 1990; Engel et al., 2011; Engel et al., 2015; Mira et al., 2017; Saggar et al., 2013; San Francisco et al., 2011; Soares et al., 2012).

Pot experiment in 2004. Soils Dürnast and Cunnersdorf used in this experiment especially differed in sand and clay content, as well as in Corg content (Table 2.3). Clay content is negatively, while sand content is positively correlated with the rate and extent of NH_3 losses (Reynolds and Wolf, 1987; San Francisco et al., 2011; Watson et al., 1994b). Rao and Ahmad (1984) observed that a higher content in soil organic matter adsorbs urea and thus reduces NH_3 loss potential. Therefore, higher NH_3 losses from urea were expected on soil Cunnersdorf. Corresponding to these expectations, yield of spring wheat, N content in grains as well as fertilizer NUE were significantly lower on soil Cunnersdorf compared to soil Dürnast, indicating higher N losses as NH_3 from this soil. Furthermore, all evaluated yield parameters were significantly lower from surface applied urea than from incorporated urea on soil Cunnersdorf.

Addition of the UI NBPT 0.3% w/w to urea significantly increased fertilizer NUE by 10% on both soils. The addition of IPAT 0.4% w/w also led to an increase in all yield parameters on both soils. However, its effect was never significant and did not attain the same level as with NBPT.

It has been shown that only low NH_3 emissions occur following surface application of CAN (Black et al., 1985; Ni et al., 2014; van der Weerden and Jarvis, 1997; Watson et al., 1990b). Corresponding to these findings, on both soils grain yield, N uptake and fertilizer NUE were slightly higher from incorporated urea than from CAN, but not significantly different.

Pot experiment in 2005. Soils Dürnast and Mintraching used in this experiment especially differed in their clay content, pH, Corg content and urease activity (Table 2.3). Soil Mintraching had a clearly higher pH and a urease activity twice as high as soil Dürnast, but at the same time a higher content in clay and Corg. While the first two parameters are positively correlated to an enhanced urea hydrolysis rate and higher NH_3 losses (Hargrove, 1988; He et al., 1999; Sommer et al., 1996; Watson et al., 1990a; Watson et al., 1994b), they are less important than the latter two (Reynolds and Wolf, 1987), which influence the retention of NH_4-N. However, as was already shown in the pot experiment in 2004 and in field experiments in winter wheat, soil Dürnast is similarly less prone to high NH_3 losses from applied urea.

As a result, when urea was surface applied, oat grain yield was significantly higher on soil Dürnast, while grain N content was significantly higher on soil Mintraching. In summary, no differences in NH_3 losses between the two soils were detected when evaluating the fertilizer NUE with 82% and 87% for soil Dürnast and Mintraching, respectively.

As NH_3 losses had obviously stayed on a low level, on both soils addition of the UI NBPT 0.3% w/w only slightly enhanced oat grain yield as well as N uptake and consequently urea NUE, but never on a significant level. As was also observed by Schuster et al. (2007), the addition of the new UI 2-NPT 0.06% w/w led to an increase in NUE, which in our work was even significant on soil Dürnast.

Conclusions from pot experiments. As we already observed in the pot experiment in 2004, IPAT 0.4% w/w showed a lower efficiency than NBPT and in this experiment also than 2-NPT, thus being the least promising UI in pot experiments. Based on these results as well as on results in field experiments (2002 - 2005) in winter wheat and on bare soil, IPAT was not further investigated as a UI. It was decided to focus on the more promising UI 2-NPT, which was consequently tested in further field experiments on grassland.

4.2.2 Tray experiments

Tray experiment on sandy soils. Soil texture of all soils used in preceding field and pot experiments were characterized by high contents in clay and silt. To evaluate the efficiency of UI IPAT 0.4% w/w on sandy soils, a first experiment with direct measurement of NH_3 emissions following application of urea was done in trays under an optimum environment for high rates of NH_3 losses in the greenhouse. The two sandy soils, Scheyern (SY) and Schrobenhausen (SO), were similar in organic carbon content as well as in soil urease activity and slightly different in their sand content (Table 2.3). Thus, higher NH_3 emissions were expected from the lighter soil SO (Reynolds and Wolf, 1987). Contrary to these expectations, higher NH_3 emissions were detected from soil SY, on which 17% of applied N got lost as NH_3, compared to 10% from soil SO. Although adjustment of soil water content was attended to with great care, there had been difficulties to guarantee optimum soil water content at the surface of the very sandy soil SO. We assume that too low water availability at the soil surface slowed down dissolution of granules as well as urea hydrolyis rate (Ernst and Massey, 1960; Herbst et al., 2005; Sommer et al., 2004). This also explains the delayed peak in NH_3 emissions on the fourth day, compared to the peak on the third day following fertilization on soil SY.

On both sandy soils, the UI IPAT 0.4% w/w showed a very good reduction potential and clearly mitigated NH_3 emissions following application of urea by 40% and 61% on soil Scheyern and Schrobenhausen, respectively. Although these reductions were not statistically significant, the UI IPAT appeared to be a potent inhibitor on sandy soils.

Tray experiment on soil under different land uses. Evaluation of a urease inhibitor poses problems when no NH_3 emissions occur to be inhibited or reduced. As tray experiments offered the possibility to exclude emission terminating rainfalls, an experiment was organized comparing soil Westermeier under different land uses, arable land (AL) and grassland (GL). A grass sward offers a higher potential for NH_3 emissions following application of urea compared to bare soil, because mass of herbage material, both living and dead, creates a physical barrier between urea granules and the soil, slowing down diffusion of urea-N into the soil (McGarity and Hoult, 1971; Hargrove, 1988). Furthermore, the plant surface contains a high urease activity, which increases urea hydrolysis rate and thus leads to rapid NH_3 emissions from the plant surface (McGarity and Hoult, 1971; Hargrove, 1988). McGarity and Hoult (1971) also observed that a major part of emitted NH_3 following application of urea was generated by the grassland sward, and was reduced when urea had access to the soil surface.

In accordance with these observations, we detected emissions twice as high from grassland than from arable land. High urease activity of the grass sward led to a quick hydrolysis of urea, and NH_3 emissions peaked on the second day after fertilizer application (Figure 3.8 b). After 7 days the NH_3 emission period had ended. Both observations were congruent with findings in literature (Black et al., 1987; Dawar et al., 2011; Forrestal et al., 2016; Sanz-Cobena et al., 2011; Sherlock et al., 1995; van der Weerden and Jarvis, 1997; Watson et al., 1994a).

Application of urea onto the bare arable soil of the same soil Westermeier, containing a urease activity half as high as on grassland, led to significantly lower N losses as NH_3. Urea hydrolysis rate was visibly lower, NH_3 losses stayed on a clearly lower level and the peak in NH_3 emissions took place on the fourth day after urea application. In return, duration of the NH_3 loss phase was prolonged compared to grassland and lasted over 9 days (Figure 3.8 b).

Soil Westermeier had similar soil characteristics as soil Dürnast on which field experiments on bare soil took place in the years 2004 and 2005. Congruent with the results of NH_3 measurements in field, the UI IPAT 0.4% showed a similar reduction potential on NH_3 emissions from urea applied to bare arable soil Westermeier. It mitigated NH_3 losses by 31% compared to on average 28% in field experiments and did not delay NH_3 losses. However, its addition significantly altered the course of NH_3 emissions (Figure 3.9 c).

When IPAT was tested on Westermeier grassland soil, which offered more favorable conditions for high NH_3 emissions, the UI showed a high efficiency by significantly mitigating 45% of NH_3 losses.

Conclusion from tray experiments. As a result of these measurements, the following field experiments for evaluation of the reduction potential of urease inhibitors were carried out on grassland.

4.3 Field experiments on grassland

Ammonia emissions from urea treated plots. All experimental periods except the first one were initiated at the beginning of an expected warmer and rainless phase after a period of precipitation. Following the fertilization of urea, an increase in NH_3 emissions could be detected immediately after fertilization. Highest emission values were detected on the second day, which is in line with common findings in literature of emission peaks occurring 1 to 3 days after fertilization (Black et al., 1987; Dawar et al., 2011; Forrestal et al., 2016; Sanz-Cobena et al., 2011; Sherlock et al., 1995; van der Weerden and Jarvis, 1997; Watson et al., 1994a). At 3 to 6 days, the duration of the loss phase also was comparable to periods of 3 to 7 days described in literature (Black et al., 1987; Dawar et al., 2011; Sanz-Cobena et al., 2011). A comparison of sums of NH_3 emissions measured within 10 or even 7 days showed no differences, and thus indicated that measurement periods of 7 days might be sufficient.

Environmental conditions. The key factors affecting NH_3 emissions from urea-fertilized grassland have been reported in literature as being temperature, precipitation, and top-soil water content at the time of fertilization (Sommer et al., 2004). Similar to observations by Black et al. (1987) and Mira et al. (2017), the dry soil surface at the start of the first experiment in 2007 resulted in reduced NH_3 losses, which amounted only to 3.3% of the applied N until the first precipitation event 6 days after fertilization (Figure 3.10 a). In the following measurement periods, fertilization was always performed immediately after a period of precipitation to ensure high soil moisture content, and, corresponding to statements by Black et al. (1987) and van der Weerden and Jarvis (1997), a rapid dissolution of the fertilizer granules and consequent hydrolysis of urea. As a result, at temperatures similar to those of the first measurement period in 2007, the NH_3 emissions were considerably higher in the following experiments.

The progress of NH_3 emissions is known to be directly influenced by temperature (Ernst and Massey, 1960; Sherlock et al., 1995). In both years, this effect can be observed as daily NH_3 emission peaks from urea coincide with the daily temperature peak. However, observations by van der Weerden and Jarvis (1997) are also supported by these experiments, i.e. the influence of soil surface humidity at the time of fertilization was greater than the temperature effect.

Furthermore, precipitation occurring after the application of urea played a decisive role in the progress of NH_3 emissions. Generally, the results of Black et al. (1987) could be confirmed: The sooner the precipitation event occurred after the date of fertilization, the higher was its emission terminating or reducing effect. This effect could be observed during the second experiment of 2007 (Figure 3.10 b) and the third experiment of 2008 (Figure 3.11 c), where precipitation amounts of 3.8 mm and 22.0 mm on the second day dramatically reduced NH_3 emissions. In the second measurement in 2008 (Figure 3.11 b), a precipitation of 32.5 mm on June 27 terminated high emissions. The enhancing effect of low precipitation on NH_3 losses (van der Weerden and Jarvis, 1997) was observed during the fourth experiment in 2007 (Figure 3.10 d), where a precipitation of 0.9 mm on day 2 induced further peaks in NH_3 emissions. Black et al. (1987) and Zhu et al. (2000) observed in pasture and in maize, respectively, that after fertilization onto an initially dry soil, a late precipitation permitted a further dissolution and hydrolysis of additional urea, and allowed the volatilization of NH_3 to start. This effect was also observed during the first measurement period in 2007, where an emission peak occurred 6 days after fertilization induced by precipitation of 3.8 mm on the previous day. Comparatively low NH_3 losses of 4.2% of the fertilized N supported observations made by Zhu et al. (2000) that a delay in NH_3 emissions, caused by dry soil conditions at the time of fertilization, led to lower NH_3 losses. In the current work, these losses were also clearly lower than those detected immediately after fertilization.

Even though effects of climatic and soil factors on NH_3 emissions were observed as described above, no significant relationships between NH_3 emissions and air temperature, soil temperature, soil humidity, maximum temperature or precipitation could be detected.

Effect of 2-NPT on NH_3 volatilization. The use of urea as a chemical fertilizer to grassland is generally considered not to be efficient because high amounts of the fertilized N can be lost as NH_3. In the current work, urea was applied to grassland under favorable conditions for high NH_3 losses to evaluate the potential of the urease inhibitor 2-NPT. The UI 2-NPT in a concentration of 0.10% w/w successfully reduced urea hydrolysis for 7 days after fertilization (Figure 3.10 c), until precipitation of 8.4 mm terminated the loss phase.

When adding 2-NPT to urea at concentrations of 0.075, 0.10 and 0.15% w/w, no delay in the effectiveness of 2-NPT could be detected. Ammonia emissions were reduced right from the start. This can be explained by the fact that the UI 2-NPT is added to the urea granule in its active form, and thus does not have to be converted to that form first. The UI did not delay the time of the maximum loss, but reliably mitigated NH_3 emissions throughout the whole loss period.

The progression of NH_3 emissions from urea + 2-NPT was similarly influenced by precipitation and by the daily course of temperature, as described above for urea alone, but it remained at a lower level.

The addition of 2-NPT successfully inhibited the hydrolysis of urea and abated NH_3 losses by 74% to 84% for a UI concentration of 0.075% w/w, by 69% to 88% for a concentration of 0.10% w/w, and by 70% to 100% for a concentration of 0.15% w/w, indicating that 0.075% w/w would already be an optimum level, which is congruent with findings by Schuster et al. (2007).

The addition of 2-NPT in a concentration of 0.10% w/w achieved a reduction in NH_3 emissions to a level not significantly different from that following the application of CAN. In absolute numbers, however, NH_3 volatilization following the application of CAN was still clearly lower, coinciding with observations made by van der Weerden and Jarvis (1997) and Watson et al. (1990b), who detected losses at the same magnitude of 0.1% to 0.8% of the fertilized N applied as CAN.

Chapter 5

General discussion

Across all experiments, NH_3 losses from surface applied urea were generally lower than reported in literature, irrespective of the crop, land use or soil type the granulated urea was surface applied to. However, in field experiments they still ranged from 0% to 3% in winter wheat, 3% to 16% on bare soil, and 4% to 14% on grassland, and led to a reduced fertilizer NUE in pot experiments.

In this work, all direct measurements of NH_3 emissions were done using a dynamic chamber system. Closed measurement systems may generally underestimate losses (Pihlatie et al., 2013), because the air exchange rates used in the ventilated enclosures (in the current case 0.8 volume changes/min) may not fully mimic real field conditions. However, static chambers are considered to be a suitable method for comparison of adjacent plots with different fertilizer treatments (Sommer et al., 2004), and they enable a close documentation of NH_3 emission response to changes in soil moisture from precipitation or drying (Ma et al., 2010). Our measurement system was thus primarily intended for comparative studies; results should be interpreted with care regarding absolute values, but, as was also stated by Misselbrook et al. (2005), they are reliable regarding the comparison of relative differences in NH_3 emissions between different fertilizers or fertilization strategies.

Application of urea to bare soil. Under similar environmental conditions, surface application of urea to bare soil led to considerably higher NH_3 emissions than when broadcasted into a standing crop. This is acknowledged in the new German fertilization legislation, prescribing that urea must be immediately incorporated or a urease inhibitor (UI) has to be used when urea is applied to bare soil.

Application of urea to winter wheat. In field experiments carried out in winter wheat, NH_3 emissions following surface application of urea were generally very low when compared to literature. This had several reasons:

1. The soil at the experimental station Dürnast does not have a high loss potential. This was not only confirmed in our pot experiments in the years 2004 and 2005, but also by Wissemeier and Weigelt (2009), who compared soil Dürnast to several other German soils concerning their NH_3 loss potential following application of urea. Low NH_3 emissions following the application of urea to winter wheat at the experimental site Dürnast were also detected by Gassner (2014) and Schmidhalter et al. (2017). In two experimental years, Gassner (2014) detected NH_3 losses of on average 5% and 7% following application of urea to winter wheat using Dräger tube and ^{15}N-isotope technique, respectively.

2. Climatic conditions at the site Dürnast shaped the NH_3 emission periods. Especially precipitation occuring during the NH_3 emission phase had a crucial effect on the extent and course of NH_3 emissions. In our experiments, regular rainfall events affected all measurement periods, indicating that, in addition to the soil's low loss characteristics, the site's climatic conditions had a generally mitigating effect on NH_3 losses following surface application of urea.

3. In all measurement periods in winter wheat, urea was applied into a standing crop (BBCH 25 to BBCH 51). A crop canopy influences extent and course of NH_3 emissions: It lowers NH_3 losses due to reduced wind speed and temperature at the soil surface (Black et al., 1989; Misselbrook et al., 2004). Furthermore, winter wheat leaves are reported to absorb on average 11% of the N volatilized from surface-applied urea (Ping et al., 2000), demonstrating that not all NH_3 emissions from applied urea can be considered as lost from the plant-soil system (Black et al., 1989; Denmead et al., 1976; Hutchinson et al., 1972; Ping et al., 2000; Schoninger et al., 2018).

Application of urea to grassland. In field measurements on grassland, NH_3 emissions from broadcasted urea were also low in comparison to losses of up to 58% reported in literature (Black et al., 1985; Black et al., 1987; Dawar et al., 2011; Forrestal et al., 2016; Sanz-Cobena et al., 2011; Sherlock et al., 1995; van der Weerden and Jarvis, 1997; Watson et al., 1994a). However, they were on a much higher level compared to NH_3 losses from surface applied urea to winter wheat, which is primarily due to the enhancing effect of the grass sward on NH_3 losses from urea (Hargrove, 1988; McGarity and Hoult, 1971; Reynolds et al., 1985). We intentionally applied urea to grassland to induce high NH_3 emissions for evaluation of 2-NPT's mitigating potential. As NH_3 losses from broadcasted urea to grassland can be high, also occur at low temperatures (Engel et al., 2011), and grassland swards have an elevated loss potential (Hargrove, 1988; McGarity and Hoult, 1971), urea seems unsuitable as a chemical fertilizer to grassland on any soil and under any climatic conditions. As incorporation is not feasible without damaging the grass sward, use of a urease inhibitor or choice of a different fertilizer is advised.

Calcium ammonium nitrate (CAN) is an alternative fertilizer which is not as prone to NH_3 losses as urea. In pot experiments as well as in field experiments in grassland, no or only very low NH_3 losses were detected following application of CAN, which was congruent with literature (Black et al., 1985; Ni et al., 2014; van der Weerden and Jarvis, 1997; Watson et al., 1990b).

Possibilities of reduction of NH_3 losses. Nevertheless, urea has many advantages over other mineral N fertilizers, which explain its worldwide importance with a market share of 58% (IFA, 2017). Therefore, under favorable soil or climatic conditions for high NH_3 losses, incorporation of urea or addition of a urease inhibitor have to be considered.

Immediate incorporation of the applied urea into the soil is known to decrease NH_3 losses distinctly (Cai et al., 2002; Ernst and Massey, 1960; Rochette et al., 2013; Sommer et al., 2004). If incorporation is not possible or not economically viable, use of a urease inhibitor is a potent way to mitigate NH_3 losses from surface applied urea. In the course of this work, the three urease inhibitors NBPT, IPAT and 2-NPT were thoroughly tested on their efficiency to reduce NH_3 losses following application of urea.

NBPT is the most common and rigorously examined urease inhibitor. Although, it has to be converted into its active form first, a good reduction potential of up to 68% in winter wheat and up to 60% on bare soil could be confirmed in our field experiments. This is congruent with a reduction in NH_3 losses from urea applied to arable land by 42% to up to 100% reported in literature (Bronson et al., 1989; Chadwick et al.; Engel et al., 2011; Gioacchini et al., 2002; Nastri et al., 2000; Pan et al., 2016; Sanz-Cobena et al., 2008).

In many of our experiments, the newly developed UI IPAT has been compared in its mitigating potential on NH_3 emissions from urea to the established urease inhibitor NBPT. In field experiments in winter wheat and on bare soil as well as in pot experiments, IPAT showed a good potential in reducing NH_3 losses by 49% to 100% following application of urea. However, in most measurements it did not attain a similar effect as NBPT. Furthermore, to attain a reliable efficacy of IPAT in reducing NH_3 losses from urea, a higher concentration in active substance of 0.4% w/w compared to NBPT with 0.3% w/w was needed. Based on these and other results, a market launch of the urease inhibitor IPAT was not further pursued.

In the course of our project, the newly developed urease inhibitor 2-NPT was added and tested in its mitigating potential in comparison to NBPT and IPAT in field and pot experiments. NH_3 losses from surface applied urea were low, but 2-NPT showed first promising results in field measurements in winter wheat in 2005. Due to application of 2-NPT to urea granules in its active form, no delay in efficiency of the UI could be observed. 2-NPT kept NH_3 emissions on a significantly lower level throughout the whole loss period. Following similarly promising results in pot experiment in 2005, 2-NPT was chosen as the only UI to be tested in field experiments in grassland, and showed a high reliability and efficiency in reducing NH_3 emissions following surface application of urea. One additional aim of these measurements was to determine an optimum concentration of the active substance. Congruent with findings of Schuster et al. (2007), a concentration of maximum 0.10% w/w was considered to be sufficient. Compared to NBPT, 2-NPT has the advantages of a clearly lower concentration together with the fact, that it is applied to urea granules in its active form. Furthermore, 2-NPT proved to be very stable in storage (Hucke et al., 2010). Based on these experiments and other results, the urease inhibitor 2-NPT was registered in the German fertilizer regulation.

Chapter 6

Conclusions

Due to its many advantages, urea will keep its worldwide importance as a chemical fertilizer. However, emissions of ammonia, which have a relevant negative impact on the environment and on human health, can be high following broadcasting of urea to any soil and regardless of land use. A site's soil characteristics and land use define its potential for NH_3 losses, while climatic conditions following the application of urea determine their actual extent. Based on our research results, a few recommendations can be made:

1. When applied onto bare soil, high NH_3 emissions of up to 16% from surface applied urea, detected in field experiments in 2004 and 2005, corresponded to the emission factor of 15.9% at a soil pH below 7.0 and in a temperate climate stated by EEA (b) for calculation of the national emission inventory. Corresponding to the new German fertilization legislation, immediate incorporation of urea or use of a UI should be generally prescribed by countries' fertilization regulations, when urea is applied onto bare soil.

2. For the experimental site Dürnast, the NH_3 emission factor of 15.9% assumed by EEA (b) is too high for urea applied into a standing crop. Many studies have shown (Black et al., 1989; Denmead et al., 1976; Hutchinson et al., 1972; Misselbrook et al., 2004; Ping et al., 2000; Schoninger et al., 2018) that a standing crop has diverse reducing effects on the extent of NH_3 losses. As EEA (b) emission factors are based on evaluation of numerous field studies, we still recommend to add a UI to urea applied into a standing crop, when the site's soil and climate characteristics are favorable for potentially high NH_3 losses.

3. Grassland is prone to high NH_3 losses, because of its high urease activity and further promoting characteristics (Hargrove, 1988; McGarity and Hoult, 1971; Reynolds et al., 1985). In our field and tray experiments, the highest NH_3 emissions were detected when urea was broadcasted onto grassland. Therefore, we do not recommend use of urea as a chemical fertilizer for grassland on any soil and in any climate.

Farmers are for the most part economically driven. Therefore, the amount of N applied to crops primarily has to ensure optimum yield. For this reason, a surplus in N is usually applied in order to cover not only the N demand of crops, but also possible losses as NH_3 and NO_3. If NH_3 losses are mitigated by addition of a urease inhibitor, an aim has to be to reduce the applied fertilizer N rate by the full amount of expected NH_3 emissions. This would be beneficial both for the farmers, who can reduce their expenses for urea containing a UI, and for society, by reducing the amount and thus negative impact of NH_3 on the environment and health. Therefore, more work similar to Kawakami et al. (2012), concentrating on enhancing NUE by reducing NH_3 losses and simultaneously reducing urea N rate, should be done to investigate a possible global reduction of urea fertilization rates, and consequently a reduction of NH_3 emissions from agriculture by means of the use of a UI.

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List of Publications

Schraml, M., Gutser, R., Schmidhalter, U., Weber, A. (2006) Potenzial von neuen Ureaseinhibitoren zur Minderung von NH_3-Verlusten nach oberflächiger Harnstoffdüngung unter verschiedenen Verlustsituationen. Proceedings of 118th VDLUFA congress “Landnutzungskonzepte heute und morgen – dargestellt am Beispiel der Region südlicher Oberrhein“, Freiburg, Germany, 324-329.

Author Contribution: SM: experimental execution and evaluation, writer; SM & WA: experimental design; GR: supervisor; SU: project leader

Schraml, M., Gutser, R., Schmidhalter, U. (2009) Abatement of NH_3 emissions following application of urea to grassland by means of the new urease inhibitor 2-NPT. Proceedings of the 18th International Symposium of CIEC “More Sustainability in Agriculture: New Fertilizers and Fertilization Management“, Rome, Italy, 312-317.

Author Contribution: SM: experimental design, execution and evaluation, writer; GR: supervisor; SU: project leader

Schraml, M., Gutser, R., Maier, H., Schmidhalter, U. (2016) Ammonia loss from urea in grassland and its mitigation by the new urease inhibitor 2-NPT. The Journal of Agricultural Science 154, 1453-1462.

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Schraml, M., Weber, A., Heil, K., Gutser, R., Schmidhalter, U. (2018) Ammonia losses from urea applied to winter wheat over four consecutive years and potential mitigation by urease inhibitors. Journal of Plant Nutrition and Soil Science 181, 914-922.

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