UNIVERSIDAD POLITÉCNICA DE MADRID

ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRONÓMICA,
ALIMENTARIA Y DE BIOSISTEMAS

SYNTHETIC INHIBITORS, A WAY TOWARDS THE NITROGEN-NEUTRALITY IN MEDITERRANEAN AGROECOSYSTEMS

TESIS DOCTORAL

JAIME RECIO HUETOS

Ingeniero Industrial

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DEPARTAMENTO DE QUÍMICA Y TECNOLOGÍA DE ALIMENTOS

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POLITÉCNICA

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RESUMEN

La agricultura del futuro se enfrenta al reto de satisfacer la demanda de alimentos de una población creciente, utilizando recursos cada vez más limitados y con la necesidad de reducir el impacto ambiental asociado a las emisiones de amoníaco (NH_3), óxido nitroso (N_2O) y óxido nítrico (NO). La tecnología de fertilizantes está promoviendo la utilización de inhibidores de la nitrificación (NIs) para reducir emisiones de óxidos de nitrógeno (N) en cultivos. Sin embargo, estos compuestos pueden aumentar las pérdidas de NH_3 debido a la mayor persistencia de amonio en el suelo. En cualquier caso, hasta la fecha existe escasa información sobre el efecto en los flujos de estos gases del nuevo inhibidor de la nitrificación 2-(3,4-dimetil-1H-pirazol-1-yl) succinato en mezcla isomérica (DMPSA), tanto en cultivos de secano como de regadío. Por ello, esta tesis plantea como **objetivo** principal el explorar los diferentes aspectos que condicionan el uso de este nuevo inhibidor con el fin de mitigar pérdidas de N reactivo al medio, particularmente NH_3 y óxidos de N , manteniendo el rendimiento y calidad de cultivos.

Para alcanzar este objetivo, se han realizado cuatro experimentos de campo en condiciones de Mediterráneo seco (Comunidad de Madrid, España). Los experimentos 1 y 2, realizados en maíz irrigado, evaluaron las emisiones de N_2O y NH_3 (utilizando la técnica micrometeorológica IHF) durante el periodo de cultivo. Los resultados indicaron claramente que el uso de IN redujo las emisiones de N_2O , sin aumentar significativamente las de NH_3 . A pesar de haber utilizado prácticas recomendadas para mitigación de NH_3 , como es el riego tras la aplicación de los fertilizantes minerales o el enterrado de purín, el rango de pérdidas se mantuvo entre el 2,5 y 6 % del N aplicado. El empleo del doble inhibidor, que incluía DMPSA y el inhibidor de la ureasa (NBPT), redujo la volatilización por debajo del 1.7% del N aplicado.

Un aspecto muy novedoso lo constituye las medidas de NH_3 perdido desde la planta durante el periodo de senescencia del maíz. Las emisiones supusieron el 1.2% del N

aplicado como fertilizante, por lo que deberá ser tenido en cuenta en la cuantificación de amoníaco volatilizado en el cultivo.

En los experimentos 3 y 4 se evaluaron las emisiones de NO, NO₂ y N₂O (con o sin IN) en trigo (Experimento 3) y en maíz con riego por goteo (Experimento 4). Se colocaron cámaras automáticas por primera vez en cultivos de zonas mediterránea, obteniendo una alta resolución temporal de los flujos de NO y NO₂. Para evaluar los datos almacenados para cada cámara, se desarrolló un software basado en lenguaje R (versión 3.2.1) (capítulo 4). Este *software* permite descargar los valores previos al “steady-state”, obtener el valor medio de “steady-state” por cámara y por ciclo, encontrar valores perdidos, calcular flujos y almacenar todos los valores correspondientes a un tratamiento en un matriz.

En el cultivo de secano (trigo, Experimento 3), el DMPSA redujo la nitrificación y por tanto la producción de NO con respecto al tratamiento sin inhibidor, siendo muy novedoso el consumo neto de NO en el suelo (-61.72 g-N ha⁻¹) observado para U+DMPSA en comparación con una producción neta (227.44 g-N ha⁻¹) para U. La explicación de los mecanismos bióticos y abióticos implicados en la deposición de NO tras la aplicación del IN, supone un reto que necesita ser estudiado en el futuro. La adición de DMPSA redujo significativamente las emisiones de N₂O un 71%. Para maíz con riego por goteo (Experimento 4), el DMPSA mitigó las emisiones totales de NO (0.21% del total de N) en comparación con U (0,81%), siendo además las emisiones de N₂O similares a las del Control (sin fertilización). El doble inhibidor aumentó la eficiencia mitigadora para el NO con respecto al IN solo, pero no para la emisión de N₂O. El IN no produjo un efecto significativo en el rendimiento o en la calidad del grano en los ensayos.

ABSTRACT

One of the biggest challenges for the agriculture of the future is to produce enough food for an increasing population from limited resources whilst at the same time reducing the environmental impacts produced by emissions of ammonia (NH_3) and N-oxides (N_2O and NO) from agrosystems. Fertilizer technology based on nitrification inhibitors (NIs) has been proposed as a useful technological approach to reduce N_2O and NO emissions, although these inhibitors can lead to large NH_3 losses due to observed increases in the pool of ammonium (N-NH_4^+) in soil. To date, scarce information exists about the effect on fluxes in either rainfed or irrigated soils of a new NI, the 2-(N-3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture, DMPSA. The main **objective** of this thesis is to explore the different aspects that condition the use of this new NI, to mitigate losses of reactive N to the environment, particularly N-oxides and NH_3 , whilst maintaining yield and crop quality. In order to achieve this objective, four field experiments were set up under dry Mediterranean conditions (Comunidad de Madrid, Spain). Experiments 1 and 2 were performed on irrigated maize and evaluated N_2O emissions and NH_3 volatilization during the crop period using the micrometeorological integrated horizontal flux (IHF) technique. The results clearly indicate that the use of a NI reduced N_2O emissions but did not significantly enhance NH_3 volatilization. Using recommended agricultural practices to mitigate NH_3 emissions, such as irrigation immediately after application of mineral N or the incorporation of pig slurry (PS), losses ranging from 2.5% to 6% of applied N were obtained. When using a double inhibitor, which included DMPSA and a urease inhibitor (NBPT), volatilization was reduced to below 1.7% of applied N. Losses of NH_3 from plant during maize senescence were also measured, obtaining emission rates of 1.2% of initial N applied as fertilizer. This is considered a novel and important value under field conditions which should be taken into account when ammonia volatilization is quantified in crops.

Experiments 3 and 4 evaluated NO, NO₂ and N₂O emissions in different fertilizer treatments (with or without NIs) in rainfed wheat (Experiment 3) and drip-irrigated maize (Experiment 4). A high temporal resolution of fluxes of NO and NO₂, obtained by using automated chambers was implemented for the first time in agricultural fertilized systems in Mediterranean areas. A specific software based on language R (version 3.2.1.1) was developed (Chapter 4) to assess the data logged from each chamber. With this program it is possible to discard the values prior to the steady-state, to obtain the steady-state value per chamber and per cycle, to find missing values, to calculate the flux and to store all the values corresponding to each treatment in one matrix.

In the rainfed crop (wheat) (Experiment 3), seventy-five days after fertilization, the effective reduction of nitrification by DMPSA had significantly decreased the production of NO with respect to the treatment without DMPSA, giving a net consumption of NO in the soil of -61.72 g-N ha⁻¹ for urea and DMPSA (U+NI) treatment in comparison to a net production of 227.44 g-N ha⁻¹ for urea treatment (U) alone, which is a novel result. The explanation of NO deposition after NI application, due to biotic and abiotic processes in the soil-plant system, supposes a challenge that needs to be studied in the future. The addition of DMPSA reduced emissions of NO by 71%. With drip-irrigated maize (Experiment 4), DMPSA also mitigated total NO emissions (0.04% of total N) in comparison with the U treatment (0.34%), obtaining similar total N₂O emissions to the Control (without fertilizer). Use of a double inhibitor treatment (DMPSA and a urease inhibitor, NBPT) increased mitigation efficiency for NO but not for N₂O emissions. Use of NI had no effect on yield or crop quality in these experiments.

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 1:

In the coming decades, agriculture will be faced with the major challenge of producing sufficient food for an increasing population from limited resources whilst at the same time reducing environmental impacts. The low N use efficiency of our agricultural systems constitutes an important problem from an environmental point of view (Spiertz, 2010). Not only the mineral sources used as N fertilizers, but also the organic N sources such as manures, contribute to the loss of N to the environment in the form of nitrates, N-oxides and ammonia (Galloway et al., 2008). Scientific and technological advances are fundamental to meet this challenge.

1. Ammonia volatilization constitutes an important loss of N in agricultural systems

Agriculture is the main sector responsible for ammonia (NH_3) emissions to the atmosphere, accounting for an estimated 94% (Fig 1.1) of total European emissions in 2015 (EEA Report, 2017). This is considered an issue of major environmental and socioeconomic concern (Van Grinsven et al., 2013). Ammonia reactions contribute to soil acidification, eutrophication and to the formation of particles of mean aerodynamic diameter smaller than $2.5\ \mu\text{m}$ ($\text{PM}_{2.5}$), which have been related to human respiratory problems (Misselbrook et al., 2014). Gas-to-particle conversion and the formation of N-NH_4^+ salts, which make up $\sim 20\%$ of the $\text{PM}_{2.5}$ in the atmosphere, are strongly dependent on the reactions between NH_3 , sulphuric acid (H_2SO_4), nitric acid (HNO_3), hydrochloric acid (HCl), and water (H_2O) (Baek and Aneja, 2004). These atmospheric compounds form new particles in the air or condense onto pre-existing ones to form secondary particulate matter (PM), which affect the Earth's radiation budget and its climate through modification of cloud formation, lifetime, and precipitation. It has been demonstrated that a reduction of NH_3 emissions effectively contributes to reduce $\text{PM}_{2.5}$ (Aneja et al., 2009).

Fig. 1.1: a) Contribution to EU-28 emissions from main source sectors in 2015 of NH_3 ; b) Agriculture sub-sectoral contribution to the total EU-28 agricultural NH_3 in 2015. Source: Air quality report (EEA Report, 2017)

Ammonia in the atmosphere plays a major role in N deposition, which is harmful to the biodiversity and productivity of natural ecosystems. Emitted nitrogen compounds give rise to acidifying components in both terrestrial and aquatic ecosystems, in addition to their role as a cause of eutrophication (Fig. 1.2). Eutrophication occurs when there is an excess of nutrients in the soil or water, with several impacts on terrestrial and aquatic ecosystems. Projections for 2020 and 2030 indicate that ecosystems exposure to eutrophication will become more and more widespread (EEA, 2018).

Fig. 1.2: Atmospheric emissions, transport, transformation and deposition of trace gases. Source (Aneja et al., 2009)

The world's population is projected to rise to over 9 billion people by 2050 (Billen et al., 2015). The agriculture sector is faced with the challenge of increasing yield, food safety and quality while increasing the sustainability of the sector and maintaining landscape use and biodiversity. According to the EEA (2018) report (Fig. 1.3), there has been some success in Europe in this respect, at least in terms of reducing pollutant emissions between 2000 and 2016. However, of these pollutants, NH_3 emissions exhibited the smallest reduction (9% in the EU-28 and 5% in the EEA-33), and even underwent an increase in the period 2013-2016 of about 3%.

Fig. 1.3: Evolution of EU-28 emissions from the agriculture sector of NO_x , NH_3 , PM_{10} , NMVOCs and CH_4 , 2000-2016 (% of 2000 levels). Sectoral activity is expressed in terms of gross value added (GVA). GVA is a measure of the value of goods and services produced in a sector.

The Gothenburg Protocol (UNECE, 2014) established 3% reduction in NH_3 emissions for 2020 and beyond in Spain, where NH_3 emissions were counted in 365 kt-N in 2005. Hence, the Spanish NH_3 emissions ceiling from 2010 to 2020 is 353 kt-N. Owing to this necessity, different abatement strategies need to be implemented in agricultural soils to mitigate NH_3 losses. According to the Advisory Code of Good Agricultural Practices of the Expert Group on Ammonia Abatement of UNECE (United Nations Economic Commission for Europe) these strategies include (Bittman et al., 2014):

- i) The mechanical incorporation of fertilizers into the soil.
- ii) Their application immediately prior to an expected light rainfall or before irrigation to incorporate the fertilizer into the upper soil.
- iii) The use of urease inhibitors or an alternative source of N, different than urea, such as ammonium nitrate (NH_4NO_3).

All of these practices are related to the main factors that control NH_3 volatilization, namely the chemistry of the gas and the strong influence of environmental conditions on the volatilization process:

a) Formation of NH_3 from NH_4^+ due to pH conditions

Volatilization is essentially a physicochemical process which results from the equilibrium (described by Henry's law) between gaseous phase NH_3 (g) and NH_3 in solution (aq), which is in turn maintained by the $\text{NH}_4^+/\text{NH}_3$ equilibrium [R1, R2] (Harrison and Webb, 2001). High pH (i.e. low $[\text{H}^+(\text{aq})]$) favours the right-hand side of R1, resulting in a greater concentration of NH_3 in solution and also, therefore, in the gaseous phase causing volatilization (Fig. 1.4).

Fig. 1.4: Relative fractions of ammonia and ammonium as a function of pH

- b) NH_4^+ adsorption and subsequent exchange processes between soil colloidal particles (clays, organic matter, sesquioxides, etc.) and various ions.

The capacity of the soil to hold cations is called cation exchange capacity (CEC) and the capacity to hold anions is called anion exchange capacity (AEC).

As clay colloids have negative charges (Fig. 1.5), cations are attracted to the clay particles and are held onto clay surfaces electrostatically, being in direct contact with the soil solution. When fertilizers are applied, elements like K^+ , Ca^{2+} , Mg^{2+} and NH_4^+ are exchanged for other cations like H^+ present in the exchange complex. If this process did not exist, the applied nutrients would be lost in drainage water.

Fig. 1.5: Schematic representation of cation exchange.

- c) The rate of urea or ammonium fertilizer applied to the soil

It has been estimated that mineral fertilizer is essential to help to feed around half of the world's population and will be fundamental to ensure global food security over the 21st

century. Natural nutrient sources and recycling (e.g. pig slurry) have been supplemented with additional fertilizer (e.g. ammonium nitrate and urea) to support increasing human consumption since the 19th century (Sutton et al., 2013).

Urea is the predominant source of inorganic N fertilizer used in agriculture throughout the world, accounting for 50% of the total world fertiliser N consumption (Sanz-Cobena et al., 2008a). In nature, urea is hydrolysed into NH₃ by an enzyme called urease (R3), a multi-subunit nickel-dependent metalloenzyme that catalyses the hydrolysis of urea at a rate approximately 10¹⁴ times the rate of the un-catalysed reaction (Kafarski and Talma, 2018). Urease is widely distributed in plants, microorganisms and soils.

d) Wind speed, soil moisture and temperature

The wind speed affects the movement of NH₃ in the atmosphere due to its influence on turbulence, friction forces, NH₃ particle trajectories from soil to sensors, etc. Wind speed also affects the interchange of NH₃ from soil pores to the atmosphere. Soil moisture and temperature alter the hydrolysis of urea, which increases as soil moisture content and temperature rise.

Fig. 1.6: Simplified overview of nitrogen (N) cycle (modified from Sutton et al., 2013)

1.2. Quantification of NH_3 volatilization in crop systems constitutes an important challenge

Determining robust NH_3 emission factors (EFs) for fertilized maize crops, as well as EFs associated with the implementation of mitigation strategies based on fertilizer application, is an important challenge for NH_3 inventories of many countries. In addition, bearing in mind that NH_3 deposition is an indirect source of N_2O emissions (Mosier et al., 1998), quantification of NH_3 is also essential to accurately estimate the potential of agricultural management practices to mitigate both NH_3 and N_2O emissions. As previously mentioned, NH_3 measurements are influenced by several environmental and management factors, including wind direction, wind speed, air moisture, solar radiation, and fertilizer type (Walker et al., 2012; Nelson et al., 2017). Utilizing an appropriate measurement method, which provides an accurate quantification of NH_3 , is therefore crucial and

necessary to develop effective best management practices. Micrometeorological methods are considered highly robust for the quantification of NH_3 volatilization under field conditions (Sanz-Cobena et al., 2008; 2010).

Of these, the integrated horizontal flux (IHF) method (Denmead et al., 1977) has generally been used as the reference (Pacholski et al., 2008; Viguria et al., 2015; Yang et al., 2017).

1.2.1. Principles of micrometeorological methods: integrated horizontal flux (IHF)

Micrometeorological methods are based on the study of the atmospheric boundary layer (ABL), the part of the troposphere that is directly influenced by the presence of the earth's surface and responds to surface forcings with a timescale of about an hour or less, i.e., small-scale phenomena (Stull, 2012). Extensive surfaces ($>2000 \text{ m}^2$) are required for the application of these methods.

The IHF method implemented by Leuning et al. (1985) and further modified by Sherlock et al. (1989), is the micrometeorological method most commonly used in Europe to quantify NH_3 emissions (Misselbrook and Hansen, 2001). Based on mass balance, it equates the vertical flux of the gas into the atmosphere from a treated area of limited upwind extent with the horizontal flux of the gas across a vertical plane at a known downwind distance (Fig 1.7).

Fig. 1.7: Diagram of NH_3 production from the soil surface. Ammonia moves upward as a result of diffusion and turbulence, and moves laterally due to convection. Masts with NH_3 traps placed at upwind and downwind boundaries. Below, vertical NH_3 flux calculation at upwind and downwind positions.

The mean total horizontal flux density of NH_3 is given by the product $u \cdot C$, where u is the mean horizontal wind speed and C is the net NH_3 concentration trapped in different passive flux samplers (PFS) or *shuttles* (Fig. 1.8), placed at different heights on a mast. Thus, airflow passes through a PFS, taking up all the NH_3 . The mass balance indicates that the NH_3 collected in shuttles (M , μg) during a time (t , s) must equate to the NH_3 horizontal flux density ($u \cdot C$) (Eq. 1.1)

$$(\overline{U \cdot C}) \cdot A_t \cdot t = M \quad \text{Eq. 1.1}$$

where,

M is the mass of collected NH_3 (μg) in the PFS during sampling period t (s),

A_t is the effective cross-sectional area of the sampler (m^2) as determined in wind tunnel calibrations.

Fig. 1.8: External view and schematic diagram of ammonia sampler

The net vertical flux (F , $\mu g \text{ m}^{-2} \text{ s}^{-1}$) from the treated surface is then calculated by integrating the horizontal fluxes over the height intervals and dividing by the fetch distance, which is the distance between the downwind and upwind boundaries (Eq. 1.2).

To calculate the mean vertical flux of NH_3 , it is necessary to integrate over the height intervals (z_i , m), the height of the uppermost sampler and divide by the fetch distance (x , m). Ammonia flux at the upwind mast position (plot) is subtracted from the downwind mast position (bg).

$$F_{\text{vert}} = \frac{1}{x} \left[\int_{z_{i-1}}^{z_i} \left(\overline{u \cdot C} \right)_{\text{plot}} \cdot dz - \int_{z_{i-1}}^{z_i} \left(\overline{u \cdot C} \right)_{\text{bg}} \cdot dz \right] \quad \text{Eq. 1.2}$$

Fig. 1.9: Five different heights above soil in the ammonia experiment.

Eq. 1.2 assumes the plot extends infinitely in the across-wind direction so the net lateral diffusion is zero; a circular treated area is often used to approach conditions nearing this assumption. In the case of a rectangular plot, as in the experiments considered in this thesis, flux estimates associated with wind directions from the corner of the plot will potentially be associated with errors due to net lateral flux of NH_3 . In these rectangular plots, fetch length was calculated according to the mean wind direction in a 1h period.

In Mediterranean areas, most of the available data on NH_3 from crop systems has been obtained by our group. We have obtained reliable information about the effectiveness of

certain agricultural practices for mitigating NH_3 emissions after urea or pig slurry application (Abalos et al., 2012). To our knowledge, very few studies have been conducted measuring NH_3 volatilization using micrometeorological methods in cereal crops, where high N fertilizers rates are normally applied (Pacholski et al., 2006, 2008; Yang et al., 2017).

1.2.2. Different methods to measure ammonia.

Apart from the IHF method, other methods can be used to quantify volatilization. These include

a) Eddy covariance technique

The eddy covariance method is a micrometeorological technique that was proposed by Montgomery (1948), Swinbank (1951) and Obukhov (1951) and is based on the fact that the vertical transport of a gas past a point is obtained by correlating the instantaneous vertical wind speed at the point with the instantaneous concentration of a specific gas. In the natural environment, eddies, irregular swirls of motion superimposed on the mean wind, occur with frequencies extending up to 5 or 10 Hz; hence a rapid response gas detector is required (Denmead, 1983).

The eddy covariance method requires a minimum number of assumptions about the nature of the transport process, and measurements at only one level above the surface are needed. However, the main disadvantages are the sophisticated and expensive recording devices that are needed and the computing facilities that are required to cope with the rapid data acquisition.

b) Backward Lagrangian stochastic (bLS) method.

The bLS is an inverse-dispersion method that simulates the transport of a trace gas (e.g., NH_3) from a source to a measurement location and predicts the ratio of the average tracer concentration to the emission rate (Sommer et al., 2005).

The bLS model is based on the Monin-Obukhov similarity theory (MOST). This states that over short time intervals (e.g., 30 min) the wind properties in the ABL are known from four variables: the atmospheric friction velocity, the Obukhov stability length, the surface roughness length and the wind direction.

1.2.3. Are there some losses of NH_3 during senescence?

Most studies that have measured NH_3 volatilization include the days or weeks after the fertilization event, usually less than a month. However, some authors have indicated that a part of NH_3 loss could be produced during crop senescence. Although most of the NH_4^+ is remobilized as amino acids from senescent leaves, a minor part is lost as NH_3 via stomata (Gregersen et al., 2008). In this respect, Schjoerring and Mattsson (2001) reported that between 1% and 4% of the amount of N present in mature shoots could be lost as NH_3 . However, up until now, no measurements of this senescent volatilization have been made using micrometeorological techniques under field conditions.

2. Understanding emission processes of N-oxides in agricultural systems

Nitrous oxide (N_2O) and nitric oxide (NO) are emitted from the soil. While N_2O is a strong greenhouse gas (GHG), NO is a chemically active gas involved in tropospheric photochemistry and is the main precursor of ground-level ozone (O_3) in rural areas (Laville et al., 2011), impacting on crop productivity and human health (Calvete-Sogo et al., 2014).

Nitrification and denitrification are considered the principal sources of the emission of N_2O and NO . Various authors have suggested that NO emitted from soils is mainly produced through nitrification, whereas the NO produced from denitrification is further reduced to N_2O before it escapes to the soil surface (Ruser et al., 2006; Loick et al., 2016; Liu et al., 2017). Nitrifier-denitrification (Ussiri and Lal, 2013) has also been considered an important microbiological source of both gases in agricultural soils. Abiotic N processes could also be an important source of both gases, although they are very dependent on soil characteristics and moisture conditions (Butterbach-Bahl et al., 2011). For example, in soils with an acid pH, NO_2^- and NO_3^- may produce NO through chemodenitrification. Likewise, the decomposition of nitrous acid (HNO_2) is another source of NO in acid soils. When the pH is neutral, the NO produced could react with humic substances to produce N_2O (Stevenson et al., 1970).

Net emissions of NO and N_2O are a consequence of biotic and abiotic processes which control the production, consumption and transport of these gases through the soil. Consumption of these gases in the soil is possible through biotic processes, such as denitrification, and through abiotic reactions, such as nitrosation, that occur with NO and organic compounds (Medinets et al., 2015). Oxidation of NO by atmospheric oxidant compounds such as O_3 , produces NO_2 , and so this reaction also contributes to consumption of NO . Additionally, plants can act as a sink for NO and NO_2 due to the possible uptake of these gases as an N source through the leaves' stomata (Schindlbacher, 2004). Since most of these processes are interrelated, an important scientific challenge is to know the contribution of each process to the production and consumption of these gases (Medinets et al., 2016). This knowledge can facilitate the implementation of mitigation measurements in agroecosystems, where N fertilizers are often the main input of N to the system.

The addition of N-fertilizers, both organic and synthetic, is considered the most important anthropogenic source of N₂O emissions.

Emissions of N₂O, NO and NO₂ are highly dependent on N inputs to soil. The IPCC (2007) established 1% as the default EF for N₂O (EF-N₂O) (i.e. the percentage of applied N fertilizer that is transformed and emitted back to the atmosphere as N₂O). In the case of NO, EF-NO ranges from 0.8 to 1.8% (Liu et al., 2017), although the soil, climatic conditions and the N source are important influences.

In general, different strategies are recommended to reduce N₂O emissions from fertilized soils. These include synchronizing applied N with the N demand of the crop (e.g. split application of fertilizers), using water-saving irrigation strategies (e.g. drip irrigation) to prevent N₂O formation through denitrification in water-saturated soils (Guardia et al., 2017b) or applying nitrification inhibitors (NIs) (Sanz-Cobena et al., 2017).

2.1.1. Chamber techniques to measure N-oxides

Chambers have been commonly used to measure gas fluxes due to their portability and low cost. The operating principle is that the chambers restrict the volume of the air with which gas exchange occurs so as to magnify gas concentration changes in the headspace (Denmead, 2008). Chambers are classified as non-flow through (closed chambers) and flow-through (open chambers) depending on whether they are open to the atmosphere or not.

2.1.1.1. Closed chambers

In most GHG emission literature, the predominant measurement technique used employs non-flow through, non-steady state chambers commonly known as static chambers (Bouwman et al., 2002). Within these chambers, the replacement of air in the headspace is negligible, hence the accumulation of GHG fluxes in the chamber's headspace

increases over time. This increase in concentration with time is considered as actual flux from soil. The rate of increase of flux density follows the equation proposed by Denmead (2008):

$$F_{gas} = \frac{V}{A} \frac{dC_g}{dt} \quad \text{Eq 1.3}$$

where

F_g is the flux density of gas at the surface in $\text{kg m}^{-2} \text{s}^{-1}$;

V is the volume of the headspace in m^3 (in this thesis 19.3 L);

C_g is the concentration of the specific gas in kg m^{-3} ;

A is the area of the soil covered by the chamber in m^2 ;

t is time in s.

Closed chambers are used more often than flow through chambers because the larger concentration changes are easier to detect and usually, closed chambers are mechanically simpler. In static chambers, there is no air circulation between chamber and sensor, and hence there are no power requirements.

The temporal and spatial variability of fluxes should be minimized as they are the main sources of uncertainties associated with flux estimation (Sapkota et al., 2014). Therefore, in the experiments presented in this thesis, the chambers were placed in specific locations on the agriculture plot in a steel ring that had been previously inserted into the soil to a depth of 5 cm, and gas samples were withdrawn at regular intervals during the experiment (Minamikawa et al., 2015).

2.1.1.2. Open Chamber

In a flow-through chamber (open chamber), a constant flow of outside air is maintained through the headspace of the chamber and the difference in concentration between the air entering and leaving the headspace is measured.

In this case, the flux (F_{chamb}) of an inert trace gas (i.e. no chemical reactions with other air constituents or with the chamber walls) between the plant-soil system and the chamber air is determined by the mass balance of the trace gas in the enclosed headspace.

$$V \cdot \rho_{air} \cdot \frac{dC_{chamber}}{dt} = A \cdot F_{chamber} - Q \cdot \rho_{air} \cdot [C_{chamber} - C_{air}] \quad \text{Eq. 1.4}$$

where:

V denotes the volume of the chamber;

A the soil surface area covered by the chamber;

Q is the purging air flow rate

$C_{chamber}$ and C_{air} are the trace gas mixing ratios of the inflowing ambient air and of the outflowing chamber air

ρ_{air} is the density of the dry air molecules

t denotes time

While for static chambers Q is zero, dynamic chambers are operated with a continuous purging of the chamber air. In this way, a dynamic equilibrium (steady-state) is developed, where the time derivative and time dependences in Eq. 1.4 vanish (Pape et al., 2009).

Under equilibrium conditions, the mass budget equation for a dynamic chamber can thus be reduced and rearranged to:

$$F_{chamber} = \frac{Q}{A} \cdot [C_{chamber} - C_{air}] \quad \text{Eq. 1.5}$$

Realisation of the dynamic chamber principle commonly follows some general assumptions and design features. Since the purging air flow (Q) through the chamber has to be known for the flux determination, it is usually produced and maintained constant by a pump or fan either at the inlet or at the outlet (Denmead, 2008).

In this thesis, an automated dynamic chamber was developed and set up on the field, providing continuous records of gas emission from the same site.

2.1.2. Automated measurements of N-oxides: a technical challenge to better understand the effect of agricultural practices

Installing an automated dynamic measuring system of N-oxides facilitates analyses of the temporal variation of the gas emissions and calculation of EFs, as well as enabling a better understanding of the climate conditions that affect these emissions and the best hours to take the most representative samples, etc. The large amount of data that is generated is beyond the capabilities of the most common calculation software applications (e.g. Microsoft Excel®), and alternative software programmes are required, such as R Core Team (2017), Matlab, Phyton, etc.

3. Nitrification and urease inhibitors as strategies to reduce the emission of N gases

3.1. Nitrification inhibitors (NIs)

Although nitrification inhibitors (NIs) were originally developed to mitigate nitrate leaching, they can also be used in combination with liquid manure, urea or ammonium-based fertilizer as an efficient strategy to reduce N₂O emissions without yield penalties (Abalos et al., 2013; Huérfano et al., 2016; Cayuela et al., 2017). These chemical

compounds deactivate the enzyme responsible for the first step of nitrification (AMO), thus maintaining the NH_4^+ in soils for a longer period (Misselbrook et al., 2014; Ruser and Schulz, 2015; Gilsanz et al., 2016). As a consequence, N_2O and NO emissions through nitrification are significantly reduced (Fig. 1.10).

NITRIFICATION INHIBITORS

Fig. 1.10: Effect of nitrification inhibitors (e.g. DMPA) in the microbiologic process of nitrification

The search for specific chemical compounds to act as NIs started in the late 1950s, but at present, only a few are available on the market. The most common in Europe are:

a) Dicyandiamide (DCD)

Dicyandiamide (DCD, Fig. 1.11) can mitigate nitrate leaching and nitrogen oxide emissions. Commercially available to farmers in New Zealand was until 2013, its use was suspended traces of DCD were detected in exported milk.

In the meta-analysis of Gilsanz et al. (2016) the average EF of DCD was $0.41\% \pm 0.05$ on average.

Fig. 1.11: Chemical structure of DCD

b) 3,4-dimethylpyrazole phosphate (DMPP)

Fig. 1.12: Chemical structure of DMPP

Some studies have shown that DMPP (Fig. 1.12) has a comparable or better inhibition effect on N_2O and NO emissions and NO_3^- leaching than DCD, as well as the advantage of a lower recommended application rate (Huérfano et al., 2015).

In the meta-analysis of Abalos et al. (2014), an average increment of 7.5% in nitrogen use efficiency (NUE) was observed when DMPP was added in comparison with fertilizers without inhibitor. Increases ranged from 1% to 16%.

c) 2-(3,4 dimethyl-1H- pyrazol-1-yl) succinic acid isomeric mixture (DMPSA)

Fig. 1.13: Chemical structure of DMPSA

The DMPSA (Fig. 1.13) will be marketed in Europe from 2019. The few studies that have been made on this novel NI include Huérfano et al. (2016), Guardia et al. (2017a), Torralbo et al. (2017), Guardia et al. (2018a, 2018b) and published papers that incorporated results reported on this thesis (Recio et al., 2018a, 2018b) on the inhibitory effect of this compound. The DMPSA differs from DMPP in the presence of the succinic group instead of phosphate (CA 2933591 A1 2015/06/18 Patent; Pacholski et al., 2016) (Fig. 1.11, Fig. 1.12). The efficiency of this NI has been shown in the mitigation of not only N_2O emissions but also NO_x emissions (Guardia et al., 2018a).

The extended retention of N in the form of NH_4^+ in soils may increase the risk of NH_3 volatilization. To date, only a small number of field studies have been conducted to quantify the effect of NIs combined with liquid manure (Aita et al., 2014) or mineral fertilizers (Ni et al., 2014) on NH_3 emissions. In most cases, the studies were carried out using DCD. Other studies have reported N_2O emission reductions with DMPP (e.g., Kou et al., 2016; Guardia et al., 2017a), although there was no effect on NH_3 volatilization in a paddy soil (Li et al., 2009). Apart from papers which have been published based on results reported in this thesis, no other information is available on NH_3 volatilization when using DMPSA combined with a source of mineral N.

3.2. Urease inhibitors

Urease inhibitors (UIs) have been highlighted as a good strategy to diminish NH_3 volatilization (Fig. 1.14). In fact, the UNECE has proposed the use of this inhibitor to minimize N losses, mainly associated with urea as the predominant source of inorganic N fertilizer used throughout the world (Harrison and Webb, 2001; Bittman et al., 2014).

Fig. 1.14: Effect of urease inhibitors (e.g. NBPT) in the process of hydrolysis of urea

Within the types of the UIs, the most studied are those derived from the phosphorodiamidates such as N-(n-butyl) thiophosphoric triamide (NBPT, Fig. 1.15).

The efficiency of NBPT has been proven under both laboratory conditions (Gill et al., 1999) and field conditions (Sanz-Cobena et al., 2011). Furthermore, recent studies have evidenced that NBPT is able to reduce N_2O emissions in Mediterranean cropping systems, showing a high mitigation potential (around 55%) in an irrigated maize field with nitrification-favouring conditions (Sanz-Cobena et al., 2011), and in a rainfed barley crop (86%).

Fig. 1.15: Chemical structure of NBPT and a representation of its activity inhibiting the urease enzyme

3.3. Combination of the two types of inhibitor: double inhibitor

As UIs delay urea hydrolysis and NIs retain N in a less mobile form as NH_4^+ , combining the two types of inhibitor with urea could be an effective way of reducing N_2O , NO and NO_3^- leaching, and NH_3 volatilization.

However, in a study by Zhao et al. (2017) at the end of the maize season, the highest mitigation of N_2O emissions was obtained using an NI alone (66% of reduction) followed by the double inhibitor (47% of reduction) and the UI alone (37% of reduction), with significant differences between the three treatments. Similar results were obtained at the

end of the wheat season, though on this occasion without statistically significant differences between an NI treatment and a UI+NI treatment (roughly 55% of reduction each).

CHAPTER 2

OBJECTIVES

CHAPTER 2:

There is an increasing concern about the large N losses that N fertilizers generate, both organic and mineral. Sustainable agricultural practices need to be implemented to mitigate the emission of these gases and at the same time maintain yield and crop quality. This constitutes one of the most important challenges for fertilized crop systems. Nitrification inhibitors (NIs) have been proposed as an useful technological approach to reduce N₂O and NO emissions. However, more efficient compounds are required to mitigate these fluxes, as well as in depth studies on the processes involved in the production and consumption of these gases. As the environmental conditions are decisive in these processes and therefore in NI efficacy, studies under rainfed and irrigated conditions are important to determine the potential use of such inhibitors with Mediterranean agricultural crops. The use of NIs could also enhance NH₃ volatilization from soils after application, due to the higher and longer NH₄⁺ concentration in the soils. In order to better understand the conditions, mechanisms and factors which affect emissions of N-oxides and NH₃ when NIs are used in semi-arid areas, the following principal objective is set out in this thesis:

TO EXPLORE THE DIFFERENT ASPECTS THAT CONDITION THE USE OF NITRIFICATION INHIBITORS BASED ON DIMETHYL PYRAZOLE (DMP) TO MITIGATE LOSSES OF REACTIVE N TO THE ENVIRONMENT, PARTICULARLY N-OXIDES AND NH₃, WHILE MAINTAINING YIELD AND CROP QUALITY.

With this objective in mind, the following novel studies were undertaken:

- The effectiveness of a new inhibitor DMPSA and of a combination of DMPSA and the urease inhibitor NBPT was studied under field conditions. To date, scarce information exists on NO_x and N₂O fluxes, especially, under Mediterranean conditions.

- Measurements of NO and NO₂ were made using automated measurement chambers for field crops. The high temporal resolution allows a better understanding of the emission processes.
- Ammonia volatilization was measured using the micrometeorological IHF technique for the first time in irrigated maize, evaluating losses of this gas after urea application with and without NI, as well as losses during senescence. Scarce information is available about the effect of NIs under field conditions, or on NH₃ losses directly from plants.

With this main objective in mind, the research work was structured in four field experiments which had a total of five specific objectives:

Objective 1: To quantify NH₃ volatilization in an irrigated maize crop when using mitigation strategies based on pig slurry, N-NH₄⁺ or urea fertilizer management.

Are zero emissions an attainable goal?

SPN: “Cuantificar la volatilización de NH₃ en un cultivo de maíz irrigado cuando se utilizan estrategias de mitigación basadas en el manejo de purín de cerdo, o fertilizantes base N-NH₄⁺ o ureicos ¿Es posible obtener emisiones cero?”.

Initial Hypothesis: *Management of fertilizers during application could reduce NH₃ volatilization, but emission zero is not possible as NH₃ is in equilibrium with NH₄⁺ in soil solution facilitating diffusion to the atmosphere.*

Objective 2: To evaluate whether the use of a nitrification inhibitor based on dimethyl pyrazole (DMP) increases NH₃ volatilization after pig slurry or calcium

ammonium nitrate (CAN) application in a maize crop. Could these compounds significantly increase indirect N₂O emissions associated to NH₃ volatilization?

SPN: “Evaluar si el uso de inhibidores de la nitrificación basados en dimetil pirazol (DMP) incrementa la volatilización de NH₃ tras la aplicación de purín de cerdo o CAN en un cultivo de maíz. ¿Aumentarían significativamente las emisiones indirectas de N₂O asociadas a la volatilización de NH₃?

Initial Hypothesis: *N₂O emissions could be reduced when the fertilizer is mixed with NI but, as the NI enlarges the pool of NH₄⁺ in soil and this could persist in the soil, there could be an increased risk of NH₃ volatilization.*

Objective 3: To evaluate the effectiveness of urea mixed with the nitrification inhibitor 3,4-dimethyl pyrazole succinic acid (DMPSA), or combined with the urease inhibitor N-(n-butyl) thiophosphorictriamide (NBPT) and DMPSA, on NH₃ volatilization as well as indirect N₂O emissions associated with NH₃ volatilization in an irrigated maize crop.

SPN: “Evaluar la efectividad de la mezcla de urea con el inhibidor de la nitrificación DMPSA o la combinada con el inhibidor de la ureasa NBPT y el DMPSA en la volatilización de NH₃ y emisiones indirectas de N₂O asociadas a la volatilización en un cultivo de maíz irrigado.

Initial Hypothesis: *The use of NBPT could mitigate NH₃ losses when NI is also applied with urea.*

Objective 4: To generate a detailed data base with a high temporal resolution on NO emissions with or without the nitrification inhibitor DMPSA in a winter wheat crop

under semi-arid conditions. The effect of this new NI on N₂O fluxes was also evaluated.

SPN: “Obtener una base de datos de NO con alta resolución temporal en un cultivo de trigo con o sin el inhibidor de la nitrificación DMPSA. Además, el efecto en los flujos de N₂O del nuevo inhibidor ha sido evaluado”.

Initial Hypothesis: *The nitrification inhibitor DMPSA reduces total NO emissions in comparison with a treatment without NI in the first 4-5 weeks after application, but these emissions could increase when other processes such as denitrification are favourable.*

Objective 5: To evaluate the effectiveness of DMPSA, NBPT and the double inhibitor DMPSA+NBPT, all of them applied with urea, in the mitigation of N₂O and NO emissions in a drip-irrigated maize crop. Obtaining a high temporal resolution of NO fluxes should enable a better understanding of the processes involved in these emissions.

SPN: “Evaluar la eficiencia del DMPSA, NBPT y del doble inhibidor (DMPSA+NBPT) aplicados con urea en la mitigación de emisiones de N₂O y NO. Una alta resolución de los flujos de NO permitiría explicar mejor los procesos de emisión que tienen lugar.”

Initial Hypothesis: *As irrigated conditions favour denitrification, the use of this NI should have low effect on N₂O and NO fluxes when mixed with urea.*

Ammonia volatilization was quantified using the IHF method in Experiments 1 and 2 for a maize crop using different N sources (pig slurry, CAN or urea). In addition, a comparison was made of, the NI DMPP for pig slurry and the NI DMPSA for CAN (Experiment 1) and of the NI DMPSA and the urease inhibitor NBPT for urea

(Experiment 2). Volatilization during senescence was also evaluated in Experiment 2. Nitrous oxide emissions and yield were evaluated in all field experiments, in both maize (Experiments 1, 2 and 4) and wheat (Experiment 3) crops. In all experiments, at least one of the treatments tested the effect of an NI. Nitric oxide and NO₂ were also measured using the novel automatic system in experiment 3 and 4 for different type of fertilizers.

The mitigation strategies based on N fertilization management are evaluated in this thesis with the goal of providing, practical recommendations about more sustainable practices for both, rainfed and irrigated cropping systems. From a methodological point of view, the software described in Chapter 4 entails an important advance in the implementation of an automated chamber system to measure NO_x emissions in real conditions for a rainfed and an irrigated crop.

It should be highlighted that one of the major outcomes of this thesis is the creation of an efficient software code based on R that is able to manage high amount of data and has allowed to perform an extensive data analysis. This tool has allowed to achieve the aforementioned objectives.

CHAPTER 3

MATERIALS & METHODS

CHAPTER 3:

Four different field experiments were arranged in the period 2015-2017 at two different experimental sites, “La Chimenea” and “CENTER” field stations, for the purpose of this thesis. A timeline of the different experiments is provided in Fig. 3.1.

The experiments carried out were as follows:

Experiment 1- Volatilization of NH_3 and N_2O emissions in an irrigated maize crop. Effect of nitrification inhibitors with pig slurry and CAN (site: “La Chimenea” field station).

Experiment 2- Volatilization of NH_3 and N_2O emissions in an irrigated maize crop. Effect of DMPSA and double inhibitor with urea (site: “Center”). Experiment 3- Emission of NO_x and N_2O in a wheat crop using automated and manual chambers. Effect of DMPSA (site: “Center”)

Experiment 4- Emission of NO and N_2O in an irrigated maize crop using automated and manual chambers. Effect of DMPSA and NBPT (site: “Center”)

Owing to the complexity involved in handling and analysing the data generated by the automated chamber measuring system used in Experiments 3 and 4, a new software was developed which is described in Chapter 4. This software has been submitted to and admitted in the General Intellectual Property Registry of the Community of Madrid (Recio 2019, number of record entry 16/2019/1001).

Fig. 3.1: Timeline diagram of the experiments carried out for the purposes of this thesis.

1. Description of the experimental sites

1.1. “La Chimenea” field station

One of the experiments carried out for the purposes of this thesis (Experiment 1) was conducted at “La Chimenea” field station (40°03’N, 3°31’W, 550 m a.s.l.) between April 2015 and September 2015 (Fig. 3.2). La Chimenea is located in the Tajo river basin near Aranjuez (Madrid, Central Spain). Soil type was a Typic Calcixerept (USDA, 2010) with a silty clay loam texture (25% clay, 49% silt, and 26% sand). Some of the physicochemical properties of the topsoil layer (0-20 cm), measured by conventional methods, were as follows: pH, 8.23; total organic C, 18.51 g kg⁻¹; bulk density, 1.36 g cm⁻³; CaCO₃, 198 g CO₃ kg⁻¹.

The area has a semi-arid Mediterranean climate with high interannual variability. The mean annual air temperature in this area is approximately 14°C. The coldest month is January with a mean temperature of 6°C, and the hottest month is August with a mean temperature of 24°C. In the last 15 years, mean annual precipitation has been

approximately 365 mm (17 mm from July to August and 102 mm from September to November).

Fig. 3.2: “La Chimenea” field site location and location of Experiment 1 at the site.

Data of wind speed, wind direction, rainfall, radiation and air temperature were obtained from the meteorological station situated at the field site (<http://portal.mapama.gob.es/websiar/SeleccionParametrosMap.aspx?dst=1>). The station was equipped with anemometers at heights of 0.8, 1.25 and 4 m (Model 05103, R.M. Young, Traverse City, MI, USA). A temperature probe (SKTS 200, Skye Instruments Ltd., Llandrin- dod Wells, UK) inserted into the soil to a depth of 10 cm was used to monitor soil temperature.

An analysis of five prior years’ worth of wind speed and wind direction data at the site was required for the experiment (Fig. 3.3). Additionally, a complete wind data analysis was performed every 30 min to assess the temporal evolution of these parameters after

the fertilization events during this experiment (Fig. 3.4). According to the results of the wind study, the predominant wind directions were SW and NE.

Fig. 3.3: Wind rose of “La Chimenea” field station during 5 years before the experiment. Vertices of the polygons represent the number of winds that took the corresponding direction. Different colours represent different wind speeds.

Fig. 3.4: Wind analysis in “La Chimenea” field experiment: a) From 20 April to 20 May 2015; b) From 23 June to 15 July 2015. Vertices of the polygons represent the number of winds that took the corresponding direction. Different colours represent different wind speeds.

1.2. “Center” field station

Three of the experiments undertaken for this thesis were carried out at the “Center” field station (40° 25' N, 3° 29' W) situated near Torrejón de Ardoz (Madrid, Spain), in the middle of the Henares river basin (Fig. 3.5) .

According to the USDA Soil Taxonomy (USDA, 2010), the soil is a Typic Xerofluvent with a silt loam texture (10% clay, 59.5% silt, and 30.5% sand) on the upper horizon (0-20 cm). Some of the physico-chemical properties of the topsoil layer (0–20 cm), measured by conventional methods, were as follows: $\text{pH}_{\text{H}_2\text{O}}$, 8.2; bulk density, 1.27 g cm^{-3} ; total organic matter, 20.7 g kg^{-1} ; total carbonate, $81.6 \pm 4.2 \text{ g kg}^{-1}$; total N, $1.64 \pm 0.12 \text{ g kg}^{-1}$; electrical conductivity, $203 \text{ } \mu\text{s cm}^{-1}$; extractable, P $28.4 \pm 0.62 \text{ mg kg}^{-1}$.

The site is under semi-arid Mediterranean climate conditions with an annual average rainfall and temperature (over the last 15 years) of 387 mm and 14°C, respectively. The coldest month is January with a mean temperature of 5°C, and the hottest month is July with a mean temperature of 25 °C. The average rainfall from November to June (winter wheat crop period) is 290 mm and from April to September (maize crop period) 149 mm.

Fig. 3.5: “Center” field site location and location of Experiment 1 at the site.

Data of wind speed, wind direction, rainfall, radiation and air temperature were obtained from the meteorological station placed at the field site (<http://portal.mapama.gob.es/websiar/SeleccionParametrosMap.aspx?dst=1>). The station was equipped with anemometers at heights 0.8, 1.25 and 4 m (Model 05103, R.M. Young, Traverse City, MI, USA). A temperature probe (SKTS 200, Skye Instruments Ltd., Llandrin- dod Wells, UK) inserted into the soil to a depth of 10 cm was used to monitor soil temperature.

An analysis of five prior years’ worth of wind speed and wind direction data at the site was required for the experiment (Fig. 3.6). Additionally, a complete analysis was performed every 30 min to assess the temporal evolution of these parameters after the fertilization events during this experiment (Fig. 3.7). According to the results of the wind study, the predominant wind directions were SW and NE.

Fig. 3.6: Wind rose of “Center” field station during 5 years before the experiment. Vertices of the polygons represent the number of winds that took the corresponding direction. Different colours represent different wind speeds

Fig. 3.7: Wind analysis in “Center” field experiment of 42 days in 2017 from 18 April (date of fertilization) to 26 July. Vertices of the polygons represent the number of winds that took the corresponding direction. Different colours represent the speed of winds.

2. Experiment 1 - Volatilization of NH₃ and N₂O emissions in an irrigated maize crop (site “La Chimenea”)

The micrometeorological IHF method was used for the first time to measure NH₃ volatilization in a maize crop, evaluating the effect of nitrification inhibitors. The principles of this method (described in Chapter 1) require extensive surfaces. According to the literature search which was performed, the number of replications is lower than three in the studies that have been published. The experimental design was adapted to the characteristics of this technique.

2.1. Experimental design and treatments applied

The experiment carried out in “La Chimenea” was carried out on four square plots (40 m × 40 m) separated by a 50 m interspace. This long distance between plots was needed in order to avoid fluxes of NH₃ from other plots. There were no wind-disturbing elements within 100 m around the plots.

Two fertilized treatments (Table 3.1) were randomly distributed with two replicates per treatment (Fig. 3.8). The first treatment comprised the application of pig slurry (PS) in the basal fertilization at a rate of 50 kg N ha⁻¹ before seeding (20 April 2015), and calcium ammonium nitrate (CAN) at a rate of 150 kg N ha⁻¹ in the top-dressing fertilization (23 June 2015) (PS-CAN). In this treatment, no nitrification inhibitors (NI) were applied. The second treatment (PS-CAN+NI) comprised application of the nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate, ENTEC®) diluted in pig slurry as basal fertilizer (50 kg N ha⁻¹), and DMPSA (2-(N-3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture), which was coated onto the CAN granules, as top-dressing fertilizer (150 kg N ha⁻¹). Fertilizers (DMPP, CAN and CAN+DMPSA) were provided by EuroChem Agro

GmbH. The separation of fertilization events in time was enough to discard the possibility of an additive effect of DMPP in the second fertilization (Zerulla et al., 2001).

Table 3.1: Treatments applied in the experiment

Treatment	Fertilizer		N rate (kg N ha ⁻¹)		
	First fertilization	Second fertilization	Basal Fertilization	Top-Dressing fertilization	Total
C	-	-	0	0	0
PS-CAN	PS	CAN	50	150	200
PS-CAN+NI	PS+DMPP	CAN+DMPSA	50	150	200

C: Control
 CAN: calcium ammonium nitrate (27% total N = 13.5% N-NH₄⁺ +13.5% N-NO₃⁻)
 PS: pig slurry (2 g-N·L⁻¹; pH = 6.8)
 *DMPP, solution of 3,4-dimethylpyrazole phosphate
 *DMPSA, 3,4 dimethyl-1H pyrazol-1-yl) succinic acid

In each plot, several areas were selected (8 m × 6 m) to collect soil samples of N₂O, ammonium (N-NH₄⁺) and nitrate (N-NO₃⁻). Four additional 8 m × 6 m plots in the non-N-fertilized area were also delimited in order to maintain a Control treatment for N₂O emissions and soil N-NH₄⁺ and N-NO₃⁻ content (Fig. 3.8).

Fig. 3.8: Experiment design. Two plots of PS-CAN and two plots of PS-CAN+NI. Masts are represented by black circle (BG for background NH_3). Numbers by arrows indicate length in meters. Control plots are dashed areas between fertilized plots. Small rectangles inside plots indicate selected areas where N_2O and soil samples were taken. The drawing is not to scale.

2.1.1. Characteristics of pig slurry and mineral fertilizers and application

Pig slurry (PS) was collected from a pig farm near the experimental site. Its composition (total N and N-NH_4^+ content) was determined prior to application. Different analyses of slurry properties were performed to determine pH, total ammoniacal nitrogen (TAN) and N-NH_4^+ (both using the Kjeldahl method), dissolved organic carbon (DOC) content using a multi EA® 3100 elemental analyser (Analytik Jena, Germany), phosphorus and potassium content (both using the acid digestion method), and dry matter (by drying at 105°C) content. These analyses were carried out by an associated laboratory (Animal

Production Department, ETSIAAB , Universidad Politécnica de Madrid). Table 3.2 shows the pig slurry characteristics and composition.

Table 3.2: Characteristics of pig slurry applied on each plot

Plot	PS1	PS2	PS+DMPP 1	PS+DMPP 2
Volume applied (m ³)	2.5	2.5	2.5	2.5
Ph	7.9	7.8	8.0	8.1
TAN applied (kg N ha ⁻¹)	55	56.25	60	61.25
Dry matter (105°C) %	0.80	0.89	0.91	0.92
Total Nitrogen (Kjeldhal) mg N L ⁻¹	1.69	1.54	1.34	1.27
DMPP relative to N-TAN (w/w)	0	0	0.81	0.81

Based on the N-NH₄⁺ content, the amount of applied pig slurry was 2.5 m³ ha⁻¹ (equivalent to 50 kg N ha⁻¹). For the treatment PS+NI, pig slurry was mixed *in situ* with a solution of DMPP at a rate of 0.8% (in relation to N applied with slurry). Pig slurry (with or without NI) was evenly surface-applied with an immediate incorporation of up to 10 cm depth with a cultivator.

The combination of PS as basal fertilization and mineral N as top-dressing is a very common fertilization practice in areas with a high density of pig farms in Mediterranean regions (Becaccia et al., 2015).

As top-dressing (23 June 2015), calcium ammonium nitrate (CAN) with 27% N at a rate of 150 kg N ha⁻¹ was used as the main source of N. In the other treatment (PS-CAN +NI), CAN included the new nitrification inhibitor DMPSA, also at a rate of 150 kg N ha⁻¹ and 0.8% of NI. This new nitrification inhibitor is not yet available on the market. These fertilizers were homogenously spread by hand, leaving the fertilizer on the soil surface. Immediately after application, an irrigation event (50 mm) was applied with the aim of incorporating the urea and inhibitors into the upper part of the soil.

2.1.2. Crop and agricultural management practices

Maize (*Zea Mays* L. FAO class 600) was sown on 27 April 2015 at a density of 75.000 seeds ha^{-1} . Plots were irrigated using a sprinkler system (12 m $\times$ 12 m frame of sprinkler, Fig. 3.9). Water application rates were estimated from the crop evapotranspiration (ET_c) of the week before application (net water requirements). This was calculated daily as $\text{ET}_c = K_c \times \text{ET}_0$, where ET_0 is the reference evapotranspiration calculated by the FAO Penman–Monteith method using data from the meteorological station located in the experimental field. The crop coefficient (K_c) was obtained for the maize crop following the FAO method (FAO, 1998). The field was kept free of weeds, pests and diseases, by application of herbicides, following local practices.

Fig. 3.9: Sprinkler system in a maize crop

Maize was harvested at physiological maturity (black-layer stage), on 23 October 2015. To obtain the grain and above-ground biomass yield, four replicates of 10 linear meters

of maize plants were harvested. Afterwards, grain and stem were separated by hand, and total grain (at 14% moisture level) and the remaining aboveground biomass were weighed. Total N content of grain and straw was analysed as described in Chapter 4.

2.2. NH₃ volatilization measurements in “La Chimenea” field experiment

As carried out previously under similar climatic conditions (Sanz-Cobena et al., 2008b; Abalos et al., 2012), five passive flux samplers (PFS, also called *shuttles*) were mounted on masts located in the middle of each plot. Two additional masts with three PFS mounted on each one were also placed in the predominant wind directions (explained in section 1.1) for the determination of background NH₃ concentration (BG) (Fig. 1.7, Fig. 3.8).

Since the irrigation system consisted of sprinkler irrigation (Fig. 3.9), there was no height limitation. The PFS, in each plot, were mounted at heights of 0.25, 0.65, 1.25, 2.05 and 3.05 m above soil or canopy surface. That is, the PFS heights were modified fortnightly according to the variable height of the maize plant after top-dressing fertilization. The PFS used for BG NH₃ were placed at heights of 0.25, 1.25, 3.05 m above ground during all the experiment. The PFS were prepared in the lab the day before use. For the preparation, 75 mL of purified acetone (CH₃COCH₃, 99.5%, Panreac Quimica SLU, Spain) was used to clean the PFS (three times with 25 mL of acetone each time), and then 30 mL of oxalic acid diluted in purified acetone (3% w/v) was applied within the *shuttle* (Fig. 3.10). The PFS were then ready to collect NH₃ and were kept isolated until their installation on the masts.

Fig. 3.10: Image of the shuttle preparation process.

Two additional *shuttles* were maintained isolated during the sampling period as blank sample.

Two different periods for quantification of NH_3 volatilization were considered: a first sampling period from 20 April to 30 May (after basal fertilization) and a second sampling period from 20 June to 13 July (after top-dressing fertilization). The sampling periods are shown in Table 3.3.

Table 3.3: Definition of sampling periods for quantification of NH_3 after basal fertilization and after top-dressing fertilization.

	Sampling Period	From	To	Hours
Sampling periods after Basal fertilization	SP1	20/04/2015	20/04/2015	4
	SP2	20/04/2015	21/04/2015	17
	SP3	21/04/2015	22/04/2015	29
	SP4	22/04/2015	24/04/2015	44
	SP5	24/04/2015	27/04/2015	72
	SP6	27/04/2015	30/04/2015	72
	SP7	30/04/2015	04/05/2015	96
	SP8	04/05/2015	11/05/2015	168
Sampling periods after Top-Dressing fertilization	SP1	23/06/2015	24/06/2015	28
	SP2	24/06/2015	26/06/2015	42
	SP3	26/06/2015	29/06/2015	75
	SP4	29/06/2015	01/07/2015	45
	SP5	01/07/2015	03/07/2015	49
	SP6	03/07/2015	08/07/2015	118
	SP7	08/07/2015	15/07/2015	168

After each sampling, the NH_3 trapped in the PFS in the form of ammonium oxalate $(\text{NH}_4)_2\text{C}_2\text{O}_4$ was collected by leaching the sampler with 40 ml of water. The ammonia collected from the sampler was subsequently mixed with a solution of Na_2EDTA and nitroprussiate. Both substances are indicator dyes which form a coloured complex with ammonia. Afterwards, each sampler was analysed with a UV-Vis double beam spectrophotometer (UV-1603, Shimadzu). The spectrophotometer was configured to measure wavelength absorption, with $\lambda = 667 \text{ nm}$. A calibration line (performed with six standard samples of ammonia with concentrations from 0.6 to 3.0 mg L^{-1}) allowed direct deduction of ammonia concentration from the absorption spectrum since the response of the spectrophotometer was linear, with $R^2 > 0.99$. Information about Eq. 1.2, used to

calculate vertical NH_3 fluxes, is given in Chapter 1 (section 1.2.1). However, Eq. 1.2 assumes that the plot extends infinitely in the across-wind direction so the net lateral diffusion is zero. In the case of a rectangular plot, as in the present study, flux estimates associated with wind directions from the corner of the plot will potentially be associated with errors due to net lateral flux of NH_3 . In these rectangular plots, fetch length was calculated according to the mean wind direction in a 1-h period. For that, a calculation program based on wind direction, plot dimension and shuttle position was used which had been developed by Dr. Misselbrook from the Rothamsted Research Centre (UK).

As the PFS were continuously replaced during the experiment, the IHF method allowed calculation of cumulative NH_3 emissions over an entire period as the sum of volatilized NH_3 at each measurement interval. Flux rates were converted to $\text{g N ha}^{-1} \text{ h}^{-1}$ for reporting purposes.

2.3. Measurement of N_2O emissions

In most GHG emission literature, the predominant measurement technique used involves the use of non-flow through, non-steady state chambers, commonly known as static chambers (Bouwman et al., 2002). Within these chambers, the replacement of air in the headspace is negligible, and hence the accumulation of GHG fluxes in the chamber's headspace increases over time. One opaque static circular chamber of 19.3 L (diameter 35.60 cm, height 19.30 cm, Fig. 3.11) was placed between plant lines in each sub-plot and fitted into stainless steel rings, which were inserted into the soil to a depth of 10 cm at the beginning of the experiment to avoid lateral diffusion of gases and disturbance of the gas fluxes (Davidson et al., 2002). A rubber stopper with a 3-way stopcock was placed in the wall of each chamber to take gas samples.

Fig. 3.11: Detailed image of a closed manual chamber that was used to collect sample of N_2O

During the first 45-60 days after fertilization (DAF), gas samples were taken 2-3 times per week (Table 3.4). Afterwards, the sampling frequency was progressively decreased. The N_2O fluxes were measured using the closed chamber technique as described by Abalos et al. (2013). Briefly, using 20 mL syringes, gas samples were taken from the headspace of each chamber fitted with 3-way stopcocks at 0, 30 and 60 min after chamber closure, and transferred to pre-evacuated vials sealed with a gas-tight neoprene septum.

Nitrous oxide concentrations were measured by gas chromatography, using an HP-6890 gas chromatograph (GC) from Agilent Technologies (Wilmington, DE, USA), equipped with a headspace sampler (TurboMatrix 110, from Perkin Elmer). HP Plot-Q capillary columns transported the gas samples, using He as carrier gas, to a ^{63}Ni electron-capture detector (ECD) to analyse the N_2O concentrations with a detection limit of 50 ppb.

The temperature of both injector and oven were programmed at 40°C, while the detector's temperature was set at 350 °C. The ECD was run with Ar-CH₄ as make-up gas. The precision of the gas chromatographic data at ambient N_2O concentrations was $\pm 1\%$ or

better. Two gas standards (2000 ± 50 and 400 ± 6 ppb N_2O) were provided by Carburos Metalicos S.A. (Barcelona, Spain). These two standards were diluted to perform a calibration curve for this gas with four different concentrations, two original standards and two diluted standards. The response of the GC was quadratic within 200–2000 ppb for N_2O . The increases in N_2O concentrations within the chamber headspace were generally linear ($R^2 > 0.95$) during the sampling period (1h).

The rate of increase of flux density follows the equation proposed by Denmead (2008) (Eq. 1.3).

In this study, emission rates of N_2O fluxes were, therefore, estimated as the slope of the linear regression between concentration and time (after corrections for temperature) and from the ratio between chamber volume and soil surface area.

Cumulative gas emissions during the experimental period were calculated by multiplying the average flux of two successive determinations by the length of the period between the samplings and adding that amount to the previous cumulative total (Menéndez et al., 2012).

2.4. Measurements of soil moisture, N-NH_4^+ and N-NO_3^- contents

Three soil cores were randomly sampled from the upper layer (0-10 cm) around each plot and mixed in the laboratory to obtain a homogeneous sample. Samples were taken on almost the same dates as those of N_2O . Soil N-NH_4^+ and N-NO_3^- concentrations were analysed using 8 g of soil extracted with 50 mL of KCl (1 M) and measured by automated colorimetric determination using a flow injection analyser (FIAS 400 Perkin Elmer) equipped with a UV-V spectrophotometer detector. Water-filled pore space (WFPS) was calculated by dividing the volumetric water content by the total soil porosity. The total soil porosity was calculated according to the following relationship (Danielson and

Sutherland, 1986): $soil\ porosity = 1 - \frac{soil\ bulk\ density}{2.65}$, assuming a particle density of $2.65\ g\ cm^{-3}$ (Klute and Page, 1982). The gravimetric water content was determined by oven-drying soil samples at $105\ ^\circ C$ with a MA30 Sartorius® moisture analyser. Information about soil samples dates are given in the Table 3.4

Table 3.4: Principal agricultural management events of the experiment and N_2O and soil sampling dates.

Basal fertilization date	20/04/2015
Planting date	27/04/2015
Top- dressing fertilization	23/06/2015
First irrigation	28/05/2015
Last irrigation	02/09/2015
Harvest date	23/10/2015
Sampling dates	
N_2O (DABF)*	7, 8, 10, 14, 16, 24, 29, 31, 36, 42, 53, 59, 64
N_2O (DADF)**	1, 3, 6, 8, 10, 15, 17, 22, 24, 29, 38, 43, 50, 66, 83
Soil (DABF)*	2, 4, 8, 14, 16, 21, 29, 31, 36, 42, 53, 59, 64
Soil (DADF)**	1, 3, 8, 10, 15, 22, 29, 38, 50, 66, 83

* DABF: days after basal fertilization

**DADF: days after top-dressing fertilization

2.5. Crop analysis and yield

Maize was harvested at physiological maturity (black-layer stage), on 23 October 2015. To obtain the grain and aboveground biomass yield, four replicates of 10 linear meters of maize plants were harvested. Afterwards, grain and stem were separated by hand, and total grain (at 14% moisture level) and the remaining aboveground biomass were

weighed. Total N content of grain and straw was analysed using a TruMac CN Leco elemental analyser. The aboveground N uptake by crop was calculated multiplying the biomass by N content in each plant fraction.

Yield-scaled N_2O emissions and the N_2O emission factor were also calculated as described in section 6 of this Chapter.

3. Experiment 2 - Volatilization of NH_3 and N_2O emissions in an irrigated maize crop. Effect of DMPSA and double inhibitor with urea (site “Center”)

The micrometeorological IHF method was used to measure NH_3 volatilization in a maize crop treated with urea with or without nitrification or urease inhibitors. This was the first time that NH_3 emissions have been quantified in an irrigated crop using urea and inhibitors. Determination of N_2O emissions was also performed for the same period.

3.1. Experiment design and treatments applied

Experiment 2 was carried out in the “Center” field station in 2017. The experimental area was comprised of six square plots ($40\text{ m} \times 40\text{ m}$) separated by a 50 m interspace. In the previous year no crop was cultivated. Three fertilized treatments were randomly distributed with 2 replicates per treatment (Fig. 3.12). The application of fertilizers was adjusted to provide $200\text{ kg total N ha}^{-1}$ for all fertilized treatments at a single dressing application (14 June 2017). The different fertilizer treatments were: 1) Urea (U); 2) Urea + DMPSA (U+NI); 3) Urea+NBPT+DMPSA (U+2I). Fertilizers (urea+DMPSA and urea+NBPT+DMPSA) were provided by EuroChem Agro GmbH and the inhibitors (UI and NI) were coated onto urea granules. The area between plots was also cultivated with maize but without N fertilizer and these plots were considered the Control treatment. There was an interspace area that was kept fallow.

In each plot, two sub-plots (8m × 6m) were selected for data collection of N₂O fluxes and crop yield. Four additional 8 m × 6 m plots in the non-N-fertilized area were also delimited in order to maintain a Control treatment for N₂O fluxes and yield properties (Fig. 3.12: C1, C2, C3, C4).

Fig. 3.12: Experiment design. Three treatments with 2 replicates per treatments: 1) Urea (U); 2) Urea + DMPSA (U+NI); 3) Urea + NBPT + DMPSA (U+2I). Masts are represented by black circle (BG for background NH₃). Numbers by arrows indicate length in meters. Control plots are areas between fertilized plots (C1, C2, C3, C4). Small squares inside plots indicate selected areas where N₂O and soil samples were taken. The drawing is not to scale

3.1.1. Characteristics of fertilizers and application

Urea plus DMPSA and urea with double inhibitor were prepared by EuroChem Agro GmbH in Germany a few months before application. The percentage of DMPSA (2-(N-3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture) in both fertilizers was 0.8% of ureic N, while the urease inhibitor NBPT (N-(n-butyl) thiophosphoric triamide)

was 0.13% of ureic N. During preparation of the double inhibitors with urea, no contact was ensured between NBPT and DMPSA in the granules in order to avoid possible reactions between them. All of them were applied to the soil surface by hand on 14 June 2017. Application of an irrigation event (10 mm) was performed immediately with the aim of incorporating the urea and inhibitors in the upper part of the soil.

3.1.2. Crop and agricultural management practices

Maize (*Zea Mays* L. FAO class 400) was sown on 18 April 2017 at a density of 95,000 seeds ha⁻¹. A single fertilization event was performed on 14 June 2017 at growth stage V5–V6. In this case, as the crop was to be used as fodder maize, only the aboveground biomass yield was obtained. Four replicates of 10 linear meters of maize plants were harvested on 20 August 2017 and the total aboveground biomass weighed. Afterwards, grain and stem were separated by hand and total N content of grain (at 14% moisture level) and straw was analysed using a TruMac CN Leco elemental analyser.

Plots were irrigated using a ranger system (Fig. 3.13). Water application rates were estimated from the crop evapotranspiration (ET_c) of the week before application (net water requirements). This was calculated daily as $ET_c = K_c \times ET_o$, where ET_o is the reference evapotranspiration calculated by the FAO Penman–Monteith method using data from the meteorological station located in the experimental field. The crop coefficient (K_c) was obtained for the maize crop following the method of Allen et al. (FAO, 2004).

Fig. 3.13: Ranger irrigation system in Experiment 2

The phosphorus (Olsen method) and potassium (K) concentrations were analysed before the beginning of the experiment. Since contents were high for both nutrients, no P or K seeding fertilization was applied. The field was kept free of weeds, pests and diseases, by application of herbicides (Spectrum, BASF, Germany), following local practices.

3.2. NH_3 volatilization measurements in “Center” field experiment

As described in Chapter 1 (section 1.2.1), the IHF method was used to measure NH_3 emissions. The measurement plots were surrounded by an unfertilized flat area. There were no wind-disturbing elements within 100 m around the plots.

Five passive flux samplers (PFS) were mounted on a mast located in the middle of each plot. Two additional masts, with three PFS mounted on each one, were also installed in the predominant wind directions (see Chapter 3, section 1.1) for determination of background NH_3 concentration (BG) (see Fig. 3.12). In this experiment, the irrigation events were performed with a ranger system (see Fig. 3.13), and hence there was a maximum height for the masts and the PFS. The PFS, located in each plot, were initially mounted at heights of 0.8, 1.1, 1.5, 2.05 and 2.8 m above the crop canopy surface. The *shuttle* height was moved fortnightly according to the height of the maize plant after top-dressing fertilization. For heights higher than 2.8 m, an additional mobile mast was used. When the ranger system was being operated, this mast was manually removed in each plot and immediately replaced again after the irrigation event. The PFS of the BG masts were placed at heights of 0.4, 1.05 and 2.8 m above ground level. The various sampling periods are shown in Table 3.5. *Shuttles* were prepared as described in Chapter 3 (section 2.2).

Table 3.5 Definition of sampling periods for quantification of NH_3 after single fertilization

Sampling Period	From	To	Hours
SP1	14/06/2017	15/06/2017	21
SP2	15/06/2017	16/06/2017	25
SP3	16/06/2017	19/06/2017	72
SP4	19/06/2017	21/06/2017	48
SP5	21/06/2017	23/06/2017	48
SP6	23/06/2017	26/06/2017	75
SP7	26/06/2017	30/06/2017	96
SP8	30/06/2017	06/07/2017	140
SP9	06/07/2017	13/07/2017	170
SP10	13/07/2017	20/07/2017	168
SP11	20/07/2017	26/07/2017	144

After each sampling, the NH_3 trapped in the PFS in the form of ammonium oxalate was collected by leaching the sampler with 40 ml of water and analysed as described in Chapter 3 (section 2.2).

Fluxes were calculated using Eq. 1.2, as described in Chapter 1 (section 1.2.1). As rectangular plots were used in this study, fetch length was calculated according to the mean wind direction in a 1-h period using a program developed by Dr. Misselbrook from the Rothamsted Research Centre (UK). Cumulative NH_3 emissions over an entire period were calculated as the sum of NH_3 volatilized at each measurement interval. Flux rates were converted to $\text{g N ha}^{-1} \text{ h}^{-1}$ for reporting purposes.

3.3. Measurement of N_2O emissions

A closed chamber system was used to measure N_2O emissions. Chamber characteristics and a description of the method for sampling and determination of N_2O concentrations are given in Chapter 1 (section 2.1.1). Chambers of 19.3 L (diameter 35.60 cm, height 19.30 cm) were placed between plants in each sub-plot and fitted into stainless steel rings. Sampling dates are shown in Table 3.6. Sampling was performed 2-3 times during the first five weeks after the fertilization event and then progressively reduced.

Cumulative N_2O emissions during the experimental period were calculated by multiplying the average flux of two successive determinations by the length of the period between the samplings and adding that amount to the previous cumulative total (Menéndez et al., 2012).

3.4. Measurements of soil moisture, N-NH₄⁺ and N-NO₃⁻ contents

Soil sampling and determination of soil moisture, N-NH₄⁺ and N-NO₃⁻ contents were performed as described in this chapter (section 2.4.). The soil sampling dates are shown in Table 3.6.

Table 3.6: Principal agricultural management events of the experiment and N₂O and soil sampling dates of Experiment 2.

Planting date	18/04/2017
First irrigation	20/04/2017
Fertilization date	14/06/2017
Last irrigation	28/07/2017
Harvest date	20/08/2017
Sampling dates (DAF*)	
N ₂ O	1, 3, 5, 7, 9, 12, 14, 16, 20, 22, 26, 28, 31, 33, 36, 42, 56, 69, 104
Soil	1, 3, 5, 9, 12, 20, 22, 26, 33, 36, 42, 56, 69, 104

**DAF: days after fertilization*

3.5. Crop analysis and yield

Plant samples were obtained after harvesting. Grain and stem were separated by hand and total grain (at 14% moisture level) and the remaining above-ground biomass were weighed. Total N content of grain and straw was analysed using a TruMac CN Leco elemental analyser. The aboveground N uptake by crop was calculated by multiplying the biomass by N content in each plant fraction.

Yield-scaled N₂O emissions (YSNE), the N₂O emission factor (EF-N₂O), yield-scaled NO_x emissions (Y-S NO_x), and nitrogen use efficiency (NUE) were calculated as

described in this Chapter (section 6). Total protein content was then calculated using a conversion factor of 6.25 by N content (FAO, 2004).

4. Experiment 3 - Winter wheat crop field experiment to determine NO, NO₂ and N₂O emissions (site “Center”)

This experiment, using automated open chambers and manual closed chambers, was performed in a wheat crop to quantify NO, NO₂ and N₂O emissions. An important methodological novelty for this experiment was the use of an automated system to measure NO and NO₂ emissions under field conditions in a wheat crop. This was the first time that this type of system has been used in soil fertilized with urea.

4.1. Characteristics of fertilizers and application

Urea plus DMPSA was prepared by EuroChem Agro GmbH in Germany several days before its application. The percentage of DMPSA (2-(N-3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture) was 0.8% of ureic N. Fertilizers were homogeneously applied by hand on 5 April 2016. No-irrigation was performed either before or after fertilization. The phosphorus (Olsen method) and potassium (K) concentrations were analysed before the beginning of the experiment. Since contents were high for both nutrients, no P or K seeding fertilization was applied.

4.2. Crop and agricultural management practices

In this field, winter wheat (*Triticum aestivum* L. 'Ingenio') was sown in rows on 27 November 2015 at a rate of 200 kg seeds ha⁻¹, and was harvested on 29 June 2016 with a combine harvester (Wintersteiger Inc, Germany, Fig. 3.14). The field was kept free of weeds, pests and diseases, by application of herbicides, following local practices.

The total grain was obtained using plant samples (aboveground biomass including the grain) that were taken by harvesting 4 randomly selected 1m² plots. Afterwards, grain was dried to approximately 14% humidity and cleaned of dust and tailings in a laboratory sieve-air separator.

Fig. 3.14: Aerial view of the experiment before harvesting (left) The harvester machine used in 29 June 2016.

4.3. Experimental design and treatments applied

Experiment 3 was carried out at the “Center” field station in 2015-2016. Nine plots (8 m × 5 m) were selected and arranged in a randomized complete block design with three replicates per treatment (Fig. 3.15). No crop was cultivated in the previous year. A single fertilizer application of 120 kg total N ha⁻¹ was performed for all treatments during the crop period. The different fertilizer treatments were 1) Control without N fertilization (Control); 2) Urea with DMPSA, (U+NI); and 3) Urea (U). The proportion of DMPSA in the fertilizers was 0.8% of the N content and was coated onto the urea granules.

This experiment was focused on acquiring a high temporal data resolution of fluxes of NO and NO₂, obtained by using automatic chambers, for the U and U+NI treatments. In addition, measurements of N₂O emissions were obtained using manual chambers for the

fertilized (U and U+NI) and unfertilized (Control) treatments. Manual chambers to measure N_2O (see Chapter 1, section 2.1.1.1.) were placed in all the plots, and automated chambers to measure NO_x (see Chapter 1, section 2.1.1.2.) only in the fertilized plots.

Fig. 3.15: Experimental design (the drawing is not to scale). Dashed surfaces represent experimental plots, white squares represent the automated chambers and black circles represent manual chambers. Solid arrows indicate tubes that connected the automated chambers with the mobile laboratory. Numbers by arrows indicate length in meters.

4.3.1. Characteristics of fertilizers and application

Urea plus DMPSA was prepared by EuroChem Agro GmbH in Germany several days before its application. The percentage of DMPSA (2-(N-3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture) was 0.8% of ureic N. Fertilizer was homogeneously applied by hand on 5 April 2016. The phosphorus (Olsen method) and potassium (K) concentrations were analysed before the beginning of the experiment. Since contents were high for both nutrients, no P or K seeding fertilization was applied.

4.4. NO, NO₂ and O₃ measurements

4.4.1. Description of automated NO_x and O₃ measurement system

Nitric oxide, NO₂ and O₃ were measured with a completely automated mobile laboratory measurement system for the cropping season of winter wheat (Fig. 3.16). This automated system was designed and constructed following the method proposed by Butterbach-Bahl et al. (1997) with some modifications, which took into account different studies (Slemr and Seiler, 1991; Schindlbacher, 2004).

The experimental setup schematically consisted of 7 dynamic flow chambers, a pump (155 W, 30 L/min, Patashnick Co, Inc.), a NO_x chemiluminescence detector (AC31 M-LCD, Environnement S.A., Poissy, France), and a UV spectrometer for O₃ (Model 42M (Environnement S.A., Poissy, France) (Fig. 3.16). Air was sucked from each dynamic chamber by a pump and was transported via Teflon tubing (length 50 m; inner diameter: 6 mm) to a solenoid valve (Flo Control®, Germany), which was followed by a PTFE manifold. The flow controllers, manifold and pump were housed in a mobile laboratory situated on the field. From the PTFE manifold, air samples were driven along PTFE tubes to the different detectors. The sample air passing through the headspace of the chamber was filtered with a charcoal and aluminium/KMnO₄ column, in order to remove O₃ and NO_x (Dickerson et al., 1984). Nitrogen dioxide (NO₂) was determined by transformation of NO_x into NO by a molybdenum converter heated to 400°C and deducting the original NO signal of the air stream (Slemr and Seiler, 1984).

Fig. 3.16: Schematic illustration of chambers and the automated measurement of O₃, NO and NO₂ flux rates in the winter-wheat experiment with 6 plots and a reference chamber

Additionally, the opening time of the valves, the sequence of chamber closure and re-opening and data collection were controlled by a PC running a specially developed computer program (SIMdas, DNOTA®, Spain). The electrovalves were closed in normal position and electrically connected to the PC interface. In the same way, chambers were opened in normal position and were closed automatically by electric motors.

4.4.2. Open chambers characteristic

The open-chambers (length=50 cm; width=50 cm; height=20 cm; vol=50 L) consisted of an aluminium frame in which polymethacrylate (PMMA) panes were fixed. The inlet pane of the chamber for ambient air was located on the opposite side to the outlet pane of the chambers and contained five holes (2.0 cm diameter). Lids, also made of PMMA,

were opened or closed by an electric motor. Chambers were inserted to a depth of 10 cm. An additional reference chamber was placed in the same experimental area, although it was isolated from the soil with a Teflon film. Electric wires and Teflon tubes, which transported the samples to the detectors housed inside the lab, each had a length of 50 m. When the chamber was closed the pump sucked out the air from inside the chamber at a flow of 30 L min⁻¹. A flow-meter inside the lab continuously controlled the flow of each chamber.

Fig. 3.17: Dimensioned drawing of front views, side views and isometric perspectives of an open chamber (top) and a closed chamber (bottom). The drawing is not to scale.

4.4.3. Automatic cycle sampling

Preliminary tests showed gas concentrations took approximately 6 min to reach steady state values after chambers were closed. As is well known, the steady state is obtained when the concentration of gas (NO, NO₂ or O₃) remains constant over time. Hence, chambers were closed for 8 min, and only the mean value of the last 90 s was used for

flux determinations. Thus, measurements took a total of 10 min for each treatment (8 min from treatment chamber plus 2 min from reference chamber). A full cycle of measurements took 1 h for all six treatments, resulting in 24 measurements per day and per chamber. For this purpose, a specific software (SIMdas, DNOTA®, Spain) controlled the sequence of chambers closure and opening connection to detectors and all data logging.

4.4.4. Determination of NO, NO₂ and O₃ concentration

Nitric oxide and NO₂ were determined by chemiluminescence using a NO_x detector. The equipment measures the sample air passing through the headspace of the chamber and was filtered with a charcoal and aluminium/KMnO₄ column, in order to remove O₃ and NO_x (Dickerson et al., 1984). Nitrogen dioxide (NO₂) was determined by transformation of NO_x into NO by a molybdenum converter heated to 400°C and deducting the original NO signal of the air stream (Slemr and Seiler, 1984). Ozone was continuously measured with a UV spectrometer detector (253 nm absorption wavelength). The NO_x and O₃ analysers were repeatedly calibrated using zero air and standard gases of 100 ppb of NO_x (v/v) in N₂ and 100 ppb of O₃ (v/v) in O₂ supplied by Carburos Metálicos SA (Barcelona, Spain).

4.4.5. Calculation of fluxes of NO and NO₂ when steady state was obtained.

Both NO and NO₂ are highly reactive gases and different (photo-) chemical reactions could take place daily in soil with time scales similar at the residence time of air in the chamber (R4 and R5). This could cause an underestimation or overestimation of the NO or NO₂ fluxes when NO is emitted by the soil (Pape et al., 2009).

Both reactions have their mathematical equations that equalize the concentrations of the reagents with the velocity of the reaction:

$$\frac{dC(NO)}{dt} = k_1 \cdot C(NO) \cdot C(O_3) \quad \text{First order reaction (Eq. 3.1)}$$

$$\frac{dC(NO)}{dt} = j(NO_2) \cdot C(NO_2) \quad \text{Photolysis reaction (Eq. 3.2)}$$

where

$j(NO_2)$ is the photolysis rate of NO_2 (depending on the chamber material),

k is the first order reaction rate constant of R_4 .

To avoid this underestimation or overestimation of these gases, equation Eq. 1.5 was adapted following the equation from Pape et al. (2009) (Eq. 3.3):

$$F = (C_{ch} - C_o) \cdot \frac{M \cdot Q \cdot 10^6}{V_m \cdot A \cdot 10^9} \cdot 60 - \frac{S_{sp}}{A} \quad \text{Eq. 3.3}$$

where:

F = flux in $\mu g - N \cdot m^{-2} \cdot h^{-1}$;

M is the atomic weight of the element ($N = 14.008 g \cdot mol^{-1}$);

V_m is the standard gaseous molar volume ($22.4 \cdot 10^{-3} m^3 \cdot mol^{-1}$);

Q is the flow with the pump ($30 L \cdot min^{-1}$);

C_{ch} is the mixing ratio (ppbv = $10^{-9} m^3 m^{-3}$) of the gas when the lid of chamber was closed and the chamber has reached steady state;

C_o is the mixing ratio of the gas in the reference chamber;

A is the soil surface area covered by the chamber ($0.5 \times 0.5 m = 0.25 m^2$);

S_{gp} is the net gas-phase source for NO within the chamber volume that can be described as:

$$S_{gp} = Vol \cdot [j(NO_2) \cdot C_{ch}(NO_2) - k_1 \cdot C_{ch}(NO) \cdot C_{ch}(O_3)] \quad \text{Eq. 3.4}$$

In this experiment, the factors k_1 and $j(NO_2)$ were calculated using a value of $4.31 \cdot 10^{-4}$ [ppb⁻¹ s⁻¹] at 1013 hPa and 298.16 K for k_1 (reaction rate constant of R4) and an effective transmissivity value of 0.48 min⁻¹ ($j(NO_2)$) for calculating the photolysis rate of NO₂ (reaction rate constant of R5) in sunlight. At night, S_{gp} was set to zero.

As radiance inside the chamber is dependent on different factors, such as weather conditions and wheat growth stage (affecting $j(NO_2)$), punctual underestimation or overestimation of NO and NO₂ fluxes using Eq. 3.4 was also possible in the measurements. As indicated in Pape et al. (2009), the S_{gp} for the O₃ and NO₂ is the same as for NO, but in the case of NO₂ the sign is the opposite:

$$S_{gp}(NO) = S_{gp}(O_3) = -S_{gp}(NO_2) \quad \text{Eq. 3.5}$$

Hence, there were two different ways to calculate the fluxes:

- i) Uncorrected NO and NO₂ fluxes were calculated setting the term S_{gp}/A as zero for all time, based on the equation from Schindlbacher et al. (2004).
- ii) Corrected NO and NO₂ fluxes were calculated taking into account the possible reaction of NO with O₃ (R4) and photolysis of NO₂ (R5) inside the chamber during daylight hours (S_{gp}/A different to zero).

Cumulative gas emissions during the experimental period were calculated by multiplying the average flux of two successive determinations by the length of the period (1 h) between sampling and adding that amount to the previous cumulative total.

4.4.6. Data analysis

Traditional software applications, such as Microsoft Excel®, were unable to handle the vast amounts of data generated during this experiment (>500,000). Consequently, a specific aim of this thesis was to build a software (see Chapter 4) to evaluate the data logged from each chamber. This specific model was created using the programming language R, version 3.2.1 (R Development Core Team, 2017). This script was able to remove data points before the steady state, to calculate the average NO/NO₂ of the last 90 s (when chambers were closed), to obtain the value of the steady state, to discard erroneous data for each plot chamber, to obtain the ambient value of the reference chamber, to calculate the absolute value of NO/NO₂ in each chamber, and to determine NO/NO₂ emission fluxes.

4.5. Measurement N₂O emissions

A manual chamber system was used to measure N₂O emissions. One chamber (diameter 35.60 cm, height 19.30 cm) per plot was fitted into stainless steel rings. Chamber characteristics and a description of the method for sampling and measuring are given in section 2.3. Plant material was included inside the chambers. When plant height was higher than that of the chamber, plants were cut. Gas sampling frequency is shown in Table 3.7. After fertilization and rainfall events, the number of samplings was increased. Afterwards, sampling frequency was progressively decreased. Calculation of N₂O fluxes and cumulative fluxes was performed as indicated in Chapter 1 (section 2.1.1.1.). The N₂O measurements were made in the three treatments, including Control.

Table 3.7: Principal agricultural management events of the experiment and N₂O and soil sampling dates of Experiment 3.

Planting date	27/11/2015
Fertilization date	05/04/2016
Harvest date	29/06/2016
Sampling dates (DAF*)	
N ₂ O	0, 1, 3, 8, 13, 17, 21, 23, 30, 38, 45, 57
Soil	0, 1, 3, 8, 13, 17, 23, 38, 57

*DAF= days after fertilization

4.6. Measurements of soil moisture, N-NH₄⁺ and N-NO₃⁻ contents

To measure soil moisture content at different depths, three lab-calibrated EnviroSCAN® probes were randomly inserted into the soil at depths of 10, 30 and 50 cm. Data were obtained from these devices every 30 min. The logged EnviroSCANS® data was uploaded using the IrriMax application, version 10.0.1 (Sentek Technologies). Volumetric moisture content was transformed into WFPS considering total soil porosity (Eq. 3.6), using equation Eq. 3.7.

$$\text{Soil porosity} = 1 - \frac{\text{soil bulk density}}{2.65} \quad (\text{Danielson and Sutherland, 1986}) \quad \text{Eq. 3.6}$$

$$\text{WFPS} = \frac{\text{Volumetric moisture content}}{\text{Soil porosity}} \quad \text{Eq. 3.7}$$

Daily WFPS values were averaged for each probe to show soil moisture results.

Soil samples from the 0-10 cm depth were taken at different dates (Table 3.7) as described in Chapter 3 (section 2.4.). Nitrate and NH₄⁺ content was analysed as also described in that section. Results were expressed as mg N kg⁻¹ soil.

4.7. Yield, plant analysis and breadmaking quality

Using all the plants collected in the 1m² plot, grain, leaves and stems were separated by hand, and the grain and biomass were weighed in order to calculate total aboveground weight and grain yield. Afterwards, grain was milled to particles <1 mm with a Udy cyclone mill (*Seedburo* Equipment. Co., IL). Total N content of grain and straw was analysed using a TruMac CN Leco elemental analyser. The aboveground N uptake by crop was calculated multiplying the biomass by N content in each plant fraction. Total protein content was calculated using a conversion factor of 5.83 by N content (FAO, 2004).

Breadmaking quality of grain was measured using the sodium dodecyl sulfate sedimentation (SDSS) test, developed by Dick and Quick (1983). This method determines the quality of gluten based on the high correlation between SDSS volume and dough deformation energy and, therefore, gluten strength. This test thus determines the swelling capacity of the protein matrix of the flour. The lower the sediment velocity, the higher the value in mm of sediment and, consequently, the greater the gluten force. It is a fast and destructive test but requires 1 g of material per sample. The lactic acid opens the structure of the gluten and allows the water to enter into it without breaking it.

Eighteen glass tubes (2 replicates per plot) with a threaded cap of 150 mm in length and 14 mm in diameter were positioned in a mm-graduated scale. One gram of whole wheat flour, ground with a Udy cyclone mill to particles <1 mm, was inserted into each glass tube with a solution of lactic acid at 85% and a solution of sodium dodecyl sulfate (SDS) at 2% in a 1:48 proportion. The test design and how the results can be viewed are shown in Figure 3.18.

Fig. 3.18: View of the results of a SDSS test.

Yield scale N_2O emission (YSNE), N_2O Emission factor (EF- N_2O), Yield scale NO_x emission (Y-S NO_x), and Nitrogen Use efficiency (NUE) were also calculated as described in the section 6 of this Chapter.

5. Experiment 4 - Effect of DMPSA and NBPT on emissions of NO , NO_2 and N_2O in a drip-irrigated maize crop using automated and manual chambers (site “Center”)

Experiment 4, using automated open chambers and manual closed chambers was carried out on a maize crop to quantified NO , NO_2 and N_2O emissions. An important methodological novelty of this experiment was that 16 automated chambers were used in the experiment, all of them controlled by a PC. This was the first time that this type of system has been used in a fertilized maize crop.

5.1. Experimental design and treatments applied

Fifteen plots of 81 m^2 ($9 \text{ m} \times 9 \text{ m}$) were set up at the “Center” field station in 2017. A randomized complete block design with three replicates was established (Fig. 3.19). The

application of fertilizers was adjusted to provide a total of 200 kg N ha⁻¹ for all treatments in a single application during the crop period. The five different fertilizer treatments were: 1) Urea (U); 2) Urea with the nitrification inhibitor 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (DMPSA) (U+NI); 3) Urea with the urease inhibitor (UI) N-(n-butyl) thiophosphoric triamide NBPT (UTEC®) (U+UI); 4) Urea + NBPT + DMPSA (U+2I); 5) Control with no N fertilization (C). The proportion of NBPT and DMPSA in the fertilizers was 0.8% of the N-NH₄⁺.

Fig. 3.19: Experimental design (the drawing is not to scale). Grey surfaces represent experimental plots, white squares represent the automated chambers and circles represent the manual chambers. Solid arrows indicate tubes that connected automated chambers with the mobile laboratory. Numbers by arrows indicate length in meters

In this experiment, a detailed database with a high temporal resolution of NO_x emission in a soil was obtained. In this case, the new automated system to measure NO_x allowed to obtain fluxes both fertilized and control plots. Manual chambers were used to measure N₂O.

5.1.1. Characteristics of fertilizers and application

Fertilizers were prepared by EuroChem Agro GmbH in Germany several days before their application. The U+NI treatment included urea and DMPSA at 0.8% of ureic N. The

U+UI included urea, and NBPT at 0.13% of ureic N and U+2I treatment included urea, NBPT and DMPSA. Throughout granule preparation, the inhibitors were isolated in order to avoid possible reactions. A commercial urea was used (46%N). Fertilizers were homogeneously applied by hand on 10 July 2017 when maize was at growth stage V5. Irrigation event was carried out immediately after fertilizer application in order to incorporate the fertilizers into the soil.

5.1.2. Crop and agricultural management practices

Maize (*Zea Mays* L. FAO class 600) was sown on 18 April 2017 at a density of 95.000 seeds ha⁻¹ and was harvested at physiological maturity, on 20 September 2017 with a research plot combine (Wintersteiger Inc.) (see Fig. 3.14). A surface drip irrigation system was installed in all plots in May, with pressure-compensated drip irrigation tubes in all maize lines. Each line had distributors of 6 L h⁻¹, capacity, placed 1 m apart from each other. Water application rates were estimated from the crop evapotranspiration (ET_c) of the week before application, as previously described in this Chapter. After the last irrigation event, all drip irrigation lines were removed in order to facilitate the maize harvesting.

The phosphorus (Olsen method) and potassium (K) concentrations were analysed at the beginning of the experiment. Since contents were high for both nutrients, no P or K seeding fertilization was applied. The field was kept free of weeds, pests and diseases following local practices (e.g. herbicides, pesticides, etc.).

As in the NH₃ volatilization field experiment (Experiment 1), the grain and aboveground biomass yield were obtained with four replicates of 10 linear meters of maize plants that were harvested at physiological maturity on 20 September 2017. Afterwards, grain and

stem were separated by hand and total grain (at 14% moisture level) and the remaining aboveground biomass weighed.

5.2. NO, NO₂ and O₃ measurements

5.2.1. Description of automated NO_x and O₃ measuring system

Nitric oxide, NO₂ and O₃ were measured with a completely new automated mobile laboratory measurement system (Fig. 3.20). This automated system incorporated nine additional automatic chambers to the six used in Experiment 3. Therefore, the experimental setup schematically consisted of 16 dynamic flow chambers, a pump (155 W, 30 L/min, Patashnick Co, Inc.), a NO_x chemiluminescence detector (Model 42i, Thermo Environmental Instruments Inc., USA) with a detection limit of 0.1 ppbv NO (Fig. 1.10), and a UV spectrometer for O₃ (Model 42M, Environnement S.A., Poissy, France) (Fig. 3.20). Air was sucked from each dynamic chamber by a pump and was transported via Teflon tubing (length varying from 25 m to 75 m; inner diameter 6 mm) to a solenoid valve (Flo Control®, Germany), which was followed by a PTFE manifold. These different lengths of Teflon tubes affected the flows of each chamber, which ranged from 20-30 L min⁻¹. In order to calculate fluxes of NO and NO₂, each flow was taking into account. The flow controllers, manifold and pump were housed in a mobile laboratory situated on the field. The characteristics of the hydraulic system were similar to those explained previously in this Chapter (Section 4.4.1)

Fig. 3.20: Schematic illustration of chambers and the automated measurement of NO and NO₂ flux rates in the maize experiment (Section 1.5) with 15 plots and a reference chamber

The chambers were similar to those used in Experiment 3 (see Fig. 3.17). In this case an emitter was situated inside of the area covered by each chamber. These chambers were placed between 2 rows of maize and did not include plant material.

Fig. 3.21: View of an automatic chamber placed between two maize rows.

5.2.2. Automatic cycle sample

Preliminary tests showed gas concentrations took approximately 7 min to reach steady state values after chambers were closed. Hence, chambers were closed for 9 minutes, and only the values of the last 90 seconds were used for flux determinations. The reference chamber was closed for 3 minutes within the period between the opening of a sampling chamber and the closure of the next one. A full cycle took 3 h to complete, resulting in eight measurements per day and per chamber.

5.2.3. Determination of NO, NO₂ and O₃ concentration

Nitric oxide and NO₂ were determined by chemiluminescence using a NO_x detector. The detectors characteristics and calibration method are given in this Chapter (section 4.4.4).

5.2.4. Calculation of fluxes of NO and NO₂ when steady state was attained.

Calculation of fluxes was performed as proposed in this Chapter (section 4.4.5). The equation used to calculate the flux was:

$$F = (C_{ch} - C_o) \cdot \frac{M \cdot Q \cdot 10^6}{V_m \cdot A \cdot 10^9} \cdot 60 \quad \text{Eq. 3.8}$$

where:

F = flux in $\mu g - N \cdot m^{-2} \cdot h^{-1}$;

M is the atomic weight of the element ($N = 14.008 g \cdot mol^{-1}$);

V_m is the standard gaseous molar volume ($22.4 \cdot 10^{-3} m^3 \cdot mol^{-1}$);

Q is the flow with the pump (variable dependent on each chamber and length of Teflon tubing),

C_{ch} is the mixing ratio (ppbv = $10^{-9} m^3 m^{-3}$) of the gas when the lid of chamber was closed and the chamber has reached the steady state;

C_o is the mixing ratio of the gas in the reference chamber;

A is the soil surface area covered by the chamber ($0.5 \times 0.5 m = 0.25 m^2$).

Since each chamber maintained a different flow during the course of the experiment, this equation (Eq. 3.8) was adapted for each chamber by modifying the Q value.

Cumulative gas emissions during the experimental period were calculated by multiplying the average flux of two successive determinations by the length of the period (3 h) between sampling and adding that amount to the previous cumulative total.

5.2.5. Data analysis

Each 30 second concentration of NO, NO₂ and O₃ was recorded in the PC. As in the Experiment 3, the amount of generated data (>350,000) meant a new challenge in terms of data analysis. Furthermore, the incorporation of nine more chambers (compared to Experiment 3) represented an added difficulty. The specific software created for Experiment 3 (Chapter 4) was modified to assess the data logged from each chamber.

This specific model was created using the programming language R version 3.2.1 (R Development Core Team, 2017). The script was able to remove data points before the steady state, to calculate the average NO/NO₂ of the last 90 s (when chambers were closed) to obtain the value of the steady state, to discard erroneous data for each plot chambers, to obtain the ambient value of the reference chamber, to calculate the absolute value of NO/NO₂ in each chamber and to determine NO/NO₂ emission fluxes.

5.2.6. Measurements of N₂O emissions

In Experiment 3, manual chambers (15) were installed to measure N₂O emissions. Due to the crop was maize, the chambers were located between two rows of maize. An emitter of the drip irrigation system was also placed under each manual chamber to simulate the moisture conditions of the rest of the plot and the automated chambers. Chambers characteristics and a description of the sampling and measuring method is given in section 2.3 (Chapter 3). A total of 15 gas sampling were performed after fertilization (Table 3.8).

Table 3.8: Principal agricultural management events of the experiment and N₂O and soil sampling dates of Experiment 4.

Planting date	18/04/2017
First irrigation	20/04/2017
Fertilization date	29/06/2017
Last irrigation	31/08/2017
Harvest date	20/09/2017
Sampling dates (DAF*)	
N ₂ O	1, 2, 4, 6, 8, 11, 13, 16, 18, 22, 27, 34, 41, 54, 89
Soil	1, 2, 4, 6, 11, 16, 18, 27, 34, 41, 54, 58, 89

*DAF= days after fertilization

Three previous samplings were taken between the beginning of May and the fertilization event to obtain a baseline data of N₂O emissions and soil properties. Gas sampling frequency was as Experiments 1, 2 and 3, i.e. 2-3 times per week after fertilization application and irrigation events because these periods are considered the most critical for N₂O emissions.

5.2.7. Measurements of soil moisture, N-NH₄⁺ and N-NO₃⁻ contents

Soil sampling and determination of soil moisture, N-NH₄⁺ and N-NO₃⁻ contents were performed as described in this chapter (section 4.6.). The soil sampling dates are shown in Table 3.8. Plant samples were obtained at harvest date. Yield-scaled N₂O emissions (YSNE), N₂O emission factor (EF -N₂O), yield-scaled NO_x emissions (Y-S NO_x), and nitrogen use efficiency (NUE) were also calculated as described in section 5.3 of this chapter. Total protein content was calculated using a conversion factor of 6.25 by N content (FAO, 2004).

6. Calculations

In addition to the estimation of cumulative gas emissions, described in the sections above, other parameters of interest that were also calculated.

- i) The yield-scaled N₂O emissions (YSNE) and yield scaled NO_x emissions (Y-S NO_x) were expressed as the ratio between the emitted N, as N₂O or NO_x (NO+NO₂), and aboveground N uptake.
- ii) The aboveground N uptake was calculated multiplying biomass by N content in each plant fraction.
- iii) The N surplus was calculated as applied N minus aboveground N uptake (Van Groenigen et al., 2010).

- iv) Nitrogen use efficiency (NUE) was calculated for the fertilized treatment as the ratio of applied N fertilizer that was taken up in aboveground biomass of the plant (Abalos et al., 2014a).
- v) The emission factor (EF) was obtained as the difference between N₂O emissions from a fertilized treatment and the non-fertilized treatment (control) divided by applied N fertilizer (Cayuela et al., 2017)

7. Statistical analysis

Each of the four Experiments described in this thesis has different features (number of treatments, number of replicates, number of samplings, etc.) which required the application of different statistical analyse.

7.1.1. Experiments 1 and 2

For daily N₂O fluxes and soil NO₃⁻ and NH₄⁺ content, eight measurements per fertilizer treatment (four per plot, corresponding to two samples per selected areas within plots) and four per control treatment for each sampling date were used. For daily NH₃ flux, two measurements were made per treatment. The analysis of data was performed using the StatgraphicsPlus 5.1 software (Statgraphics Technologies, Inc, USA).

Analyses of variance (ANOVA) or the Student's t-test (for NH₃ fluxes) were performed for all variables (except for climatic ones). Previously, the assumption of independence between treatments, normal distribution of samples and equal variances were checked by calculation of covariance (equal to zero), Q-Q plot and Bartlett-test, respectively (P>0.05 in all cases). No transformation was necessary to assure the normality of samples. For all variables, except for NH₃ emissions, the least significant difference (LSD) test was used for multiple comparisons between means. Although the IHF method is often used as reference method for its advantages of non-destructive sampling and good

representativeness of environmental conditions (Denmead, 2008), most of the studies using the IHF method to measure NH_3 volatilization have been carried out with one or two replications (Ni et al., 2015; Sun et al., 2016; Bai et al., 2017). This is a statistical limitation when comparing different treatments.

7.1.2. Experiments 3 and 4

The analysis of data was performed by using the StatGraphics Centurion XVI software (StatPoint Technologies Inc, Warrenton, VA, USA). Analyses of variance (ANOVA) were carried out for grain and biomass yield, N-ratio in grain, cumulative values of N_2O , average values of N-NH_4^+ and N-NO_3^- in soil, SDSS test and YSNE, using treatment and replicate as factors. Student's t-tests were performed for cumulative flux of NO and NO_2 emissions, Y-S NO_x , and EF of the U and U+NI treatments using the same factors. The normality and variance uniformity of the data distribution were previously assessed by Q-Q plot and Levene's statistic, respectively. Averages were separated by t-test and LSD test at $P < 0.05$. A search was also made for correlations between hourly NO values, hourly soil temperature and WFPS values.

A repeated measures ANOVA (RM-ANOVA) was performed using the R software program, version 3.2.1 (R Development Core Team, 2017), to analyse the temporal evolution of fluxes of N_2O and soil content of N-NO_3^- and N-NH_4^+ . These RM-ANOVA were performed using sampling days and treatments as factors, as well as the interaction between these two factors. In the case of NO and NO_2 fluxes, the RM-ANOVA was calculated for weekly average NO (and NO_2) fluxes, using treatment, week and their interaction as factors ($P < 0.05$). In addition, a principal components analysis (PCA) was done to assess correlations between fluxes of N_2O , NO and NO_2 , N-NH_4^+ and N-NO_3^- content in soil, soil temperature and WFPS. The data available to perform the PCA corresponded to 6 different sampling days (0-38 DAF) for the fertilized treatments only.

Pearson correlations were studied for these same variables and same sampling days. A Pearson correlation matrix was also constructed for all treatments (U; U+NI and Control) for the variables N_2O , N-NH_4^+ , N-NO_3^- , soil temperature and WFPS, and another for yield and quality parameters: grain and aboveground yield, ratio of N and protein in grain, grain N uptake, NUE and cumulative N_2O .

CHAPTER 4

**AN EFFICIENT SOFTWARE
BASED ON R-CODE TO OBTAIN
NO_x AND O₃ FLUXES FROM
AUTOMATIC OPEN CHAMBERS**

CHAPTER 4:

This chapter includes a briefly description of the R code necessary to measure NO_x and O₃ using automated chambers, as a result of this thesis. Therefore, it has been included before Results and particular discussion chapter (Chapter 5), instead of in Chapter 3 (Materials and Methods). This code was used for the data analysis of Experiments 3 and 4.

This software has been submitted to and admitted in the General Intellectual Property Registry of the Community of Madrid (Recio 2019, number of record entry 16/2019/1001)

1. Database obtained through NO_x measurements device

As described in sections 4.4. and 5.2. of the Chapter 3 the NO_x measurements were acquired with an automated system designed specifically for the Experiments 3 and 4 of this thesis. All the system was controlled with the software SIMDAS (DNOTA®) which obtained and stored a database.

This database was formed by:

- A file folder per chamber
- A comma separated values file (.csv) per day, stored in the folder of each chamber.
- In each .csv file, two measurements (NO_x molar fraction) per minute per chamber were saved during all the days that the system was working out.

It should be reminded that the desired value within all these data was the value of the steady state (the value when concentration of gas (NO, NO₂ or O₃) maintains constant along time). Preliminary tests were performed to find the time when the steady state was reached. For the Experiments 3 and 4, this time was 6 and 7 minutes respectively.

In the Experiment 3, data of NO_x and O₃ molar fraction (concentrations) were logged during 8 minutes for each chamber, and the reference chamber (ambient chamber) during 2 minutes. Therefore, the period of measurements were 10 minutes per chamber (2 minutes for reference chamber + 8 minutes), being the cycle time for all the chambers equal to 1 hour (6 chambers × 10 minutes). Since one value of each cycle was taken for the flux calculation (corresponding to the steady-state value of each chamber minus the average value of the reference chamber), in total, 24 net molar fraction values were taken per day.

On the other hand, in the Experiment 4, the number of chambers was increased to 16 chambers (15 chambers + 1 reference chamber). In this case, the cycle time was 3 hours for all the chambers, as it takes 12 minutes for calculating the steady state of each chamber (9 minutes per chamber and 3 minutes per reference chamber)

Hence, the programming script developed by the author of this thesis using the programme R version 3.2.1 (R Development Core Team 2017) is able:

1. To discard the values previous to the steady-state
2. To obtain the steady-state value per chamber and per cycle.
3. To find out missing values, both ambient and plot chambers.
4. To relate the mean value of the last 2 minutes of the plot chambers, with the mean value of the reference chamber measured before each chamber.
5. To calculate the net value of concentrations, subtracting the value of the chamber to the average of the reference chamber (ambient).
6. To calculate the flux for each concentration ($\mu\text{g m}^{-2} \text{ h}^{-1}$) applying the conversion factor represented in Eq. 3.3 and 3.8.
7. To store all the values corresponding to each treatment in one matrix, calculating the average and standard deviation values and cumulative values.

2. Scripts of the programme.

2.1. Checking out the reach of the steady-state

Firstly, it was necessary to check if the steady-state was reached for all the cycles. This was checked for all the chambers and during all the days of the experiment. A plot was created per chamber to show the values of NO_x molar fractions for every hour, the minutes throughout the chamber was measuring. The Fig. 4.1 represents two examples of one day of experiment 3 (a) and 4 (b), respectively. Despite the steady state could be visually identified, the assumption taken to determine when the steady state was reached, was a variation of the concentrations of ± 1 ppb.

Fig. 4.1: a) Example of all the values logged for chamber 5 (urea) of experiments 3, 9 DAF; b) Example of all the values logged for chamber 1 (control) of experiments 4, 21 DAF.

The scripts of this part of the software appear in the file **1_CHK_SteadyState_HourMin.R** included in Recio (2019, number of record entry 16/2019/1001).

2.2. Checking out the definitive value of each chamber, each measurement period, each day.

To prove the efficiency of the script, it was necessary to check if the value of the steady state obtained via plots was the mean value of the last minutes in which the chamber was measuring.

In Fig. 4.2, the values measured in any chamber during any day are represented in green. Besides, red lines indicate the value selected as steady state by the script. In this figure is represented the same than the Fig. 4.1 once the code has selected the value of the steady state.

Fig. 4.2: **a)** Example of how the R code calculates the value of the steady state of the chamber 5 (urea), of Experiment 3, 9 DAF; **b)** Example of the steady-state calculation of chamber 1 (control) of Experiment 4, 21 DAF.

The scripts of this part of the software are in the file **2_CHK_SteadyState_HourDay.R** included in Recio (2019, number of record entry 16/2019/1001).

2.3. Obtaining the definitive database of each plot chamber.

Once the value of the steady-state of the plot chambers was selected and checked, a new matrix (database) was created with the steady-state values of every chamber (one per cycle) each day (Fig. 4.3 and 4.4) .

For Experiment 3, this matrix had 1957 rows corresponding to 83 days and 24 data per day, taking into account missing values.

For Experiment 4, this matrix had 439 rows corresponding to 59 days and 8 data per day, taking into account missing values.

The scripts of this part of the software are in the file **3_ConstruirMatrizPorCadaCaja.R** included in the Recio (2019, number of record entry 16/2019/1001).

Time when the last value were measured

Number of the chamber

Values of the steady-state of concentration

Date and time parameters

Chamber 04 Definitive concentrations values matrix

Fecha	Foco	O3	NO	NOx	NO2	YMONDOY	MONDOY	DOY	DOM	HourMinu	HOD	Minute
9001	2016-04-25 00:40:00	Caja_04	1.30	2	0	0	20160425	425	116	25	40	0
9017	2016-04-25 01:40:00	Caja_04	0.83	3	1	0	20160425	425	116	25	140	1
9033	2016-04-25 02:40:00	Caja_04	0.68	4	2	0	20160425	425	116	25	240	2
9049	2016-04-25 03:40:00	Caja_04	2.70	4	3	0	20160425	425	116	25	340	3
9065	2016-04-25 04:40:00	Caja_04	2.64	3	2	0	20160425	425	116	25	440	4
9081	2016-04-25 05:40:00	Caja_04	1.81	3	1	0	20160425	425	116	25	540	5
9097	2016-04-25 06:40:00	Caja_04	1.84	12	15	2	20160425	425	116	25	640	6
9113	2016-04-25 07:40:00	Caja_04	0.32	15	20	4	20160425	425	116	25	740	7
9129	2016-04-25 08:40:00	Caja_04	0.70	7	8	1	20160425	425	116	25	840	8
9145	2016-04-25 09:40:00	Caja_04	0.35	10	15	4	20160425	425	116	25	940	9
9161	2016-04-25 10:40:00	Caja_04	2.54	9	16	6	20160425	425	116	25	1040	10
9177	2016-04-25 11:40:00	Caja_04	4.31	5	7	2	20160425	425	116	25	1140	11
9193	2016-04-25 12:40:00	Caja_04	8.09	4	4	0	20160425	425	116	25	1240	12
9209	2016-04-25 13:40:00	Caja_04	3.77	4	3	0	20160425	425	116	25	1340	13
9225	2016-04-25 14:40:00	Caja_04	2.23	4	5	1	20160425	425	116	25	1440	14
9241	2016-04-25 15:40:00	Caja_04	3.41	5	7	2	20160425	425	116	25	1540	15
9257	2016-04-25 16:40:00	Caja_04	3.51	4	7	2	20160425	425	116	25	1640	16
9273	2016-04-25 17:40:00	Caja_04	2.45	4	7	2	20160425	425	116	25	1740	17
9289	2016-04-25 18:40:00	Caja_04	3.02	5	8	3	20160425	425	116	25	1840	18
9305	2016-04-25 19:40:00	Caja_04	5.09	4	7	2	20160425	425	116	25	1940	19
9321	2016-04-25 20:40:00	Caja_04	3.62	3	4	0	20160425	425	116	25	2040	20
9337	2016-04-25 21:40:00	Caja_04	2.52	4	2	0	20160425	425	116	25	2140	21
9353	2016-04-25 22:40:00	Caja_04	1.87	5	5	0	20160425	425	116	25	2240	22
9369	2016-04-25 23:40:00	Caja_04	0.25	5	7	1	20160425	425	116	25	2340	23

Chamber 04_Dimensions= 1957 rows × 13 columns

Fig. 4.3: Snip of chamber 4 matrix of experiment 3 (U+NI). Values inside of red square are the steady-state values of this specific chamber.

Chamber 10_ Definitive concentrations values matrix

	Fecha	Foco	O3	NO	NO2	NOx	MONDOY	MONDOY	DOY	HourMinu	HOD	Minute
	236	2017-07-02 01:59:30	Caja_10	0	21	5	26	20170702	702	183	159	1 59
	255	2017-07-02 04:59:30	Caja_10	0	11	6	17	20170702	702	183	459	4 59
	273	2017-07-02 07:59:30	Caja_10	0	19	7	26	20170702	702	183	759	7 59
	290	2017-07-02 10:59:30	Caja_10	0	12	8	20	20170702	702	183	1059	10 59
	308	2017-07-02 13:59:30	Caja_10	0	30	19	49	20170702	702	183	1359	13 59
	326	2017-07-02 16:59:30	Caja_10	0	14	7	21	20170702	702	183	1659	16 59
	344	2017-07-02 19:59:30	Caja_10	0	9	5	14	20170702	702	183	1959	19 59
	362	2017-07-02 22:59:30	Caja_10	0	9	9	18	20170702	702	183	2259	22 59
	381	2017-07-03 01:59:30	Caja_10	0	11	8	19	20170703	703	184	159	1 59
	400	2017-07-03 04:59:30	Caja_10	0	10	5	15	20170703	703	184	459	4 59
	418	2017-07-03 07:59:30	Caja_10	0	58	12	70	20170703	703	184	759	7 59
	436	2017-07-03 10:59:30	Caja_10	0	18	14	32	20170703	703	184	1059	10 59
	454	2017-07-03 13:59:30	Caja_10	0	51	50	101	20170703	703	184	1359	13 59
	472	2017-07-03 16:59:30	Caja_10	0	26	26	52	20170703	703	184	1659	16 59
	490	2017-07-03 19:59:30	Caja_10	0	16	14	30	20170703	703	184	1959	19 59
	508	2017-07-03 22:59:30	Caja_10	0	13	12	25	20170703	703	184	2259	22 59
	526	2017-07-04 01:59:30	Caja_10	0	10	8	18	20170704	704	185	159	1 59
	544	2017-07-04 04:59:30	Caja_10	0	7	6	13	20170704	704	185	459	4 59
	561	2017-07-04 07:59:30	Caja_10	0	17	16	33	20170704	704	185	759	7 59
	580	2017-07-04 10:59:30	Caja_10	0	12	13	24	20170704	704	185	1059	10 59
	598	2017-07-04 13:59:30	Caja_10	0	18	20	38	20170704	704	185	1359	13 59
	616	2017-07-04 16:59:30	Caja_10	0	13	14	27	20170704	704	185	1659	16 59
	634	2017-07-04 19:59:30	Caja_10	0	9	9	18	20170704	704	185	1959	19 59
	652	2017-07-04 22:59:30	Caja_10	0	6	7	14	20170704	704	185	2259	22 59

Chamber 10_ Dimensions= 439 rows × 12 columns

Fig. 4.4: Snip of chamber 10 matrix of experiment 4 (U+NI). Values inside of red square are the steady-state values of this specific chamber.

2.4. Obtaining the definitive database of the reference chamber (ambient)

In a similar way to the previous section, a matrix with the mean values taken by the reference chamber was constructed. At the end of experiments, the database of the ambient concentrations consisted of 46967 and 40462 values for experiment 3 and 4, respectively.

For Experiment 3 (Fig. 4.5.a), the matrix created had 11734 rows, as a result of 6 values per cycle (1 value each 10 minutes) × 24 cycles per day × 83 days of experiment, taking into account missed values.

For Experiment 4 (Fig. 4.5.b), the result was a matrix of 7932 rows, 15 values per cycle (1 value each 12 minutes) × 8 cycles per day × 59 days of measurements.

The code related to this part is included in Recio (2019, number of record entry 16/2019/1001): **4_ArchivoGeneral_Amb.R**

a) Experiment 3's ambient chamber

Fecha	Foco	O3	NO	NOx	NO2
2016-05-04 00:02:00	Ambiente	1.90	8	12	3
2016-05-04 00:12:00	Ambiente	1.92	11	15	3
2016-05-04 00:22:00	Ambiente	3.45	8	11	3
2016-05-04 00:32:00	Ambiente	0.83	19	30	10
2016-05-04 00:42:00	Ambiente	2.45	15	16	1
2016-05-04 00:52:00	Ambiente	0.69	15	25	10
2016-05-04 01:02:00	Ambiente	2.89	14	23	8
2016-05-04 01:12:00	Ambiente	0.37	16	25	8
2016-05-04 01:22:00	Ambiente	0.60	15	25	10
2016-05-04 01:32:00	Ambiente	0.73	37	51	13
2016-05-04 01:42:00	Ambiente	0.18	41	54	12
2016-05-04 01:52:00	Ambiente	0.29	44	56	12
2016-05-04 02:02:00	Ambiente	0.45	38	50	12
2016-05-04 02:12:00	Ambiente	0.09	34	46	11
2016-05-04 02:22:00	Ambiente	0.41	30	43	12
2016-05-04 02:32:00	Ambiente	0.19	26	37	10
2016-05-04 02:42:00	Ambiente	0.69	22	27	5
2016-05-04 02:52:00	Ambiente	0.55	22	29	7
2016-05-04 03:02:00	Ambiente	0.56	23	33	9
2016-05-04 03:12:00	Ambiente	0.33	25	34	9
2016-05-04 03:22:00	Ambiente	0.35	24	35	11
2016-05-04 03:32:00	Ambiente	1.52	22	32	10
2016-05-04 03:42:00	Ambiente	0.42	18	27	9
2016-05-04 03:52:00	Ambiente	0.00	21	30	8

b) Experiment 4's ambient chamber

Fecha	Foco	O3	NO	NO2	NOx
2017-08-03 00:15:00	Ambiente	0	2	7	9
2017-08-03 00:27:00	Ambiente	0	4	9	13
2017-08-03 00:39:00	Ambiente	0	2	8	10
2017-08-03 00:50:30	Ambiente	0	2	7	9
2017-08-03 00:51:00	Ambiente	0	2	15	17
2017-08-03 01:03:00	Ambiente	0	3	14	17
2017-08-03 01:15:00	Ambiente	0	3	6	9
2017-08-03 01:27:00	Ambiente	0	3	5	8
2017-08-03 01:39:00	Ambiente	0	2	6	8
2017-08-03 01:50:30	Ambiente	0	2	5	7
2017-08-03 02:03:00	Ambiente	0	2	5	7
2017-08-03 02:15:00	Ambiente	0	2	5	7
2017-08-03 02:27:00	Ambiente	0	2	6	8
2017-08-03 02:39:00	Ambiente	0	2	5	7
2017-08-03 02:50:30	Ambiente	0	2	5	7
2017-08-03 02:51:00	Ambiente	0	2	6	8
2017-08-03 03:03:00	Ambiente	0	2	5	7
2017-08-03 03:15:00	Ambiente	0	2	4	6
2017-08-03 03:27:00	Ambiente	0	3	5	8
2017-08-03 03:39:00	Ambiente	0	2	4	6
2017-08-03 03:50:30	Ambiente	0	3	5	8
2017-08-03 03:51:00	Ambiente	0	3	10	13

Fig. 4.5: Snips of part of the database of reference chamber for: a) Experiment 3 and b) Experiment 4.

2.5. Function to find missed values between the reference and the plot chambers.

An auxiliary function was programmed to identify the number of missed values each day, comparing the number of values of any chamber with the number of values of the reference chamber associated with that chamber. Therefore, this function creates a matrix whose first and third column is the date (one day per row); the second column is the number of values of the plot chamber each day; and the fourth one is the number of values of the reference chamber each day. Thus, the user could find out which values had been missed and if these missed values were from the ambient or the plot chambers.

The programming of this function is shown below.

```
FindMissValues <- function (box,amb) {  
  
  SrchBox <- unique(box[['YMONDOY']]); length(SrchBox)  
  
  SrchAmb <- unique(amb[['YMONDOY']]); length(SrchAmb)  
  
  DatosDiaCaja <- matrix(data=NA, nrow=length(SrchBox),ncol = 2);  
  
  DatosDiaAmb <- matrix(data=NA, nrow=length(SrchAmb),ncol = 2);  
  
  for (q in 1:length(SrchBox)) {  
  
    ay1 <- which(box[['YMONDOY']]==SrchBox[q]) #ay=ayudante  
  
    ay2 <- which(amb[['YMONDOY']]==SrchAmb[q])  
  
    DatosDiaCaja[q,1] <- SrchBox[q]  
  
    DatosDiaCaja[q,2] <- length(ay1)  
  
    DatosDiaAmb[q,1] <- SrchBox[q]  
  
    DatosDiaAmb[q,2] <- length(ay2) }  
  
    Comp1 <- cbind(DatosDiaCaja,DatosDiaAmb);  
  
    Comp1 <- cbind(Comp1,DatosDiaCaja[,2]==DatosDiaAmb[,2])  
  
    print(Comp1)  
  
  }  
}
```

2.6. Calculating the absolute values

Firstly, the code read the ambient data and identified each value to the corresponding chamber. This is done considering that, the ambient data is measured at a fixed interval

within each cycle just before each chamber is measured. (Fig. 4.6). For example, the ambient values matrix for chamber 2 in Experiment 3 would be all the values measured between minutes 10 and 12 of all the hours of the day. In the case of Experiment 4, the ambient values matrix for chamber 2 would be all the values within the ambient database measured between minutes 12 and 15 each 3 hours, i.e., in the hours 0,3,6,9,12,15,18 and 21.

a) Experiment 3

b) Experiment 4

Fig. 4.6: Schedule of experiment 3 (a) and 4 (b). Grey squares are the period of time per cycle when the reference chamber was measured. Colour squares are the period per cycle when the plot chambers were measured (closures).

Once the different ambient values for each chamber are obtained, the function explained aforementioned in section 2.5 of this Chapter, allowed to identify the missed values for each chamber during all the experiments (Table 4.1).

Table 4.1: Example of the auxiliary function to find the missed values per day.

Day	Values for the plot	Day	Values for the ambient	
20170629	3	20170629	2	0
20170630	6	20170630	3	0
.	.	.	.	.
.	.	.	.	.
20170903	8	20170903	8	1
20170904	3	20170904	4	0

3 missed value of ambient

1 missed value of plot

Secondly, the scripts achieved to equalise the dimensions of the ambient matrix and its corresponding chamber matrix, inserting (mean, minimum, median, etc.) or deleting values.

Finally, the code calculated the net NO_x molar fraction values, subtracting the value of a specific chamber to the mean value of the reference chamber (ambient) measured, for all the chambers during the experiment (Fig. 4.7).

All this code is presented in the file **5_ObtenerValoresABS.R** in the Recio (2019, number of record entry 16/2019/1001).

Fecha	Foco	NO	NO2	NOx
2017-06-29 21:12:00	Caja_01	7	6	13
2017-06-30 00:12:00	Caja_01	6	5	10
2017-06-30 03:12:00	Caja_01	5	5	11
2017-06-30 06:12:00	Caja_01	6	5	11
2017-06-30 09:12:00	Caja_01	10	9	19
2017-06-30 12:12:00	Caja_01	5	5	10
2017-06-30 15:12:00	Caja_01	6	6	12

Fecha	Foco	NO	NO2	NOx
2017-06-29 21:03:00	Ambiente	5	7	12
2017-06-30 00:03:00	Ambiente	3	5	8
2017-06-30 03:03:00	Ambiente	2	5	7
2017-06-30 06:03:00	Ambiente	5	6	11
2017-06-30 09:03:00	Ambiente	8	10	18
2017-06-30 12:03:00	Ambiente	4	5	9
2017-06-30 15:03:00	Ambiente	5	6	11

Fecha	Foco	NO_ABS	NO2_ABS	NOx_ABS
2017-06-29 21:12:00	Caja_01	2	-1	1
2017-06-30 00:12:00	Caja_01	3	0	2
2017-06-30 03:12:00	Caja_01	3	0	4
2017-06-30 06:12:00	Caja_01	1	-1	0
2017-06-30 09:12:00	Caja_01	2	-1	1
2017-06-30 12:12:00	Caja_01	1	0	1
2017-06-30 15:12:00	Caja_01	1	0	1

Fig. 4.7: Example of how the script calculates the absolute values of NO_x.

#chamber 1 for the experiment 4

```
AMB_chamber1.net <- subset(chambAMB_Gnal, subset= (chambAMB_Gnal$HOD
%in% seq(0,21,3) & chambAMB_Gnal$HourMinu %% 100==3
),select=names(chambAMB_Gnal))
```

```

nrow(AMB_chamber1.net)

nrow(chamb1_Gnal)

FindMissValues(chamb1_Gnal,AMB_chamber1.net) # Auxiliary function

ayx2 <- which(AMB_chamber1.net[['YMONDOY']]==20170629 &
AMB_chamber1.net[['HOD']]!=21)

AMB_chamber1.net <- AMB_chamber1.net[-ayx2,]

Find.min <- which(AMB_chamber1.net$NOx==min(AMB_chamber1.net$NOx));

Find.min

rdiff<- nrow(chamb1_Gnal)-nrow(AMB_chamber1.net); rdiff

r.extra <- AMB_chamber1.net[Find.min,]; r.extra

AMB_chamber1.net[diff(AMB_chamber1.net$Fecha)==6,]

VectLost <- which(diff(AMB_chamber1.net$Fecha)==6)

#este vector me da la posicion de los miss values

# AMB_chamber1 <- AMB_chamber1.net

w=0

for (v in 1:length(VectLost)) {

  AMB_chamber1.net <- AMB_chamber1.net [c(1:(VectLost[v]+w), (VectLost[v]+w),
  (VectLost[v]+w+1):nrow(AMB_chamber1.net)) ,]

  w=w+1

}

AMB_chamber1 <- AMB_chamber1.net

```

```
rm(AMB_chamber1.net)
```

```
chamb1_Gnal$NO_ABS <- (chamb1_Gnal$NO-AMB_chamber1$NO)
```

```
chamb1_Gnal$NO2_ABS <- (chamb1_Gnal$NO2-AMB_chamber1$NO2)
```

```
chamb1_Gnal$NOx_ABS <- (chamb1_Gnal$NOx-AMB_chamber1$NOx)
```

```
head(chamb1_Gnal)
```

2.7. Calculation of flux NOx emissions [$\mu\text{g m}^{-2} \text{h}^{-1}$] from molar fractions [ppb].

According to Eq. 3.3 and 3.8, the change from ppb to fluxes needs the molecular weights, the air flow inside the chamber, the surface covered by the chamber, etc. New columns were added to each chamber matrix (Fig. 4.8). Furthermore, cumulative values were calculated and lastly, fluxes were saved in a matrix per treatment formed by the fluxes of the three replicates, the average, the standard deviation, the corrected factor of the fluxes (only for the Experiment 3; Eq. 3.3), etc. Another similar matrix was constructed with cumulative values. Finally, the daily, weekly or monthly mean values were calculated (Table 4.2).

Fecha	Foco	YMONDOY	HourMinu	fluxNOx	Flux.NO	Flux.NO2	NOxCum	NOxCum	NO2Cum	PeriodDay	SunHours
2017-06-29 11:03:30	Caja_11	20170629	1103	0.00	0.00	0.00	0.00000	0.00000	0.0000	morning	light
2017-06-29 17:12:00	Caja_11	20170629	1712	17.10	31.35	48.45	52.51125	96.27063	148.7819	afternoon	light
2017-06-29 20:12:00	Caja_11	20170629	2012	-11.40	5.70	34.20	61.06125	151.84563	272.7569	evening	dark
2017-06-29 23:12:00	Caja_11	20170629	2312	-17.10	5.70	17.10	18.31125	168.94563	349.7069	evening	dark
2017-06-30 02:12:00	Caja_11	20170630	212	17.10	17.10	0.00	18.31125	203.14562	375.3569	night	dark
2017-06-30 05:12:00	Caja_11	20170630	512	11.40	8.55	0.00	61.06125	241.62062	375.3569	night	dark
2017-06-30 08:12:00	Caja_11	20170630	812	48.45	31.35	14.25	150.83625	301.47062	396.7319	morning	light
2017-06-30 11:12:00	Caja_11	20170630	1112	11.40	2.85	11.40	240.61125	352.77062	435.2069	morning	light
2017-06-30 14:11:30	Caja_11	20170630	1411	5.70	2.85	2.85	266.19000	361.29688	456.5225	afternoon	light

Number of the chamber

Time parameters

Fluxes calculated

Cumulative values calculated

Fig. 4.8: Different columns of a chamber matrix with the fluxes calculated

The code below is an example of the R code developed by Recio (2019, number of record entry 16/2019/1001).

$$\text{FluxNOx11} = k1 * \text{Qaudal}[11] * \text{chamb11_Gnal\$NOx_ABS}; \text{chamb11_Gnal\$FluxNOx} \\ = \text{FluxNOx11}$$

$$\text{FluxNO11} = k1 * \text{Qaudal}[11] * \text{chamb11_Gnal\$NO_ABS}; \text{chamb11_Gnal\$Flux.NO} \\ = \text{FluxNO11}$$

$$\text{FluxNO211} = k1 * \text{Qaudal}[11] * \text{chamb11_Gnal\$NO2_ABS}; \text{chamb11_Gnal\$Flux.NO2} = \\ \text{FluxNO211}$$

Table 4.2: A treatment matrix after calculating absolute values of concentrations and fluxes. Three replicates per treatment

		Replicate 1			Replicate 2			Replicate 3			Average and standard deviation of Urea					
Date	YMONDOY	FluxNOx_ch5	FluxNO_ch5	FluxNO2_ch5	FluxNOx_ch9	FluxNO_ch9	FluxNO2_ch9	FluxNOx_ch12	FluxNO_ch12	FluxNO2_ch12	Mean.Urea.NOx	SD.Urea.NOx	Mean.Urea.NO	SD.Urea.NO	Mean.Urea.NO2	SD.Urea.NO2
2017-08-02 17:59:30	20170802	22.800	9.690	11.970	9.600	6.000	3.000	3.400	2.280	0.570	11.940	24.704	5.990	4.592	5.180	7.912
2017-08-03 13:29:30	20170803	23.513	14.250	8.550	17.250	13.125	4.125	24.225	14.963	9.619	21.663	33.271	14.113	9.895	7.431	11.176
2017-08-04 13:29:30	20170804	13.181	7.481	6.094	6.750	5.250	1.875	-2.494	-3.206	0.712	5.812	24.455	3.175	8.287	2.881	8.163
2017-08-05 13:29:30	20170805	23.869	14.250	9.975	9.000	6.750	1.875	0.356	0.000	0.712	11.075	16.693	7.000	2.858	4.188	4.719
2017-08-06 13:29:30	20170806	31.350	14.963	15.675	10.875	7.125	3.750	-1.069	0.356	-1.069	13.719	19.026	7.481	4.807	6.119	8.941
2017-08-07 13:29:30	20170807	35.269	18.325	17.100	16.125	10.500	6.375	15.675	10.331	6.056	22.356	30.437	13.119	10.697	9.844	5.782
2017-08-08 13:29:30	20170808	23.869	7.838	15.319	4.875	3.375	1.875	-2.850	-4.275	2.138	8.631	17.085	2.312	4.814	6.444	3.596
2017-08-09 13:29:30	20170809	9.619	4.275	6.056	1.500	2.625	-0.750	4.275	1.425	2.850	5.131	8.966	2.775	2.714	2.719	6.311
2017-08-10 13:29:30	20170810	9.975	2.494	7.838	3.750	2.625	1.500	-2.138	-0.356	-2.494	3.962	7.940	1.507	1.150	2.281	3.480
2017-08-11 13:29:30	20170811	12.469	3.206	8.095	3.750	1.500	2.250	6.056	1.425	4.275	7.425	6.328	2.544	0.796	5.144	3.454
2017-08-12 13:29:30	20170812	16.387	4.631	12.113	2.250	2.250	0.375	5.563	1.781	2.138	7.460	12.239	2.888	1.782	4.875	4.179
2017-08-13 13:29:30	20170813	13.894	4.631	6.262	-0.375	1.875	-2.250	7.125	3.562	3.562	6.881	14.247	3.356	1.019	2.525	3.780
2017-08-14 13:29:30	20170814	13.181	3.206	6.262	1.500	1.875	0.000	-1.425	1.069	-2.850	4.419	21.491	2.000	6.074	2.137	10.138

CHAPTER 5

RESULTS & DISCUSSION

CHAPTER 5:

1. Experiment 1 - Volatilization of NH₃ and N₂O emissions in an irrigated maize crop (site “La Chimenea”)

This section reports the results of this experiment, previously published in the journal Science of the Total Environment (ISSN: 0048-9697; JIF (2017) = 4.610).

1.1. Soil moisture and meteorological conditions

The mean soil temperature (0-10 cm) throughout the experiment was 25°C and ranged from 13.6 to 34.2°C (Fig. 5.1). The experimental period ran from the date of basal fertilization (beginning of Period I) to harvest day (end of Period II). During Period II, daily soil temperature was >30°C in the 4 weeks following fertilization.

The mean daily wind speed during the experiment was 0.91 m s⁻¹ with a speed variation between maximum and minimum of 2.37 m s⁻¹ (Fig. 5.2). Higher mean wind speeds were observed during Period II (1.46 m s⁻¹) compared to Period I (0.64 m s⁻¹). The predominant wind directions during the experimental period were NE and SW, as expected.

Total rainfall was 100.7 mm from April to October. The total amount of irrigated water was c. 900 mm, with irrigation starting on 28 May and ending on 27 August.

After basal fertilization, WFPS was >40% for one week (Fig. 5.3). It then decreased to c. 20% until the first irrigation event, one month after the first fertilization. However, WFPS was generally maintained above 60% during 40 days after the second fertilization through irrigation.

Fig. 5.1: Precipitation (mm), irrigation (mm) on the right-hand vertical axis; air and soil temperature ($^{\circ}\text{C}$) on the left-hand vertical axis during the experimental period. Solid arrows indicate the times of N fertilization and the sowing and harvest days. Vertical and horizontal segments indicate time period of NH_3 measurements.

Fig. 5.2: Mean wind speed (m s^{-1}) during the experimental period. Solid arrows indicate the times of N fertilization and the sowing and harvest days.

Fig. 5.3: Evolution of soil water-filled pore space (WFPS, %) on the left-hand vertical axis and precipitation (mm) and irrigation (mm) on the right-hand vertical axis during the experimental period. Dotted arrows indicate the times of N fertilization and solid arrows the sowing and harvest days.

1.2. Soil mineral N

Ammonium (NH_4^+) content of the topsoil (0-10 cm) increased both in the N fertilized treatments and the control, but markedly due to N fertilizer application as shown by comparing the soil content for the 2nd and the 5th week after PS application (data not shown). The mean value of NH_4^+ for Period I was 2.32 (± 0.46) and 7.03 (± 1.50) mg N kg^{-1} for PS-CAN and PS-CAN+NI, respectively (Fig. 5.4). Ammonium concentration dropped below 10 mg N kg^{-1} in PS-CAN within the first week after application, whereas as PS-CAN-NI was above 10 mg N kg^{-1} for three weeks (data not shown).

Fig. 5.4: a) N-NO_3^- ; b) N-NH_4^+ ; average concentrations in the 0–10 cm soil layer during the two experimental periods for the different treatments (PS-CAN, pig slurry applied in the basal fertilization and CAN applied in the top-dressing fertilization; PS-CAN+NI, pig slurry with DMPP applied in the basal fertilization and CAN with DMPSA applied in the top-dressing fertilization; C, Control without applied fertilizer). Different letters indicate significant differences within each period of year after applying the LSD test at $P < 0.05$. Vertical bars indicate standard errors of the ANOVA.

1.3. Ammonia fluxes

1.3.1. Ammonia fluxes following basal fertilization

Ammonia fluxes increased following application of PS (Fig. 5.5). In the case of PS without NI (PS-CAN treatment), fluxes of NH_3 ranged between 570 and $10 \text{ g N ha}^{-1} \text{ h}^{-1}$ on basal fertilization day (20 April) and 15 days later, respectively. The highest NH_3 volatilization was measured within the first four hours after application. A similar pattern was observed when PS was applied with DMPP. However, the emission of NH_3 peaked immediately after application, reaching a maximum flux of $677 \text{ g N ha}^{-1} \text{ h}^{-1}$. Throughout the 2 weeks following PS application, the minimum value was $4.6 \text{ g N ha}^{-1} \text{ h}^{-1}$.

Average cumulative NH_3 emissions for Period I (Table 5.1) was 7.3 kg N ha^{-1} in PS-CAN, representing c. 14% of applied N. In the case of pig slurry applied with NI (DMPP) the cumulative NH_3 value was 5.0 kg N ha^{-1} (10% of total N applied), although the difference between these two treatments was not significant.

*Table 5.1: Mean cumulative NH_3 fluxes for the different treatments (PS-CAN, pig slurry applied in the basal fertilization and CAN applied in the top-dressing fertilization; PS-CAN+NI, pig slurry with DMPP applied in the basal fertilization and CAN with DMPSA applied in the top-dressing fertilization). N.S. indicates non-significant differences applying a *t*-test at $P < 0.05$. Standard deviations are given in brackets.*

Treatment	Mean NH_3 Cumulative Emissions (kg-N ha^{-1})		
	Period I (22 DABF*)	Period II (22 DADF*)	TOTAL
PS-CAN	7.28 (1.73)	3.77 (1.35)	10.9 (2.91)
PS-CAN+NI	4.98 (0.54)	2.81 (0.32)	7.8 (0.86)
t-student	1.27 (P=0.21)	0.97 (P=0.43)	1.45 (P=0.28)
	N.S.	N.S.	N.S.
*DABF (Days after basal fertilization)			
*DADF (Days after dressing fertilization)			

A *t*-test was performed to compare the NH_3 emission rates of the PS-CAN and PS-CAN +NI treatments during the four first hours following fertilization. The peak of NH_3 in the PS-CAN+NI treated soil was numerically higher than that measured in PS-CAN, but the differences were not statistically different (Fig. 5.5).

Fig. 5.5: Period I. Daily ammonia fluxes the following three weeks after basal fertilization (50 kg N ha^{-1}) for the different fertilized treatments (PS-CAN, PS-CAN+NI). Vertical bars indicate standard errors.

1.3.2. Ammonia fluxes following top-dressing fertilization

Application of CAN immediately produced a detectable flux of NH_3 , with maximum values detected 24 h after fertilizer application in both the PS-CAN and PS-CAN+NI treatments ($44.3 \text{ g N ha}^{-1} \text{ h}^{-1}$ and $47.9 \text{ g N ha}^{-1} \text{ h}^{-1}$, respectively) (Fig. 5.6). During the 20 days following fertilization, volatilized NH_3 ranged from 0.7 to $44.3 \text{ g N ha}^{-1} \text{ h}^{-1}$ and from 0.64 to $47.9 \text{ g N ha}^{-1} \text{ h}^{-1}$ for the PS-CAN and PS-CAN+NI treatments, respectively (Fig. 5.6), without significant differences ($P < 0.05$). Mean cumulative emissions of NH_3 20

days after top-dressing fertilization were 2.8 and 3.8 kg N ha⁻¹ for the treatments with and without NI, respectively (Table 5.1). This difference was not significant at $P < 0.05$, representing 1.8% and 2.5% of total applied N, respectively.

When considering NH₃ volatilization in the entire cropping period (fertilization with PS and CAN), N losses through this pathway were 7.8 and 10.9 kg N ha⁻¹ for treatments with and without NI, respectively (Table 5.1).

The ratio of N losses and total N applied, considering the entire crop period, were 5.5% and 4% for PS-CAN and PS-CAN + NI treatment, respectively, a difference that was not significant.

Fig. 5.6: Period II. Daily ammonia fluxes the following three weeks after top-dressing fertilization (150 kg N ha⁻¹) for the different treatments (PS-CAN; PS-CAN+NI). Vertical bars represent the standard errors

1.4. Fluxes of nitrous oxide

As expected, N_2O fluxes were influenced by fertilizers inputs (Fig. 5.7). Daily mean fluxes of N_2O during Period I ranged from 0.008 to $0.56 \text{ g N ha}^{-1} \text{ h}^{-1}$, with the highest flux measured 53 days after PS fertilization for the PS-CAN treatment. Soil N_2O fluxes were lower in plots where DMPP was added. Fluxes remained lower than $0.12 \text{ g N ha}^{-1} \text{ h}^{-1}$ throughout experimental period for this treatment.

Fig. 5.7: Daily N_2O emissions during the entire experimental period for the different treatments (PS-CAN, Control, PS-CAN+NI). Dotted arrows indicate the times of N fertilization and the solid arrow the sowing date. Vertical bars indicate standard errors.

The application of CAN (top-dressing fertilization) rapidly increased N_2O fluxes, peaking 10 days after the fertilization event ($1.60 \text{ g N ha}^{-1} \text{ h}^{-1}$). The incorporation of the nitrification inhibitor DMPPA significantly ($P < 0.05$) reduced fluxes during a period of 2 weeks following application (see Fig. 5.7). The highest flux of N_2O for the PS-CAN+NI

treatment was $0.90 \text{ g N ha}^{-1} \text{ h}^{-1}$, with fluxes falling below $0.35 \text{ g N ha}^{-1} \text{ h}^{-1}$ 3 weeks after fertilization, despite frequent irrigation.

The use of DMPP and DMPSA significantly reduced cumulative N_2O emissions respectively from PS (basal fertilization) and CAN (dressing fertilization) for 20 and 22 days after application, respectively (Table 5.2). However, after 53 days no significant effect was observed for CAN application in the treatment without NI compared to the treatment with NI (0.36 vs. $0.32 \text{ kg N-N}_2\text{O ha}^{-1}$). When considering the entire PS-CAN experimental period, NI reduced mean N_2O emissions but this difference was not significant ($P > 0.05$). The control treatment emitted the lowest N_2O fluxes.

Table 5.2: Mean cumulative N_2O fluxes for the different treatments (PS-CAN, pig slurry applied in the basal fertilization and CAN applied in the top-dressing fertilization; PS-CAN+NI, pig slurry with DMPP applied in the basal fertilization and CAN with DMPSA applied in the top-dressing fertilization; C, Control without fertilizer). N.S. indicates no significant differences applying a t-test at $P < 0.05$ for the treatment with and without NI. Standard deviations are given in brackets.

	Mean N ₂ O Cumulative Emissions (kg-N ha ⁻¹)			Total
	Period I	Period II		
Treatment	20 DABF*	22 DADF**	53 DADF**	Harvest Day
PS-CAN	0.18 (0.05)	0.30 (0.11)	0.36 (0.22)	0.57 (0.37)
PS-CAN + NI	0.05 (0.03)	0.18 (0.07)	0.32 (0.18)	0.40 (0.17)
CONTROL	0.01 (0.005)	0.17 (0.11)	0.27 (0.14)	0.29 (0.14)
t-student	P<0.05	P<0.05	P>0.05 (N.S.)	P>0.05(N.S.)
*DABF: days after basal fertilization				
**DADF: days after top-dressing fertilization				

1.5. Discussion of Experiment 1

1.5.1. Effect of nitrification inhibitors on nitrous oxide emissions

This study showed that the use of DMPP could be considered an effective strategy to reduce direct N₂O emissions from PS (reduction of c. 70% of N₂O emissions) mainly because, as reported by Chadwick et al. (2011), under the study conditions, an important fraction of N₂O emitted after DMPP application was produced through nitrification. Our results also confirm those of Guardia et al. (2017b) who observed a 49% inhibitory effect of DMPP on N₂O emissions when 120 kg N ha⁻¹ of liquid PS fraction was applied to a maize crop. Additionally, DMPSA mitigated 40% of total N₂O fluxes within the first three weeks after top-dressing fertilizer application, with N₂O losses in DMPSA plots similar to those of the unfertilized treatment (Table 5.2). Guardia et al. (2017b) found a significant inhibitory effect of 57% when applying CAN and DMPSA (180 kg N ha⁻¹), also in a maize crop in a clay soil, suggesting that nitrification was the dominant N₂O production process at the peaking time. The nitrification inhibitory effect of DMPSA is not well known yet, but probably its effect is based on the presence of dimethylpyrazole (DMP), which is released through the degradation of succinic acid in soil. A technical advantage of DMPSA, in comparison with DMPP (also a precursor of DMP), is the possibility of its combination with any mineral N fertilizer such as CAN. Three weeks after NI application the effectivity of DMPSA was low, and consequently most of the NH₄⁺ was nitrified (NH₄⁺ <10 mg N kg⁻¹, similar to the treatment without NI). Due to the frequent irrigation events in this period (twice per week), moisture conditions were maintained above 60% WFPS most of the time and, consequently, a small amount of NO₃⁻ may have been lost through denitrification, thus producing similar N₂O emissions in all treatments, even in the Control plots. Therefore, the percentage of N₂O reduction using the new NI was lower (30%) and differences were not statistically significant. Menéndez

et al. (2012) described that the effectiveness of the NI DMPP in an incubation experiment was conditioned by the magnitude of the losses from the fertilizer without NI, being lower for low emission rates. This would have been the case in Experiment 1, where N_2O emissions from CAN were very low ($0.36 \text{ kg N ha}^{-1}$).

The higher concentration of NO_3^- in soil for Period I when NI was used could also be considered a beneficial effect of the DMPP. As plant N requirements are not high in this period, it is possible that higher NO_3^- losses could also be produced when NI is not used, contributing to indirect emissions of N_2O .

1.5.2. Effect of nitrification inhibitors on ammonia volatilization

Ammonia emissions were not increased by the use of NIs (DMPP or DMPSA), despite both inhibitors enlarging the pool of NH_4^+ in soil within the period of 6 to 30 days after fertilization (Fig. 5.4.b). Overall, compared with PS alone, DMPP reduced average NH_3 cumulative emissions by 2.3 kg N ha^{-1} , but this difference was not significant ($P>0.05$) and mainly occurred within the first 24 h after application. The rapid mechanical incorporation of pig slurry into the soil and the subsequent adsorption of NH_4^+ onto soil colloids reduced the risk of NH_3 volatilization in such a way that the presence and effect of NI on the pool of NH_4^+ , the main precursor of NH_3 , did not affect these gaseous losses. Additionally, the necessary mechanical agitation of PS to mix it with DMPP prior to application may have facilitated homogenization of the slurry composition in relation to NH_4^+ distribution over the soil, thus probably favouring a faster adsorption of NH_4^+ onto soil particles. Our results are consistent with the meta-analysis performed by Pan et al. (2016) which found no significant impact of DMPP on NH_3 emissions, although in the review of these authors slurry with DMPP was not used under field conditions.

The NI DMPSA did not increase NH_3 losses, in contrast to the results of the review by Qiao et al. (2015) for other NIs. In this review, 78 experimental observations were analysed, but none applying PS or DMPSA. In the case of CAN+DMPSA, despite the fertilizer being applied over the soil surface, the addition of water the day after application might have facilitated the dissolution of CAN and its distribution in a wider soil volume. This practice may also improve the absorption of NH_4^+ by clay and humus present within the soil colloid, thus limiting desorption of NH_3 to the soil atmosphere and solution. It may be assumed that if irrigation is high, and NH_4^+ is introduced several cm below the upper soil, NH_3 released into the soil atmosphere could be re-adsorbed in other locations before escaping to the atmosphere by gaseous diffusion. This assumption is in agreement with Holcomb et al. (2011) who reported a significant reduction in cumulative NH_3 losses in winter wheat as the amount of irrigation water increased. Sanz-Cobena et al. (2008) also proposed 10 mm irrigation immediately after urea application as a possible explanation of low NH_3 fluxes measured with IHF under field conditions in central Spain.

2. Experiment 2 - Volatilization of NH_3 and N_2O emissions in an irrigated maize crop. Effect of DMPSA and double inhibitor with urea (site “Center”)

2.1. Soil moisture and meteorological conditions

The average daily mean soil temperature during the maize cropping cycle was 21°C (23°C the first month after N fertilization) (Fig. 5.8), and the maximum and minimum air temperatures were 30°C and 13.5°C , respectively. Total precipitation was 108 mm (60 mm the first 30 days after N fertilization). The WFPS (Fig. 5.9) ranged from 15% to 38%.

Fig. 5.8: Daily rainfall and irrigation (mm), mean soil temperature at 10 cm depth and mean air temperature ($^{\circ}\text{C}$) during the experimental period. Fertilizer application was on 10 June (solid arrow). Dotted arrows represent sowing and harvest day.

Fig. 5.9: Soil water-filled pore space (WFPS, %) during the April-August period. Vertical bars indicate standard errors. Fertilizer application was on June 10 (solid arrow). Dotted arrow indicates sowing day.

2.2. Soil Mineral N

Average N-NH_4^+ and N-NO_3^- values were calculated from the soil samples that were taken during 45 days after fertilization. Ammonium content increased immediately after the fertilization event in the Urea and U+NI treatments, peaking at 173.91 and 161.76 $\text{mg-N kg}^{-1}_{\text{soil}}$, respectively (Fig. 5.10). Because of the irrigation, urea rapidly dissolved and was incorporated into the 0-10 cm upper soil layer. During 5 days after fertilization (DAF), average soil and air temperature were 24°C and 29°C, respectively. Therefore, under these warm conditions, the rapid hydrolysis of urea explains the highest N-NH_4^+ concentrations in both the Urea and U+NI treatments. The significant delay in the hydrolysis process caused by NBPT in the U+2I treatment can be observed during the first 20 DAF, when NH_4^+ concentrations increased slightly. Afterwards, the effect of DMPSA in this treatment (U+2I) explains the large N-NH_4^+ concentration of 142.61 $\text{mg-N kg}^{-1}_{\text{soil}}$ at 20 DAF (Fig. 5.10).

The mean NO_3^- concentration during the irrigation period from 0 to 60 DAF decreased in the following treatment order: Urea $\geq$ U+2I $\geq$ U+NI $>$ Control. As can be seen in Fig 5.11, these differences were significant at $P < 0.05$ between the Control and the fertilizer treatments. The U+NI also gave a lower mean N-NO_3^- concentration than the Urea treatment, but not the double inhibitor treatment (U+2I) (Fig. 5.11).

Fig. 5.10: Temporal evolution of topsoil N-NH₄⁺ content (0-10 cm). Vertical bars indicate standard errors. Solid arrow indicates fertilization event. Vertical bars indicate standard errors

Fig. 5.11: N-NO₃⁻; average concentrations in the 0-10 cm soil layer during the experimental period for the different treatments. Different letters indicate significant differences after applying the LSD test at $P < 0.05$. Vertical bars indicate standard errors of the ANOVA.

2.3. Ammonia fluxes

2.3.1. Ammonia fluxes after fertilization

Ammonia fluxes during 40 DAF ranged from 0.8 to 84.0 g-N ha⁻¹ h⁻¹ (Fig. 5.12). The largest emissions were observed during the first 48 h after fertilization for the treatments without urease inhibitor (Urea and U+NI treatments). The maximum peak of NH₃ was observed in the U+NI treatment, though this was not statistically significantly higher than the maximum peak of NH₃ in the Urea treatment ($P > 0.05$).

Fig. 5.12: Mean hourly ammonia fluxes per day over the 40 days following top-dressing fertilization (200 kg-N ha⁻¹) for the different treatments (Urea, U+NI and U+2I). Vertical bars indicate standard errors.

Total NH₃ losses in the Urea treatment were 5.5% of applied N, with this value corresponding to roughly half the value of 10% used in the IPCC accounting methodology to estimate NH₃ volatilization from fertilizer applications (IPCC, 2014). The highest cumulative emissions were produced in the U+NI treatment, at 6.1% of applied N, though the difference compared to the Urea treatment was not statistically significant. The

combination of the urease inhibitor NBPT and DMPSA (U+2I), kept the level of NH_3 fluxes lower than $14 \text{ g-N ha}^{-1} \text{ h}^{-1}$. The important reduction in NH_3 fluxes from 0 to 10 DAF in the U+2I treatment gave a significant total reduction of ammonia volatilization (Table 5.3) of 70% compared to the Urea treatment, and 74% compared with the U+NI treatment ($P < 0.05$).

Table 5.3: $N\text{-NH}_4^+$ and $N\text{-NO}_3^-$ content in soil, cumulative NH_3 emissions, cumulative N_2O , ratio of emitted NH_3 and applied N (200 kg ha^{-1}), emission factors for N_2O (EF- N_2O), Cumulative NH_3 emissions during senescence and biomass yield for the different treatments (Control, Urea, U+NI and U+2I). Different letters within columns indicate significant differences after applying the LSD test at $P < 0.05$. Standard error (SE) is given for each effect

Treatment	NH_4^+ ($\text{mg-N kg}^{-1}_{\text{soil}}$)	NO_3^- ($\text{mg-N kg}^{-1}_{\text{soil}}$)	NH_3 (kg-N ha^{-1})	N_2O (kg-N ha^{-1})	NH_3 emissions (%N applied)	EF- N_2O (%)	NH_3 senescence (kg-N ha^{-1})	Biomass Yield (kg ha^{-1})
CONTROL	1.13 c	42.08 c	–	0.07 b	–	–	–	27432 b
UREA	38.45 b	80.51 a	11.08 a	0.75 a	5.5 a	0.34 a	2.95	31178 a
U+NI	55.98 a	63.22 b	12.23 a	0.16 b	6.1 a	0.05 b	1.92	31762 a
U+2I	42.41 ab	66.46 ab	3.26 b	0.15 b	1.6 b	0.04 b	2.18	30450 a
SE	4.65	5.24	1.38	0.01	0.5	0.007	0.43	652.1
P-value	0.0001	0.0003	0.0357	0.0000	0.04	0.001	0.19	0.006
Different letters within columns indicate significant differences after applying the LSD test at $P < 0.05$. SE is the standard error of the mean. The variables “ NH_4^+ ” and “ NO_3^- ” were log-transformed.								

2.3.2. Ammonia emissions associated to the senescence and biomass yield.

Emissions of NH_3 were measured during 27 days before the harvest (Table 5.3). Overall, there were no significant differences between treatments ($P > 0.05$). The highest emissions were observed in the Urea ($2.95 \text{ kg-N ha}^{-1}$) and U+2I ($2.18 \text{ kg-N ha}^{-1}$) treatments. The U+NI treatment emitted $1.95 \text{ kg-N ha}^{-1}$ in this period.

Aboveground biomass yield significantly increased with the application of fertilizers ($P < 0.05$, Table 5.3) and decreased in the following treatment order: Urea $\sim$ U+2I $\sim$ U+NI $>$ Control.

2.4. GHG emissions

With regards to the Urea treatment, the fertilizers with DMPSA (U+NI and U+2I treatments) significantly decreased the nitrous oxide fluxes (Fig. 5.13). In the Urea treatment, N_2O fluxes ranged from 0.03 to $6.42 \text{ mg-N m}^{-2} \text{ d}^{-1}$. Three different peaks were observed in the Urea treatment, the highest peak at 3 DAF ($6.42 \text{ mg-N m}^{-2} \text{ d}^{-1}$), the second at 12 DAF ($5.5 \text{ mg-N m}^{-2} \text{ d}^{-1}$) and the last on 26 DAF ($2.4 \text{ mg-N m}^{-2} \text{ d}^{-1}$). Total N_2O emissions for the Urea treatment amounted $745.5 \text{ g-N ha}^{-1}$ (see Table 5.3).

The addition of DMPSA (U+NI) resulted in daily fluxes ranging from 0.02 to $1.03 \text{ mg-N m}^{-2} \text{ d}^{-1}$, significantly lower ($P < 0.05$) than those in Urea treatment in most of the samples taken from 1 to 30 DAF. Apart from the Control treatment, whose fluxes ranged from -0.1 to $0.23 \text{ mg-N m}^{-2} \text{ d}^{-1}$, the minimum fluxes in the fertilized plots were found in the U+2I treatment (0.02 – $0.7 \text{ mg-N m}^{-2} \text{ d}^{-1}$). Considering the cumulative N_2O emissions, the addition of DMPSA was very effective at reducing these losses.

The two treatments containing DMPSA (U+NI and U+2I) produced statistically similar N_2O emissions. That is, similar N_2O emissions were obtained when the DMPSA was applied alone (U+NI) and when the urease inhibitor NBPT was applied as well as the

DMPSA (U+2I), giving values of 164.5 and 149.2 g-N ha⁻¹, respectively. When compared to the Urea treatment, the U+2I treatment decreased cumulative N₂O emissions by 80% and the U+NI treatment by 78%.

Fig. 5.13: N₂O emissions during the experimental period in the Urea, U+NI (urea + DMPSA), U+2I (urea + DMPSA + NBPT) and Control treatments. Solid arrow indicates N fertilization date. Vertical bars indicate standard errors.

Emission factors were lower than the IPCC Tier I default value (1%), with the highest value obtained in the Urea treatment (0.39%) followed by the U+NI and U+2I treatments (0.05% and 0.04%, respectively).

2.5. Discussion of Experiment 2

2.5.1. Effect of nitrification and urease inhibitors on mineral N and N₂O emissions

Environmental conditions after top-dressing fertilization in the irrigated maize crop rapidly dissolved the urea and favoured nitrification. A higher N-NH₄⁺ concentration was maintained in the soil from 20-30 DAF with DMPSA than without this NI (Fig. 5.10). Although, according to the literature search which was performed, this field experiment was the first in which urea mixed with DMPSA was evaluated in a maize crop, similar results in a maize crop were reported by Guardia et al. (2018b), when NH₄NO₃ was mixed with this new inhibitor, and also in Experiment 1 (this Thesis), using CAN with DMPSA, at least during the first 22 days after the second fertilization (150 kg-N ha⁻¹ of CAN). For non-irrigated crops such as rainfed wheat, Guardia et al. (2018a) also observed a significant reduction of nitrification when DMPSA was added to urea. The combination of NBPT and DMPSA (U+2I) under the environmental conditions of this study produced an interesting pattern in the release of N from fertilizer, maintaining a progressive increase of N-NH₄⁺ concentration during close to 4 weeks after fertilizer application (Fig. 5.10).

The effectiveness of DMPSA in reducing N₂O emissions in comparison to urea without inhibitor was very important in this experiment, mitigating 78-80% (Table 5.3) of total N₂O emissions. This mitigation efficacy was mainly produced during the 4 weeks after fertilizer application. In this critical period, the presence of DMPSA in soil decreased nitrification, maintaining higher NH₄⁺ concentrations in this treatment (U+NI) than in the Urea treatment. Owing to this significant delay in nitrification, lower N₂O emissions were observed in the U+NI treatment. These results concur with those of Guardia et al. (2018b) using different sources of N (¹⁵NH₄⁺ and ¹⁵NO₃⁻) in an irrigated maize crop. These authors

found that DMPSA effectively reduced $^{15}\text{N}_2\text{O}$ from $^{15}\text{NH}_4$ in comparison to a source of $^{15}\text{NH}_4$ without the NI. Additionally, the high crop demand for N in this period maintained lower NO_3^- concentration in soil in the NI treatment, leaving a lower N substrate for denitrifiers. Although, the effect of DMPSA on denitrifiers could be indirect (i.e. limitation of N- NO_3^- availability via the inhibition of nitrification), some authors have reported a direct effect of DMPSA on denitrification using ^{15}N (Guardia et al., 2018b) or measuring the abundance of denitrifiers (Torralbo et al., 2017). Using urea and DMPSA in a rainfed wheat crop, Guardia et al. (2018a) found a lower mitigation capacity of DMPSA mixed with urea than in this experiment, since the reduction was roughly 35%.

Comparing the results of this Experiment with those of Experiment 1 (using CAN with or without DMPSA), a lower effect was found for CAN (close to 30%) than for urea. This lower effect can be put down to the fact that in Experiment 1 half of N was applied as NO_3^- , reducing the substrate of nitrifiers and therefore the N_2O from nitrification. Guardia et al. (2017a) also found lower mitigation effectiveness (49%-58%) when DMPSA was added with CAN.

The effect of NBPT with urea was previously evaluated by Sanz-Cobena et al. (2011). They found considerable N_2O mitigation. However, to date, no studies have been published using the double inhibitor (DMPSA and NBPT) with urea. Our results suggest that, in maize, using the double inhibitor treatment does not improve the effect on direct N_2O emissions compared to using only DMPSA.

2.5.2. Effect of nitrification and urease inhibitors on ammonia volatilization

Ammonia volatilization was not enhanced by the use of DMPSA. As can be seen in Fig. 5.12, NH_3 losses from 0-10 DAF were similar when comparing the urea treatments with and without DMPSA. These results are justified because the concentration of N- NH_4^+

was also similar for these two treatments during this period. The main difference occurred when the DMPSA maintained a significantly higher N-NH_4^+ concentration, with a high flux of NH_3 observed for the inhibitor treatment, although this did not have any significant effect on total NH_3 losses. These results agree with those found in Experiment 1, where CAN with or without DMPSA was compared. The possible explanation for this fact was that in the irrigated conditions of the study, urea was rapidly diluted in water and incorporated into the soil. Under these circumstances, the urea hydrolysis occurred inside the soil favouring the adsorption of NH_4^+ onto soil colloids and reducing the losses of NH_3 . In fact, total NH_3 emissions to the air atmosphere were 5.5% and 6.1% for the U and U+NI treatments, respectively. These amounts are considerably lower than the 14% used by the IPCC as NH_3 emission factor for urea under summer conditions (soil temperature $>20^\circ\text{C}$).

The addition of NBPT and DMPSA effectively reduced volatilization of NH_3 after application, maintaining fluxes of NH_3 below $20 \text{ g N-NH}_3 \text{ ha}^{-1} \text{ h}^{-1}$. This important flux reduction resulted in cumulative NH_3 losses close to 1.6% of total applied N in the experimental period. This effect was mainly associated to the urease inhibitor N-(n-butyl) thiophosphorictriamide (NBPT). It is known that this inhibitor does not affect urea hydrolysis in the first hours after fertilization (i.e. 24 h) because of an incomplete oxidation to its active form, NBPT_o, and insufficient diffusion through the soil micropores (Manunza et al., 1999). Probably, in this experiment, this “activation period” was shorter than 24 h, as also occurred in the experiment of Abalos et al. (2012) using U+NBPT in barley, possibly due to an enhanced distribution of NBPT within the active sites of the soil enzyme, associated to high soil moisture. As urease is an enzyme present in a wide variety of fungi and bacteria species of soil, the efficiency of NBPT to decrease urease activity may depend on its diffusion from the application point and the

concentration of inhibitor maintained in microsites. The inhibition of hydrolysis allowed more time for urea to diffuse into the soil, lowering the amount of NH_4^+ susceptible to be transformed into NH_3 and thus volatilized from the upper soil (0-5 cm) (Grant et al., 1996). Added to this, and despite its NH_3 -abating effect, NBPT did not completely eliminate urea hydrolysis as there was a pool of NH_4^+ measured throughout the entire experiment (Fig. 5.10). The higher NH_4^+ concentration maintained in the soil from 20-30 DAF (similar to the U+NI treatment), and associated to the inhibition of nitrification by DMPSA, did not result in any significant increase in ammonia volatilization in this period. A high amount of this NH_4^+ was probably adsorbed onto soil colloids, hindering the release of NH_3 to the atmosphere.

3. Experiment 3 - Winter wheat crop field experiment to determine NO , NO_2 and N_2O emissions (site “Center”)

The results shown below have been published in the journal Environmental Pollution (ISSN: 0269-7491; JIF (2017) = 4.358).

3.1. Soil moisture and meteorological conditions

Mean air temperature ranged from 35°C to -7°C during the wheat cropping period, while the average daily mean soil temperature was 11°C (Fig. 5.14). Total rainfall was 305 mm (69 mm in the first month after fertilization). The average WFPS at a depth of 10 cm was 23.0% during the experimental period, and ranged from 7.3% to 75.4%. At a depth of 30 cm, the WFPS remained in the range 23-50% (Fig. 5.15).

Fig. 5.14: Daily mean air and soil temperatures ($^{\circ}\text{C}$) and rainfall (mm) during the wheat-cropping period. The arrows indicate agricultural practices during the cropping period.

Fig. 5.15: Evolution of soil water-filled pore space (WFPS, %) on the left vertical axis and precipitation (mm) on the right-hand vertical axis during the experimental period.

3.2. Soil mineral N

Soil ammonium (N-NH_4^+) concentration in the 0-10 cm soil depth increased markedly after N fertilization (Fig. 5.16.a). The maximum peak of the N-NH_4^+ concentration ($66.9 \text{ mg N-NH}_4^+ \text{ kg}^{-1}_{\text{soil}}$) occurred 13 DAF in the U+NI treatment. The RM-ANOVA indicated a significant higher concentration of N-NH_4^+ ($P < 0.05$) in the U+NI than in U at 13 DAF.

From 40 DAF to the end of the experiment, soil N-NH_4^+ concentrations were roughly similar and below $5.0 \text{ mg N-NH}_4^+ \text{ kg}^{-1}_{\text{soil}}$ in all treatments.

Fig. 5.16: a) $N-NH_4^+$ and b) $N-NO_3^-$ contents in the 0–10 cm soil layer during the first 75 days after N fertilization for the Urea (U), U+DMPSA (U+NI) and Control (C) treatments; Vertical bars indicate standard errors.

The topsoil nitrate ($N-NO_3^-$) content ranged from 2.7 to 54.3 mg $N-NO_3^-$ kg⁻¹ soil (Fig. 5.16.b). In the case of U, the $N-NO_3^-$ content began increasing at 8 DAF, just when the $N-NH_4^+$ content started decreasing. The RM-ANOVA showed significant differences in means $N-NO_3^-$ soil content between the two fertilized treatments for the samples taken 13, 17 and 38 DAF ($P < 0.0001$). The maximum concentrations of $N-NO_3^-$ in soil took

place 17 DAF in the U treatment, 54.3 mg N-NO₃⁻ kg⁻¹soil (Fig. 5.16. b), significantly higher than the value obtained in the U+NI. In the case of U+NI, the maximum peak was at 38 DAF (29.3 mg N -NO₃⁻ kg⁻¹soil) and was significantly higher than in the U treatment.

3.3. Nitrous oxide emissions

Daily N₂O fluxes from April to mid-June ranged from -2.9 to 23.5 g N ha⁻¹ d⁻¹ (Fig. 5.17). Outside this period, N₂O fluxes were generally negligible (-1 to +1 g N ha⁻¹ d⁻¹) and are not shown to improve data readability. The application of U rapidly increased the levels of N₂O emissions, peaking twice. The first peak was observed at 17 DAF, with a daily mean flux of 22.8 ± 0.8 g N ha⁻¹ d⁻¹, and the second peak took place 30 DAF, with a daily mean flux of 23.5 ± 9.7 g N ha⁻¹ d⁻¹. The U+NI treatment significantly reduced emissions of N₂O, maintaining mean daily fluxes lower than 5 g N ha⁻¹ d⁻¹ during the crop period. The highest fluxes of this treatment were < 5 g N ha⁻¹ d⁻¹ (Fig. 5.17). The Control treatment had the lowest fluxes (< 2.0 g N ha⁻¹ d⁻¹). Cumulative N₂O emissions (Table 5.4) were 498.8, 143.2 and 43.7 g-N ha⁻¹ for the U, U+NI and Control treatments, respectively. Therefore, the treatment with NI decreased N₂O emissions by 71% in comparison to the treatment without NI. The Control treatment released the significantly lowest N₂O emissions during the crop period.

Fig. 5.17: Daily N_2O emissions for the different treatments (Control, U, U+NI). Vertical bars indicate standard errors.

The EF for cumulative N_2O emissions (Table 5.4) in U+NI (0.1) was significantly lower than in the U treatment (0.4), although in both cases it was below the default IPCC Tier I emission factor (1%).

Table 5.4: Grain and biomass yield, N content in grain, sedimentation test for bread-making quality (SDSS), cumulative N-N₂O; yield-scaled N₂O emissions (YSNE), emission factors for N₂O (EF-N₂O) and nitrogen use efficiency (NUE) for the different treatments (Control, U, U+NI). NO-N fluxes corrected NO₂-N fluxes corrected and yield-scaled NO_x emissions (Y-S NO_x) for the different fertilized treatments (U, U+NI). Different letters within columns indicate significant differences after applying the LSD test at $P < 0.05$. Standard error (S.E.) is given for each effect. The letters n.s. mean not significant ($P > 0.05$)

Treatment	Grain Yield (kg ha ⁻¹)	Biomass Yield (kg ha ⁻¹)	% N grain	SDSS (mm)	N ₂ O (g N ha ⁻¹)	YSNE (g N ₂ O kg ⁻¹ N uptake)	EF-N ₂ O (%)	NUE (kg N uptake kg ⁻¹ N applied)	NO Corrected (g N ha ⁻¹)	NO ₂ Corrected (g N ha ⁻¹)	Y-S NO _x (g NO _x kg ⁻¹ N uptake)
Control	2199 b	6649 b	2.02 b	74 b	43.7 c	0.67 b	—	—	—	—	—
U	3415 a	13520 a	2.93 a	98 a	498.8 a	2.63 a	0.4 a	1.20	227.4 a	-3.2	1.1 a
U+NI	3278 a	12896 a	2.99 a	92 a	143.2 b	0.70 b	0.1 b	1.19	-61.7 b	-1.1	-0.3 b
SE	215	804	0.087	3	27.8	0.29	0.02	0.18	7.6	5.1	0.06
P-value	0.001	0.001	0.002	0.002	0.0001	0.021	0.01	n.s.	0.001	n.s.	0.004

3.4. Nitric oxide emissions

Three days after urea application, positive NO fluxes were detected in the U treatment (Fig. 5.18.a) obtaining a first peak at 10 DAF ($34 \pm 3 \mu\text{g m}^{-2} \text{h}^{-1}$ daily average), then a higher peak at 14 DAF, with a daily mean flux of $78 \pm 17 \mu\text{g m}^{-2} \text{h}^{-1}$ (using Eq. 3.3, corrected values of Table 5.5). Rainfall occurred 15 DAF (22 mm), which increased the WFPS to 70% and immediately reduced NO emission fluxes close to 0. Nitric oxide peaked again at 23 DAF at $93 \pm 4 \mu\text{g m}^{-2} \text{h}^{-1}$ (Eq. 3.3), coinciding with a WFPS below 35%. After May 7 (>35 DAF), net NO fluxes were zero or even negative. These negative fluxes (from 35-45 DAF) for the U treatment occurred immediately after several days of rainfall events. Cumulative NO emissions for the U treatment in the experimental period were $227.4 \text{ g-N ha}^{-1}$, which corresponds to 0.19% of applied N (Table 5.4).

Fig. 5.18: a) Comparison of temporal evolution of NO fluxes in U and U+NI treatments during experimental period. b) Comparison of temporal evolution of NO_2 fluxes in the U and U+NI treatments during the experimental period. Coloured points represent the corrected value of the average the hourly NO (a) and NO_2 (b) fluxes of the different replicates of the two treatments. Grey points represent the uncorrected value of the same average (Eq. 3.3). Lines represent the corrected daily mean NO (a) and NO_2 (b) fluxes. Vertical bars indicate the standard errors of the daily averages.

The application of urea + DMPSA significantly reduced (RM-anova, $P < 0.05$) NO fluxes from 5 to 35 DAF in comparison to the U treatment, with frequent negative fluxes measured in this period (see Fig. 5.18.a). the tendency changed 35 DAF and positive NO fluxes were observed for this treatment, peaking at 40 DAF at $35 \pm 11 \mu\text{g m}^{-2} \text{h}^{-1}$. The frequent negative NO fluxes observed for this treatment (51% of all measured fluxes) also induced negative total emissions of $-61.7 \text{ g-N ha}^{-1}$ (see Table 5.4 and see Table 5.5).

Table 5.5: N-NO fluxes corrected and uncorrected, N-NO₂ fluxes corrected and uncorrected for the different fertilized treatments (U, U+NI). Different letters within columns indicate significant differences after applying the LSD test at $P < 0.05$. Standard error (S.E.) is given for each effect. The letters n.s. mean not significant ($P > 0.05$)

Treatment	NO (g N ha ⁻¹)	NO Corrected (g N ha ⁻¹)	Difference NO (g N ha ⁻¹)	NO ₂ (g N ha ⁻¹)	NO ₂ Corrected (g N ha ⁻¹)	Difference NO ₂ (g N ha ⁻¹)
U	223.94 a	227.44 a	3.5	2.36	-3.20	5.5
U+NI	-67.05 b	-61.72 b	5.3	3.06	-1.09	4.1
<i>SE</i>	22.8	7.6	1.9	4.2	5.1	0.7
<i>P-value</i>	0.001	0.001	n.s.	n.s.	n.s.	n.s.

The reactions of NO with O₃ and the photolysis of NO₂ during daylight produced NO inside the chamber with slightly modified fluxes (see Fig. 5.18.a). The Sgp (NO)/A ranged from -2 to $1 \mu\text{g m}^{-2} \text{h}^{-1}$ (Fig. 5.19). Comparing uncorrected with corrected values the difference was, on average, 4 g-N ha^{-1} . In this Experiment, an underestimation of NO was produced in uncorrected fluxes.

Fig. 5.19: $S_{gp}(NO)$ indicates the net chemical gas phase source in the chamber for O_3 , NO , and NO_2 due to reactions (R3) and (R4). Points represent the hourly mean value of S_{gp} recorded by the automated chamber NO measurement system deployed in the experiment.

3.5. Nitrogen dioxide fluxes

The daily mean NO_2 flux ranged from -42 to $109 \mu g m^{-2} h^{-1}$ (Fig. 5.18.b). Although frequent deposition of NO_2 was observed in both treatments, the highest daily mean deposition occurred for the DMPSA treatment at 29 DAF ($-13 \pm 4 \mu g m^{-2} h^{-1}$). Positive net fluxes of NO_2 were observed in both treatments when positive fluxes of NO were also observed. In the U treatment, maximum fluxes were observed from 26 to 30 DAF, and in U+NI, from 35 to 40 DAF.

Total NO_2 emissions were $-3.2 g-N ha^{-1}$ and $-1.1 g-N ha^{-1}$ for U and U+NI, respectively. The mean difference of the total NO_2 flux considering the correction done by the S_{gp}/A factor was $4.8 g-N ha^{-1}$ (see Table 5.5)

3.6. Yield, yield-scaled N₂O and NO and bread-making quality

In general, Control grain and biomass yields were significantly smaller regarding the fertilized treatments ($P < 0.05$) (see Table 5.4). In the case of grain, both N fertilized treatments (U and U+NI) increased yield by 35%, on average, with respect to the Control. The U+NI treatment did not significantly affect the grain and biomass yields.

The scaling of N₂O losses to N uptake (YSNE) averaged from 0.67 (Control) to 2.63 (U) g N₂O-N kg⁻¹ aboveground N uptake. The inhibitor significantly decreased this parameter, being significantly similar to the unfertilized treatment (Control). With regard to Y-S NO_x (the scaling of NO + NO₂ losses to N uptake), the urea treatment alone gave 1.1 g NO_x kg⁻¹ N uptake on average, whereas the treatment with DMPSA gave a negative value, indicating that this crop system absorbed 0.3 g NO_x kg⁻¹ of applied N.

Compared to the Control, the fertilized treatments obtained significantly higher values for grain N content and SDSS volume. As expected, a significant correlation was found between grain N content and SDSS volume ($R^2 = 0.8621$; $n=9$; $P = 0.0003$; see section 3.7). The NUE was not significantly affected by the addition of DMPSA (U+NI) compared with the urea treatment alone.

3.7. Correlation and principal components analysis.

A significant linear regression was found between daily N₂O fluxes and daily N-NO₃⁻ soil content for the 3 treatments (U; U+NI and Control) ($R^2 = 0.732$; $n=54$; $p < 0.0001$). The correlations among these variables are presented in Table 5.6.

Table 5.6: Pearson correlation matrix of N_2O fluxes and soil parameters ($N-NH_4^+$ and $N-NO_3^-$ content, WFPS and soil temperature) for all replicates and all treatments (U, U+NI, Control) measured on 6 different days ($n=54$; $P < 0.05$) Numbers indicate r -Pearson coefficients (*, ** and *** indicate $P < 0.0001$; $P < 0.001$ and $P < 0.01$ respectively). Letters NS mean not significant.

Variables	N_2O	NH_4^+	NO_3^-	Tsoil	WFPS
N_2O	1				
$N-NH_4^+$	NS	1			
$N-NO_3^-$	0.732***	NS	1		
Tsoil	0.293*	NS	0.44**	1	
WFPS	NS	NS	NS	NS	1

A significant multilinear regression was found only for the U treatment with NO fluxes > 0 (9-35 DAF) among hourly mean NO fluxes, hourly mean WFPS and hourly mean soil temperature in the first 10 cm ($R^2 = 0.4036$; $n=360$; $P < 0.0001$; Fig. 5.20). Consumption of NO was observed in a large range of WFPS (20-70%) and in the range of 11-18°C for the U+DMPSA, and it was not correlated with WFPS or temperature.

Fig. 5.20: Significant multilinear regression model among the positive hourly average NO fluxes from 9 to 36 DAF for the U treatment. This model explains the 40% of the NO fluxes (z-axis) related to hourly WFPS (x-axis) and soil temperature (y-axis). Points represent the values and the plane represents the multilinear regression model

The Pearson correlation matrix (Table 5.7) showed a significant correlation between N₂O fluxes and N-NO₃⁻ soil content ($r = 0.856$; $P < 0.0001$; $n=36$). NO fluxes was correlated with N-NO₃⁻ ($r = 0.702$; $P < 0.0001$; $n=36$) and N₂O emissions ($r=0.691$; $P < 0.0001$; $n=36$) for the days that all the samples were taken.

Table 5.7: Pearson correlation matrix of measured variables for only fertilized treatments and the days for which there were available data for all variables ($n=36$; $P < 0.05$). Numbers indicate

r-Pearson coefficients (*, ** and *** indicate $P < 0.05$; $P < 0.001$ and $P < 0.0001$, respectively). Letters NS mean not significant.

Variables	N ₂ O	NO	NO ₂	N-NH ₄ ⁺	N-NO ₃ ⁻	Tsoil	WFPS
N ₂ O	1						
NO	0.691***	1					
NO ₂	NS	NS	1				
N-NH ₄ ⁺	NS	NS	NS	1			
N-NO ₃ ⁻	0.856***	0.702***	NS	NS	1		
Tsoil	0.365*	NS	NS	NS	0.516**	1	
WFPS	NS	NS	NS	NS	NS	-0.348*	1

The principal component analysis (Fig. 5.21) showed that the first 3 principal components (PC) accounted for 73% of the total variance (Table 5.8). PC1 accounted for 38.36% of the observed variability and showed higher correlation with NO₃⁻ ($r = 0.933$), N₂O emissions ($r = 0.899$), and NO fluxes ($r = 0.811$). Likewise, PC2 explained 19.21% of the variability and was strongly correlated with WFPS ($r = 0.863$), as well as with soil temperature and NO₂ fluxes but with a weaker correlation (Table 5.8); $r = -0.392$ and $r = -0.579$, respectively). Finally, PC3 accounted for 15% of the total variance and was mainly correlated with N-NH₄⁺ content in soil ($r = 0.695$).

Fig. 5.21: Principal components analysis (PCA) of variables WFPS (%), soil temperature (°C), N-NH₄⁺ content in soil (mg kg⁻¹ soil), N-NO₃⁻ content in soil (mg kg⁻¹ soil), and N₂O, NO and NO₂ fluxes only for the 6 different days (from 0 to 38 DAF) when all these variables were sampled in the field. a) Representation of all the variables mentioned in the plane shaped by the first two principal components (PC1 and PC2); b) Representation of the variables in the plane PC1 and PC3; c) Representation of the variables in the plane PC2 and PC3. The first three PCs explained 73% of the variability.

Table 5.8: Correlation matrix of variables and principal components of the PCA analysis of N_2O , NO , NO_2 , NH_4^+ , NO_3^- , soil temperature (T_{soil}) and $WFPS$. Numbers indicate Pearson's coefficient between the studied variable and the principal component. Standard deviation, proportion of variance and cumulative proportion are shown below.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7
N_2O	0.899	0.286	0.169	0.050	0.031	-0.163	0.227
NO	0.811	0.164	-0.258	0.102	-0.437	-0.128	-0.178
NO_2	0.045	-0.392	-0.636	0.632	0.191	-0.027	0.049
NH_4^+	-0.177	-0.023	0.695	0.675	-0.166	0.016	-0.029
NO_3^-	0.933	-0.040	0.026	0.036	0.050	0.352	-0.001
T_{soil}	0.563	-0.579	0.326	-0.121	0.441	-0.132	-0.120
$WFPS$	-0.016	0.863	-0.057	0.190	0.454	-0.006	-0.101
Importance of components							
Standard deviation	2.685	1.344	1.094	0.921	0.660	0.185	0.111
Proportion of variance (%)	38.36	19.21	15.62	13.15	9.42	2.65	1.58
Cumulative proportion (%)	38.36	57.57	73.18	86.34	95.76	98.41	100

Table 5.9: Pearson correlation matrix of measured variables for only fertilized treatments and the days for which there were available data for all variables (n=36). Numbers indicate r-Pearson values (*, ** and *** indicate $P < 0.05$; $P < 0.001$ and $P < 0.0001$ respectively). Letters NS mean not significant.

Variables	Grain yield	Cumulative N ₂ O	%N grain	SDSS	Aboveground yield
Grain yield	1				
Cumulative N ₂ O	NS	1			
%N grain	0.779*	NS	1		
SDSS	0.750*	NS	0.928**	1	
Aboveground yield	0.948***	NS	0.914**	0.864**	1

3.8. Discussion

3.8.1. NO flux after urea application

According to the literature search which was performed, this was the first time that NO fluxes were continuously measured with an automated dynamic chamber system under field conditions in a fertilized winter wheat crop. This system offered the opportunity to continually measure net NO fluxes (positive or negative) from the soil to the atmosphere. Since the main pathway of NO production is via nitrification (Wolf and Russow, 2000; Russow et al., 2008), positive fluxes of NO were expected in the days following urea application. Urea was rapidly hydrolysed by soil urease, increasing the net soil N-NH₄⁺ concentration during the first 13 DAF (Fig. 5.16.a). The high pH of this soil (8.2) favoured nitrification of N-NH₄⁺ (Pilegaard, 2013) and consequently fluxes of NO (mainly from 7 to 34 DAF).

However, the highest fluxes were very dependent on WFPS, with a considerable reduction in fluxes observed after rainfall events. For instance, a rainfall event of 22 mm which occurred 15 DAF resulted in a WFPS close to 70% (Fig. 5.15). Denitrification could have been activated under these soil moisture conditions, favouring the

consumption of NO in microsites before its release to the atmosphere. Under these conditions, NO could be produced via denitrification of NO_3^- , but only to a small degree, as confirmed by Wolf and Russow (2000) and Russow et al. (2008). The highest NO emissions were produced when the WFPS ranged from 30-60% and the soil temperature was in the 14-18°C range. These results are in agreement with those of Medinets et al. (2016), who reported peak NO emissions in a 2012-2014 field experiment with different crops over a range of soil moisture rates from 25% to 80%, and when soil temperature was mostly in a 10-22°C range.

Maximum NO emission peaks in this experiment reached 1 g-N ha⁻¹ h⁻¹ (Fig. 5.18.a). In an experiment performed under similar environmental conditions with barley crop (Abalos et al., 2012), the maximum observed peak was around four times higher than in Experiment 3, despite application of the same N source and amount.

3.8.2. NO fluxes after addition of the nitrification inhibitor

Our results confirm that the addition of DMPSA to urea reduces the nitrification rate. Thus, the N-NH₄⁺ concentration in the upper soil layer was higher than that of the U treatment from 7 to 35 DAF, but not significantly in all sampling dates. The nitrification inhibitory effect of DMPSA is produced from dimethylpyrazole (DMP), which is released by degradation of succinic acid in soil. This effect had an important impact on NO fluxes during this period. with frequent negative NO fluxes observed in plots with DMPSA. Only from 35 to 45 DAF were positive NO fluxes measured. This behaviour was very different from that observed for the U treatment, where positive fluxes were measured from 7-35 DAF.

Since net NO flux is a consequence of the production and consumption of this gas by the plant-soil system, negative values indicate that production of this gas is less important

than the sink of NO. The possible mechanisms affecting this sink will be discussed in the general discussion (Chapter 6).

Cumulative emissions associated with this period for the U+NI treatment were negative, $-61.7 \text{ g-N ha}^{-1}$ (Table 5.4), which is not a common result in fertilized soils. Unfortunately, the number of automated chambers did not allow the study of the NO fluxes emitted by the Control treatment (without the addition of urea or NI). However, it is possible that the sink effect in the Control treatment would have been lower than in the U+NI treatment because the population of nitrifiers would not have been affected by fertilization in the Control, and hence nitrification would not have been inhibited, producing NO at a higher rate than in the U+NI treatment. This is a question that needs to be further considered in future studies.

Guardia et al. (2018a) performed a wheat crop trial during two consecutive years, in rainfed conditions and with manual chambers, in which NO emissions were also compared between treatments with calcium ammonium nitrate (CAN) and U+DMPSA (among others). In that study, the mean inhibitory effect during the two years was 71-81%, with a significant reduction of NO emissions. However, in that study, and although some negative fluxes of NO were observed for the NI treatment, the estimated NO emission was positive.

3.8.3. Fluxes of NO₂

Frequent NO₂ deposition was expected in both the U and U+NI treatments, associated with the NO₂ taken up by leaf stomata. Total NO₂ consumption was similar in the two treatments.

Positive fluxes were also observed in both treatments, although they coincided with positive fluxes of NO and low WFPS. Most likely, part of the NO produced during

nitrification was oxidized inside the soil and released to the atmosphere. In any case, consumption was higher than emission, and total emissions were -3.2 g-N ha^{-1} and -1.1 g-N ha^{-1} for U and U+NI, respectively (corrected values, Table 5.4). The percentage of NO_2 emitted or deposited in the soil-plant system was very low in comparison to NO and, therefore, the principal variation in NO_x was due to NO.

3.8.4. Effect of DMPSA on N_2O emissions

Cumulative N_2O emissions were significantly reduced by the effect of DMPSA, showing an inhibitory effectiveness of 71%. This mitigation efficacy was higher than those found by Guardia et al. (2018a) in a rainfed wheat crop (30-43%) where DMPSA was applied with urea, but conditions favoured nitrification after application. The high correlation between N_2O , NO and NO_3^- observed in the PCA analysis done for the period 0-38 DAF confirmed the assumption that nitrification would be an important pathway producing N_2O in this period, both for urea and urea with DMPSA. This assumption was made because NO fluxes are mainly produced during nitrification as an intermediate compound in the oxidation step from NH_2OH to NO_2^- (Ludwig et al., 2001), and NO_3^- is also the main compound obtained during nitrification. The favourable nitrification conditions in this period explain the lower N_2O production when DMPSA was used. This has been discussed in Experiment 2.

An interesting result was that the N_2O fluxes of the U+NI treatment were similar to those of Control for the first 21 DAF. The slight increase in N_2O fluxes occurred between 21 and 40 DAF, which was when the NI reduced its efficiency in the inhibition of the nitrification rate.

In the U treatment, the maximum N_2O fluxes took place from 13 to 30 DAF, coinciding with the maximum content of N-NO_3^- in the soil. It is widely known that nitrate content,

soil temperature and moisture are the main factors that control the denitrification process (Firestone and Davidson, 1989; Skiba, 2008). The first peak of N₂O occurred 17 DAF and reached $22.8 \pm 0.9 \text{ g-N ha}^{-1} \text{ d}^{-1}$. This peak was produced the day after an important rainfall event of 22 mm, provoking a WFPS above 70%, while NO fell close to zero. It is likely that denitrification was the main source of N₂O after this event. Fluxes of N₂O increased at low WFPS in the period 25-30 DAF when the nitrification rate was high.

3.8.5. NUE and bread-making quality

Nitrification inhibitors form part of the technological solutions proposed to increase the NUE of N fertilizers. As has been discussed previously, the new inhibitor DMPSA effectively reduced NO and N₂O emissions when applied with urea. The low concentration of N-NO₃⁻ in soil for 30 DAF could also have contributed to diminishing the risk of NO₃⁻ leaching losses from N fertilizers, although in this period total rainfall was 69 mm and losses from leaching would not be expected. Therefore, the NUE value, expressed as kg N uptake by aboveground biomass related to N fertilizer, was similar in both fertilizer treatments but higher than 1 (1.2 kg N uptake kg⁻¹ N applied), indicating that part of the N used by the plant was obtained from mineralization of organic N in the soil.

Previous literature findings have shown that N-NH₄⁺ based plant nutrition increases protein content (Fuertes-Mendizábal et al., 2013). The NI DMPSA maintained the grain protein, in agreement with Huérfano et al. (2016). No significant differences in SDSS were observed between the U and U+NI treatments. Conversely, the Control treatment gave significantly lower grain yield, grain protein and lower quality for bread-making (Table 5.4), confirming that N fertilization is necessary to maintain yield and quality.

4. Experiment 4 - Effect of DMPSA and NBPT on emissions of NO, NO₂ and N₂O in a drip-irrigated maize crop using automated and manual chambers (site “Center”)

4.1. Soil moisture and meteorological conditions

The average daily mean soil temperature during maize cropping cycle was 21 °C (24 °C the first month after N fertilization) (Fig. 5.22), and the maximum and minimum air temperatures were 30°C and 13.5°C respectively. Total precipitation was 108 mm (53 mm the first 30 days after N fertilization). Water filled pore-space (Fig. 5.23) ranged from 15% to 48%.

Fig. 5.22: Daily rainfall and irrigation (mm), mean soil temperature at 10 cm depth (°C) and mean air temperature (°C) during the experimental period. Fertilizers were applied on 29 June (solid arrow). Dotted arrows represent sowing and harvest day.

Fig. 5.23: Soil water-Filled Pore Space (WFPS, %) during the April-August period. Vertical bars indicate standard errors.

4.2. Soil mineral N

The average N-NH_4^+ content in soil throughout 54 DAF was significantly greater in U+NI treatment than in the other treatments (37.18 mg kg^{-1} soil, Table 5.10). The urea treatment (U) obtained the second highest average content of N-NH_4^+ in soil, $18.94 \text{ mg N-NH}_4^+ \text{ kg}^{-1}$ soil, followed by the rest of the fertilized treatments, U+2I and U+UI, 14.93 and $10.89 \text{ mg N-NH}_4^+ \text{ kg}^{-1}$ soil, respectively. Finally, the Control treatment averaged $1.31 \text{ mg N-NH}_4^+ \text{ kg}^{-1}$ soil. According to the statistical analysis performed (Fisher, LSD, $P < 0.05$) N-NH_4^+ average content decrease in the order: $\text{U+NI} > \text{U} \geq \text{U+2I} \approx \text{U+UI} \geq \text{C}$.

Table 5.10: Average $N-NH_4^+$ and $N-NO_3^-$ contents in the 0–10 cm soil layer of the first 54 days after N fertilization for the different treatments: Urea (U), U+DMPSA (U+NI); U+NBPT (U+UI); U+NBPT+DMPSA (U+2I) and Control (C).

Treatments	N- NH_4^+ content (mg kg ⁻¹ soil)	N- NO_3^- content (mg kg ⁻¹ soil)
C	1.31 c	4.73 c
U	18.94 b	35.37 a
U+UI	10.89 bc	22.92 b
U+NI	37.18 a	18.93 b
U+2I	14.93 bc	18.55 b
<i>S.E.</i>	4.89	5.29
<i>P value</i>	<0.0001	<0.001
Different letters within columns indicate significant differences after applying the LSD test at P<0.05. SE is the standard error of the mean. Both variables were log-transformed-		

The concentration of $N-NH_4^+$ in soil increased markedly after N fertilization (DAF) (Fig. 5.24.a). Regarding the temporal evolution throughout sampling days, significant differences were observed among the treatments with inhibitors (U+NI, U+2I and U+UI) with respect to the U and C treatments (RM-ANOVA, $P<0.01$) the first week after fertilization (until July 5). The maximum peak of the $N-NH_4^+$ concentration (166.5 mg $N-NH_4^+$ kg⁻¹soil) occurred 11 DAF (7 July) in the U+NI treatment. The same day, a spike in the U treatment was observed (122.62 mg $N-NH_4^+$ kg⁻¹soil), but this spike was significantly lower than the peak of the U+NI treatment (RM-ANOVA, $P<.0001$). However, there were no significant differences between the other fertilized treatments (U+NI and U+UI) the same day, though both were significantly higher than C and lower than in U and U+NI. Subsequently, $N-NH_4^+$ concentrations fell until July 26 when $N-NH_4^+$ content was lower than 10 mg $N-NH_4^+$ kg⁻¹soil for all treatments (Fig. 5.24.a).

Regarding the soil $N-NO_3^-$ content, the U treatment obtained the significantly highest value of this parameter, 35.37 mg $N-NO_3^-$ kg⁻¹soil (see Table 5.10). The same analysis of variance (ANOVA) was performed, and the diminishing order for these concentrations

was: $U > U+NI \approx U+UI \approx U+2I > C$. Therefore, the application of inhibitors, both urease and nitrification, significantly decreased nitrate content in soil.

In the evolution of topsoil nitrate ($N-NO_3^-$) content during the experimental period (Fig. 5.24.b) a significant increase was observed in the U+UI and U treatments at 7 DAF (5 July). Subsequently, the $N-NO_3^-$ content in the U treatment kept increasing until July 10, when it reached its highest value ($158.30 \text{ mg } N-NO_3^- \text{ kg}^{-1} \text{ soil}$), significantly higher than the other treatments. On July 17 another peak was observed in the U+2I and U+UI treatments, with significantly higher concentrations than the other treatments (35.6 and $43.9 \text{ mg } N-NO_3^- \text{ kg}^{-1} \text{ soil}$, respectively, $P < 0.05$). Overall, the nitrate content ranged from 2.7 to $158.3 \text{ mg } N-NO_3^- \text{ kg}^{-1} \text{ soil}$ and from the first month after fertilization, $N-NO_3^-$ contents were lower than $15 \text{ mg } N-NO_3^- \text{ kg}^{-1} \text{ soil}$ without significant differences between treatments.

Fig. 5.24: a) $N-NH_4^+$ and b) $N-NO_3^-$ contents in the 0–10 cm soil layer during the first 54 days after N fertilization for the different treatments: Urea (U); U+DMPSA (U+NI); U+NBPT (U+UI); U+NBPT+DMPSA (U+2I); and Control (C); Vertical bars indicate standard errors. Solid arrow indicate the application of fertilizers.

4.3. Nitric oxide emissions

Daily average nitric oxide fluxes ranged from -0.02 to $3.64 \text{ g-N ha}^{-1} \text{ h}^{-1}$ (Fig. 5.25). The highest emissions were observed in the U treatment, with daily mean fluxes ranging from 0 to $3.64 \text{ g-N ha}^{-1} \text{ h}^{-1}$. Maximum NO fluxes in this treatment were observed on July 3 and July 12 (3.64 ± 0.62 and $2.65 \pm 0.2 \text{ g-N ha}^{-1} \text{ h}^{-1}$, respectively). The results of the RM-ANOVA ($P < 0.05$) showed that the weekly average NO fluxes related to the first peak was significantly higher than the other treatments except for the U+UI and, with respect to the second peak, was significantly the highest (Fig. 5.25). Total NO emissions in the U treatment were $677.72 \text{ g-N ha}^{-1}$, which correspond to 0.34% of applied N (Table 5.11).

Fig. 5.25: Mean NO fluxes in periods of three hours (points) and daily mean fluxes (line with points) by treatment the first 35 DAF. Solid arrow indicates N fertilization. Vertical bars indicate standard errors

The same NO emissions pattern was observed in the U+UI treatment during the first 5 DAF, reaching a maximum peak of $2.1 \pm 0.3 \text{ g-N ha}^{-1} \text{ h}^{-1}$ (July 3), 42% lower than the peak of the U treatment (Fig. 5.25). Although the urease inhibitor (UI) reduced the maximum peak in comparison to the U treatment, this decrease was not significant in that week (RM-ANOVA, $P > 0.05$). Furthermore, there was another spike in NO fluxes on 12 July for this treatment of $0.65 \pm 0.05 \text{ g-N ha}^{-1} \text{ h}^{-1}$. Thus, the addition of NBPT (U+UI)

significantly diminished weekly average NO fluxes compared with the U treatment from 9 July to 26 July, when all NO fluxes were $<0.5 \text{ g-N ha}^{-1} \text{ h}^{-1}$ for all treatments.

Regarding the treatment with both inhibitors (U+2I), total NO emissions were $169.63 \text{ g-N ha}^{-1}$, lower than U+UI ($271.51 \text{ g-N ha}^{-1}$) but greater than U+NI ($82.77 \text{ g-N ha}^{-1}$), though these were not statistically significant differences (Table 5.11). The NO fluxes were $<0.5 \text{ g-N ha}^{-1}$ throughout the experiment, and only 7% of hourly mean NO fluxes were negative. The highest NO fluxes for the U+2I treatment were also seen on 3 July and 12 July, with values of 0.44 ± 0.17 and $0.41 \pm 0.27 \text{ g-N ha}^{-1} \text{ h}^{-1}$, respectively (Fig. 5.25).

The DMPSA nitrification inhibitor reduced NO fluxes to levels lower than $0.21 \text{ g-N ha}^{-1} \text{ h}^{-1}$ during the experimental period (Fig. 5.25). This treatment resulted in the lowest NO emissions with the exception of the Control treatment (C), but significantly similar to them, 82.77 and $59.20 \text{ g-N ha}^{-1}$ for U+NI and C, respectively (Table 5.11). In addition, the maximum number of negative NO fluxes was observed in the U+NI treatment (31% of measured data). Total NO emissions decreased in the following treatment order: $U > U+UI \geq U+2I \geq U+NI \approx C$ ($P < 0.05$) (Table 5.11).

In order to enhance data visibility, in Fig. 5.25 only the NO fluxes from 0 to 36 DAF are shown. During the rest of the experimental period the fluxes were negligible.

Table 5.11: Grain and biomass yield, %N in grain, cumulative NO_x and N₂O emissions, emission factors (EF-NO_x and EF-N₂O), nitrogen use efficiency (NUE) and yield-scaled NO_x (Y-S NO_x) and N₂O emissions (YSNE) for the different treatments: control (C), urea (U), urea with NBPT (U+UI), urea with DMPSA (U+NI), urea with double inhibitor (U+2I). Different letters within columns indicate significant differences after applying the LSD test at $P < 0.05$. Standard error (SE) is given for each effect. The letters n.s. mean not significant ($P > 0.05$).

Treatments	Grain yield (kg ha ⁻¹)	Biomass yield (kg ha ⁻¹)	N grain (%)	NO (g N ha ⁻¹)	NO ₂ (g N ha ⁻¹)	N ₂ O (g N ha ⁻¹)	EF-NO _x (%)	EF-N ₂ O (%)	NUE (kg Nuptake kg ⁻¹ N applied)	Y-S NO _x (g NO _x kg ⁻¹ Nuptake)	YSNE (g N ₂ O kg ⁻¹ Nuptake)
C	13958 b	36611 c	1.28	59.20 c	23.75 b	341.76 c	–	–	–	0.32 b	1.33 b
U	15415 a	44000 ab	1.27	677.72 a	219.72 a	1153.73 a	0.40 a	0.40 a	0.16 b	3.08 a	4.04 a
U+UI	15525 a	39333 bc	1.39	271.51 b	55.50 b	743.66 b	0.15 b	0.20 b	0.38 a	0.96 b	2.25 b
U+NI	16644 a	46333 a	1.22	82.77 c	0.55 b	503.58 bc	0.03 b	0.08 b	0.25 ab	0.28 b	1.63 b
U+2I	15530 a	44833 ab	1.26	169.63 bc	82.92 ab	490.19 bc	0.08 b	0.08 b	0.29 ab	0.78 b	1.59 b
<i>S.E.</i>	168.81 a	768.92	0.02	65.12	42.85	80.53	0.05	0.04	6.53	0.33	0.37
<i>P value</i>	0.013	0.019	n.s.	0.003	0.002	0.001	0.01	0.005	n.s.	0.01	0.005
Different letters within columns indicate significant differences after applying the LSD test at $P < 0.05$. SE is the standard error of the mean. The variables “NO”, “EF-NO” and “Y-S NO _x ” were log-transformed.											

4.4. Nitrogen dioxide fluxes

The maximum NO₂ fluxes were observed during the weeks 1-8 July and 9-16 July, for all treatments (Fig. 5.26). The maximum daily mean NO₂ fluxes was observed in the U treatment on July 12 (0.88 ± 0.12 g-N ha⁻¹ h⁻¹), with the average of the NO₂ fluxes of this week significantly higher than the rest of treatments (RM-ANOVA, $P < 0.05$). Conversely, the maximum deposition of NO₂ was observed in the U+UI treatment (-0.08 ± 0.03 g-N ha⁻¹ h⁻¹), although the most frequent deposition was detected in the U+NI treatment with a rate of negative hourly mean fluxes which corresponded to 51% of total measured data.

In this experiment, the cumulative NO₂ emissions were positive for all treatments, with emissions decreasing in the following treatment order: $U \geq U+2I \geq U+UI \approx C \approx U+NI$ ($P < 0.01$; Table 5.11).

In order to enhance data visibility, in Fig. 5.26 only the NO₂ fluxes from 0 to 36 DAF are shown. During the rest of the experimental period the fluxes were negligible.

Fig. 5.26: Mean NO_2 fluxes in periods of three hours (points) and daily mean fluxes (line with points) by treatment the first 35 DAF. Solid arrow indicates N fertilization. Vertical bars indicate standard errors.

4.5. Nitrous oxide emissions

The U treatment presented daily N_2O fluxes ranging from -0.75 to $214.3 \text{ g-N ha}^{-1} \text{ d}^{-1}$. The U treatment presented daily N_2O fluxes ranging from -0.75 to $214.3 \text{ g-N ha}^{-1} \text{ d}^{-1}$ (Fig. 5.27). The addition of DMPSA induced daily mean fluxes from 0.62 to $29.77 \text{ g-N ha}^{-1} \text{ d}^{-1}$ in the U+NI treatment and from 0.10 to $13.71 \text{ g-N ha}^{-1} \text{ d}^{-1}$ in U+2I. The Control treatment produced daily average N_2O fluxes from 0.67 to $8.72 \text{ g-N ha}^{-1} \text{ d}^{-1}$. Finally, the

addition of NBPT alone (U+UI) also reduced the range of N_2O fluxes compared to the U treatment ($1.14\text{--}170.22 \text{ g-N ha}^{-1} \text{ d}^{-1}$).

A maximum flux peak was observed in the U treatment on July 10 (11 DAF) of $214.3 \text{ g-N ha}^{-1} \text{ d}^{-1}$, which was significantly higher than in the other treatments (RM-ANOVA, $P < 0.001$). On this day, the maximum flux of the U+NI treatment also took place with a value of $29.77 \text{ g-N ha}^{-1} \text{ d}^{-1}$, which was significantly higher than in the U+UI, U+2I and C treatments (RM-ANOVA; $P < 0.05$). The NBPT treatment (U+UI) produced a spike sooner than in the U treatment (8 DAF), and was significantly higher than the other treatments ($44.91 \text{ g-N ha}^{-1} \text{ d}^{-1}$) for this day (Fig. 5.27).

Fig. 5.27: N_2O emissions during the experimental period in urea, urea + DMPSA, U+NI, urea + NBPT; U+UI; urea + DMPSA +NBPT, U+2I and Control. The solid arrow indicates N fertilization. Vertical bars indicate standard errors. Zoom view of the N_2O emissions without urea treatment

Cumulative N_2O emissions decreased in the following treatment order: $U > U+UI \geq U+NI \approx U+2I \geq C$ ($P < 0.05$) (Table 5.11). The treatment with the NI alone (U+NI) significantly

diminished total N₂O emissions by approximately 56%. Likewise, the combination of the two inhibitors (U+2I) significantly decreased (by an average 57.5%) cumulative N₂O emissions. The total N₂O emissions were 1153.73 g-N ha⁻¹ for U, 743.66 g-N ha⁻¹ for U+UI, 503.58 g N ha⁻¹ for U+NI and 490.16 g N ha⁻¹ for U+2I (Table 5.11). The N-fertilized treatment with UI alone (U+UI) mitigated cumulative N₂O emissions by 35%. Finally, the non-fertilizer treatment (C) emitted a total of 341.76 g-N(N₂O) ha⁻¹.

4.6. Yield and yield-scaled N₂O and NO

Grain and biomass yield (Table 5.11) were significantly increased by the application of N fertilizers with respect to the Control treatment, but without significant differences between them. Interestingly, the U+NI treatment produced non-significant increases of grain and biomass yield (16% and 21%, respectively) with respect to the U treatment.

The N₂O and NO_x (NO+NO₂) emissions are also represented in this study as the ratio between cumulative emissions and grain yield (YSNE and Y-S NO_x). The results show that the application of any inhibitor significantly reduced the yield-scaled parameters when compared to the U treatment. Nevertheless, the treatment with both inhibitors (U+2I) did not decrease significantly these variables despite minimum values for YSNE and Y-S NO_x of 1.59 and 0.78, respectively (Table 5.11).

The ratio of N₂O and NO_x (NO+NO₂) emissions to the amount of N applied subtracting the emissions of the C treatment (EF-N₂O and EF-NO_x) was significantly affected by the application of inhibitors. Thus, the U treatment obtained an EF of 0.4% for both N₂O and NO_x. The addition of NBPT (U+UI treatment) significantly lowered this value to 0.15% and 0.20% for EF-N₂O and EF-NO_x, respectively. The U+2I treatment reduced these factors even more than the U+UI (to 0.08%), although without significant differences. Finally, the highest reduction for EF-NO_x was observed in the U+NI treatment (0.03%).

For EF-N₂O, the U+NI and U+2I treatments had the same value of 0.08%. With respect to the NUE, expressed as the percentage of applied N fertilizer that was taken up by the aboveground biomass of the plant, no significant differences were detected.

4.7. Correlations and principal components analysis.

The Pearson's correlation matrix (Table 5.12) showed a significant correlation between N₂O fluxes and N-NH₄⁺ soil content ($r = 0.651$; $P < 0.0001$; $n=131$). The NO fluxes was correlated with N-NO₃⁻ ($r = 0.375$; $P < 0.0001$; $n=131$) and NO₂ emissions ($r=0.809$; $P < 0.0001$; $n=131$) for the nine days on which all the samples were taken (0-54 DAF).

Table 5.12: Pearson correlation matrix of measured variables for all treatments and the days for which there were available data for all variables (9 days; $n=131$; $P < 0.05$). Numbers indicate r -Pearson values (, ** and *** indicate $P < 0.05$; $P < 0.001$ and $P < 0.0001$ respectively). Letters NS mean not significant.*

Variables	NO	NO ₂	N ₂ O	N-NH ₄ ⁺	N-NO ₃ ⁻	Tsoil	WFPS
NO	1						
NO ₂	0.809***	1					
N ₂ O	NS	NS	1				
N-NH ₄ ⁺	NS	NS	0.651***	1			
N-NO ₃ ⁻	0.375***	0.518***	NS	0.526*	1		
Tsoil	-0.263*	-0.341**	NS	-0.181*	-0.329**	1	
WFPS	NS	-0.220*	NS	NS	0.182*	0.223*	1

The principal component analysis (Fig. 5.28) showed that the first 3 principal components (PC) accounted for 73% of the total variance (Table 5.13). PC1 accounted for 36.7% of the observed variability and showed higher correlation with NO₂ ($r = -0.537$), NO fluxes ($r = -0.505$) and NO₃⁻ ($r = -0.426$). Likewise, PC2 explained 23% of the variability and was correlated with N₂O ($r = 0.682$) and NH₄⁺ ($r = 0.606$). Finally, PC3 explained 13%

of the total variance and was mainly correlated with WFPS ($r=-0.668$) and soil temperature ($r = -0.475$).

Table 5.13: Correlation matrix of variables and principal components of the PCA analysis of N_2O , NO , NO_2 , $N-NH_4^+$, $N-NO_3^-$, soil temperature (T_{soil}) and $WFPS$. Numbers indicate Pearson's coefficient between the studied variable and the principal component. Standard deviation, proportion of variance and cumulative proportion shown below.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7
N_2O	-0.177	0.682	0.029	0.163	0.105	0.675	0.085
$N-NH_4^+$	-0.264	0.606	0.225	0.062	-0.253	-0.665	-0.016
NO	-0.505	-0.074	-0.419	0.270	0.248	-0.061	-0.651
NO_2	-0.537	-0.175	-0.314	0.135	0.091	-0.091	0.739
$N-NO_3^-$	-0.426	-0.155	0.019	-0.415	-0.725	0.274	-0.139
T_{soil}	0.361	0.051	-0.475	0.566	-0.564	0.009	-0.039
$WFPS$	0.203	0.321	-0.668	-0.620	0.100	-0.111	-0.027
Importance of components							
Standard deviation	1.603	1.271	0.980	0.862	0.802	0.548	0.407
Proportion of variance (%)	36.71	23.07	13.73	10.62	9.19	4.29	2.39
Cumulative proportion (%)	36.71	59.78	73.51	84.13	93.32	97.61	100

Fig. 5.28: Principal components analysis (PCA) of variables WFPS (%), soil temperature (°C), $N-NH_4^+$ content in soil (mg kg⁻¹ soil), $N-NO_3^-$ content in soil (mg kg⁻¹ soil), and N_2O , NO and NO_2 fluxes only for the 9 different days (0-54 DAF) when all these variables were sampled in the field. a) Representation of all the variables mentioned in the plane shaped by the first two principal components (PC1 and PC2); b) Representation of the variables in the planes PC1 and PC3; c) Representation of the variables in the planes PC2 and PC3. The first three PCs explain 73% of the variability.

4.8. Discussion

4.8.1. NO flux after urea application

According to the literature search which was performed, this was the first time that NO fluxes were continuously measured with an automated dynamic chamber system under field conditions in a fertilized irrigated maize crop. Since urea was applied on the soil surface under a drip irrigation system, distribution of urea and subsequent hydrolysis mainly occurred in the upper part of the soil and, therefore, NH_4^+ accumulated mainly on the soil surface. As the mean WFPS in the 0-10 cm soil depth was below 50% throughout the crop period (Fig. 5.23), nitrification of NH_4^+ was favoured. The highest NO emissions were often produced when the WFPS ranged from 30-60%. The high soil temperature in the weeks following fertilization (22-24°C) also favoured the nitrification process.

When considering NO emissions in the U treatment, it was found that decreasing emissions coincided with increasing WFPS, with values close to 50%. Peaks emissions occurred when the WFPS was 37% (4 DAF) and 40% (13 DAF), NO fluxes were similar to the Control treatment when N-NH_4^+ concentration was below 5 mg kg⁻¹ and so nitrification was little importance in the soil.

4.8.2. NO fluxes after addition of the nitrification and urease inhibitors

The addition of urease or nitrification inhibitors significantly reduced NO emissions in this crop (Table 5.11). The effectiveness of NBPT at reducing NO losses, with reductions of 55% compared to the U treatment, was lower than that reported by Sanz-Cobena et al. (2012) in an irrigated maize crop (67% reduction) and by Abalos et al. (2012) in a rainfed cereal crop (74% reduction). As discussed in Abalos et al. (2012), in the presence of the UI most urea hydrolysis takes place in the soil, as the movement of urea with irrigation water reduces the concentration of N-NH_4^+ in the upper part of the soil. These authors

speculate that this reduction in the size of the N-NH_4^+ pool might reduce the nitrification rate in such a way that the production of NO is also affected during this initial period. In this study, the irrigation system (drip irrigation) favoured the horizontal movement of water from the dripper to the drier soil areas. In this case, high N-NH_4^+ concentrations were maintained in the upper part of the soil (Fig. 5.24.a). Nonetheless, the effect of NBPT was significant, and a considerable reduction of NO emissions was observed in comparison to the urea treatment alone.

The use of the double inhibitor (DMPSA+NBPT) reduced total NO emissions by 70%, which was a greater inhibitory effect than with the DMPSA alone. Based on results obtained in Experiment 2, a possible explanation for this behaviour could be that a higher N mineral content (especially total N-NH_4^+) was maintained in the soil in the days after fertilization, which could have favoured NO emissions through nitrification. However, no significant enhancement was observed of N-NH_4^+ in the 0-10 cm soil depth in the U+2I treatment compared to the U+NI treatment. Therefore, another plausible hypothesis could be that the mobility in the soil of the urea and DMPSA with irrigation water could have produced a spatial dislocation of the DMPSA and urea. Probably, the urea (which is more soluble and mobile than DMPSA) was transported to microsites whereas the DMPSA was not, impacting on the effectiveness of the NI. This is a question that needs to be further considered in future studies.

4.8.3. Effect of DMPSA in N₂O and NO₂ emissions

Emissions of NO₂ was significantly lower (more than 10 times lower) than those of NO, but an important indirect effect of DMPSA and NBPT was obtained. Due to the high correlation between NO and NO₂ fluxes, the reduction of total NO supposed an important reduction in total NO₂. Probably, during emission of NO, part of this gas was transformed by reaction with O₂ or other oxidant sources into NO₂.

In the case of N₂O, DMPSA had a high mitigation effect, reducing total N₂O emissions by roughly 56% in comparison to urea alone. Similar to Experiment 2, the combination of the two inhibitors, in the U+2I treatment, did not improve the mitigation effect obtained with DMPSA alone. However, the type of irrigation affected the obtained efficiency. The reduction obtained by this NI with drip irrigation was lower than when using the ranger irrigation system. It is possible that the allocation of both DMPSA and NH₄⁺ obtained from urea hydrolysis could have affected the efficiency rate. The different water distributions obtained with drip and ranger systems may promote different mobility patterns of chemicals in soils. In the case of drip irrigation, an important horizontal diffusion will have occurred.

Our results demonstrated the high efficacy of DMPSA when applied with urea, with and without NBPT, in the reduction of yield-scaled N₂O losses. The use of nitrification and/or urease inhibitors can be recommended to minimize the ratio of N₂O emissions per kg of grain yield, and consequently to increase the sustainability of irrigated maize cropping systems in Mediterranean areas, without apparent yield penalties.

CHAPTER 6

GENERAL DISCUSSION

CHAPTER 6

One of the challenges for the agricultural systems in the next decades is to change to a greater sustainable, cost-effective and environmental friendly way, maintaining high crop yields and food quality. Nowadays, the globally demand of N fertilizers is approximately 119 millions of tons of N per year (FAO, 2015). Therefore, using the IPCC default values (IPCC, 2014) from total N applied (TAN), i.e. 1% in direct N₂O emissions and 1% in the indirect emissions (EF₄, e.g. NH₃ and NO), this means that roughly 1.2 millions of tons of fertilizers are lost as N₂O in the world each year. On the other hand, the UNECE (Bittman et al., 2014) established an average value of 14% for NH₃ volatilization from applied N. In order to face these environmental risks, several agricultural practices should be adequately assessed under each specific cropping system and under each climate condition.

Within the 4R nutrient stewardship developed by Bruulsema et al. (2009), i.e. right N sources, right rates, right timing, and right placement; this thesis has been focused on the effect of N sources, accomplishing the rest of the best management practices (BMPs). Thus, a comparison between inhibitors (specially the novel NI, DMPSA) and some N losses has been assayed in different ways: i) Volatilization of NH₃ (Experiments 1 and 2); ii) NO_x emissions (Experiments 3 and 4) and iii) the N₂O emissions (all the experiments). In this Chapter, the importance of mitigating pollutants and the enhancement of the efficacy of N fertilizers is discussed for some of the most common Mediterranean crops.

1. Efficiency of DMPSA in mitigation of N₂O and NO_x

1.1. N₂O emissions

According to the results afore described (Chapter 5), the inhibitor DMPSA got reduce significantly N₂O emissions (Table 6.1 and 6.2).

Table 6.1: Summary of the N_2O , NH_3 and NO_x emissions of each experiment reported in this thesis

Experiment	Crop	Irrigation System	N rate (kg-N ha ⁻¹)	Fertilizer type	Split N	NIs/UIs	Area scaled N_2O (kg-N ha ⁻¹)	Area scaled NH_3 (kg-N ha ⁻¹)	Area scaled NO_x (kg-N ha ⁻¹)	IPCC N_2O eq. (kg-N ha ⁻¹)
1	Maize	Sprinkler	200	PS/CAN	YES	YES/NO	0.29-0.57	7.8-10.9	–	0.48-0.68
2	Maize	Sprinkler	200	U	NO	YES/YES	0.15-0.75	1.6-6.1	–	0.18-0.86
3	Wheat	Rainfed	120	U	NO	YES/NO	0.14-0.50	–	-0.06-0.22	0.14-0.50
4	Maize	Drip	200	U	NO	YES/YES	0.49-1.15	–	0.25-0.96	0.49-1.16

PS=pig slurry; CAN=calcium ammonium nitrate; U=Urea

IPCC N_2O eq. = Sum of total emissions applying the IPCC conversion factor of 1% for NH_3 and NO_x

N rate= Following the regional fertilization practice

Table 6.2: Comparison of N_2O , NH_3 and NO_x emissions observed in this thesis as function of type of crop and fertilizers.

	Crop	N rate (kg-N ha ⁻¹)	Fertilizer type	Area scaled N_2O (kg-N ha ⁻¹)	Area scaled NH_3 (kg-N ha ⁻¹)	Area scaled NO_x (kg-N ha ⁻¹)	IPCC N_2O eq. (kg-N ha ⁻¹)
Treatments without inhibitors	Maize	200	PS/CAN/U	0.57-1.15	10.9-11.1	0.97	0.68-1.16
	Wheat	120	U	0.50	–	0.22	0.50
Treatments with NIs	Maize	200	PS/CAN/U	0.29-0.57	7.8-12.2	0.08	0.28-0.50
	Wheat	120	U	0.14	–	-0.06	0.14
Treatments with UIs	Maize	200	U	0.74	–	0.33	0.74
Treatments with 2Is	Maize	200	U	0.15-0.49	3.3	0.25	0.18-0.49

PS=pig slurry; CAN=calcium ammonium nitrate; U=Urea

IPCC N_2O eq. = Sum of total emissions applying the IPCC conversion factor of 1% for NH_3 and NO_x

N rate= Following the regional fertilization practice

In all the trials performed in this thesis, the cumulative N₂O emissions were significantly lower in the treatment with DMPSA than the treatment without it, with the exception of Experiment 1, that applied CAN as N source. The question was if the N source was the key point in the effectiveness of NI or it was an indirect effect associated with the total amount of N₂O emission. Comparing the results of the treatments without NI and N application of 200 kg ha⁻¹, the PS-CAN treatment emitted 570 g-N ha⁻¹ (Experiment 1, Table 5.2), whereas with the urea treatments 750 g-N ha⁻¹ and 1150 g-N ha⁻¹ were emitted (Experiment 3 and 4, respectively) (Table 5.4 and 5.11). Although these experiments were performed in different soils or management conditions, these results were in line with those of Guardia et al. (2017), who found higher emission with urea than CAN. This is owing to the half of applied N, was NO₃⁻ so reducing the substrate of nitrifiers and therefore, the N₂O from nitrification. This specific effect of N source is very dependent on the main processes involved in the emissions of this gas, and only when nitrification had an important weight in N₂O emissions, this assumption should be observed.

A plausible explanation of the effect of DMPSA was that the total N₂O emission when NI was activated, affected the effectivity of this compound on N₂O mitigation, diminishing its influence when N₂O fluxes were low in treatments without NI. In fact, the low efficiency of the DMPSA in Experiment 1 could have been associated with the low N₂O emission using CAN. Therefore, an hypothesis for future experiments may be that DMPSA could mitigate N₂O emissions from CAN or other N sources if the N₂O emission during several weeks after application is considerably high and WFPS and soil temperature favour nitrification. Results of Guardia et al. (2017) endorse this explanation because they found a significant mitigation efficiency (close to 58%) when DMPSA was added with CAN, but in this case the total N₂O emission was higher than 1.14 kg N ha⁻¹ for CAN without NI (more than double than in Experiment 1).

Another question was if climatic conditions could affect the efficacy of DMPSA on N_2O mitigation. Overall, the inhibitory effect of NIs is affected by soil conditions, since the nitrification and denitrification processes are very dependent on soil moisture and temperature (Gilsanz et al., 2016; Yang et al., 2016). Comparing results of urea alone, the mean %N applied (Table 6.3) was 0.41% and 0.25% for irrigated (maize) and rainfed (wheat) crops, respectively. The application of the NI reduced significantly this parameter up to 0.17% and 0.07%, also for irrigated and rainfed conditions, respectively. This represented an average of 55% and 72% of reduction in these fluxes, respectively. Although information of the performance of this new inhibitor (DMPSA) is scarce, our results revealed even higher mitigation efficiency of DMPSA using with urea than that reported by Gilsanz et al. (2016) for DCD (average 42%) and DMPP (average 40%) in a meta-analysis from 39 recent studies. In addition, this inhibitory effect was higher than the observed in the meta-analysis of Akiyama et al. (2010), where a maximum of 55% of inhibition was reported in DMPP, or in Thapa et al. (2016), who reported an average N_2O reduction of 40% for NIs, being the inhibitory effect greater in corn (50%) than in wheat (30%). In the case of Mediterranean crops, the meta-analysis performed by Cayuela et al. (2017) indicated an average reduction on EFs of 65% for the NIs, which was very similar for which obtained in the experiments of this thesis. Hence, the new NI, DMPSA may be a good mitigation option for N_2O emissions in Mediterranean areas. In the case of fertilizers without NI, Cayuela et al. (2017) obtained similar EFs for rainfed crops (0.2-0.3%) but slightly higher for irrigated crops (0.6%).

Table 6.3: Summary of %N applied, Yield-scaled emissions and NUE of the different experiments performed in this thesis

Experiment	N-fertilizers	% N applied				Yield scaled emissions (g-N kg ⁻¹ Nuptake)			NUE (kg Nuptake kg ⁻¹ N applied)
		N ₂ O	NH ₃	NO _x	IPCC eq.	N ₂ O	NH ₃	NO _x	
1	Treatment without inhibitors	0.28%	17.07%	–	0.34%	1.57	30.0	–	0.60
	Treatment with NIs	0.20%	11.83%	–	0.24%	1.02	19.94	–	0.90
2	Treatment without inhibitors	0.37%	5.54%	–	0.43%	1.78	26.32	–	0.62
	Treatment with DMPSA	0.08%	6.11%	–	0.14%	0.34	25.90	–	0.88
	Treatment with DMPSA+NBPT	0.07%	1.63%	–	0.07%	0.16	11.01	–	0.76
3	Treatment without inhibitors	0.25%	–	0.19%	0.25%	2.37	–	0.62	1.20
	Treatment with DMPSA	0.07%	–	0.05%	0.07%	0.68	–	-0.16	1.19
4	Treatment without inhibitors	0.58%	–	0.48%	0.58%	4.05	–	2.30	0.16
	Treatment with DMPSA	0.25%	–	0.16%	0.25%	1.63	–	0.69	0.26
	Treatment with DMPSA+NBPT	0.24%	–	0.12%	0.24%	2.25	–	0.26	0.29
	Treatment with NBPT	0.37%	–	0.16%	0.37%	1.59	–	0.75	0.38

1.2. NO and NO₂ emissions.

An interesting beneficial effect of NIs is the mitigation of NO emission (Qiao et al., 2015), which is an important N reactive compound emitted after urea or NH₄⁺-based fertilizers application (Pilegaard, 2013). In Experiments performed in this thesis, NO emissions were only studied with a single N source, urea, and the %N applied emitted was 0.19% and 0.48% for rainfed and irrigated crop systems, respectively (Table 6.1).

The total NO lost from U in Experiment 3 was 226.7 g-N ha⁻¹ (0.19% N_{applied}), which was below the results of Garrido et al. (2002) that ranged from 0.6-2.5% in a study in five agricultural soils where a rotation of wheat, rapeseed and maize was performed over more than 20 years. Guardia et al. (2018) observed higher losses of NO when U was used (>1.5%) in a wheat crop under similar edaphic, climatic and management conditions.

In Experiment 4, the total NO emissions from U the first 30 DAF was 0.48% of applied N (Table 6.1), which were higher than the emissions observed for wheat in Experiment 3, but lower than the ones observed by Guardia et al. (2017), who reported a percentage of 1.30% of applied N in a drip irrigated maize fertilized with urea. In Experiments 3 and 4, NO emissions were measured with manual chambers instead of automated ones.

Our findings not only have demonstrated the effectiveness of DMPSA in NO reduction but also have improved the knowledge of N oxides emissions in Mediterranean areas from a mechanistic point of view. Overall, the new DMPSA seems to be a good NO mitigation strategy for both summer irrigated and rainfed crops. However, further researches are needed to confirm these results. The EFs for NO with DMPSA ranged from 0.08 to 0.44% in Experiment 4 (where data for control treatment were available, see Table 5.11). Concerning the ratio of applied N for NO_x emissions, the treatment with DMPSA

achieved 0.16% for drip-irrigated maize, being significantly lower than urea (0.48%). The same mitigation pattern was observed in Experiment 3 with rainfed wheat.

An interesting result of this thesis was that losses of U+NI in the maize experiment were statistically similar to those of Control, although U+NI was 28% greater. Furthermore, frequent negative fluxes were observed for DMPSA treatment as occurred in Experiment 3. However, the total NO emission was positive for maize, contrarily to what happened for winter-wheat. Discussion of possible processes involved in this sink effect was included in the Chapter.

On the other hand, apart from the aforementioned studies of Guardia et al. (2017, 2018), the results of this thesis surpassed the inhibitory effect of NIs for the meta-analysis of Akiyama et al. (2010) with DMPP, since these authors found a mitigation efficiency of 65%, being our mitigation efficiency of 127% and 91% in Experiment 3 and 4, respectively. Furthermore, Yang et al. (2016) evaluated the efficiency of DMPP abating NO (among others) and they obtained an average reduction of 25%. The Fig. 6.1 shows the mitigation effect of DMPSA on NO emissions in the Experiments 3 and 4 and the mitigation effect of previous studies.

Fig. 6.1: Relative NO emissions as ratio of emissions abated by NIs. Positive values indicate a reduction on NO emissions compared with the treatment without inhibitor

2. Pathways of NO and N₂O production and consumption.

Regarding the main pathways of N transformation in soils, both biotic (nitrification, nitrifier and heterotrophic denitrification) and abiotic (chemodenitrification). Many studies support the idea that in a wide range of soils nitrification is the dominant process for soil NO production as an intermediate in the oxidation of NH₂OH to NO₂⁻, catalized by *hao*, hydroxylamine oxidoreductase (Firestone, M. K. and Davidson, E. A., 1989; Conrad, 1996; Cui et al., 2012; Medinets et al., 2015). Also, the N₂O emitted is attributed to this reaction, despite the majority of N₂O emitted is primarily attributed to denitrification (Pilegaard, 2013; Hallin et al., 2018).

In Mediterranean rainfed systems, several authors state that nitrification is the most usual via for N_2O and NO production, because of the low rainfall, low organic matter and well-aerated soils that is unsuitable for denitrification (Menéndez et al., 2008; Aguilera et al., 2013; Abalos et al., 2017; Guardia et al., 2018a). On the other hand, in Mediterranean irrigated systems, some authors observed that a combination of nitrification and denitrification as the major vias of NO and N_2O production exists (Abalos et al., 2012, 2014b; Guardia et al., 2017a). According to the results of this thesis, the nitrification process could have promoted the main N_2O and NO emission. Our hypothesis is based on the pattern under which these emissions took place. Thus, the maximum peaks of N_2O and NO happened in the period from 5 to 40 DAF, with a soil temperature ranged between 20-35°C for maize and 10-25°C for wheat. Temperature is a key factor for microbial activity and influences on the soil water evaporation rate (WFPS) (Aguilera et al., 2013). A positive significant correlation between T_{soil} and N_2O or NO fluxes has been observed by some authors (Vallejo et al., 2006; Meijide et al., 2007). In our case, only a weak significant correlation was found in Experiment 3 ($R^2=0.13$; $n=36$; $p < 0.05$, Table 5.7) for N_2O and in the Experiment 4 for NO ($R^2=0.06$, $n=131$; $p < 0.05$). In addition, the maximum peaks of N_2O and NO occurred when WFPS ranged from 20 to 70%, which is in line with the researches of Aguilera et al. (2013) and Pilegaard (2013). Finally, the amount of substrate for nitrification and denitrification, i.e. N-NH_4^+ and N-NO_3^- content, indicated conversely results. On the one hand, N_2O and NO emissions should be triggered by nitrification because WFPS levels were not enough high for denitrification. Furthermore, a positive significant correlation was found between N-NH_4^+ and N_2O in Experiment 4 ($R^2=0.42$; $n=131$; $p < 0.0001$, Table 5.12) confirming the nitrification as major source of this gas. Nevertheless, in Experiment 3 other correlations were found between N-NO_3^- and N_2O emissions and between N-NO_3^- and NO , $R^2= 0.73$ and 0.49 ,

respectively ($n=36$; $p < 0.001$). This may indicate that several emissions in rainfed wheat crop could be caused by denitrification, due to the regular rainfalls during that year.

Nitrification is the main mechanisms proposed to explain the inhibition effect of NIs. These compounds (1) interacts with AMO, the enzyme catalyser of the first step of nitrification, by indiscriminate binding to the complex of membrane-bound proteins (for DMPP) or blocking the electron transport (for DCD) (Benckiser et al., 2013), and (2) the removal of the co-factors of AMO (i.e. metals, particularly Cu), thus behaving as chelating compounds (Ruser and Schulz, 2015). The DMPSA had a succinic residue bonded to the pyrazole ring that confer more stability than the phosphate of DMPP (Torrallbo et al., 2017), allowing the DMPSA to be used with other synthetic fertilizers, such as CAN, ammonium sulphate, etc.

An important finding of this thesis is the sink effect of NO promoted by DMPSA both in wheat and maize crop. The possible mechanisms that promoted this sink could be due to different biotic and abiotic processes that could be involved in the consumption of NO, although its magnitude in soils remains uncertain (Medinets et al., 2015). Under our experimental conditions (Experiment 3), a rise in the WFPS, could activate denitrification, such as what occurred 15 DAF, when the sink of NO was also reduced, indicating that this process had low importance in this sink.

Some heterotrophic bacteria can oxidize rather than reduce NO via aerobic co-oxidation reactions (Dunfield and Knowles, 1999; Conrad, 2002). Additionally, NO is also consumed by different organisms due to the function as a free diffusive signalling molecule in higher organisms, which is favoured when O₂ concentration increases in soil (Thomas et al., 2008). The low production of NO through nitrification as a consequence of NI and the high O₂ concentration at low WFPS could have facilitated the consumption of exogenous NO. Abiotic nitrosation by humic substances reactions could also have

acted as a sink for NO in the soil (Medinets et al., 2015). Furthermore, a reversible reaction of NO with OH⁻ producing HONO is a possible pathway that consumes NO from the air inside the soil. The high pH of this soil probably facilitated this reaction. Additionally, we speculated that this sink effect of NO promoted by abiotic processes was easier at low WFPS because it facilitates the interchange between NO from the air and soil. Our results also indicated that the sink effect diminished. It was not detectable even when NI lost its potential to inhibit nitrification, as occurred after 35 DAF (Experiment 3).

In addition, direct diffusive NO uptake by leaves of plants constitutes a canopy sink (Medinets et al., 2015) although, due to the low solubility of NO in the aqueous solution of the apoplastic state, this mechanism is of lower importance than the uptake of NO₂. Comparing NO₂ and NO consumption (Fig. 5.18.a and b) for the U+NI treatment, the sink of NO was greater than that of NO₂ in the period 7-35 DAF. Due to the complexity of the possible processes involved in the deposition of NO in the soil-plant system, additional experiments need to be implemented, also evaluating the effect of other NIs

3. NH₃ volatilization: Is it possible to obtain zero emission?

3.1. Management of pig slurry and ammonia volatilization

Regarding the application of organic fertilizers (Experiment 1) the maximum amount of N was lost with this type of fertilizers, in terms of ratio of N applied (Fig. 6.2). The selection of the adequate source of N fertilizer must be a priority to prevent reactive N losses from crops with high N demand (Aguilera et al., 2013). Combination of pig manure, used at sowing, and synthetic fertilizer as top-dressing is the most common fertilization strategy in irrigated crops in areas with high density of pigs. Application of 50 kg N ha⁻¹ of liquid manure before seeding led to direct NH₃ losses of 14.5% and 10%

of total N applied, for PS and PS+DMPP, respectively (Fig. 6.2). The majority of NH_3 emissions occurred during the first day of PS application with a peak four hours after fertilizing.

Fig. 6.2: Ratio of N applied volatilized as NH_3 for the three different N sources applied in this Thesis. Vertical bars represent standard errors. Capital letters over the standard errors indicate significant differences (LSD, $p < 0.05$). N.S. mean not significant.

This is a common pattern of emission already observed after surface application of slurry in semiarid cropping soils (Gericke et al., 2011). Nevertheless, this amount was considerably lower than that measured by Nicholson et al. (2017) (i.e. 20-40% of N applied), possibly due to specific management practices and more unfavourable weather conditions than in our case (i.e., application over stubble in autumn; higher soil moisture in spring and low wind speed at surface level), where slurry was immediately incorporated into soil using a cultivator, a recommended NH_3 -abating application method (Bittman et al., 2014). Although not considered as a treatment in our study, an immediate mechanical incorporation of pig slurry into the soil facilitates the quick adsorption of N-NH_4^+ to soil colloid thus enhancing the potential of NH_3 abatement (Bittman et al., 2014).

Similarly to the findings made by Tiwary et al. (2015), although incorporation of PS enhances the contact of NH_4^+ within the soil, it was difficult to fully prevent N volatilization in the following hours after incorporation. This could be explained by the high NH_3 (g)/ NH_4^+ ratio found under the pH conditions of the liquid manure and the soil solution (c. 8.2), which may have promoted desorption of NH_3 from the soil solution, provoking rapid diffusion to the atmosphere of the upper soil.

Our results, in line with previous related research, raise an important challenge for the use of manures as fertilizers, a pillar of circular economy in agro-food systems at the regional scale. Sanz-Cobena et al., (2014a) estimated a 57% reduction of manure incorporation without yield penalties at the Spanish national scale (i.e., 113 Gg NH_3 yr⁻¹). Therefore, in highly fertilized areas, incorporation cannot be enough for ensuring the achievement of environmental targets of fertilizing practices. Our results indeed show that, even with the implementation of abatement application practices such as slurry incorporation (Bittman et al., 2014), emissions could remain as high as c. 14% of applied N. Based on this, other reactive N abating initiatives, beyond technical approaches, need to be explored. Most of the livestock producing macro regions of the world are net importers of feed and net exporters of animal products (Lassaletta et al., 2014). This disconnection hampers circular economy since nutrients in manure are hardly recirculated under sustainable conditions within the local cropping systems. Relocation of livestock systems close to cropping systems may facilitate the recycling of manures in an optimal way thus avoiding over application of these materials, and hence reducing losses (Garnier et al., 2016). Again at a larger scale, reduction of the share of animal protein in the human diet might also help finding a better equilibrium between manure production and crop capacity to absorb it, thus reducing N losses to the environment (Westhoek et al., 2013; Billen et al., 2014).

3.2. Management of synthetic fertilizers and NH_3 volatilization

In the case of N synthetic fertilizers, CAN was the better option to mitigate the N losses via volatilization with regards to urea. In the case of CAN and CAN+DMPSA, losses were 2.5% and 1.87% of N applied, respectively. When urea was applied in an irrigated crop (Experiment 2), NH_3 losses were 5.5% of N applied (see Table 5.3), which mainly occurred for 10 days after fertilizer application. The incorporation of fertilizer with a cultivator via mean of irrigation is highly recommended. For top-dressing fertilization in maize, when incorporation of fertilizer is not possible, irrigation is a useful strategy to mitigate NH_3 emissions, because it favours urea incorporation into the soil. Although these losses were considerably lower than those proposed by IPCC (2014) for this fertilizer in summer crops, 5% of N loss to the atmosphere should be considered an important and worrying value.

The substitution of urea by CAN, or non-urea fertilizers, is a recommended strategy to mitigate NH_3 emission (Pan et al., 2016). Furthermore, the incorporation of fertilizer with a cultivator or by mean via irrigation is highly recommended. In Experiment 1, c. 50 mm of water were applied immediately following CAN fertilization thus facilitating its dilution and incorporation into the soil. The pattern of NH_3 emission after CAN application was different than that from PS and also than urea. There was a minor peak of NH_3 emission ($370 \text{ g N ha}^{-1} \text{ d}^{-1}$) 8 days after the irrigation event (Fig. 4.6). Under this conditions, 2.5% of N applied was lost to the atmosphere. Other studies using only CAN gave similar level of losses, i.e- Bussink (1994) observed N losses amounting 1.5 % of total N applied (250 kg N ha^{-1} applied) in grassland.

From a higher scale (country) and according to Sanz-Cobena et al. (2014b), irrigation of synthetic fertilizer may mitigate emission in irrigated crops, but still contributing to $231 \text{ Gg NH}_3 \text{ y}^{-1}$ at the Spanish level. Therefore, similar to the case of manure fertilizers, the

potential of any mitigation strategy addressed to synthetic fertilizers management must be assessed focusing not only on technical solutions but also including potential structural initiatives at regional basis (Sanz-Cobena et al., 2017).

3.3. Ammonia volatilization during senescence

During senescence of leaves, part of NH_4^+ remobilized as amino acids could be lost via stomata (Gregersen et al., 2008). In this experiment, NH_3 losses during this stage of crop with a micrometeorological method were quantified for the first time, constituting an important novelty. Our results indicated that NH_3 loss could be close to 1.2 % on average, which is in the range indicated by Schjoerring and Mattsson (2001). These losses affected all the urea fertilized treatments, both with inhibitors and without. Therefore, this constitutes an important way of NH_3 losses to the air atmosphere. In our Experiments these losses were of the same order to those observed after fertilizer application in the treatment with the double inhibitor or with CAN.

4. Effect of nitrification inhibitor on NH_3 volatilization

In this thesis, the effect of different NIs have been studied on NH_3 volatilization. The NIs delayed nitrification, increasing the pool of N-NH_4^+ in soil, the main precursor of NH_3 . Therefore, it was expected that the use of NIs increased significantly the volatilization. However, our results rejected this hypothesis because no significant differences were found between treatments with and without NIs.

Application of 50 kg N ha^{-1} of liquid manure before seeding led to direct NH_3 losses of 14.5% and 10% of total N applied, for PS and PS+DMPP, respectively. As said, the application of DMPP did not change the pattern or the amount of this emission. The application of DMPSA did not significantly increased or decreased NH_3 volatilization. Hence, losses for CAN were 2.5% and 1.9% of N applied without and with NI,

respectively (see Fig. 6.2). For U treatment, losses were 5.5% and 6.1% when NI was applied.

However, contradictory results could be found in literature. For instance, in the meta-analysis of Pan et al. (2016) the effect of NIs on NH_3 emissions was an increment of 36% on average in these emissions. The study of DMPP isolated of the rest of NIs, showed a mitigation of 32% on NH_3 volatilization. Our results of PS applied with DMPP supported this statement (-31%). The meta-analysis of Yang et al. (2016) indicated that the effect of fertilizer with the nitrification inhibitor, DMPP, diminished NH_3 emissions with regards to the fertilizer alone. Our results about the effect of PS with DMPP (-31%) and of CAN with DMPSA (-25%) endorsed these results. However a clear explanation of NI behaviour was not found. On contrary, only an increment of 9% in ratio of NH_3 emitted and N applied was observed in urea applied with DMPSA (not significantly).

Kim et al. (2012) analysed statically the number of studies that had reported an increase, a decrease or no changes in NH_3 volatilization. The number of studies with an increment in NH_3 losses due to the NIs, was clearly higher than those with a decrease or no changes. Mainly, their results are in accordance with our results related to Urea+DMPSA (Fig. 6.3). A weak point of this meta-analysis was that an important part of studies was done under laboratory conditions (11), even without plants. Only scarce number of the studies included in that study were measured under field conditions (6) using micrometeorological techniques.

Fig. 6.3: Effect of NIs on NH_3 volatilization (%). Negative values indicate that the addition of NIs reduced NH_3 volatilization and positive values indicate an increase in these losses. Horizontal bars represent the standard errors. Pig slurry (PS), CAN and urea indicate the results of this Thesis about the effect of NIs in these different N sources, in comparison with the same N soured but without NI.

5. Efficiency of urease inhibitor and the combination of urease and nitrification inhibitor. Is the double inhibitor a technological solution to simultaneously abate N oxides and NH_3 emission?

Apart from an only nitrification inhibitor, there were treatments in this Thesis where an urease inhibitor was used and assessed. Additionally, the combination of the new DMPSA and the NBPT was assayed.

The most widespread UI (N-butyl thiophosphorictriamide, NBPT) inhibits competitively the enzyme urease (Manunza et al., 1999), thus allowing more time for urea to diffuse into the soil, lowering the amount of N-NH_4^+ susceptible to be transformed in NH_3 and thus volatilized from the upper soil (0–5 cm). Therefore, these compounds effectively abate NH_3 emissions. (Sanz-Cobena et al., 2008a, 2012).

Some authors have observed that urease inhibitors could be used to effectively mitigate the release of other N forms (i.e. NO , N_2O and NO_3^-) from urea-based fertilizers. In trials that an irrigated maize crop was fertilized with urea under similar soil and environmental conditions, the results were rather similar than the results of Experiment 4, where the treatment U+UI significantly lessened N_2O and NO emissions by 35% and 59%, respectively. Approximately, the same inhibitory effect was observed by Sanz-Cobena et al. (2012), 35% for N_2O and 61% for NO . In a more recent study of Guardia et al. (2017), the urea +NBPT reduced significantly these emissions by 50% for N_2O , higher than our results, and 62% for NO , roughly the same. The N_2O and NO abatement effect of NBPT could be due to NBPT, which delayed urea hydrolysis, diminishing N-NH_4^+ content, limiting nitrification rate and thereby, reducing the amount of N-NO_3^- (substrate required for denitrification). This hypothesis is supported by Lam et al. (2018).

Furthermore, in two of the experiments of this Thesis, urea with the double DMPSA + NBPT form was added to assess the capacity of these inhibitors acting together. The mixture of UI and NI has been proposed as a way to reduce total N losses combining the beneficial effect of UI on NH_3 volatilization abatement and that of NIs on the reduction of emissions of N oxides and N leaching (Pan et al., 2016).

In this thesis, U+2I generally resulted in statistically similar N_2O than U+DMPSA (experiment 2 and 4). This result was in agreement with the study of Guardia et al. (2018), which reported that the effectiveness of DMPSA alone was similar than obtained using

DMPSA+NBPT. The low N₂O fluxes as consequence of NI masked the possible benefit effect of NBPT.

In the Experiment 2, there was a significant mitigation by the U+2I treatment, both on N₂O and NH₃. In terms of N₂O, the double inhibitor treatment was statistically similar than U+NI and Control treatment. In terms of NH₃, the amount of NH₃ volatilized was drastically lower than urea and urea with DMPSA (c. 70%). Even higher mitigation than which Lam et al. (2017) reported (40%), although their experiment was based on pastures.

Lam et al. (2018) performed a trial similar than Experiment 2, i.e. they measured NH₃ volatilization comparing urea, urea + DMPP and urea + NBPT instead of urea, urea + DMPSA and urea + DMPSA + NBPT, as our case. The treatment with NBPT in this study mitigated the NH₃ by 44%. Taking into account the findings of this Thesis, we hypothesizes that only the UI is the responsible of the abatement of NH₃ emissions, since there was not a significant effect of NIs in these emissions. Hence, the results of Lam et al. (2018) may support the NH₃-mitigation effect of the double inhibitor (by 70 %) in Experiment 2. Also, in this study the NI, DMPP did not increase ammonia volatilization, just like in Experiment 1 and 2 of this Thesis.

Other authors, such as, Drury et al. (2017), observed a mean reduction of NH₃ volatilization by 67(±9)% in the U+2I treatment during 2 years of experiment in a maize crop, using DCD instead of DMPSA as NI. These outcomes were more in accordance with our results. Furthermore, they observed N₂O emission mitigation in this treatment too, but not significantly.

On the other hand, the effect of the double inhibitor on NO_x emissions have not been widely studied (Sanz-Cobena et al., 2012; Guardia et al., 2018a). The automatic NO_x measurements system presented in this Thesis, helped to take a global information about when these emissions were produced. In Experiment 4, the use of the double inhibitor

reduced a 70% of the total NO emission, which was a greater inhibitory effect than DMPSA alone. As it has been discussed in chapter 3 a plausible hypothesis would be that the mobility of urea in soil and DMPSA with irrigation water could have produced a spatial dislocation of DMPSA and urea. Likely, urea (which is more soluble and mobile than DMPSA) was located in microsites where the NI was not placed, affecting the effectiveness of the NI.

6. Integrating yield, crop quality and mitigation of gaseous N losses

In general, grain, biomass yield and crop quality were significantly increased by the application of N fertilizers with respect to Control treatment in all experiments, but there was not significant difference among fertilizer treatments. Only in the experiment 4 on drip irrigated maize grain yield increased 16% when U was mixed with NI, but this was not significant at $P < 0.05$. Neither N protein content nor quality for bread-making in wheat was improved using NI or the double inhibitor.

The main value of this fertilizer technology (using nitrification or urease inhibitor) is based on reducing yield scaled of N_2O , NO and NH_3 . In Table 6.3., it has been included the mean values of these factors. It could be concluded that $YS-NH_3$ was mainly reduced with the double inhibitor in maize reducing 2.3 times this negative effect. In the case of $YS-N_2O$ the most efficient was the use of a nitrification inhibitor: 1.5, 5.2, 3.5 and 2.5 times for experiments 1, 2, 3 and 4, respectively. In the case of the double inhibitor $YS-N_2O$ was reduced 11.1 and 1.8 times in relation to U for experiment 2 and 4, respectively. For the $YS-NO$, NI reduced 3.3 times its value in drip irrigate maize, whereas the double inhibitor was mitigated 8.8 times in this experiment. Under a ranger irrigation system, NI gave negative $YS-NO$, which is not frequent in agricultural systems (see Table 6.3).

CHAPTER 7

CONCLUSIONS

CHAPTER 7:

- 1 The agronomic strategies implemented in this study focusing on reducing NH_3 losses through volatilization (fertilizer incorporation, irrigation after fertilizer application, urease inhibitors) led to a level of NH_3 emission that was very dependent on soil environmental conditions and N source. In the case of PS, the rapid losses occurred in the following hours after application constitutes an important challenge for the use of these manures as fertilizers, even if abating practices are implemented. In the case of N-NH_4^+ based-fertilizers the possibilities of mitigation are higher but emission zero is not possible. Based on this conclusion, the threshold of direct emissions could be revised.
- 2 Contrarily to that it has been hypothesized in this thesis, the use of NIs (DMPP or DMPSA) did not increase NH_3 volatilization in irrigated maize, probably because the benefit of incorporating PS into the soil as well as the irrigation applied immediately after CAN or urea fertilization. Both strategies favoured the quick adsorption of N-NH_4^+ to the soil colloid. In fact, a significant higher N-NH_4^+ concentration in soils was observed as consequence of NIs for several weeks, but fluxes of NH_3 were similar than these from the soil without NI in the same period.
- 3 It has been confirmed that a detectable amount of NH_3 was emitted directly by plants during senescence, independent if crops were or not fertilized. In the case of maize, these N losses ranged from 2-4 kg N ha⁻¹.
- 4 The new inhibitor DMPSA applied with urea significantly reduced direct N_2O , both under rainfed or under irrigated conditions. In both cases, nitrification of N-NH_4^+ was an important source of N_2O . Mitigation option with DMPSA was reduced when N_2O fluxes after fertilizer application was low, as occurred when DMPSA was mixed with CAN in maize. Also, NI did reduce the total N_2O emission, which included indirect N_2O emission associated to NH_3 volatilization.

- 5 A software based on R programme was implemented to measure efficiently NO, NO₂ and O₃ fluxes using an automated chambers system, where a high number of data was obtained along experimental period. The code has allowed to obtain the steady state concentration in each chamber to compare with concentration obtained in an ambient chamber and to calculate fluxes and cumulative emission in each replicate and, hence in each treatment.
- 6 Cumulative NO fluxes, measured for the first time at high temporal resolution using an automated system in a rainfed wheat crop were negative for the DMPSA treatment. In this case, NO production through nitrification was lower than consumption through different biotic and abiotic processes for 5 weeks after application. For irrigated maize, negative values were also frequent, but cumulative NO fluxes were positive and similar to the Control treatment.
- 7 The use of the double inhibitor (DMPSA plus NBPT) constitute an interesting agronomic and environmental option, because effectively reduced NH₃ volatilization, N₂O and NO emission, maintaining crop yield in irrigated maize.
- 8 Most of the fluxes of NO₂ measured with an automatic chamber system were negative. Its contribution to NO_x emission was lower than 2% in all treatments. In general, the correction of NO fluxes using the possible reactions of NO with O₃ given NO₂, and the photolysis of this last gas during sunlight hours resulted in a total amount lower than 5% of uncorrected NO. In these experiments, underestimation of NO was produced when fluxes where not corrected.
- 9 In general, grain and biomass yield were not affect by the use of NI or UI. However, an important reduction of Yield-N₂O and yield NO was obtained when these inhibitor was used with urea, both in rainfed or irrigated soils.

- 1 Las estrategias agronómicas implementadas en este estudio para reducir pérdidas por volatilización de NH_3 , como la incorporación de los fertilizantes, el riego posterior a su aplicación o el uso de inhibidores de la ureasa, generaron emisiones muy dependientes de las condiciones ambientales y de la fuente de N. En el caso del purín de cerdo (PS) las rápidas pérdidas tras su incorporación constituyen un importante reto para su uso, incluso cuando se utilizan estrategias de mitigación. En el caso de fertilizantes amónicos o ureicos las estrategias son más efectivas, pero emisión cero no es posible. Este comportamiento debería ser tenido en cuenta en los niveles a alcanzar en cada una de las estrategias.
- 2 Contrariamente a la hipótesis planteada en la Tesis, el uso de los inhibidores de la nitrificación DMPSA y DMPP no incrementó significativamente la volatilización de NH_3 en maíz irrigado. Probablemente debido al beneficio de la incorporación del PS en el suelo, así como el riego inmediatamente posterior al uso de CAN o urea. Ambas estrategias favorecieron la rápida adsorción de N-NH_4^+ en el coloide del suelo, detectándose una significativa mayor concentración de este ion en las semanas siguientes a la aplicación del inhibidor de la nitrificación (IN), que no tuvo consecuencias en las emisiones de amoníaco.
- 3 Se ha comprobado que existen pérdidas de NH_3 por volatilización durante la senescencia de maíz, independientemente de si el cultivo había sido fertilizado o no. Estas pérdidas estaban en el rango de $2\text{-}4 \text{ kg N ha}^{-1}$.
- 4 El nuevo inhibidor DMPSA aplicado junto a urea redujo significativamente las emisiones directas de N_2O , tanto en condiciones de secano como de regadío. En ambos casos, la nitrificación del N-NH_4^+ fue una importante fuente de N_2O . Las opciones de mitigación con DMPSA se redujeron cuando los flujos de N_2O eran bajos, como ocurrió con el DMPSA mezclado con CAN en maíz. De igual forma,

- el NI redujo las emisiones totales de N_2O , que incluyen las indirectas de N_2O asociadas a la volatilización del NH_3
- 5 Se ha implementado un software basado en código R para medir de manera eficiente los flujos de NO , NO_2 and O_3 con cámaras automáticas, donde un elevado número de datos se obtenían en cada tratamiento. El código ha permitido obtener el ‘steady state’ en cada cámara, su comparación con la concentración obtenida en la cámara ambiente y el cálculo de flujos y de emisiones acumuladas en cada tratamiento.
 - 6 Midiendo por primera vez flujos acumulados de NO con un sistema de cámaras automáticas y de alta resolución temporal se obtuvieron flujos negativos en un cultivo de trigo en el tratamiento con DMPSA. En este caso, la producción de NO era inferior al consumo de este gas a través de otros procesos bióticos o abióticos durante 5 semanas. Para maíz irrigado también hubo frecuentes flujos negativos, aunque el valor acumulado fue positivo, y muy similar al del tratamiento control.
 - 7 El empleo del doble inhibidor, obtenido por mezcla del DMPSA y NBPT, constituye un interesante estrategia agronómica y ambiental, ya que se reduce efectivamente la volatilización de NH_3 y las emisiones de N_2O y NO , manteniendo a su vez el rendimiento del cultivo de maíz irrigado.
 - 8 La mayor parte de los flujos de NO_2 , medidos con cámaras automáticas, fueron negativos. Su contribución al NO_x total fue menor al 2% en todos los tratamientos. En general, la corrección de flujos de NO considerando las reacciones del NO con O_3 para dar NO_2 y la fotólisis de este último durante las horas de luz solar fue inferior al 5% del NO no corregido. En estos experimentos, una subestimación del NO se producía al no considerar la corrección de flujos.

- 9 En general, el uso de inhibidores de la nitrificación y de la ureasa con urea tanto en trigo en secano como maíz en regadío no afectó a los rendimientos de grano y biomasa, pero a los correspondientes a rendimientos escalados a N_2O

REFERENCES

- Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., Vallejo, A., 2014a. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agric. Ecosyst. Environ.* 189, 136–144. <https://doi.org/10.1016/j.agee.2014.03.036>
- Abalos, D., Sanchez-Martin, L., Garcia-Torres, L., van Groenigen, J.W., Vallejo, A., 2014b. Management of irrigation frequency and nitrogen fertilization to mitigate GHG and NO emissions from drip-fertigated crops. *Sci. Total Environ.* 490, 880–888. <https://doi.org/10.1016/j.scitotenv.2014.05.065>
- Abalos, D., Sanz-Cobena, A., Andreu, G., Vallejo, A., 2017. Rainfall amount and distribution regulate DMPP effects on nitrous oxide emissions under semiarid Mediterranean conditions. *Agric. Ecosyst. Environ.*, Quantification and mitigation of greenhouse gas emissions in Mediterranean cropping systems 238, 36–45. <https://doi.org/10.1016/j.agee.2016.02.003>
- Abalos, D., Sanz-Cobena, A., Garcia-Torres, L., Groenigen, J.W. van, Vallejo, A., 2013. Role of maize stover incorporation on nitrogen oxide emissions in a non-irrigated Mediterranean barley field. *Plant Soil* 364, 357–371. <https://doi.org/10.1007/s11104-012-1367-4>
- Abalos, D., Sanz-Cobena, A., Misselbrook, T., Vallejo, A., 2012. Effectiveness of urease inhibition on the abatement of ammonia, nitrous oxide and nitric oxide emissions in a non-irrigated Mediterranean barley field. *Chemosphere* 89, 310–318. <https://doi.org/10.1016/j.chemosphere.2012.04.043>
- Aguilera, E., Lassaletta, L., Sanz-Cobena, A., Garnier, J., Vallejo, A., 2013. The potential of organic fertilizers and water management to reduce N₂O emissions in Mediterranean climate cropping systems. A review. *Agric. Ecosyst. Environ.* 164, 32–52. <https://doi.org/10.1016/j.agee.2012.09.006>
- Aita, C., Gonzatto, R., Miola, E.C.C., B, D., Santos, dos, Rochette, P., Angers, D.A., Chantigny, M.H., Pujol, S.B., Giacomini, D.A., Giacomini, S.J., 2014. Injection of Dicyandiamide-Treated Pig Slurry Reduced Ammonia Volatilization without Enhancing Soil Nitrous Oxide Emissions from No-Till Corn in Southern Brazil. *J. Environ. Qual.* 43, 789. <https://doi.org/10.2134/jeq2013.07.0301>
- Akiyama, H., Yan, X., Yagi, K., 2010. Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N₂O and NO emissions from agricultural soils: meta-analysis. *Glob. Change Biol.* 16, 1837–1846. <https://doi.org/10.1111/j.1365-2486.2009.02031.x>
- Allen, R.G., Pereira, L.S., Raes, D., Smith, M., n.d. Crop evapotranspiration - Guidelines for computing crop water requirements - FAO Irrigation and drainage paper 56 15.
- Aneja, V.P., Schlesinger, W.H., Erisman, J.W., 2009. Effects of Agriculture upon the Air Quality and Climate: Research, Policy, and Regulations. *Environ. Sci. Technol.* 43, 4234–4240. <https://doi.org/10.1021/es8024403>
- Back, B.H., Aneja, V.P., 2004. Measurement and Analysis of the Relationship between Ammonia, Acid Gases, and Fine Particles in Eastern North Carolina. *J. Air Waste Manag. Assoc.* 54, 623–633. <https://doi.org/10.1080/10473289.2004.10470933>

- Bai, M., Sun, J., Denmead, O.T., Chen, D., 2017. Comparing emissions from a cattle pen as measured by two micrometeorological techniques. *Environ. Pollut.* 230, 584–588. <https://doi.org/10.1016/j.envpol.2017.07.012>
- Becaccia, A., Ferrer, P., Ibañez, M.A., Estellés, F., Rodríguez, C., Moset, V., De Blas, C., Calvet, S., García-Rebollar, P., 2015. Relationships among slurry characteristics and gaseous emissions at different types of commercial Spanish pig farms. *Span. J. Agric. Res.* 13, e0602. <https://doi.org/10.5424/sjar/2015131-6575>
- Benckiser, G., Christ, E., Herbert, T., Weiske, A., Blome, J., Hardt, M., 2013. The nitrification inhibitor 3,4-dimethylpyrazole-phosphat (DMPP) - quantification and effects on soil metabolism. *Plant Soil* 371, 257–266. <https://doi.org/10.1007/s11104-013-1664-6>
- Billen, G., Lassaletta, L., Garnier, J., 2014. A biogeochemical view of the global agro-food system: Nitrogen flows associated with protein production, consumption and trade. *Glob. Food Secur., SI: GFS Conference 2013* 3, 209–219. <https://doi.org/10.1016/j.gfs.2014.08.003>
- Billen, G., Lassaletta, L., Garnier, J., 2015. A vast range of opportunities for feeding the world in 2050: trade-off between diet, N contamination and international trade. *Environ. Res. Lett.* 10, 025001. <https://doi.org/10.1088/1748-9326/10/2/025001>
- Bittman, S., Dedina, M., Howard, C.M. (Clare), Oenema, O., Sutton, M.A., UNECE, 2014. Options for ammonia mitigation: guidance from the UNECE Task Force on Reactive Nitrogen. Centre for Ecology & Hydrology, on behalf of Task Force on Reactive Nitrogen, of the UNECE Convention on Long Range transboundary Air Pollution, Edinburgh.
- Bouwman A. F., Boumans L. J. M., Batjes N. H., 2002. Modeling global annual N₂O and NO emissions from fertilized fields. *Glob. Biogeochem. Cycles* 16, 28–1. <https://doi.org/10.1029/2001GB001812>
- Bruulsema, T., Lemunyon, J., Herz, B., 2009. Know your fertilizer rights 6.
- Bussink, D.W., 1994. Relationships between ammonia volatilization and nitrogen fertilizer application rate, intake and excretion of herbage nitrogen by cattle on grazed swards. *Fertil. Res.* 38, 111–121. <https://doi.org/10.1007/BF00748771>
- Butterbach-Bahl, K., Gasche, R., Breuer, L., Papen, H., 1997. Fluxes of NO and N₂O from temperate forest soils: impact of forest type, N deposition and of liming on the NO and N₂O emissions. *Nutr. Cycl. Agroecosystems* 48, 79–90. <https://doi.org/10.1023/A:1009785521107>
- Butterbach-Bahl, K., Gundersen, P., Ambus, P., Augustin, J., Beier, C., Boeckx, P., Dannenmann, M., Sanchez Gimeno, B., Ibrom, A., Kiese, R., Kitzler, B., Rees, R.M., Smith, K.A., Stevens, C., Vesala, T., Zechmeister-Boltenstern, S., 2011. Nitrogen processes in terrestrial ecosystems, in: *The European Nitrogen Assessment: Sources, Effects and Policy Perspectives*. Cambridge University Press, pp. 99–125.
- Calvete-Sogo, H., Elvira, S., Sanz, J., González-Fernández, I., García-Gómez, H., Sánchez-Martín, L., Alonso, R., Bermejo-Bermejo, V., 2014. Current ozone levels threaten gross primary production and yield of Mediterranean annual

- pastures and nitrogen modulates the response. *Atmos. Environ.* 95, 197–206. <https://doi.org/10.1016/j.atmosenv.2014.05.073>
- Cayuela, M.L., Aguilera, E., Sanz-Cobena, A., Adams, D.C., Abalos, D., Barton, L., Ryals, R., Silver, W.L., Alfaro, M.A., Pappa, V.A., Smith, P., Garnier, J., Billen, G., Bouwman, L., Bondeau, A., Lassaletta, L., 2017. Direct nitrous oxide emissions in Mediterranean climate cropping systems: Emission factors based on a meta-analysis of available measurement data. *Agric. Ecosyst. Environ.* 238, 25–35. <https://doi.org/10.1016/j.agee.2016.10.006>
- Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B., Misselbrook, T., 2011. Manure management: Implications for greenhouse gas emissions. *Anim. Feed Sci. Technol.* 166–167, 514–531. <https://doi.org/10.1016/j.anifeedsci.2011.04.036>
- Conrad, R., 1996. Metabolism of Nitric Oxide in Soil and Soil Microorganisms and Regulation of Flux into the Atmosphere, in: Murrell, J.C., Kelly, D.P. (Eds.), *Microbiology of Atmospheric Trace Gases*, NATO ASI Series. Springer Berlin Heidelberg, pp. 167–203.
- Conrad, R., 2002. Control of microbial methane production in wetland rice fields. *Nutr. Cycl. Agroecosystems* 64, 59–69. <https://doi.org/10.1023/A:1021178713988>
- Cui, F., Yan, G., Zhou, Z., Zheng, X., Deng, J., 2012. Annual emissions of nitrous oxide and nitric oxide from a wheat–maize cropping system on a silt loam calcareous soil in the North China Plain. *Soil Biol. Biochem.* 48, 10–19. <https://doi.org/10.1016/j.soilbio.2012.01.007>
- Danielson, R.E., Sutherland, P.L., 1986. Porosity. *Methods Soil Anal. Part 1—Physical Mineral. Methods* sssabookseries, 443–461. <https://doi.org/10.2136/sssabookser5.1.2ed.c18>
- Davidson, E.A., Savage, K., Verchot, L.V., Navarro, R., 2002. Minimizing artifacts and biases in chamber-based measurements of soil respiration. *Agric. For. Meteorol., FLUXNET 2000 Synthesis* 113, 21–37. [https://doi.org/10.1016/S0168-1923\(02\)00100-4](https://doi.org/10.1016/S0168-1923(02)00100-4)
- Denmead, O.T., Simpson, J.R., Freney, J.R., 1977. A Direct Field Measurement of Ammonia Emission After Injection of Anhydrous Ammonia. *Soil Sci. Soc. Am. J.* 41, 1001. <https://doi.org/10.2136/sssaj1977.03615995004100050039x>
- Denmead, O.T., 1983. Micrometeorological methods for measuring gaseous losses of nitrogen in the field, in: Freney, J.R., Simpson, J.R. (Eds.), *Gaseous Loss of Nitrogen from Plant-Soil Systems*, Developments in Plant and Soil Sciences. Springer Netherlands, Dordrecht, pp. 133–157. https://doi.org/10.1007/978-94-017-1662-8_5
- Denmead, O.T., 2008. Approaches to measuring fluxes of methane and nitrous oxide between landscapes and the atmosphere. *Plant Soil* 309, 5–24. <https://doi.org/10.1007/s11104-008-9599-z>
- Dick, J., Quick, J., 1983. A Modified Screening-Test for Rapid Estimation of Gluten Strength in Early-Generation Durum-Wheat Breeding Lines. *Cereal Chem.* 60, 315–318.

- Dickerson, R.R., Delany, A.C., Wartburg, A.F., 1984. Further modification of a commercial NO_x detector for high sensitivity. *Rev. Sci. Instrum.* 55, 1995–1998. <https://doi.org/10.1063/1.1137694>
- Drury, C.F., Yang, X., Reynolds, W.D., Calder, W., Oloya, T.O., Woodley, A.L., 2017. Combining Urease and Nitrification Inhibitors with Incorporation Reduces Ammonia and Nitrous Oxide Emissions and Increases Corn Yields. *J. Environ. Qual.* 46, 939–949. <https://doi.org/10.2134/jeq2017.03.0106>
- Dunfield, P.F., Knowles, R., 1999. Nitrogen monoxide production and consumption in an organic soil. *Biol. Fertil. Soils* 30, 153–159. <https://doi.org/10.1007/s003740050602>
- EEA Report. Air quality in Europe - 2018 [WWW Document], n.d. European Environmental. Agency. URL <https://www.eea.europa.eu/publications/air-quality-in-europe-2018> (accessed 1.3.19).
- EEA Report. Air quality in Europe -2017 report [WWW Document], n.d. European Environmental. Agency. URL <https://www.eea.europa.eu/publications/air-quality-in-europe-2017> (accessed 1.3.19).
- FAO (Ed.), 2004. Food energy: methods of analysis and conversion factors ; report of a technical workshop, Rome, 3 - 6 December 2002, FAO food and nutrition paper. Food and Agriculture Organization of the United Nations, Rome.
- FAO. (2015). World fertilizer trends and outlook to 2018. Food and Agriculture organization of the United Nations (FAO), Rome, ISBN 978-92-5-108692-6, Annual Report 14.
- Firestone, M. K., Davidson, E. A., 1989. Microbiological basis of NO and N₂O production and consumption in soil. *Exch. Trace Gases Terr. Ecosyst. Atmosphere* 47, 7–21.
- Fuertes-Mendizábal, T., González-Torralba, J., Arregui, L.M., González-Murua, C., González-Moro, M.B., Estavillo, J.M., 2013. Ammonium as sole N source improves grain quality in wheat: Ammonium as sole N source improves grain quality in wheat. *J. Sci. Food Agric.* 93, 2162–2171. <https://doi.org/10.1002/jsfa.6022>
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science* 320, 889–892. <https://doi.org/10.1126/science.1136674>
- Garnier, J., Anglade, J., Benoit, M., Billen, G., Puech, T., Ramarson, A., Passy, P., Silvestre, M., Lassaletta, L., Trommenschlager, J.-M., Schott, C., Tallec, G., 2016. Reconnecting crop and cattle farming to reduce nitrogen losses to river water of an intensive agricultural catchment (Seine basin, France): past, present and future. *Environ. Sci. Policy* 63, 76–90. <https://doi.org/10.1016/j.envsci.2016.04.019>
- Garrido, F., Hénault, C., Gaillard, H., Pérez, S., Germon, J.C., 2002. N₂O and NO emissions by agricultural soils with low hydraulic potentials. *Soil Biol. Biochem.* 34, 559–575. [https://doi.org/10.1016/S0038-0717\(01\)00172-9](https://doi.org/10.1016/S0038-0717(01)00172-9)

- Gericke, D., Pacholski, A., Kage, H., 2011. Measurement of ammonia emissions in multi-plot field experiments. *Biosyst. Eng.* 108, 164–173. <https://doi.org/10.1016/j.biosystemseng.2010.11.009>
- Gill, J.S., Bijay-Singh, Khind, C.S., Yadvinder-Singh, 1999. Efficiency of N-(n-butyl) thiophosphoric triamide in retarding hydrolysis of urea and ammonia volatilization losses in a flooded sandy loam soil amended with organic materials. *Nutr. Cycl. Agroecosystems* 53, 203–207. <https://doi.org/10.1023/A:1009702707389>
- Gilsanz, C., Báez, D., Misselbrook, T.H., Dhanoa, M.S., Cárdenas, L.M., 2016. Development of emission factors and efficiency of two nitrification inhibitors, DCD and DMPP. *Agric. Ecosyst. Environ.* 216, 1–8. <https://doi.org/10.1016/j.agee.2015.09.030>
- Gothenburg Protocol - Air Pollution - Environmental Policy - UNECE [WWW Document], n.d. URL <http://www.unece.org/environmental-policy/conventions/air/guidance-documents-and-other-methodological-materials/gothenburg-protocol.html> (accessed 1.3.19).
- Grant, C.A., Brown, K.R., Bailey, L.D., Jia, S., 1996. SHORT COMMUNICATION: Volatile losses of NH₃ from surface-applied urea and urea ammonium nitrate with and without the urease inhibitors NBPT or ammonium thiosulphate. *Can. J. Soil Sci.* 76, 417–419. <https://doi.org/10.4141/cjss96-050>
- Gregersen, P.L., Holm, P.B., Krupinska, K., 2008. Leaf senescence and nutrient remobilisation in barley and wheat. *Plant Biol.* 10, 37–49. <https://doi.org/10.1111/j.1438-8677.2008.00114.x>
- Guardia, G., Cangani, M.T., Andreu, G., Sanz-Cobena, A., García-Marco, S., Álvarez, J.M., Recio-Huetos, J., Vallejo, A., 2017a. Effect of inhibitors and fertigation strategies on GHG emissions, NO fluxes and yield in irrigated maize. *Field Crops Res.* 204, 135–145. <https://doi.org/10.1016/j.fcr.2017.01.009>
- Guardia, G., Cangani, M.T., Sanz-Cobena, A., Junior, J.L., Vallejo, A., 2017b. Management of pig manure to mitigate NO and yield-scaled N₂O emissions in an irrigated Mediterranean crop. *Agric. Ecosyst. Environ.* 238, 55–66. <https://doi.org/10.1016/j.agee.2016.09.022>
- Guardia, G., Sanz-Cobena, A., Sanchez-Martín, L., Fuertes-Mendizábal, T., González-Murua, C., Álvarez, J.M., Chadwick, D., Vallejo, A., 2018a. Urea-based fertilization strategies to reduce yield-scaled N oxides and enhance bread-making quality in a rainfed Mediterranean wheat crop. *Agric. Ecosyst. Environ.* 265, 421–431. <https://doi.org/10.1016/j.agee.2018.06.033>
- Guardia, G., Vallejo, A., Cardenas, L.M., Dixon, E.R., García-Marco, S., 2018b. Fate of 15 N-labelled ammonium nitrate with or without the new nitrification inhibitor DMPSA in an irrigated maize crop. *Soil Biol. Biochem.* 116, 193–202. <https://doi.org/10.1016/j.soilbio.2017.10.013>
- Hallin, S., Philippot, L., Löffler, F.E., Sanford, R.A., Jones, C.M., 2018. Genomics and Ecology of Novel N₂O-Reducing Microorganisms. *Trends Microbiol.* 26, 43–55. <https://doi.org/10.1016/j.tim.2017.07.003>

- Harrison, R., Webb, J., 2001. A review of the effect of N fertilizer type on gaseous emissions, in: *Advances in Agronomy*. Elsevier, pp. 65–108. [https://doi.org/10.1016/S0065-2113\(01\)73005-2](https://doi.org/10.1016/S0065-2113(01)73005-2)
- Holcomb, J.C., Sullivan, D.M., Horneck, D.A., Clough, G.H., 2011. Effect of Irrigation Rate on Ammonia Volatilization. *Soil Sci. Soc. Am. J.* 75, 2341. <https://doi.org/10.2136/sssaj2010.0446>
- Huérfano, X., Fuertes-Mendizábal, T., Duñabeitia, M.K., González-Murua, C., Estavillo, J.M., Menéndez, S., 2015. Splitting the application of 3,4-dimethylpyrazole phosphate (DMPP): Influence on greenhouse gases emissions and wheat yield and quality under humid Mediterranean conditions. *Eur. J. Agron.* 64, 47–57. <https://doi.org/10.1016/j.eja.2014.11.008>
- Huérfano, X., Fuertes-Mendizábal, T., Fernández-Diez, K., Estavillo, J.M., González-Murua, C., Menéndez, S., 2016. The new nitrification inhibitor 3,4-dimethylpyrazole succinic (DMPSA) as an alternative to DMPP for reducing N₂O emissions from wheat crops under humid Mediterranean conditions. *Eur. J. Agron.* 80, 78–87. <https://doi.org/10.1016/j.eja.2016.07.001>
- IPCC, 2007. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press Cambridge, UK and New York, NY, USA, pp. 996.
- IPCC, 2014. *Climate Change 2014: Mitigation of climate change. Contribution of working group III to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK and New York, NY, USA.
- Kafarski, P., Talma, M., 2018. Recent advances in design of new urease inhibitors: A review. *J. Adv. Res., Biotechnological and medical relevance of ureases* 13, 101–112. <https://doi.org/10.1016/j.jare.2018.01.007>
- Kim, D.-G., Saggar, S., Roudier, P., 2012. The effect of nitrification inhibitors on soil ammonia emissions in nitrogen managed soils: a meta-analysis. *Nutr. Cycl. Agroecosystems* 93, 51–64. <https://doi.org/10.1007/s10705-012-9498-9>
- Klute, A., Page, A.L. (Eds.), 1982. *Methods of soil analysis*, 2nd ed. ed, Agronomy. American Society of Agronomy : Soil Science Society of America, Madison, Wis.
- Kou, Y., Wei, K., Gx, C., Zy, W., Xu, H., 2016. Effects of 3,4-dimethylpyrazole phosphate and dicyandiamide on nitrous oxide emission in a greenhouse vegetable soil. *Plant Soil Environ.* 61, 29–35. <https://doi.org/10.17221/762/2014-PSE>
- Lam, S.K., Suter, H., Bai, M., Walker, C., Davies, R., Mosier, A.R., Chen, D., 2018. Using urease and nitrification inhibitors to decrease ammonia and nitrous oxide emissions and improve productivity in a subtropical pasture. *Sci. Total Environ.* 644, 1531–1535. <https://doi.org/10.1016/j.scitotenv.2018.07.092>
- Lam, S.K., Suter, H., Mosier, A.R., Chen, D., 2017. Using nitrification inhibitors to mitigate agricultural N₂O emission: a double-edged sword? *Glob. Change Biol.* 23, 485–489. <https://doi.org/10.1111/gcb.13338>
- Lassaletta, L., Billen, G., Grizzetti, B., Anglade, J., Garnier, J., 2014. 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield

- and nitrogen input to cropland. *Environ. Res. Lett.* 9, 105011. <https://doi.org/10.1088/1748-9326/9/10/105011>
- Laville, P., Lehuger, S., Loubet, B., Chaumartin, F., Cellier, P., 2011. Effect of management, climate and soil conditions on N₂O and NO emissions from an arable crop rotation using high temporal resolution measurements. *Agric. For. Meteorol.* 151, 228–240. <https://doi.org/10.1016/j.agrformet.2010.10.008>
- Leuning, R., Freney, J.R., Denmead, O.T., Simpson, J.R., 1985. A sampler for measuring atmospheric ammonia flux. *Atmospheric Environ.* 19, 1117–1124. [https://doi.org/10.1016/0004-6981\(85\)90196-9](https://doi.org/10.1016/0004-6981(85)90196-9)
- Li, H., Chen, Y., Liang, X., Lian, Y., Li, W., 2009. Mineral-nitrogen Leaching and Ammonia Volatilization from a Rice–Rapeseed System as Affected by 3,4-Dimethylpyrazole Phosphate. *J. Environ. Qual.* 38, 2131. <https://doi.org/10.2134/jeq2008.0476>
- Liu, S., Lin, F., Wu, S., Ji, C., Sun, Y., Jin, Y., Li, S., Li, Z., Zou, J., 2017. A meta-analysis of fertilizer-induced soil NO and combined NO+N₂O emissions. *Glob. Change Biol.* 23, 2520–2532. <https://doi.org/10.1111/gcb.13485>
- Loick, N., Dixon, E.R., Abalos, D., Vallejo, A., Matthews, G.P., McGeough, K.L., Well, R., Watson, C.J., Laughlin, R.J., Cardenas, L.M., 2016. Denitrification as a source of nitric oxide emissions from incubated soil cores from a UK grassland soil. *Soil Biol. Biochem.* 95, 1–7. <https://doi.org/10.1016/j.soilbio.2015.12.009>
- Ludwig, J., Meixner, F.X., Vogel, B., Förstner, J., 2001. Soil-air exchange of nitric oxide: An overview of processes, environmental factors, and modeling studies. *Biogeochemistry* 52, 225–257. <https://doi.org/10.1023/A:1006424330555>
- Manunza, B., Deiana, S., Pintore, M., Gessa, C., 1999. The binding mechanism of urea, hydroxamic acid and N-(N-butyl)-phosphoric triamide to the urease active site. A comparative molecular dynamics study. *Soil Biol. Biochem.* 31, 789–796. [https://doi.org/10.1016/S0038-0717\(98\)00155-2](https://doi.org/10.1016/S0038-0717(98)00155-2)
- Medinets, S., Gasche, R., Skiba, U., Medinets, V., Butterbach-Bahl, K., 2016. The impact of management and climate on soil nitric oxide fluxes from arable land in the Southern Ukraine. *Atmos. Environ.* 137, 113–126. <https://doi.org/10.1016/j.atmosenv.2016.04.032>
- Medinets, S., Skiba, U., Rennenberg, H., Butterbach-Bahl, K., 2015. A review of soil NO transformation: Associated processes and possible physiological significance on organisms. *Soil Biol. Biochem.* 80, 92–117. <https://doi.org/10.1016/j.soilbio.2014.09.025>
- Meijide, A., Díez, J.A., Sánchez-Martín, L., López-Fernández, S., Vallejo, A., 2007. Nitrogen oxide emissions from an irrigated maize crop amended with treated pig slurries and composts in a Mediterranean climate. *Agric. Ecosyst. Environ.* 121, 383–394. <https://doi.org/10.1016/j.agee.2006.11.020>
- Menéndez, S., Barrena, I., Setien, I., González-Murua, C., Estavillo, J.M., 2012. Efficiency of nitrification inhibitor DMPP to reduce nitrous oxide emissions under different temperature and moisture conditions. *Soil Biol. Biochem.* 53, 82–89. <https://doi.org/10.1016/j.soilbio.2012.04.026>
- Menéndez, S., López-Bellido, R.J., Benítez-Vega, J., González-Murua, C., López-Bellido, L., Estavillo, J.M., 2008. Long-term effect of tillage, crop rotation and N

- fertilization to wheat on gaseous emissions under rainfed Mediterranean conditions. *Eur. J. Agron.* 28, 559–569. <https://doi.org/10.1016/j.eja.2007.12.005>
- Minamikawa, K., Tokida, T., Sudo, S., Padre, A., Yagi, K. (2015) Guidelines for measuring CH₄ and N₂O emissions from rice paddies by a manually operated closed chamber method. National Institute for Agro-Environmental Sciences, Tsukuba, Japan.
- Misselbrook, T., Hansen, M., 2001. Field evaluation of the equilibrium concentration technique (JTI method) for measuring ammonia emission from land spread manure or fertiliser. *Atmos. Environ.* 35, 3761–3768. [https://doi.org/10.1016/S1352-2310\(01\)00169-8](https://doi.org/10.1016/S1352-2310(01)00169-8)
- Misselbrook, T.H., Cardenas, L.M., Camp, V., Thorman, R.E., Williams, J.R., Rollett, A.J., Chambers, B.J., 2014. An assessment of nitrification inhibitors to reduce nitrous oxide emissions from UK agriculture. *Environ. Res. Lett.* 9, 115006. <https://doi.org/10.1088/1748-9326/9/11/115006>
- Montgomery, R.B., 1948. Vertical eddy flux of heat in the atmosphere. *J. Meteorol.* 5, 265–274. [https://doi.org/10.1175/1520-0469\(1948\)005<0265:VEFOHI>2.0.CO;2](https://doi.org/10.1175/1520-0469(1948)005<0265:VEFOHI>2.0.CO;2)
- Mosier, A., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S., Cleemput, O. van, 1998. Closing the global N₂O budget: nitrous oxide emissions through the agricultural nitrogen cycle. *Nutr. Cycl. Agroecosystems* 52, 225–248. <https://doi.org/10.1023/A:1009740530221>
- Nelson, A.J., Koloutsou-Vakakis, S., Rood, M.J., Myles, L., Lehmann, C., Bernacchi, C., Balasubramanian, S., Joo, E., Heuer, M., Vieira-Filho, M., Lin, J., 2017. Season-long ammonia flux measurements above fertilized corn in central Illinois, USA, using relaxed eddy accumulation. *Agric. For. Meteorol.* 239, 202–212. <https://doi.org/10.1016/j.agrformet.2017.03.010>
- Ni, K., Köster, J.R., Seidel, A., Pacholski, A., 2015. Field measurement of ammonia emissions after nitrogen fertilization—A comparison between micrometeorological and chamber methods. *Eur. J. Agron.* 71, 115–122. <https://doi.org/10.1016/j.eja.2015.09.004>
- Ni, K., Pacholski, A., Kage, H., 2014. Ammonia volatilization after application of urea to winter wheat over 3 years affected by novel urease and nitrification inhibitors. *Agric. Ecosyst. Environ.* 197, 184–194. <https://doi.org/10.1016/j.agee.2014.08.007>
- Nicholson, F., Bhogal, A., Cardenas, L., Chadwick, D., Misselbrook, T., Rollett, A., Taylor, M., Thorman, R., Williams, J., 2017. Nitrogen losses to the environment following food-based digestate and compost applications to agricultural land. *Environ. Pollut.* 228, 504–516. <https://doi.org/10.1016/j.envpol.2017.05.023>
- Obukhov AM (1951) Charakteristiki mikrostruktury vetra v prizemnom sloje atmosfery (Characteristics of the micro-structure of the wind in the surface layer of the atmosphere). *Izv AN SSSR ser Geofiz* 3:49–68
- Pacholski, A., Berger, N., Bustamante, I., Ruser, R., Guardia, G., Mannheim, T., 4 – 8 December 2016. Effects of the novel nitrification inhibitor DMPSA on yield, mineral N dynamics and N₂O emissions. Proceedings of the 2016 International

- Nitrogen Initiative Conference, “Solutions to improve nitrogen use efficiency for the world” (Melbourne, Australia). www.ini2016.com.
- Pacholski, A., Cai, G., Nieder, R., Richter, J., Fan, X., Zhu, Z., Roelcke, M., 2006. Calibration of a simple method for determining ammonia volatilization in the field – comparative measurements in Henan Province, China. *Nutr. Cycl. Agroecosystems* 74, 259–273. <https://doi.org/10.1007/s10705-006-9003-4>
- Pacholski, A., Cai, G.-X., Fan, X.-H., Ding, H., Chen, D., Nieder, R., Roelcke, M., 2008. Comparison of different methods for the measurement of ammonia volatilization after urea application in Henan Province, China. *J. Plant Nutr. Soil Sci.* 171, 361–369. <https://doi.org/10.1002/jpln.200625195>
- Pan, B., Lam, S.K., Mosier, A., Luo, Y., Chen, D., 2016. Ammonia volatilization from synthetic fertilizers and its mitigation strategies: A global synthesis. *Agric. Ecosyst. Environ.* 232, 283–289. <https://doi.org/10.1016/j.agee.2016.08.019>
- Pape, L., Ammann, C., Nyfeler-Brunner, A., Spirig, C., Hens, K., Meixner, F.X., 2009. An automated dynamic chamber system for surface exchange measurement of non-reactive and reactive trace gases of grassland ecosystems. *Biogeosciences* 6, 405–429. <https://doi.org/10.5194/bg-6-405-2009>
- Pilegaard, K., 2013. Processes regulating nitric oxide emissions from soils. *Philos. Trans. R. Soc. B Biol. Sci.* 368, 20130126–20130126. <https://doi.org/10.1098/rstb.2013.0126>
- Qiao, C., Liu, L., Hu, S., Compton, J.E., Greaver, T.L., Li, Q., 2015. How inhibiting nitrification affects nitrogen cycle and reduces environmental impacts of anthropogenic nitrogen input. *Glob. Change Biol.* 21, 1249–1257. <https://doi.org/10.1111/gcb.12802>
- R Core Team, 2017. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Recio, J., Álvarez, J.M., Rodríguez de Quijano, M., Vallejo, A., 2018a. Nitrification inhibitor DMPSA mitigated N₂O emission and promoted NO sink in rainfed wheat. *Environ. Pollut.* <https://doi.org/10.1016/j.envpol.2018.10.135>
- Recio, J., Vallejo, A., Le-Noë, J., Garnier, J., García-Marco, S., Álvarez, J.M., Sanz-Cobena, A., 2018b. The effect of nitrification inhibitors on NH₃ and N₂O emissions in highly N fertilized irrigated Mediterranean cropping systems. *Sci. Total Environ.* 636, 427–436. <https://doi.org/10.1016/j.scitotenv.2018.04.294>
- Ruser, R., Flessa, H., Russow, R., Schmidt, G., Buegger, F., Munch, J.C., 2006. Emission of N₂O, N₂ and CO₂ from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. *Soil Biol. Biochem.* 38, 263–274. <https://doi.org/10.1016/j.soilbio.2005.05.005>
- Ruser, R., Schulz, R., 2015. The effect of nitrification inhibitors on the nitrous oxide (N₂O) release from agricultural soils-a review. *J. Plant Nutr. Soil Sci.* 178, 171–188. <https://doi.org/10.1002/jpln.201400251>
- Russow, R., Spott, O., Stange, C.F., 2008. Evaluation of nitrate and ammonium as sources of NO and N₂O emissions from black earth soils (Haplic Chernozem) based on ¹⁵N field experiments. *Soil Biol. Biochem.* 40, 380–391. <https://doi.org/10.1016/j.soilbio.2007.08.020>

- Sanz-Cobena, A., García-Marco, S., Quemada, M., Gabriel, J.L., Almendros, P., Vallejo, A., 2014a. Do cover crops enhance N₂O, CO₂ or CH₄ emissions from soil in Mediterranean arable systems? *Sci. Total Environ.* 466, 164–174. <https://doi.org/10.1016/j.scitotenv.2013.07.023>
- Sanz-Cobena, A., Lassaletta, L., Aguilera, E., Prado, A. del, Garnier, J., Billen, G., Iglesias, A., Sánchez, B., Guardia, G., Abalos, D., Plaza-Bonilla, D., Puigdueta-Bartolomé, I., Moral, R., Galán, E., Arriaga, H., Merino, P., Infante-Amate, J., Meijide, A., Pardo, G., Álvaro-Fuentes, J., Gilsanz, C., Báez, D., Doltra, J., González-Ubierna, S., Cayuela, M.L., Menéndez, S., Díaz-Pinés, E., Le-Noë, J., Quemada, M., Estellés, F., Calvet, S., van Grinsven, H.J.M., Westhoek, H., Sanz, M.J., Gimeno, B.S., Vallejo, A., Smith, P., 2017. Strategies for greenhouse gas emissions mitigation in Mediterranean agriculture: A review. *Agric. Ecosyst. Environ.* 238, 5–24. <https://doi.org/10.1016/j.agee.2016.09.038>
- Sanz-Cobena, A., Lassaletta, L., Estellés, F., Prado, A.D., Guardia, G., Abalos, D., Aguilera, E., Pardo, G., Vallejo, A., Sutton, M.A., Garnier, J., Billen, G., 2014b. Yield-scaled mitigation of ammonia emission from N fertilization: the Spanish case. *Environ. Res. Lett.* 9, 125005. <https://doi.org/10.1088/1748-9326/9/12/125005>
- Sanz-Cobena, A., Misselbrook, T., Camp, V., Vallejo, A., 2011. Effect of water addition and the urease inhibitor NBPT on the abatement of ammonia emission from surface applied urea. *Atmos. Environ.* 45, 1517–1524. <https://doi.org/10.1016/j.atmosenv.2010.12.051>
- Sanz-Cobena, A., Misselbrook, T., Sanz, M.J., Vallejo, A., 2010. Use of an inverse dispersion technique for estimating ammonia emission from surface-applied slurry. *Atmos. Environ.* 44, 999–1002. <https://doi.org/10.1016/j.atmosenv.2009.08.044>
- Sanz-Cobena, A., Misselbrook, T.H., Arce, A., Mingot, J.I., Diez, J.A., Vallejo, A., 2008a. An inhibitor of urease activity effectively reduces ammonia emissions from soil treated with urea under Mediterranean conditions. *Agric. Ecosyst. Environ.* 126, 243–249. <https://doi.org/10.1016/j.agee.2008.02.001>
- Sanz-Cobena, A., Misselbrook, T.H., Arce, A., Mingot, J.I., Diez, J.A., Vallejo, A., 2008b. An inhibitor of urease activity effectively reduces ammonia emissions from soil treated with urea under Mediterranean conditions. *Agric. Ecosyst. Environ.* 126, 243–249. <https://doi.org/10.1016/j.agee.2008.02.001>
- Sanz-Cobena, A., Sánchez-Martín, L., García-Torres, L., Vallejo, A., 2012. Gaseous emissions of N₂O and NO and NO₃– leaching from urea applied with urease and nitrification inhibitors to a maize (*Zea mays*) crop. *Agric. Ecosyst. Environ.* 149, 64–73. <https://doi.org/10.1016/j.agee.2011.12.016>
- Sanz-Cobena, Alberto, Lassaletta, L., Garnier, J., Smith, P., 2017. Mitigation and quantification of greenhouse gas emissions in Mediterranean cropping systems. *Agric. Ecosyst. Environ.* 238, 1–4. <https://doi.org/10.1016/j.agee.2016.12.032>
- Sapkota, T.B., Rai, M., Singh, L.K., Gathala, M.K., Jat, M.L., Sutaliya, J.M., Bijarniya, D., Jat, M.K., Jat, R.K., Parihar, C.M., Kapoor, P., Jat, H.S., Dadarwal, R.S., Sharma, P.C. and Sharma, D.K. 2014. Greenhouse Gas Measurement from Smallholder Production Systems: Guidelines for Static Chamber Method.

- International Maize and Wheat Improvement Center (CIMMYT) and Indian Council of Agricultural Research (ICAR), New Delhi, India. p. 18
- Schindlbacher, A., 2004. Effects of soil moisture and temperature on NO, NO₂, and N₂O emissions from European forest soils. *J. Geophys. Res.* 109. <https://doi.org/10.1029/2004JD004590>
- Schindlbacher, A., Zechmeister-Boltenstern, S., Butterbach-Bahl, K., 2004. Effects of soil moisture and temperature on NO, NO₂, and N₂O emissions from European forest soils. *J. Geophys. Res. Atmospheres* 109, D17302. <https://doi.org/10.1029/2004JD004590>
- Schjoerring, J.K., Mattsson, M., 2001. Quantification of ammonia exchange between agricultural cropland and the atmosphere: Measurements over two complete growth cycles of oilseed rape, wheat, barley and pea. *Plant Soil* 228, 105–115. <https://doi.org/10.1023/A:1004851001342>
- Sherlock, R.R., Freney, J.R., Smith, N.P., Cameron, K.C., 1989. Evaluation of a sampler for assessing ammonia losses from fertilized fields. *Fertil. Res.* 21, 61–66. <https://doi.org/10.1007/BF01080531>
- Skiba, U., 2008. Denitrification, in: Jorgensen, S.E., Fath, B.D. (Eds.), *Encyclopedia of Ecology*. Elsevier, Oxford, pp. 866–871.
- Slemr, F., Seiler, W., 1984. Field measurements of NO and NO₂ emissions from fertilized and unfertilized soils. *J. Atmospheric Chem.* 2, 1–24. <https://doi.org/10.1007/BF00127260>
- Slemr, F., Seiler, W., 1991. Field study of environmental variables controlling the NO emissions from soil and the NO compensation point. *J. Geophys. Res.* 96, 13017. <https://doi.org/10.1029/91JD01028>
- Sommer, S.G., McGinn, S.M., Flesch, T.K., 2005. Simple use of the backwards Lagrangian stochastic dispersion technique for measuring ammonia emission from small field-plots. *Eur. J. Agron.* 23, 1–7. <https://doi.org/10.1016/j.eja.2004.09.001>
- Spiertz, H., 2010. Food production, crops and sustainability: restoring confidence in science and technology. *Curr. Opin. Environ. Sustain.* 2, 439–443. <https://doi.org/10.1016/j.cosust.2010.10.006>
- Stevenson, F.J., Harrison, R.M., Wetselaar, R., Leeper, R.A., 1970. Nitrosation of Soil Organic Matter: III. Nature of Gases Produced by Reaction of Nitrite with Lignins, Humic Substances, and Phenolic Constituents Under Neutral and Slightly Acidic Conditions1. *Soil Sci. Soc. Am. J.* 34, 430. <https://doi.org/10.2136/sssaj1970.03615995003400030024x>
- Stull, R.B., 2012. *An Introduction to Boundary Layer Meteorology*. Springer Science & Business Media.
- Sun, J., Bai, M., Shen, J., Griffith, D.W.T., Denmead, O.T., Hill, J., Lam, S.K., Mosier, A.R., Chen, D., 2016. Effects of lignite application on ammonia and nitrous oxide emissions from cattle pens. *Sci. Total Environ.* 565, 148–154. <https://doi.org/10.1016/j.scitotenv.2016.04.156>
- Sutton, M.A. et al., 2013. Our nutrient world: the challenge to produce more food and energy with less pollution. Centre for Ecology and Hydrology, Edinburgh, on

- behalf of the Global Partnership on Nutrient Management and the International Nitrogen Initiative.
- Swinbank, W.C., 1951. The measurement of vertical transfer of heat and water vapor by eddies in the lower atmosphere. *J. Meteorol.* 8, 135–145. [https://doi.org/10.1175/1520-0469\(1951\)008<0135:TMOVTO>2.0.CO;2](https://doi.org/10.1175/1520-0469(1951)008<0135:TMOVTO>2.0.CO;2)
- Tallec, T., Brut, A., Joly, L., Dumelié, N., Serça, D., Mordelet, P., Claverie, N., Legain, D., Barrié, J., Decarpenterie, T., Cousin, J., Zawilski, B., Ceschia, E., Guérin, F., Le Dantec, V., 2019. N₂O flux measurements over an irrigated maize crop: A comparison of three methods. *Agric. For. Meteorol.* 264, 56–72. <https://doi.org/10.1016/j.agrformet.2018.09.017>
- Thapa, R., Chatterjee, A., Awale, R., McGranahan, D.A., Daigh, A., 2016. Effect of Enhanced Efficiency Fertilizers on Nitrous Oxide Emissions and Crop Yields: A Meta-analysis. *Soil Sci. Soc. Am. J.* 80, 1121–1134. <https://doi.org/10.2136/sssaj2016.06.0179>
- Thomas, D.D., Ridnour, L.A., Isenberg, J.S., Flores-Santana, W., Switzer, C.H., Donzelli, S., Hussain, P., Vecoli, C., Paolocci, N., Ambs, S., Colton, C.A., Harris, C.C., Roberts, D.D., Wink, D.A., 2008. The chemical biology of nitric oxide: implications in cellular signaling. *Free Radic. Biol. Med.* 45, 18–31. <https://doi.org/10.1016/j.freeradbiomed.2008.03.020>
- Tiwary, A., Williams, I.D., Pant, D.C., Kishore, V.V.N., 2015. Assessment and mitigation of the environmental burdens to air from land applied food-based digestate. *Environ. Pollut.* 203, 262–270. <https://doi.org/10.1016/j.envpol.2015.02.001>
- Torralbo, F., Menéndez, S., Barrena, I., Estavillo, J.M., Marino, D., González-Murua, C., 2017. Dimethyl pyrazol-based nitrification inhibitors effect on nitrifying and denitrifying bacteria to mitigate N₂O emission. *Sci. Rep.* 7, 13810. <https://doi.org/10.1038/s41598-017-14225-y>
- USDA, N., 2010. Keys to soil taxonomy. Soil Surv. Staff Wash.
- Ussiri, D., Lal, R., 2013. Soil Emission of Nitrous Oxide and its Mitigation. Springer Netherlands, Dordrecht. <https://doi.org/10.1007/978-94-007-5364-8>
- Vallejo, A., Skiba, U., Garcíatorres, L., Arce, A., Lopezfernandez, S., Sanchezmartin, L., 2006. Nitrogen oxides emission from soils bearing a potato crop as influenced by fertilization with treated pig slurries and composts. *Soil Biol. Biochem.* 38, 2782–2793. <https://doi.org/10.1016/j.soilbio.2006.04.040>
- Van Grinsven, H.J.M., Holland, M., Jacobsen, B.H., Klimont, Z., Sutton, M. a., Jaap Willems, W., 2013. Costs and Benefits of Nitrogen for Europe and Implications for Mitigation. *Environ. Sci. Technol.* 47, 3571–3579. <https://doi.org/10.1021/es303804g>
- Van Groenigen, J.W., Velthof, G.L., Oenema, O., Van Groenigen, K.J., Van Kessel, C., 2010. Towards an agronomic assessment of N₂O emissions: a case study for arable crops. *Eur. J. Soil Sci.* 61, 903–913. <https://doi.org/10.1111/j.1365-2389.2009.01217.x>
- Viguria, M., Sanz-Cobena, A., López, D.M., Arriaga, H., Merino, P., 2015. Ammonia and greenhouse gases emission from impermeable covered storage and land application of cattle slurry to bare soil. *Agric. Ecosyst. Environ.* 199, 261–271. <https://doi.org/10.1016/j.agee.2014.09.016>

- Walker, J.T., Jones, M.R., Bash, J.O., Myles, L., Meyers, T., Schwede, D., Herrick, J., Nemitz, E., Robarge, W., 2012. Processes of ammonia air-surface exchange in a fertilized *Zea mays* canopy. *Biogeosciences Discuss.* 9, 7893–7941. <https://doi.org/10.5194/bgd-9-7893-2012>
- Westhoek, H.J., Overmars, K.P., van Zeijts, H., 2013. The provision of public goods by agriculture: Critical questions for effective and efficient policy making. *Environ. Sci. Policy, Special Issue on Ecosystem services and rural land management* 32, 5–13. <https://doi.org/10.1016/j.envsci.2012.06.015>
- Wolf, I., Russow, R., 2000. Different pathways of formation of N₂O, N₂ and NO in black earth soil. *Soil Biol. Biochem.* 32, 229–239. [https://doi.org/10.1016/S0038-0717\(99\)00151-0](https://doi.org/10.1016/S0038-0717(99)00151-0)
- Yang, M., Fang, Y., Sun, D., Shi, Y., 2016. Efficiency of two nitrification inhibitors (dicyandiamide and 3, 4-dimethylpyrazole phosphate) on soil nitrogen transformations and plant productivity: a meta-analysis. *Sci. Rep.* 6, 22075. <https://doi.org/10.1038/srep22075>
- Yang, W., Zhu, A., Zhang, J., Xin, X., Zhang, X., 2017. Evaluation of a backward Lagrangian stochastic model for determining surface ammonia emissions. *Agric. For. Meteorol.* 234–235, 196–202. <https://doi.org/10.1016/j.agrformet.2017.01.001>
- Zerulla, W., Barth, T., Dressel, J., Erhardt, K., Locquenghien, K.H. von, Pasda, G., Rädle, M., Wissemeier, A., 2001. 3,4-Dimethylpyrazole phosphate (DMPP) – a new nitrification inhibitor for agriculture and horticulture. *Biol. Fertil. Soils* 34, 79–84. <https://doi.org/10.1007/s003740100380>
- Zhao, Z., Wu, D., Bol, R., Shi, Y., Guo, Y., Meng, F., Wu, W., 2017. Nitrification inhibitor's effect on mitigating N₂O emissions was weakened by urease inhibitor in calcareous soils. *Atmos. Environ.* 166, 142–150. <https://doi.org/10.1016/j.atmosenv.2017.07>