TESIS DE LA UNIVERSIDAD
DE ZARAGOZA

2022

172

Noemí Mateo Marín

Efficacy of nitrification and urease inhibitors to reduce nitrogen losses under optimal management practices in irrigated Mediterranean agrosystems

Director/es
Isla Climente, Ramón
Quílez Sáez de Viteri, Dolores

ISSN 2254-7606

Prensas de la Universidad
Universidad Zaragoza

ISSN 2254-7606

Tesis Doctoral

EFFICACY OF NITRIFICATION AND UREASE INHIBITORS TO REDUCE NITROGEN LOSSES UNDER OPTIMAL MANAGEMENT PRACTICES IN IRRIGATED MEDITERRANEAN AGROSYSTEMS

Autor

Noemí Mateo Marín

Director/es

Isla Climente, Ramón
Quílez Sáez de Viteri, Dolores

UNIVERSIDAD DE ZARAGOZA
Escuela de Doctorado

Programa de Doctorado en Ciencias Agrarias y del Medio Natural

2020

DOCTORAL THESIS

2020

Efficacy of nitrification and urease inhibitors to reduce nitrogen losses under optimal management practices in irrigated Mediterranean agrosystems

Noemí Mateo Marín

Centro de Investigación y Tecnología Agroalimentaria de Aragón

Gobierno de Aragón

Programa de Doctorado en Ciencias Agrarias y del Medio Natural

Universidad de Zaragoza

1542

TESIS DOCTORAL

Programa de Doctorado en Ciencias Agrarias y del Medio Natural
de la Universidad de Zaragoza

Efficacy of nitrification and urease inhibitors to reduce nitrogen losses under optimal management practices in irrigated Mediterranean agrosystems

Memoria presentada por:

NOEMÍ MATEO MARÍN

en satisfacción de los requisitos necesarios para la obtención del título de Doctora
por la Universidad de Zaragoza.

Directores: Dr. Ramón Isla Climente
Dra. Dolores Quílez Sáez de Viteri

Zaragoza, 2020

Financiación

Esta Tesis Doctoral ha sido realizada en la Unidad de Suelos y Riegos (asociada a EEAD-CSIC) del Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA, Gobierno de Aragón) y se ha desarrollado gracias a un contrato predoctoral de Formación de Personal Investigador (FPI-INIA CPD-2015-0044) financiado por el Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria y el Ministerio de Economía y Competitividad, y cofinanciado por el Fondo Social Europeo.

Los trabajos se han realizado en el marco de tres proyectos de investigación:

- "Prácticas agrícolas sostenibles para la reducción de emisiones de gases de efecto invernadero en zonas Mediterráneas" (AGL2013-49062-C4-3-R) financiado por el Ministerio de Economía y Competitividad.
- "Prácticas de manejo agrícola y de la fertilización orgánica en la dinámica del nitrógeno en cultivos de cereal: Aspectos agronómicos y ambientales" (RTA2013-0057-C05-04) financiado por el Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria.
- "Nuevos productos y metodologías para incrementar la eficiencia de uso de los nutrientes en las materias orgánicas de origen animal" financiado por el Fondo de Inversiones de Teruel (FITE 2016, Fondo Europeo de Desarrollo Regional).

"[...] ¡Ay, loco para el que la felicidad sólo estará en el deseo,
goza al menos el instante presente,
déjate embriagar por este instante único en el que,
suspendido entre el cielo y la tierra,
casi flotando en la caricia del viento,
dominas el mundo!
¡Embriágate de cielo, que es lo único que detiene tu mirada!
Bajo tus pies, y hasta el infinito, emergiendo apenas del mar de nubes,
a miles se elevan hacia ti flechas de rocas y hielo..."

LIONEL TERRAY

LOS CONQUISTADORES DE LO INÚTIL

Agradecimientos · Acknowledgements

Esta historia no se ha construido sola, muchas personas se merecen como poco un ¡gracias!.

Muchas gracias a mis directores por aceptarme en esta aventura. Lola, gracias por toda la libertad que me has dado, por intentar sacar tiempo para mi, por aguantarme cuando iba metiendo presión, por tus minuciosas revisiones. Ramón, gracias por dejarme heredar y confiar, por enseñarme a darle dos vueltas a las cosas y a tomármelo *algo* más tranquilo, por tu paciencia con mis problemas estadísticos, por tus eficientes y rápidas revisiones.

Gracias a *los de campo* por proporcionarme el suelo sobre el que crece esta tesis. Miguel, gracias por estar siempre pendiente y saber organizar así de bien todas las tareas. Vicente, gracias por la templanza manejando el tractor a la hora de aplicar el purín, por la delicadeza con la cosechadora y por todos los ánimos que me diste desde el primer día. Pablo, gracias por hacer los muestreos mucho más amenos.

Mónica, gracias por enseñarme todo desde cero y acompañarme en todo momento.

Marta, Laura, Lola y Tere, gracias por registrar, pesar y analizar, por vuestras manualidades con las bayetas y por soportar mis prisas.

Gracias a los mejores compañeros de despacho. Nacho, gracias por los ánimos, consejos y apoyo, por resolver mis dudas agronómicas, por estar ahí para charrar un poquico. Cristina, gracias por tu positividad y por esa macro maravillosa que me ahorró tanto tiempo.

Gracias al resto de la unidad. Loli, gracias por todos los trámites. Bea, gracias por marcar el camino y por tu ayuda. María, gracias por tomar el mando en el maíz. Daniel, gracias por tu ayuda con la estadística. Raquel, gracias por ayudarme con el riego enterrado. Eva, gracias por estar siempre preguntando y animando. Farida, gracias por amenizar las comidas. Rosa, gracias por estar siempre dispuesta a solucionar cualquier cosilla. José Manuel, gracias por tu ayuda con el inglés. Auxi, Wafa, Maite y Marian, gracias por los ánimos y estar ahí si necesitaba algo.

Begoña Escuaín, gracias por los trámites administrativos con el INIA.

Dr Søren O. Petersen, thank you so much for giving me the chance of visiting the Section and collaborating with you. Drishya, thanks a lot for letting me be part of your adventure in Denmark. Diego, gracias por cuidar de mí, por preocuparte en todo momento y por confiarme ensayos y datos. Khagendra, thank you for teaching me how to use the HMR package. Thanks to the people of the laboratories, especially Karen and Bodil. Thanks to the whole Soil Fertility Section (Department of Agroecology, Aarhus University) for the warm hospitality. Tak! to everyone for making my stay a *hygge* experience.

Gracias al Grupo Nitrógeno de la Universidad Politécnica de Madrid por la genial acogida y vuestras enseñanzas sobre el amoniaco.

Samuel, gracias por tu tiempo en el croma. Jorge, gracias por tus sugerencias para los muestreos de amoniaco y de gases de efecto invernadero.

Muchísimas gracias a mis padres ya que son los que han permitido que llegue hasta aquí. Víctor, gracias por poner música a estos 4 años. Y gracias a Fata por hacerme desconectar ().

Gracias a todos aquellos que de una u otra manera han hecho que me moviera en esta dirección. Ángel Velamazán, gracias por hacerme disfrutar con las Ciencias Naturales durante tantos años. José Manuel Nicolau, gracias por enseñarme que debía moverme en vertical (grado-máster-doctorado). Clara Martí, muchas gracias por transmitirme tu pasión por el suelo. Àngela Bosch, muchas gracias por iniciarme en la investigación. Rosa Poch, gracias por tu ejemplaridad y vitalidad.

Dra. Lacal, mi psiquiatra con consulta en los bares, siempre al lado de una buena tapa, gracias por escucharme y por el apoyo. Arturo, gracias por resolverme al instante todas mis dudas legislativas. Jenny y Jorge, simplemente gracias por estar ahí.

Ambientólogas, gracias por creer en mi desde el principio y mostrármelo en vuestras borracheras a través de vídeos inesperados.

Masterianos, gracias por los buenos momentos. Tania y Robert, gracias por preocuparos por mi. Antonio, muchas gracias por tu ayuda y ánimos, gracias por ser el mejor ejemplo de cómo afrontar el doctorado y hacer una tesis de 10.

Diana, desahogo y comprensión mutua, gracias por ser uno de mis pañuelos de lágrimas y por enseñarme a ver las cosas de otra manera (véase huitlacoche o chilitos).

Marta Vilarrasa, gracias por tus ánimos y optimismo infinito.

Gracias a esos compañeros de congresos que hicieron de ellos unas experiencias superdivertidas; gracias en especial a *the Spanish crew*.

Gracias a aquellos otros que se preocuparon y creyeron por mí (Stefania, Asier, Sergio, Javi, amante, Abi, Kike y muchos otros que se me olvida nombrar).

Gracias al resto de familia que ha preguntado y confiado, aunque sin entender muy bien qué he estado haciendo.

Millones de gracias al mejor compañero de cordada, gracias por aguantarme, por estar a mis pies asegurándome y motivándome, gracias por enseñarme el camino, por abrir traza, por marcar el ritmo y por hacerme desconectar entre montañas.

A mis padres

Table of contents

Chapter 5

Agronomic and environmental implications of substituting synthetic nitrogen for pig slurry in a wheat crop

Resumen

En el contexto actual de cambio climático y crecimiento exponencial de la población se han desarrollado nuevas tecnologías como los inhibidores de la nitrificación y de la ureasa que aplicados junto a fertilizantes nitrogenados buscan cubrir la demanda alimentaria con un menor impacto en el medio ambiente. En sistemas agrícolas de regadío donde las pérdidas de nitrógeno (emisión de óxido nitroso, lavado de nitrato, entre otras) son potencialmente importantes, el uso de esta tecnología podría ser relevante. La eficacia de estos productos depende en gran medida de factores climáticos, edáficos y de manejo agrícola, y por ello sus ventajas deben ser evaluadas bajos las condiciones específicas en las que van a ser utilizados.

Por ello, el objetivo principal de esta Tesis es evaluar, en términos productivos y de pérdidas de nitrógeno (emisión de óxido nitroso, volatilización de amoniaco, lavado de nitrato), el uso de inhibidores de la nitrificación y de la ureasa cuando son utilizados con fertilizantes sintéticos y orgánicos en cereales cultivados bajo condiciones mediterráneas de regadío con manejo óptimo del riego y la fertilización nitrogenada. Además, como objetivo adicional, evaluar la influencia de diversos aspectos metodológicos de la técnica de cámaras estáticas cerradas para la determinación de los flujos de óxido nitroso. Para el desarrollo de la Tesis se establecieron tres ensayos de campo entre los años 2015 y 2018 en los que se evaluó la dinámica del nitrógeno en el sistema suelo-planta-atmósfera, cuantificando su uso por parte del cultivo, las transformaciones en el suelo y las pérdidas del sistema agrícola. El ensayo 1 fue una rotación maíz-maíz-trigo llevada a cabo en 24 lisímetros de drenaje con dos suelos con diferente capacidad de retención de agua disponible ("Profundo" y "Somero"), donde se evaluaron cuatro tratamientos consistentes en urea, urea estabilizada con inhibidores de la ureasa (NBPT: ureasa triamida N (n-butil) tiofosfórica o MCDHS: monocarbamida dihidrógeno sulfato) o con un inhibidor de la nitrificación (DMPP: 3,4-dimetilpirazol fosfato). El ensayo 2 consistió en un cultivo de trigo en el que se aplicó urea, purín porcino o purín porcino estabilizado mediante el inhibidor MCDHS. En el ensayo 3, también desarrollado en un cultivo

de trigo, se compararon tres aditivos (potenciador de la microbiología del suelo, inhibidor MCDHS e inhibidor DMPP en forma de Vizura®) añadidos al purín porcino. La información relativa a los flujos de emisión de óxido nitroso (N_2O) de los ensayos 2 y 3 fue también utilizada para estudiar los aspectos metodológicos planteados en el segundo objetivo.

El uso de los inhibidores, al ser aplicados junto a urea o purín porcino y con un manejo eficiente de la dosis de agua de riego, no afectó a la productividad de los cultivos ni a la eficiencia en el uso del nitrógeno por parte de la planta; aunque permitió una reducción del número de aplicaciones nitrogenadas en cobertera en maíz sin comprometer los citados parámetros. El inhibidor DMPP fue capaz de mitigar las emisiones de N_2O independientemente del tipo de suelo (73% en suelo Profundo, 60% en suelo Somero) o fuente de nitrógeno (67% en urea, 70% en purín de cerdo). Además, en el ensayo 1 los inhibidores de la ureasa redujeron las emisiones de N_2O escaladas al rendimiento, aunque únicamente en el suelo Profundo (68 g N Mg^{-1} grano en el tratamiento con MCDHS, 76 g N Mg^{-1} grano en el tratamiento con NBPT vs. 131 g N Mg^{-1} grano en la aplicación tradicional de urea). Ninguno de los inhibidores fue capaz de disminuir la masa de nitrato perdida por drenaje en ninguno de los dos suelos, probablemente debido al ajuste óptimo de las dosis de nitrógeno y agua de riego a las necesidades de los cultivos. El ensayo 2 demostró que el purín porcino puede sustituir completamente a la urea en un cultivo de trigo, manteniendo el rendimiento y sin afectar a la eficiencia del uso de nitrógeno, aunque se observó un descenso en el contenido de proteína en grano. No se observó ningún efecto del inhibidor MCDHS añadido al purín porcino sobre la productividad del cultivo y las pérdidas de nitrógeno. En el ensayo 3, los aditivos (potenciador de la actividad microbiana e inhibidor MCDHS) no lograron reducir las pérdidas de nitrógeno por volatilización de amoniaco.

En relación con el segundo objetivo de la tesis, se ha propuesto una metodología de análisis de imagen para estimar el volumen ocupado por las plantas en las cámaras estáticas cerradas de medida de flujo de gases hacia la atmósfera. A partir de la misma se ha determinado que la no consideración de dicho volumen supone un error sistemático en las estimaciones de las emisiones de N_2O, cuantificado en un 0,9% para el cultivo y año analizado. Por otro lado, la emisión de N_2O, un 35% mayor en las cámaras sin plantas respecto a las que tenían plantas, ha demostrado que la eliminación o corte de plantas en el interior de las cámaras para facilitar la medida de los flujos de emisión no es recomendable. Esta gran diferencia en las emisiones de N_2O se atribuye al menor contenido en nitrógeno mineral y a la menor temperatura en los primeros centímetros del suelo en las cámaras donde las plantas están presentes. Además, el cálculo de las emisiones de N_2O mediante el modelo lineal dio estimaciones un 18% más bajas que mediante la utilización del paquete HMR que elige entre modelo lineal o exponencial; sin embargo, no se pudo observar un descenso en los flujos de emisión al aumentar el contenido de N_2O dentro de las cámaras con tiempos de cierre más largos, por lo que no se justificaría la elección del modelo exponencial. Asimismo, se considera viable reducir el número de tiempos de muestreo de cuatro (0, 20, 40, y 60 min) a dos (0 y 60 min), con una pérdida de precisión de menos de un 0,3% en la estimación de los flujos de N_2O en las condiciones estudiadas.

Abstract

New technologies, such as nitrification and urease inhibitors, have been developed in the current context of climate change and exponential population growth to ensure food supply and reduce the environmental impacts of nitrogen fertilisation. The use of these products might be more relevant in irrigated agricultural systems where nitrogen losses (nitrous oxide emission, nitrate leaching, among others) are potentially important. The efficacy of these products depends largely on climatic and edaphic factors, as well as agricultural practices; therefore, they should be evaluated under the conditions that they will be used.

Thus, the main objective of this Thesis is to evaluate, in terms of crop productivity and nitrogen losses (nitrous oxide emission, ammonia volatilisation, nitrate leaching), the use of nitrification and urease inhibitors with synthetic and organic fertilisers in irrigated cereal crops under optimal management practices and Mediterranean climate. An additional objective is to evaluate the influence of different methodological aspects of measuring nitrous oxide fluxes when closed chamber technique is used. Accordingly, three field experiments were established from 2015 to 2018 and nitrogen dynamics in the soil-plant-atmosphere system were assessed (crop uptake, soil transformations, and losses from the agrosystem). A maize-maize-wheat rotation (trial 1) was conducted in 24 drainage lysimeters with two soils with contrasting water holding capacity ("Deep" and "Shallow"), and four fertiliser treatments were evaluated: i) urea; ii) urea stabilised with the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP); iii) urea stabilised with the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT); and iv) urea stabilised with the urease inhibitor monocarbamide dihydrogen sulphate (MCDHS). Trial 2 was performed in a wheat crop and treatments of urea, pig slurry, and pig slurry stabilised with MCDHS were assessed. In trial 3, three additives (a soil microbial activator, MCDHS, and DMPP as Vizura®) mixed with pig slurry were compared in a wheat crop. Information from trials 2 and 3 about nitrous oxide (N_2O) emission fluxes was used to respond to the second objective related to methodological aspects of the closed chamber technique.

The use of inhibitors did not affect crop productivity nor nitrogen use efficiency when they were applied with urea or pig slurry under efficient irrigation doses, although they allowed saving a second nitrogen side-dress application in maize crop without compromising these parameters. The inhibitor DMPP was able to mitigate N_2O emissions whichever it was the soil type (73% for Deep soil, 60% for Shallow soil) or the nitrogen source (67% for urea, 70% for pig slurry). Moreover, in trial 1, urease inhibitors reduced yield-scaled N_2O emissions in the Deep soil (68 g N Mg^{-1} grain in MCDHS treatment, 76 g N Mg^{-1} grain in NBPT treatment vs. 131 g N Mg^{-1} grain in the traditional application of urea). None of the inhibitors was able to reduce the mass of nitrate lost by drainage for any soil type, presumably because of the optimal adjustment of nitrogen and irrigation water to crop needs. In the wheat crop of trial 2, urea was substituted for pig slurry maintaining yield and nitrogen use efficiency; although grain protein was reduced after pig slurry fertilisation. No effect of MCDHS addition to pig slurry was detected in crop productivity neither in nitrogen losses. The additives tested in trial 3 (soil microbial activator and MCDHS) were not able to reduce nitrogen losses by ammonia volatilisation.

Regarding the second objective of the Thesis, a methodology based on image analysis have been proposed to estimate plant volume displaced inside the closed chambers for measuring gas fluxes to the atmosphere. This methodology has proved that disregarding the plant volume leads to a systematic overestimation in N_2O fluxes, quantified in 0.9% for the analysed crop and season. On the other hand, the 35% higher N_2O emissions in chambers with plant absence than in chambers with plant presence have demonstrated that removing or trimming plants inside chambers, to facilitate the measurements, is not a recommended practice. This large difference in N_2O emissions is attributed to lower mineral nitrogen content and temperature in the first soil centimetres under plant presence. Moreover, using the linear model to calculate N_2O emissions led to 18% lower fluxes estimates than those using the HMR package, which chooses between linear and exponential models. However, a reduction in N_2O fluxes was not observed for longer closures times as N_2O increased inside the chamber headspace; therefore, the election of the exponential model could not be justified. Besides, reducing the number of air sampling times from four (0, 20, 40, and 60 min) to two (0 and 60 min) is feasible with a minor loss of precision for fluxes estimation, 0.3% under the studied conditions.

List of abbreviations

AN — Ammonium nitrate

DMPP — 3,4–dimethyl-1H-pyrazole phosphate or 3,4-dimethylpyrazole phosphate

ECD — Electron-capture detector

EEA — European Environment Agency

EENF — Enhanced efficiency N fertiliser

EF — Emission factor

ELD — Economics of Land Degradation Initiative

ETo — Mean annual reference evapotranspiration

FAO — Food and Agriculture Organization of the United Nations

FID — Flame-ionisation detector

GHG — Greenhouse gas

GWP — Global warming potential

HMR — Regression-based extension of Hutchinson/Mosier method

I — Irrigation

IPCC — Intergovernmental Panel on Climate Change

LR — Linear regression

MAPA — Ministerio de Agricultura, Pesca y Alimentación

MCHDS — Monocarbamide dihydrogen sulphate

NBPT — N-(n-butyl) thiophosphoric triamide

NI — Nitrification inhibitor

NUE — Nitrogen use efficiency

P — Precipitation

Pm — Mean annual precipitation

$PM_{2.5}$ — Fine particulate matter (diameter of less than 2.5 micrometres)

PS — Pig slurry

PS-A	Pig slurry with a microbial activator
PSI	Pig slurry with MCDHS
PS-NI	Pig slurry with Vizura®
PS-UI	Pig slurry with MCDHS
RE_N	Apparent recovery efficiency of applied N
SMN	Soil mineral nitrogen content
SOC	Semi-opened free static chambers
T	Mean half-hourly temperature
Tm	Mean annual air temperature
U	Urea
UI	Urease inhibitor
UNEP	United Nations Environment Programme
W	Mean half-hourly wind speed
WFPS	Soil water-filled pore space
WHO	World Health Organization
YS_{N2O}	Yield-scaled N_2O emissions

Note:

In the present Thesis, the term 'flux' is used for an instantaneous emission, expressed per day generally; whereas the term 'emission' is used for a cumulative emission over an extended period, especially per cropping season.

Chapter 1

General introduction

1. General context of the agricultural sector

The accelerated human population growth and our changing lifestyle subject natural resources to an unprecedented pressure (Kopittke et al., 2019). By 2050, the world population is expected to reach almost ten billion people (United Nations, 2019) and to meet its global food demand, the food production must grow by 70% compare with 2005 production (ELD Initiative, 2015). This increase should guaranty nutrition security which encompasses food security (Ingram, 2020) and it is linked to agricultural productivity (Cole et al., 2018). The challenges of raising agricultural productivity and reducing associated environmental degradation should be based on increasing resource use efficiency (energy, water, nutrients) (Pinstrup-Andersen and Pandya-Lorch, 1998). Thus, the objective of the agricultural sector for the next decades is to accomplish a sustainable intensification (Smith, 2013), but in a context of low prices of basic agricultural products.

2. Environmental effects of agrosystems: losses of reactive nitrogen

The combination of fertilisation, irrigation and high-yielding varieties during the Green Revolution was the key driver that allowed feed people in many countries (Roy et al., 2006). However, the use of nitrogen (N) as fertiliser has affected the functioning of ecosystems and the human health, especially due to the mismanagement that led to significant N losses to the environment (EU Nitrogen Expert Panel, 2015; Vitousek et al., 1997). The main N loss pathways from agricultural systems are the leaching of nitrate (NO_3^-), the volatilisation of ammonia (NH_3), and the emissions of different nitrogen compounds such as dinitrogen (N_2), nitric oxide (NO), nitrogen dioxide (NO_2), and nitrous oxide (N_2O) (EU Nitrogen Expert Panel, 2015).

2.1. Nitrate leaching

The leaching of a substance responds to the quantity of water that leaves the root zone and the concentration of that substance in the leaching solution (Clothier and Green, 2005). According to IPCC (2019a), the N leaching is roughly estimated as 24% of the total N applied as fertiliser.

Nevertheless, this estimation could be improved considering that nitrate leaching responds exponentially rather than linearly to increasing N inputs (Wang et al., 2019). The N leached that reaches return flows of agrosystems is the major diffuse contributor to nitrate contamination in water bodies, which is especially important under irrigated conditions (Isidoro et al., 2006). This N can reach ground and surface water bodies, decreasing their quality and increasing the risk of eutrophication or even originating human health problems (Gold and Sims, 2005). In 1991, the European Commission approved the Nitrates Directive (91/676/EEC) to protect ground and surface waters from pollution originated from agriculture. The EC-countries were compelled to designate as Nitrate Vulnerable Zones all the agricultural areas 'which are or could be affected by pollution and which contribute to pollution by intensive use of fertilisers or intensive livestock production'. Especial measures to reduce nitrate leaching are applied in these areas.

2.2. Nitrogen atmospheric emissions

2.2.1. Reactive nitrogen forms

Gaseous N forms (N_2, N_2O, and NO) are released from agricultural systems during nitrification and denitrification processes (EU Nitrogen Expert Panel, 2015); processes that are predominantly microbial and provide energy to specialised groups of organisms (Ussiri and Lal, 2013). The importance of these emissions lies in the reactive N forms (N_2O and NO) which have a critical role in atmospheric chemistry (Liu et al., 2017). Nitrous oxide and nitrogen oxides (NO_x = NO + the derivate NO_2) are the pillars of many important environmental problems since are involved in atmospheric warming (direct or indirectly), in production and consumption of atmospheric oxidants (such as ozone and hydroxyl radical), and in the formation of nitric acid, responsible for terrestrial ecosystems acidification and eutrophication (Williams et al., 1992).

Nitrous oxide losses from the agricultural sector are, by far, the largest source of anthropogenic N_2O emissions (66% of total gross anthropogenic emissions) (UNEP, 2013). Human-influenced N_2O is the third-largest climate-forcing agent today due to its positive radiative forcing (Global Warming Potential over a 100-year time horizon of 265) and its long atmospheric lifetime (currently estimated in 121 years) (Myhre et al., 2013; UNEP, 2013). Furthermore, this gas is the most significant ozone-depleting substance emission to the atmosphere (Ravishankara et al., 2009). This gas together with the carbon dioxide (CO_2) and the methane (CH_4) contributes more than 85% of the total forcing from well-mixed greenhouse gases (UNEP, 2013). The contribution of the agricultural sector to the release of these gases is mainly related to the decomposition of the organic matter and fossil fuel burning for the CO_2 emission, and manure and waste management, enteric fermentation and rice cultivation for the CH_4 emission (IPCC, 2014).

2.2.2. Ammonia volatilisation

Ammonia is a volatile compound emitted as a gas that pollutes the atmosphere and surrounding environment (Sigurdarson et al., 2018). Ammonia reacts in the atmosphere with condensation nuclei (nitric and sulphuric acids) producing fine particulate matter ($PM_{2.5}$) (Hristov, 2011) that represents a serious environmental risk to human health (WHO, 2005). The European Environmental Agency (EEA, 2019a) evaluated the health impacts attributable to exposure to air pollution and indicated that, in the year 2016, $PM_{2.5}$ was responsible for 412,000 premature deaths in Europe. Moreover, volatilised ammonia is deposited in the environment by dry or wet route (Fangmeier et al., 1994) contributing to acidification, eutrophication and indirect N_2O emissions (EUROSTAT, 2016; IPCC, 2019b). In this context, political strategies are paying attention to NH_3 volatilisation, and regulations of NH_3 emissions have been implemented in several countries trying to reduce the emissions significantly (Sigurdarson et al., 2018). One of the target sectors to act on is the agriculture since it is the main source of atmospheric ammonia (Morán et al., 2016), representing the 92% of the total NH_3 emitted in the EU-28 during 2017 (EEA, 2019a). Within this sector, the largest contributors are crop fertiliser application and livestock-excrement management, which made up 54% of total EU-28 NH_3 emissions in 2017 (EEA, 2019b).

3. Practices to reduce nitrogen losses from agrosystems

Nitrogen use efficiency (NUE) of crops can improve through the 4R Nutrient Stewardship: applying the Right Source of nutrients, at the Right Rate, at the Right Time and in the Right Place (Johnston and Bruulsema, 2014), although new technological developments might help to achieve further gains (slow-release fertilisers, inhibitors, fertigation, and precision agriculture) (Ferguson, 2015). The practical limitation to accomplish the interesting 4R approach is the difficulty to balance the potential good agricultural practices to the undeniable need to deliver benefits to farmers in a global economic context.

3.1. Efficient management

Adjusting N fertilisation to needs of crops and improving irrigation efficiencies with an accurate schedule of N fertilisation and irrigation could reduce N export (Cavero et al., 2012; Isidoro et al., 2006; Malik et al., 2019). On the one hand, the simultaneous efficient management of water irrigation and N fertiliser applied to crops may provide more advantages enhancing the use efficiency of these resources than the separated optimisation of water and nitrogen inputs (Quemada and Gabriel, 2016). On the other hand, the timing and splitting of fertiliser application could be more important than the optimum rate to use (López-Bellido et al., 2005). Thus, splitting is a common strategy followed by farmers in arable crops to improve NUE (EU Nitrogen Expert Panel, 2015). Moreover, optimising both application timing and N source can allow for a moderate reduction in N rate that does not affect grain yield but decreases N_2O fluxes to the atmosphere (Venterea et al., 2016). Therefore, adjusting N fertilisation to crop needs have been proposed as an agronomic measure to mitigate direct N_2O emissions with positive side-effect potential on the emission of indirect N_2O due to reduction of NO_3^- leaching and NH_3 volatilisation (Sanz-Cobena et al., 2017).

In a context of circular economy where organic fertilisers, like manures and slurries, are applied to the soil, their efficient management is essential (Daudén et al., 2004), although that could be a challenge, especially in areas with a high density of farms (Guillaumes et al., 2006).

Thus, the 'The International Code of Conduct for the Sustainable Use and Management of Fertilizers' (FAO, 2019) recommends establishing evidence-based application limits for nutrients from fertilisers, including inorganic and organic fertilisers, sewage sludge, animal waste and organic residues to avoid damaging effects on the environment and the human, animal and soil health. Negative nutrient balances should also be avoided to prevent the risk of soil fertility decline due to nutrient mining.

3.2. Nitrification and urease inhibitors

Stabilised N fertilisers are new forms of N fertilisers that contain inhibitor compounds. They reduce or control the rate at which urea- or ammonium-N in fertilisers is transformed into less stable N forms making N less transferable into the environment (Ussiri and Lal, 2013). The Regulation (EU) 2019/1009 of the European Parliament and the Council defines 'Inhibitor' as 'an EU fertilising product the function of which is to improve the nutrient release patterns of a product providing plants with nutrients by delaying or stopping the activity of specific groups of micro-organisms or enzymes'. This Regulation considers nitrification inhibitors (NIs) such as substances that inhibit the biological N oxidation of ammonium to nitrate, and urease inhibitors (UIs) such as substances that inhibit hydrolytic action on urea by enzyme urease.

Therefore, inhibitors are added to fertilisers (nitrification and urease inhibitors with ammonium and urea N fertilisers, respectively) to extend the time the N component remains in the soil in more stable forms (Fig. 1) (Ussiri and Lal, 2013). The addition can be simple (one inhibitor type) or double (combining both types of inhibitors to complement strengths).

Maintaining N in the ammonium (NH_4^+) form in the soil through the NIs application can prevent its loss by drainage, nitrification, and denitrification; therefore, it prevents NO_3^- leaching and production of NO and N_2O by these processes (Ussiri and Lal, 2013). Besides, reducing NO_3^- leaching contributes to reducing eutrophication and indirect N_2O emissions are avoided (Tian et al., 2019). However, some studies (Lam et al., 2017) have proved that NIs may increase NH_3 volatilisation.

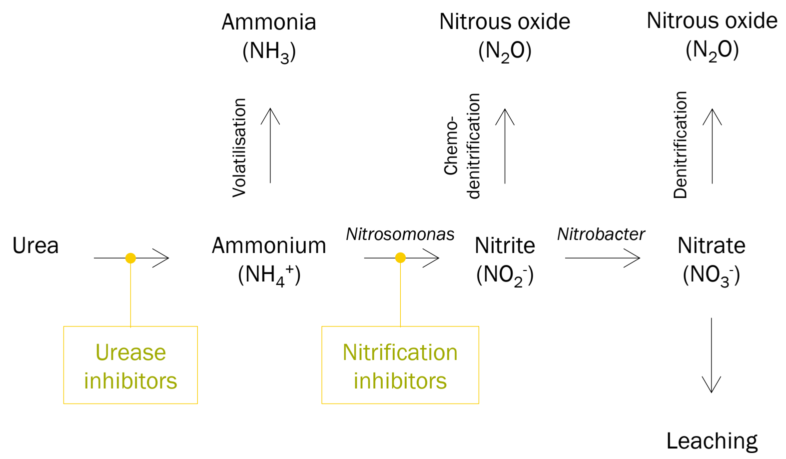


Figure 1. Some important biochemical reactions of N forms relevant to the use of urease and nitrification inhibitors.

Inactivating the urease enzyme in the soil through the UIs application slows down the rate of hydrolysis of urea to NH_4^+ and that can minimise NH_3 volatilisation and nitrification (Ussiri and Lal, 2013) because of the lesser concentration of NH_4^+ in the soil surface. Indirect N_2O emissions could be avoided after reducing NH_3 volatilisation (Martins et al., 2017) and direct N_2O emissions might be abated if the uptake of NH_4^+ by plants increases (Akiyama et al., 2010). Nevertheless, conflicting results have been reported about the reduction of N_2O emission when a UI is applied, e.g., no significant effect on this emission was reported by Akiyama et al. (2010), whereas N_2O emissions were significantly reduced under nitrification-favouring conditions according to Sanz-Cobena et al. (2012).

Increasing or decreasing the concentration of soil NH_4^+ after applying NIs and UIs, respectively, could have consequences on plants. Ammonium concentrations can be toxic to germinating seeds and young seedlings at ammonium N concentrations prevalent in the soil solution of fertiliser application zones, especially at neutral and higher pH (Kissel et al., 2008). On the contrary, the energetic cost for NH_4^+ uptake and assimilation by plants is considerably lower than that for NO_3^- (Britto and Kronzucker, 2005). Thus, application of urease inhibitors could reduce the seed damage (Malhi et al., 2003) and nitrification inhibitors may enhance the ammonium nutrition of plants lessening the required energy to incorporate it into aminoacids to the plants (Trenkel, 2010).

A reduction in N losses would increase the crop nitrogen use efficiency, the increment of productivity, and the reduction of inputs. Because of the reduction in N losses, inhibitors have been proposed to enhance the efficiency of N use. Furthermore, accordingly with this premise, a positive effect on crop yield should be expected, but no consistent results have been found across different experiments (Abalos et al., 2014).

The major barrier to the implementation and broad use of the stabilised fertilisers is their cost, greater than that of standard fertilisers (Timilsena et al., 2015), which should be counterbalanced by savings in applications and/or crop yield increases. The use of inhibitors could simplify the task of fertilisation by reducing the N rate, allowing for greater flexibility in the timing of application, or decreasing the number of applications that reduces the number of field operations and the labour and fuel cost requirements (Huérfano et al., 2015; Linzmeier et al., 2001; Ussiri and Lal, 2013).

Nowadays, according to current legislation in the European Union (Commission Regulation (EC) 1107/2008; Commission Regulation (EU) 223/2012; Commission Regulation (EU) 1257/2014; Commission Regulation (EU) 2016/1618), NIs that can be used are dicyandiamide (DCD), dicyandiamide + 1,2,4-triazole (DCD/TZ), 1,2,4-triazole + 3-methylpyrazole (TZ/MP), and 3,4–dimethyl-1H-pyrazole phosphate (DMPP); and UIs that can be used are N-(n-butyl) thiophosphoric triamide (NBPT), N-(2-nitrophenyl)phosphoric triamide (2-NPT), N-butylphosphorothioic triamide + N-propylphosphorothioic triamide (NBPT/NPPT). Besides, the Spanish Government recognises as NIs the dicyandiamide, 3,4-dimethylpyrazole phosphate, and the isomeric mixture of 2-(3,4-dimethylpyrazole-1-yl)-succinic acid and 2-(4,5-dimethylpyrazole-1-yl)-succinic acid (DMPSA) and as UI the monocarbamide dihydrogen sulphate (MCDHS) (Real Decreto 999/2017; Orden APA/161/2020).

Although most of the mentioned additives have been extensively studied in different agrosystems, the effect with other management factors (e.g., soil type, irrigation system, fertiliser management, fertiliser source) can have a great impact on their effectiveness to increase the nitrogen use efficiency and additional studies under particular conditions are of considerable interest to evaluate their potential under specific management conditions adequately.

References

Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., Vallejo, A., 2014. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. Agric. Ecosyst. Environ. 189, 136–144. doi:10.1016/j.agee.2014.03.036

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. doi:10.1111/j.1365-2486.2009.02031.x

Britto, D.T., Kronzucker, H.J., 2005. Nitrogen acquisition, PEP carboxylase, and cellular pH homeostasis: new views on old paradigms. Plant, Cell Environ. 28, 1396–1409. doi:10.1111/j.1365-3040.2005.01372.x

Cavero, J., Barros, R., Sellam, F., Topcu, S., Isidoro, D., Hartani, T., Lounis, A., Ibrikci, H., Cetin, M., Williams, J.R., Aragüés, R., 2012. APEX simulation of best irrigation and N management strategies for off-site N pollution control in three Mediterranean irrigated watersheds. Agric. Water Manag. 103, 88–99. doi:10.1016/j.agwat.2011.10.021

Clothier, B.E., Green, S., 2005. Leaching processes. Encycl. Soils Environ. 336–343 doi:10.1016/B0-12-348530-4/00388-X

Cole, M.B., Augustin, M.A., Robertson, M.J., Manners, J.M., 2018. The science of food security. Nat. Partn. Journals - Sci. Food 2, 14. doi:10.1038/s41538-018-0021-9

Daudén, A., Quílez, D., Vera, M.V., 2004. Pig slurry application and irrigation effects on nitrate leaching in Mediterranean soil lysimeters. J. Environ. Qual. 33, 2290–2295. doi:10.2134/jeq2004.2290

ELD Initiative, 2015. Report for policy and decision makers. Reaping economic and environmental benefits from sustainable land management. Stewart, N. and Etter H. (Eds.), Germany, 23 pp.

EU Nitrogen Expert Panel, 2015. Nitrogen Use Efficiency (NUE) - an indicator for the utilization of nitrogen in agriculture and food systems. Wageningen University, Netherlands, 47 pp.

EEA, 2019a. Air quality in Europe - 2019 report. Publications Office of the European Union, Luxembourg, 99 pp.

EEA, 2019b. European Union emission inventory report 1990-2017 under the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP). Publications Office of the European Union, Luxembourg, 143 pp.

EUROSTAT, 2016. Agriculture, forestry and fishery statistic. 2016 edition. Publications Office of the European Union, Luxembourg, 224 pp.

Fangmeier, A., Hadwiger-Fangmeier, A., Van der Eerden, L., Jäger, H.J., 1994. Effects of atmospheric ammonia on vegetation - A review. Environ. Pollut. 86, 43–82. doi:10.1016/0269-7491(94)90008-6

Ferguson, R.B., 2015. Groundwater Quality and Nitrogen Use Efficiency in Nebraska's Central Platte River Valley. J. Environ. Qual. 44, 449–459. doi:10.2134/jeq2014.02.0085

FAO, 2019. The International Code of Conduct for the Sustainable Use and Management of Fertilizers. Italy, 43 pp.

Gold, A.J., Sims, J.T., 2005. Eutrophication. Encycl. Soils Environ. 486–494 doi:10.1016/B0-12-348530-4/00093-X

Guillaumes, E., Carrasco, I., Villar, J.M., 2006. Response of wheat to additional nitrogen fertilizer application after pig slurry on over-fertilized soils. Agron. Sustain. Dev. 26, 127–133. doi:10.1051/agro:2006006

Hristov, A.N., 2011. Technical note: Contribution of ammonia emitted from livestock to atmospheric fine particulate matter (PM2.5) in the United States. J. Dairy Sci. 94, 3130–3136. doi:10.3168/jds.2010-3681

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. doi:10.1016/j.eja.2014.11.008

Ingram, J., 2020. Nutrition security is more than food security. Nat. Food 1, 2–2. doi:10.1038/s43016-019-0002-4

IPCC, 2019a. Chapter 11: N2O Emissions from Managed Soils, and CO2 Emissions From Lime and Urea Application, in: Calvo-Buendia, E., Tanabe, K., Kranjc, A., Baasansuren, J., Fukuda, M., Ngarize S., Osako, A., Pyrozhenko, Y., Shermanau, P., and Federici, S. (Eds.), 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Volume 4: Agriculture, Forestry and Other Land Use. Switzerland, 48 pp.

IPCC, 2019b. Overview, in: Calvo-Buendia, E., Tanabe, K., Kranjc, A., Baasansuren, J., Fukuda, M., Ngarize S., Osako, A., Pyrozhenko, Y., Shermanau, P., and Federici, S. (Eds.), 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Switzerland, 15 pp.

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. Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Farahani, E., Kadner, S., Seyboth, K., Adler, A., Baum, I., Brunner, S., Eickemeier, P., Kriemann, B., Savolainen, J., Schlömer, S., von Stechow, C., Zwickel, T., and Minx, J.C. (Eds.), Cambridge University Press, United Kingdom and United States of America, 1,435 pp.

Isidoro, D., Quílez, D., Aragüés, R., 2006. Environmental Impact of Irrigation in La Violada District (Spain): II. Nitrogen Fertilization and Nitrate Export Patterns in Drainage Water. J. Environ. Qual. 35, 776–785. doi:10.2134/jeq2005.0065

Johnston, A.M., Bruulsema, T.W., 2014. 4R Nutrient stewardship for improved nutrient use efficiency. Procedia Eng. 83, 365–370. doi:10.1016/j.proeng.2014.09.029

Kissel, D.E., Cabrera, M.L., Paramasivam, S., Schepers, J.S., Raun, W.R., 2008. Ammonium, Ammonia, and Urea Reactions in Soils, in: Nitrogen in Agricultural Systems. Agronomy Monographs 49, pp. 101–155. doi:10.2134/agronmonogr49.c4

Kopittke, P.M., Menzies, N.W., Wang, P., McKenna, B.A., Lombi, E., 2019. Soil and the intensification of agriculture for global food security. Environ. Int. 132, 105078. doi:10.1016/j.envint.2019.105078

Lam, S.K.K., Suter, H., Mosier, A.R.R., Chen, D.L., 2017. Using nitrification inhibitors to mitigate agricultural N 2 O emission: a double-edged sword? Glob. Chang. Biol. 23, 485–489. doi:10.1111/gcb.13338

Linzmeier, W., Gutser, R., Schmidhalter, U., 2001. Nitrous oxide emission from soil and from a nitrogen-15-labelled fertilizer with the new nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). Biol. Fertil. Soils 34, 103–108. doi:10.1007/s003740100383

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. Chang. Biol. 23, 2520–2532. doi:10.1111/gcb.13485

López-Bellido, L., López-Bellido, R.J., Redondo, R., 2005. Nitrogen efficiency in wheat under rainfed Mediterranean conditions as affected by split nitrogen application. F. Crop. Res. 94, 86–97. doi:10.1016/j.fcr.2004.11.004

Malhi, S.S., Oliver, E., Mayerle, G., Kruger, G., Gill, K.S., 2003. Improving Effectiveness Of Seedrow-placed Urea With Urease Inhibitor And Polymer Coating For Durum Wheat And Canola. Commun. Soil Sci. Plant Anal. 34, 1709–1727. doi:10.1081/CSS-120021307

Malik, W., Isla, R., Dechmi, F., 2019. DSSAT-CERES-maize modelling to improve irrigation and nitrogen management practices under Mediterranean conditions. Agric. Water Manag. 213, 298–308. doi:10.1016/j.agwat.2018.10.022

Martins, M.R., Sant'Anna, S.A.C., Zaman, M., Santos, R.C., Monteiro, R.C., Alves, B.J.R., Jantalia, C.P., Boddey, R.M., Urquiaga, S., 2017. Strategies for the use of urease and nitrification inhibitors with urea: Impact on N2O and NH3 emissions, fertilizer- 15 N recovery and maize yield in a tropical soil. Agric. Ecosyst. Environ. 247, 54–62. doi:10.1016/j.agee.2017.06.021

Morán, M., Ferreira, J., Martins, H., Monteiro, A., Borrego, C., González, J.A., 2016. Ammonia agriculture emissions: From EMEP to a high resolution inventory. Atmos. Pollut. Res. 7, 786–798. doi:10.1016/j.apr.2016.04.001

Myhre, G., Shindell, D., Bréon, F.M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Stephens, G., Takemura, T., Zhang, H., 2013. Anthropogenic and Natural Radiative Forcing, in: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P.M. (Eds.), Climate Change 2013 - The Physical Science Basis. Cambridge University Press, United Kingdom, pp. 659–740.

Pinstrup-Andersen, P., Pandya-Lorch, R., 1998. Food security and sustainable use of natural resources: a 2020 Vision. Ecol. Econ. 26, 1–10. doi:10.1016/S0921-8009(97)00067-0

Quemada, M., Gabriel, J.L., 2016. Approaches for increasing nitrogen and water use efficiency simultaneously. Glob. Food Sec. 9, 29–35. doi:10.1016/j.gfs.2016.05.004

Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous Oxide (N2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. Science. 326, 123–125. doi:10.1126/science.1176985

Roy, R.N., Finck, A., Blair, G.J., Tandon, H.L.S., 2006. Plant nutrition for food security - A guide for integrated nutrient management. FAO Fertil. Plant Nutr. Bull. 16, Italy, 349 pp.

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. doi:10.1016/j.agee.2016.09.038

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. doi:10.1016/j.agee.2011.12.016

Sigurdarson, J.J., Svane, S., Karring, H., 2018. The molecular processes of urea hydrolysis in relation to ammonia emissions from agriculture. Rev. Environ. Sci. Bio/Technology 17, 241–258. doi:10.1007/s11157-018-9466-1

Smith, P., 2013. Delivering food security without increasing pressure on land. Glob. Food Sec. 2, 18–23. doi:10.1016/j.gfs.2012.11.008

Tian, L., Cai, Y., Akiyama, H., 2019. A review of indirect N2O emission factors from agricultural nitrogen leaching and runoff to update of the default IPCC values. Environ. Pollut. 245, 300–306. doi:10.1016/j.envpol.2018.11.016

Timilsena, Y.P., Adhikari, R., Casey, P., Muster, T., Gill, H., Adhikari, B., 2015. Enhanced efficiency fertilisers: a review of formulation and nutrient release patterns. J. Sci. Food Agric. 95, 1131–1142. doi:10.1002/jsfa.6812

Trenkel, M.E., 2010. Slow- and controlled-release and stabilized fertilizers. International Fertilizer Industry Association, France, 160 pp.

United Nations (Department of Economic and Social Affairs, Population Division), 2019. World Population Prospects 2019: Highlights (ST/ESA/SER.A/423). United States of America, 39 pp.

UNEP, 2013. Drawing Down N2O to Protect Climate and the Ozone Layer. A UNEP Synthesis Report. Kenya, 57 pp.

Ussiri, D., Lal, R., 2013. Soil Emission of Nitrous Oxide and its Mitigation. Springer, Netherlands, 378 pp.

Venterea, R.T., Coulter, J.A., Dolan, M.S., 2016. Evaluation of intensive “4R” strategies for decreasing nitrous oxide emissions and nitrogen surplus in rainfed corn. J. Environ. Qual. 45, 1186. doi:10.2134/jeq2016.01.0024

Vitousek, P.M., Aber, J., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, G.D., 1997. Human alteration of the global nitrogen cycle: causes and consequences issues in ecology. Issues Ecol. 1, 1–17. doi:10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2

Wang, Y., Ying, H., Yin, Y., Zheng, H., Cui, Z., 2019. Estimating soil nitrate leaching of nitrogen fertilizer from global meta-analysis. Sci. Total Environ. 657, 96–102. doi:10.1016/j.scitotenv.2018.12.029

Williams, E.J., Hutchinson, G.L., Fehsenfeld, F.C., 1992. NOx and N2O Emissions From Soil. Global Biogeochem. Cycles 6, 351–388. doi:10.1029/92GB02124

WHO, 2005. Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide. Global update 2005: Summary of risk assessment. WHO Press, Switzerland, 20 pp.

Chapter 2

Objectives and structure

The main objective of this Thesis (1) is to assess the agronomic and environmental effects of nitrification and urease inhibitors added to synthetic and organic fertilisers applied to cereal crops under Mediterranean irrigated conditions. This general objective was addressed through the following specific objectives:

a) To evaluate, in two soil types with contrasting water holding capacity, the effect of three different stabilised nitrogen fertilisers (urea mixed with the nitrification inhibitor 3,4-dimethyl pyrazole phosphate (DMPP) and with the urease inhibitors N-(n-butyl) thiophosphoric triamide (NBPT) and monocarbamide dihydrogen sulphate (MCDHS)) in comparison with a traditional urea application on crop productivity, nitrogen use efficiency, and nitrate losses by leaching.

The hypothesis was that delaying the transformation in the soil of urea and ammonium forms to nitrate form could allow the reduction in the number of fertiliser applications, maintaining crop productivity and without increasing nitrate leaching, but the effect could be related to the type of soil (mainly to its water retention capacity).

b) To assess under the above-mentioned experimental conditions, if the inhibitors DMPP, NBPT, and MCDHS are efficient to reduce greenhouse gas emissions and whether their efficiencies depend on the type of soil.

The initial hypothesis was that some urease and nitrification inhibitors, through the delay in nitrogen transformations and the preservation of nitrogen in more stable forms, could mitigate greenhouse gas emissions, but the reduction could be soil-type dependent.

c) To evaluate the effect of substituting synthetic urea for pig slurry and the effect of the addition the urease inhibitor MCDHS to pig slurry on crop productivity and greenhouse gas emissions, to contribute and improve the circular economy.

The hypothesis was that pig slurry could substitute synthetic fertilisers without affecting crop productivity and greenhouse gas emissions and that the addition of MCDHS could modify the soil nitrogen dynamics due to the micro-acidification produced in the hydrolysis of the molecule.

d) To test the effectivity of the inhibitory molecules MCHDS and DMPP (Vizura®) and a soil microbial activator mixed with pig slurry to reduce gaseous nitrogen losses to the atmosphere.

The hypothesis was that DMPP (as Vizura®) could slow down the transformation of pig slurry ammonium to nitrate and the microbial activator could enhance the efficiency of plant nitrogen absorption, in both cases promoting a decrease in nitrous oxide emissions. The MCDHS could reduce ammonia volatilisation, even when the pig slurry urea has been transformed to ammonium, due to the micro-acidification produced during the hydrolysis of the molecule.

An additional objective (2) is to evaluate the influence of different methodological aspects of the closed chamber technique to improve the estimation of nitrous oxide fluxes according to the experimental conditions.

The hypothesis was that changes in methodological aspects (considering plants inside the chamber, adjusting the fluxes to a linear or exponential model, reducing the number of sampling times) could correct systematic errors for nitrous oxide emission estimation improving the comparison of studies.

To accomplish the mentioned objectives three experimental field trials were carried out. Experiment 1 was conducted in a battery of 24 drainage lysimeters (12 filled with a deep soil with absence of stones and 12 with a shallow soil with frequent stoniness), where a rotation maize-maize-wheat was grown. In this experiment, Objectives 1a and 1b were evaluated and the information is presented in Chapter 3 and Chapter 4 of the document, respectively. Experiment 2 was conducted in a wheat crop during three consecutive growing seasons and collected the information necessary to answer Objective 1c and some information relevant for Objective 2. The information is presented in Chapter 5 (Objective 1c) and Chapter 7 (Objective 2). Experiment 3 was conducted in miniplots cultivated to wheat to answer Objectives 1d and 2, and it was carried out during two consecutive years with pig slurry applications. The information relative to Objective 1d is exposed in Chapter 6 and the information relative to methodological aspects (Objective 2) is presented in Chapter 7. Additional information about nitrous oxide emission from a previous wheat experiment was gathered and included in the analysis of methodological aspects, Objective 2 (Chapter 7).

In addition to the five above-cited chapters, the document includes a General introduction (Chapter 1), Objectives and structure (Chapter 2), General discussion (Chapter 8), and Conclusions (Chapter 9).

Chapter 3

Utility of stabilised nitrogen fertilisers to reduce nitrate leaching under optimal management practices

Abstract

The inadequate application of nitrogen (N) to crops has increased the reactive N in the atmosphere and in the surface and ground waters. Stabilised N-fertilisers with nitrification (NI) and urease (UI) inhibitors have been proposed to reduce these environmental problems without affecting or even increasing crop productivity. The objective of this study was to evaluate, in a maize-maize-wheat rotation, if the use of the NI 3,4-dimethylpyrazole phosphate (DMPP) and the UIs N-(n-butyl) thiophosphoric triamide (NBPT) and monocarbamide dihydrogen sulphate (MCDHS) reduces N leaching without compromising yield under optimal management of N and water. The experiment was conducted in 24 drainage lysimeters with two soil types with contrasting water holding capacity under Mediterranean irrigated conditions. The fertiliser treatments were urea, urea with DMPP, urea with NBPT, and urea with MCDHS. For the maize crop, conventional fertiliser application was split at 6- and 13-leaf stages, whereas stabilised fertilisers were applied as a single application at the 6-leaf stage. All fertiliser treatments were applied at late tillering in the wheat crop. The soil mineral N was measured at the beginning and the end of each cropping season, but no differences were found among fertiliser treatments. Differences in the volume of water drained or the cumulative mass of nitrate depending on the fertiliser were not significant (three-year treatment average of 200 L m^{-2} and 22 kg N ha^{-1} in the Deep soil, and 334 L m^{-2} and 40 kg N ha^{-1} in the Shallow type, respectively). No consistent significant differences were found in agronomic parameters (chlorophyll measurements, yield, and total N uptake) between the fertiliser treatments. Based on the results, the use of stabilised N-fertiliser could be recommended to reduce the number of N applications in maize without compromising grain yield but with no advantages to reduce nitrate-leaching losses if N rates are managed properly under efficient irrigation management practices.

1. Introduction

Food production depends on the addition of nitrogen (N) fertilisers to obtain profitable crop yields (Timilsena et al., 2015), especially under irrigated conditions (Berenguer et al., 2009). Nevertheless, excessive N application causes environmental problems such as contamination of surface and ground waters by nitrate (Peña-Haro et al., 2010) or atmospheric contamination through the release of nitrogen oxides and ammonia (Huérfano et al., 2015; Timilsena et al., 2015). In semiarid Mediterranean conditions, high nitrate (NO_3^-) concentrations are found in irrigation return flows (Barros et al., 2012) and have been related to mismanagement of N application, inadequate irrigation practices, or inefficient irrigation systems that lead to water pollution (Cavero et al., 2012).

Enhancing nitrogen use efficiency (NUE) seems to be a good approach for addressing the triple challenge of environmental degradation, climate change, and food security (Zhang et al., 2015) and for reaching the Sustainable Development Goals of the 2030 Agenda (United Nations, 2015). This increase of NUE can be accomplished by improving the synchronisation between the N supply and crop demand and by reducing N losses using stabilised N-fertilisers that include nitrification and urease inhibitors (Abalos et al., 2014). Nitrification inhibitors (NIs) are compounds that delay the bacterial oxidation of ammonium (NH_4^+) to nitrite in the soil for a certain period by depressing the activity of *Nitrosomonas* bacteria (Zerulla et al., 2001). Urease inhibitors (UIs) inactivate the urease enzyme; consequently, the enzymatic hydrolysis of urea is slowed down or even stopped (Snyder et al., 2009), delaying the conversion of urea to ammonium and thus to nitrate. Different studies in maize (Díez-López et al., 2008; Díez et al., 2010), wheat (Carrasco and Villar, 2001), and other crops (Serna et al., 2000; Egea and Alarcón, 2004) have described potential reductions in nitrate losses by leaching using different NIs under irrigated conditions. Nitrate leaching reduction by NIs has been estimated at approximately 17%, with an increment in yield production of 3% according to the meta-analysis of Quemada et al. (2013). UIs have also shown to be effective in reducing NO_3^- leaching (Abalos et al., 2014), yet Rawluk et al. (2001) indicate the risk of rapid movement of urea deeper into the soil profile due to its high solubility.

Rose et al. (2018), in a re-evaluation of the effectiveness of nitrification inhibitors, found that they achieve higher yields over conventional fertilisers at sub-optimal N rates, and the key question that arose is whether N loss can be reduced by applying inhibitors without loss of yield while being economically viable. Moreover, the general utility of these stabilised N-fertilisers in increasing NUE have been also questioned (Yang et al., 2016; Rose et al., 2018) due to their interactions with other climatic, edaphic, and management factors. Further studies across a range of crops and environments are needed to obtain such information.

Two of the most commonly used nitrification and urea inhibitors are, respectively, 3,4-dimethylpyrazole phosphate (DMPP) and N-(n-butyl) thiophosphoric triamide (NBPT) (Abalos et al., 2014). More recently, a Spain-based fertiliser company released the technology DURAMON® based on the addition of the molecule monocarbamide dihydrogen sulphate (MCDHS; international patent WO 2007/132032 A1) to urea fertilisers with the potential to stabilise the urea-N through the inhibition of the urease enzyme. No information is available in the scientific literature about the effectiveness of this product to improve NUE compared to the above-mentioned and more-studied inhibitors.

For conventional fertilisers, the most extended and recommended practice in irrigated maize is to split the N into two side-dress applications to increase its efficiency. Stabilised N-fertilisers might reduce the number of N applications, which would decrease fuel needs and operation time (Huérfano et al., 2015). Nevertheless, most of the studies addressing the effectiveness of inhibitors consider neither this issue nor the importance of irrigation management practices to increase NUE and reduce N losses. Besides, there is an absence of studies developed during a complete crop rotation established with N fertilisers managed at near-optimal management rates with rational irrigation management. Accordingly, the objective of this research is to assess, under semiarid irrigated conditions in a 3-year rotation (maize-maize-wheat), the effect of three stabilised N-fertilisers (urea with DMPP, NBPT, and MCDHS) on crop productivity, nitrogen use efficiency, and nitrate losses by leaching in two soil types with contrasting water holding capacity. The hypothesis was that, in the case of maize, a single application of stabilised urea could reduce nitrate leaching compared to the conventional two side-dress urea applications, maintaining crop productivity.

2. Materials and methods

2.1. Site and experimental design

This study was conducted from 2015 to 2017 in the CITA experimental field 'Soto Lezcano' in the middle Ebro river basin (Zaragoza, Spain), where the climate is semiarid Mediterranean-continental (mean annual maximum and minimum air temperatures of 21.4 and 8.3 ºC, respectively; yearly average precipitation of 319 mm; and yearly average reference evapotranspiration of 1,239 mm, period 2004-2018).

An experimental facility with 24 concrete-made drainage lysimeters (size 2.0 m × 2.5 m, and 1.5-m depth) was used for the research. Lysimeters were filled in 2013 with disturbed soil from two different fields, twelve lysimeters with each soil type, to represent two contrasting soil types that appear in the Ebro valley area frequently. The soils are denominated in the study as "Deep" (restricted to 1.25-m soil depth) and "Shallow" (restricted to 0.50-m soil depth). The soil in the lysimeters was over a layer of gravel of 1 m for Shallow soil and 0.25 m for Deep soil. The physicochemical characteristics of the two soils are presented in Table 1. The main differences between both soils are the soil depth and the soil stoniness, which confer contrasting soil water holding capacity (223.3 mm in Deep soil and 63.2 mm in Shallow soil).

A crop rotation of maize-maize-wheat (*Zea mays* L. hybrid 'Pioneer P1758' and bread-making wheat *Triticum aestivum* L. cv. 'Rimbaud') was followed according to the management description in Table 2. The areas surrounding the lysimeters were also sowed to the same crop to avoid border effects. Previously to this experiment, the lysimeters were cropped with sunflower (2014) and barley (2015) with no differences in fertilisation among lysimeters.

Table 1. Main physicochemical characteristics of Deep and Shallow soils at the different depths.

	Deep soil			Shallow soil	
	0-30 cm	30-60 cm	60-125 cm	0-25 cm	25-50 cm
Soil texture	Silt Loam	Silt Loam	Loam	Clay Loam	Clay Loam
Sand (%)	29	31	33	24	30
Silt (%)	52	51	48	40	36
Clay (%)	19	18	19	36	34
Stoniness ($\%_{vol.}$)	3.1	0.9	7.0	11.4	15.2
Available water (mm)	54.5	54.5	114.3	32.1	31.1
P (Olsen) (mg kg^{-1})	30.7	7.8	12.4	14.5	17.5
K (NH_4Ac) (mg kg^{-1})	499	236	72	225	202
Organic matter (%)	1.46	0.94	0.79	2.04	1.24
Soil pH ($1:2.5_{H2O}$)	8.04	7.71	7.65	8.27	8.65

Table 2. General crop management description in field trials.

	Maize 1	Maize 2	Wheat
Sowing date	04/05/2015	14/04/2016	10/11/2016
Harvest date	05/10/2015	13/09/2016	03/07/2017
Plant density (plants ha^{-1})	88,083	87,000	286[a]
Date N preplanting	30/04/2015	13/04/2016	-
Date N side-dress 1	15/06/2015	06/06/2016	27/02/2017
Date N side-dress 2	20/07/2015	05/07/2016	-
Total N applied (kg N ha^{-1})			
Deep soil	211	173	150
Shallow soil	236	211	150
Irrigation + Rain (mm)[b]	985	945	609
Crop E.T. (mm)[c]	918	866	578

[a]- kg seed ha^{-1}; [b]- From sowing to harvest; [c]- Obtained from soil water balance.

The experiment for each soil type had a completely randomised block design with three replicates for each treatment. The side-dressing fertiliser treatments evaluated along the three-year rotation were a) standard urea (Urea), b) urea with the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP), c) urea with the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT), and d) urea with the urease inhibitor monocarbamide dihydrogen sulphate (MCDHS). In maize crops, Urea treatment was split into two applications at the V6 and V13 stages (Ritchie et al., 1986) as is usual among local farmers, whereas in treatments with stabilised urea fertilisers were applied in a single application at the V6 stage, as is normally recommended to farmers by the companies that commercialize these products. In the wheat crop, N was applied at tillering in a single N dose for all treatments (Table 2). Directly after N application, fertilisers were incorporated into the soil through a short irrigation event to reduce N losses by ammonia volatilisation.

The proportion of inhibitor substance relative to nitrogen was established by the fertiliser companies as 0.8, 0.13, and 1.5% for DMPP, NBPT, and MCDHS, respectively. Only one of the three stabilised fertilisers (NBPT) used in the experiment is a commercial product (UTEC®), and the other two (DMPP and MCDHS) were prepared *ad hoc* for the study by the manufacturing companies.

At pre-sowing, 50-100-150 kg ha^{-1} (N-P_2O_5-K_2O) was applied to maize crops and 0-229-154 kg ha^{-1} (N-P_2O_5-K_2O) to wheat. The total N rates, showed in Table 2, were calculated each year taking into account the soil mineral nitrogen (SMN) at preplanting in the upper part of the soil profile (0-25 cm in Shallow soil and 0-30 cm in Deep soil) and considering previous studies in the area showing that maize requires 250 kg N ha^{-1} (Isla et al., 2006) of available N (SMN at preplanting + N from fertiliser). Wheat received a constant rate of 150 kg N ha^{-1} in both soil types.

The weekly irrigation requirements were calculated from the Penman-Monteith reference evapotranspiration and the crop coefficients of maize and wheat according to Martínez-Cob (2008) and FAO procedures (Allen et al., 1998), respectively. The salinity of the irrigation water (average of 1.5 dS m^{-1} over the three seasons) was over the threshold maize salt tolerance (Ayers and Westcot, 1994), and a 15-20% surplus of irrigation water was added over the irrigation requirements to avoid salt accumulation in the soil and the associated yield reduction due to salt stress. Crops were sprinkler irrigated and the total water received in each lysimeter was measured every irrigation with a rain gauge. When necessary, due to the wind effect on water distribution, the irrigation dose was corrected using a drip irrigation system to ensure that all lysimeters received the same amount of water. The total water applied to each crop is presented in Table 2.

Weeds and pests were controlled according to standard practices of the area to guarantee adequate growth of maize and wheat and no special problems were observed during the study.

2.2. Soil mineral nitrogen

Soil sampling was performed at the beginning of each season and after harvest to evaluate the SMN content. Shallow soil was sampled in two depth intervals (0-25 and 25-50 cm) and Deep soil in three depth intervals (0-30, 30-60, and 60-120 cm). At each lysimeter and for each soil depth, two-soil core samples were taken using an auger (5-cm diameter) and combined for further analyses. A subsample was used to calculate soil water content by gravimetry (drying at 105 ºC until constant weight). Another subsample of 10 g of fresh soil was extracted with 30 mL of 2 N KCl, shaken for 30 min, and filtered through a cellulose filter. The nitrate and ammonium concentrations in the extracts were analysed by colourimetry using a segmented flow analyser (AutoAnalyser 3, Bran+Luebbe, Germany).

2.3. Drainage water

Drainage from each lysimeter was collected weekly in 50 L graduated tanks set in an underground gallery and the volume was measured. A 30-mL subsample was collected from each tank to analyse nitrate and ammonium concentrations using a segmented flow analyser. The mass of nitrate leached was calculated for each sampling date as the product of drainage volume by nitrate concentration. The ammonium concentration was analysed only during the first year (2015) because it was extremely low (average of 0.10 mg N L^{-1}; n=310) compared to that of nitrate (18.1 mg N L^{-1}).

2.4. Crop nitrogen status and yield

The nutritional status of maize and wheat plants was evaluated using a portable chlorophyll meter (SPAD-502®, Minolta Camera Co., Ltd., Japan) at different growth stages. In maize, SPAD readings were taken on the youngest fully developed leaf at the sixth leaf (V6) and tenth leaf (V10), and on the ear leaf at the thirteenth leaf (V13), tasseling (VT), and milky grain (R3) stages according to Ritchie et al. (1986) scale. In wheat, SPAD readings were taken on the previous to the last unfolded leaf at anthesis half-way (GS-65), caryopsis water ripe (GS-71), and medium milk (GS-75) stages according to the Zadoks et al. (1974) scale.

At maize maturity (2nd October 2015 and 13th September 2016), all ears in each lysimeter were hand-harvested to determine grain yield (reported on the basis of 140 g kg^{-1} moisture content) and number of grains per square meter. The rest of the aerial parts (stem + leaves) were harvested, and a subsample was dried to determine the total dry aboveground biomass.

At wheat maturity (3rd July 2017), a 0.73-m^2 subsample was randomly hand-harvested from each plot to determine biomass yield and number of grains per square meter. The rest of the plot was mechanically harvested by an experimental combine to determine grain yield (reported on the basis of 120 g kg^{-1} moisture content).

Nitrogen content was analysed from dry (at 65 ºC) and finely ground grain and plant samples of maize and wheat by dry combustion (TruSpec CN, LECO, St. Joseph, MI, USA). NUE was calculated as the ratio between total N extracted in the aboveground biomass and the N applied by fertilisation.

2.5. Statistical analysis

The effect of fertiliser treatments on the different variables was analysed separately for Deep and Shallow soil and for the three experimental years: maize 1 (from sowing maize 1 to sowing maize 2), maize 2 (from sowing maize 2 to sowing wheat) and wheat (from sowing to end of September). Some variables were also analysed for maize crop (as the sum of both maize seasons) and for the whole rotation (from sowing maize 1 to end of September 2017).

Data were subjected to analysis of variance and differences among fertiliser treatment means were established with Tukey's test. Analysis of variance was also used to evaluate differences between soils, although without stressing on it since soil type is not controlled by the farmer. In the case of repeated measurements over time (nitrate mass in drainage), a repeated measure analysis was performed with the MIXED procedure considering a first-order autoregressive structure covariance model AR(1). A paired Student's t-test was used to compare the average nitrate concentration among treatments when there were matches in time in drainage events. Linear regression was used to relate yield with yield components. In all tests, the default level of significance considered was 0.05, although differences at p-values between 0.05 and 0.1 were indicated.

Statistical analyses were performed using SAS® software (University Edition, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Soil mineral nitrogen

No differences in SMN content among fertiliser treatments were found in the two types of soil during the development of the experiment (Table 3). Overall, the different treatments presented a similar coefficient of variation (18% in Deep soil and 20% in Shallow soil on average), indicating a reasonable variability in SMN content among replicated plots.

Table 3. Average soil mineral nitrogen content (kg N ha^{-1}; n=3) in the different fertiliser treatments (Urea, DMPP, NBPT, and MCDHS) for the whole soil profile (0-120 cm in Deep soil and 0-50 cm in Shallow soil) at different times during the maize-maize-wheat rotation.

	Maize 1		Maize 2		Wheat
	Preplant (21/04/15)	Harvest (19/10/15)	Preplant (08/04/16)	Harvest (20/09/16)	Harvest (10/07/17)
			Deep soil		
Urea	64.7	36.2	165.8	38.9	66.0
DMPP	68.0	36.1	161.3	40.9	62.2
NBPT	54.6	37.4	137.2	37.4	61.4
MCDHS	59.6	36.5	156.6	40.0	42.2
p-value	0.413	0.995	0.826	0.725	0.209
			Shallow soil		
Urea	18.9	28.0	65.0	26.3	42.8
DMPP	10.6	21.5	51.7	28.1	31.0
NBPT	15.6	28.7	49.1	24.3	58.3
MCDHS	18.1	21.1	43.0	27.8	41.4
p-value	0.067	0.223	0.056	0.692	0.415
Soil type	**	**	**	**	**

* Significant at p<0.05; ** Significant at p<0.01.

A high increase in SMN content was observed between the harvest of maize 1 and the sowing of maize 2 in both soil types. Thus, averaging across treatments, the SMN content increased from 37 to 155 kg N ha^{-1} in Deep soil and from 24 to 52 kg N ha^{-1} in Shallow soil.

3.2. Nitrate losses by drainage

The volume of drainage was not affected by fertiliser treatments in any of the three seasons in the two soil types (Table 4). Averaging over the seasons and soil types, the volume of water drained during the wheat crop was approximately 36% of that drained during maize crops. A high proportion of drainage (74%) happened during the period from seeding to harvest, i.e., during the crop cycle (Supplementary material, Fig. S1). Overall, using the measured volumes of drainage, rain, and irrigation, the leaching fraction for the whole rotation was 0.07 for the Deep soil and 0.13 for the Shallow soil.

Table 4. Average cumulative drainage (mm; n=3) in the different fertiliser treatments (Urea, DMPP, NBPT, and MCDHS) for the three crops (maize 1, maize 2, and wheat) and the two soil types (Deep and Shallow). For maize, the period includes the crop period (sowing to harvest) and the intercrop period (harvest to the following crop sowing). For wheat, the period goes from sowing to the end of September.

	Maize 1	Maize 2	Wheat
		Deep soil	
Urea	68	98	48
DMPP	80	81	40
NBPT	71	54	25
MCDHS	82	94	60
p-value	0.993	0.680	0.427
		Shallow soil	
Urea	163	119	90
DMPP	157	120	108
NBPT	151	121	90
MCDHS	154	127	97
p-value	0.950	0.976	0.063
Soil type	**	*	**

* Significant at $p<0.05$; ** Significant at $p<0.01$.

The weekly mass of nitrate leached (Fig. 1), analysed using a repeated measure procedure, did not show differences (p>0.05) among treatments for any of the three crops in the two soil types.

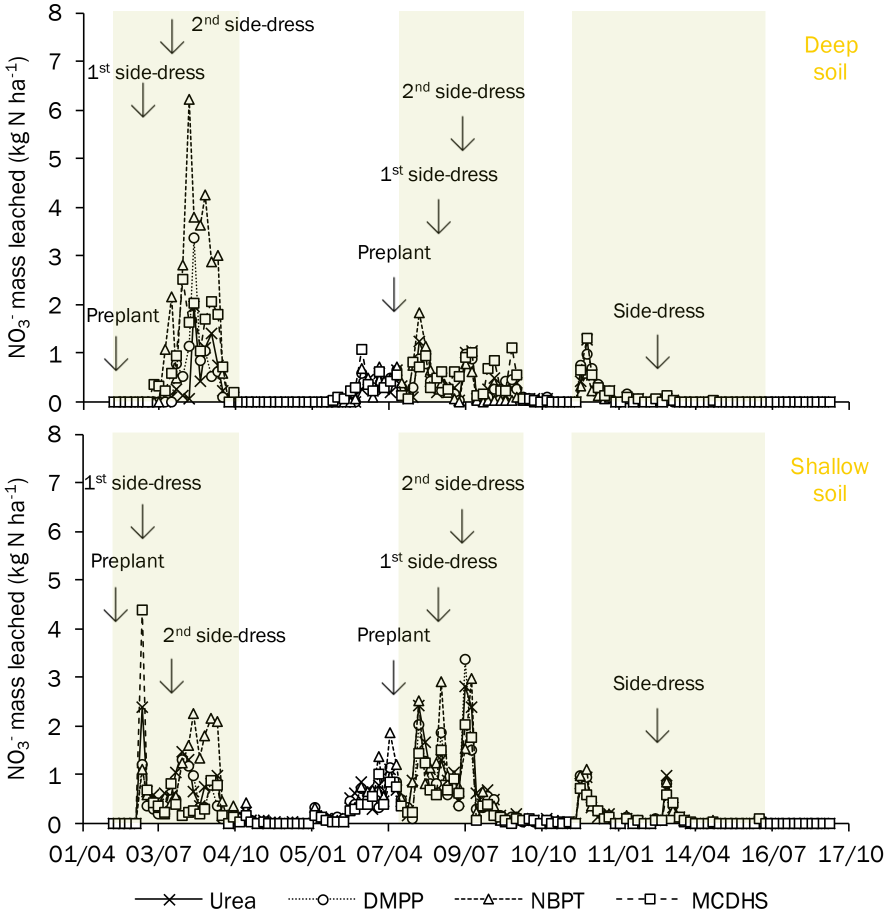


Figure 1. Average weekly nitrate mass leached (kg N ha^{-1} week^{-1}, n=3) for the different fertiliser treatments (Urea, DMPP, NBPT, and MCDHS) for the Deep and Shallow soil. Green areas show the period between seeding and harvest for each crop (maize 1, maize 2, and wheat).

No differences among fertiliser treatments in the mass of nitrate leached were found for the crop period, intercrop period, or 30-day post-fertilisation period (data not shown). Considering the whole 3-year rotation, higher cumulative losses of nitrate were observed in the Shallow (40.4 kg N ha^{-1}) compared to the Deep soil (22.0 kg N ha^{-1}). However, no significant differences were observed in the cumulative mass of nitrate leached among treatments in any of the two soil types (Table 5). In both soils, most of the nitrate was leached during the crop period (82% and 84% for Deep and Shallow soil, respectively) and the period within a month after the fertilisation date accounted for 33% (Deep soil) and 44% (Shallow soil) of the total N leached.

Table 5. Average cumulative mass of nitrate (kg N ha^{-1}; n=3) leached in the different fertiliser treatments (Urea, DMPP, NBPT, and MCDHS). The results are presented separately by soil type (Deep and Shallow) and periods[a].

	Urea	DMPP	NBPT	MCDHS	p-value
			Deep soil		
Maize 1 (2015)	6.8	7.2	15.3	11.4	0.857
Maize 2 (2016)	8.3	7.5	7.8	11.5	0.864
Wheat (2017)	3.3	3.7	1.7	3.6	0.542
Maize 1 + Maize 2	15.1	14.7	23.0	23.0	0.901
Whole rotation	18.4	18.4	24.7	26.5	0.926
			Shallow soil		
Maize 1 (2015)	19.8	14.5	25.7	14.3	0.256
Maize 2 (2016)	19.2	16.5	19.6	13.7	0.237
Wheat (2017)	4.5	4.8	5.0	4.1	0.563
Maize 1 + Maize 2	39.0	31.1	45.3	28.0	0.201
Whole rotation	43.5	35.9	50.2	32.0	0.182

[a]- 'Maize 1', 'Maize 2' and 'Wheat' include the period from sowing to the following sowing. 'Maize 1+2' includes from maize 1's sowing to wheat's sowing. 'Whole rotation' includes from maize 1's sowing to end September.

3.3. Nutritional status of maize and wheat

No significant differences among fertiliser treatments were observed in SPAD meter readings for the first maize crop (2015) for the five sampling dates in any of the two soil types (Fig. 2). However, in the second maize crop (2016) there were significant differences among treatments on some sampling dates. In Deep soil, MCDHS and DMPP showed lower SPAD values than NBPT and Urea at later growth stages, although only the MCDHS (on average 11% lower than NBPT and Urea) was significantly different at the VT stage. In Shallow soil, the SPAD values of the MCDHS treatment were 14% and 16% lower ($p<0.05$) than those of the NBPT treatment at the VT and R3 stages, respectively. In wheat crop, no significant differences were found in SPAD values among treatments at any time for the two soil types (Fig. 2).

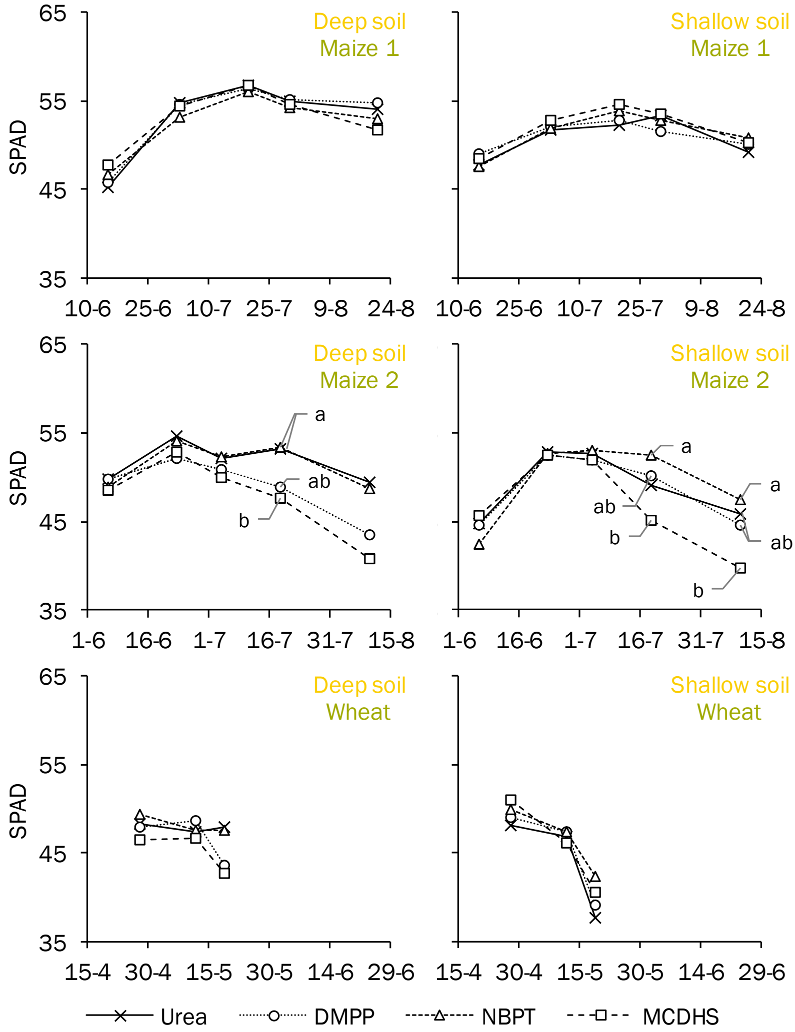


Figure 2. Averages chlorophyll meter readings (SPAD, n=3) for the different fertiliser treatments (Urea, DMPP, NBPT, and MCDHS) in different maize (V6, V10, V13, VT, and R3) and wheat (GS-65, GS-71, and GS-75) stages. Different letters indicate significant differences between treatments.

3.4. Total aboveground biomass and grain yield

The maize yield averaged 19.0 and 15.4 Mg ha^{-1} in 2015 (maize 1) and 2016 (maize 2), respectively (Table 6). Grain yield in Deep soil was significantly higher (averaging 18%) than in Shallow soil. Variations in maize grain yield among plots across years and soil types were significantly related to kernel weight (R^2=0.74) and number of grains per square meter (R^2=0.70). The grain yield of wheat averaged 7.5 Mg ha^{-1} and was 39% higher in Deep soil than in Shallow soil (p<0.05).

Table 6. Average grain yield and total aboveground biomass (Mg ha^{-1}; n=3) of maize (maize 1, maize 2, maize 1+2) and wheat in the different fertiliser treatments (Urea, DMPP, NBPT, and MCDHS) for the two soil types (Deep and Shallow). Values followed by the same letter in a column are not different (p>0.05; Tukey's test).

	Grain yield				Total aboveground biomass			
	Maize 1	Maize 2	Wheat	Maize 1+2	Maize 1	Maize 2	Wheat	Maize 1+2
	Deep soil							
Urea	20.9	17.2	8.7	38.1	35.3	30.8	18.2	65.2
DMPP	20.7	16.3	8.9	36.9	33.9	30.0	19.2	63.9
NBPT	21.1	18.0	8.8	39.1	35.3	31.5	19.7	66.8
MCDHS	20.1	16.4	8.5	36.3	33.3	28.9	19.1	62.2
p-value	0.419	0.225	0.796	0.117	0.333	0.267	0.487	0.124
	Shallow soil							
Urea	17.5	14.6	6.7 a	32.1 ab	28.7	26.7	15.1	55.4 ab
DMPP	18.8	14.4	6.0 b	33.0 ab	28.6	26.6	14.8	55.1 ab
NBPT	19.6	15.4	6.3 ab	34.8 a	29.3	28.1	15.2	57.3 a
MCDHS	17.3	12.4	6.2 ab	29.7 b	27.7	23.8	14.6	51.4 b
p-value	0.052	0.069	0.032	0.015	0.224	0.065	0.563	0.029
Soil type	**	**	**	**	**	**	**	**

* Significant at p<0.05; ** Significant at p<0.01.

Differences in yield performance were observed among fertiliser treatments in Shallow soil but not in Deep soil (Table 6). No significant differences in maize grain yield among treatments were observed in Deep soil, but differences (p<0.1) were observed in Shallow soil in both seasons. Thus, when maize yield in Shallow soil was pooled, MCDHS treatment had a 15% lower grain yield than NBPT. Similarly, total aboveground biomass was 10% lower in MCDHS compared to NBPT. In the case of wheat, Urea showed a 10% higher yield than the treatment with DMPP (Shallow soil; p<0.05), although no significant differences among treatments were observed in aboveground biomass.

3.5. Plant nitrogen concentration and nitrogen use efficiency

The grain N content of maize ranged between 1.23% and 1.38% depending on the year, soil type, and treatment (Table 7). There was a significant tendency for higher grain N content in Deep soil (1.36% on average) compared to that in Shallow soil (1.28%).

Table 7. Average (n=3) N in grain (%), N in total aboveground biomass (kg ha^{-1}), and NUE (kg N kg^{-1} N applied) for maize (maize 1 and maize 2) and wheat in the different fertiliser treatments (Urea, DMPP, NBPT, and MCDHS) and the two soil types (Deep and Shallow). Values followed by the same letter in a column are not significantly different (p>0.05; Tukey's test).

	Grain N			Total aboveground biomass N			NUE		
	Maize 1	Maize 2	Wheat	Maize 1	Maize 2	Wheat	Maize 1	Maize 2	Wheat
					Deep soil				
Urea	1.37	1.37	1.61	353	303	155	1.67	1.75	1.03
DMPP	1.36	1.36	1.61	328	272	157	1.55	1.57	1.04
NBPT	1.36	1.36	1.73	360	315	171	1.71	1.82	1.14
MCDHS	1.33	1.38	1.60	318	276	149	1.51	1.59	1.00
p-value	0.936	0.998	0.042§	0.177	0.181	0.278	0.160	0.187	0.287
					Shallow soil				
Urea	1.30	1.30	1.41 b	270	241 a	103	1.14	1.15 a	0.69 ab
DMPP	1.26	1.26	1.47 ab	248	229 ab	93	1.10	1.09 ab	0.62 ab
NBPT	1.25	1.31	1.56 a	269	247 a	104	1.15	1.18 a	0.70 a
MCDHS	1.23	1.23	1.43 b	254	195 b	90	1.08	0.93 b	0.60 b
p-value	0.647	0.616	0.008	0.132	0.012	0.033§	0.288	0.011	0.026
Soil type	*	*	**	**	**	**	**	**	**

§ indicates significant effect of treatments (p<0.05) from the analysis of variance procedure, but Tukey's test did not show differences.

* Significant at p<0.05; ** Significant at p<0.01.

No significant differences in maize grain N content among fertiliser treatments were observed in the two years for the two soil types. Some minor, although statistically significant, differences were found in the wheat grain N content since it was slightly higher in NBPT than in Urea (11% lower) and MCDHS (9% lower) in the Shallow soil.

No significant differences among fertiliser treatments were observed in the total N uptake (total aboveground biomass N) of maize and wheat in the Deep soil (Table 7). However, some significant differences were found in the Shallow soil for maize. MCDHS treatment presented (in the second maize crop) lower N uptake than Urea (19%) and NBPT (21%) treatments. NBPT treatment always ranked as the top treatment in terms of total N uptake in the two soils, although the differences were not always significant.

In maize, NUE was higher than 1 for all fertiliser treatments (except for MCDHS in Shallow soil - maize 2; Table 7), indicating a relevant contribution of the soil to maize N nutrition. In the Deep soil, this contribution is remarkable because the soil contribution is equivalent, at least, to 51-82% of that of N fertiliser. Averaging over crops and years, NUE was significantly higher in the Deep soil (1.44 kg N kg^{-1} N applied) than in the Shallow soil (0.94 kg N kg^{-1} N applied). No significant differences in NUE among fertiliser treatments were observed in the Deep soil for maize or wheat. In the Shallow soil, averaging over the two maize years, NBPT presented a 17% higher NUE than MCDHS, although the difference was significant only in 2016 (maize 2). Similarly, Urea also showed a 15% higher NUE than MCDHS in maize, but the difference was only significant in 2016. In wheat, the NUE of NBPT was 17% higher than that of the MCDHS treatment ($p<0.05$).

4. Discussion

Soil mineral nitrogen responded according to the management practices. SMN content increased from harvest to the subsequent seeding, as in the study of Arregui and Quemada (2006), presumably due to organic matter mineralisation. The Deep soil presented higher SMN change (119 kg N ha^{-1}) during the intercrop period from the harvest of maize 1 (October 2015) to the sowing of maize 2 (April 2016) than Shallow soil (27 kg N ha^{-1}). That important increase in SMN content, especially in the Deep soil, could be explained by the high number of short rainfall events (51 days with precipitation lower than 5 mm and one day with precipitation higher than 25 mm) and scarcity of events of drainage. The high soil water content in the topsoil during spring could promote high N mineralisation rates. This happened despite the removal of maize crop residues each year, which indicates the high mineralisation rate that could be expected in some soils under irrigated Mediterranean conditions. SMN values after the harvest were prone to be small, which indicates a good adjustment of N fertiliser rates. This good adjustment may be the reason for the absence of significant differences in residual SMN among treatments at the end of the experiment, in contrast to the residual SMN effect of inhibitors reported by other studies. Alonso-Ayuso et al. (2016) found, in two consecutive years of maize cultivation a higher residual SMN after the application of ammonium nitrate sulphate blended with the nitrification inhibitor DMPP compared to the application of the same N fertiliser without NI. According to that study, the higher long-term life of NH_4^+ in the soil solution associated with NIs produced a larger non-exchangeable NH_4^+ fixation that could be conserved and released in the subsequent years to meet crop demands. The three-year rotation of this study does not suggest a significant effect in residual SMN using NI coupled with urea compared to standard urea. The considerably higher maize grain yields observed in this study (17.5 Mg ha^{-1}) compared to 10 Mg ha^{-1} in the study of Alonso-Ayuso et al. (2016) could drive to higher N crop uptake decreasing the chance for ammonium fixation by the clay particles of the soil, even with the comparatively higher doses of N applied in this experiment (average 208 kg N ha^{-1} vs. 170 kg N ha^{-1} in the above-mentioned study). Besides, the total N plant uptake after three years of cropping in the DMPP treatment (664 kg N ha^{-1}) was similar to or even lower than that in the Urea treatment (712 kg N ha^{-1}).

No significant differences in the mass of leached nitrate were observed with the addition of inhibitors. The good adjustment of irrigation to crop needs using very well defined crop coefficient values may explain the non-significant differences in N leaching among fertiliser treatments. As suggested by Diez et al. (2000), the mass of N leached depends strongly on the amount of drainage and, to a lesser extent, on the variation in drainage nitrate concentration. However, in this study, a high percentage of the variability in the mass of nitrate drained among treatments and crops (97% and 72% in Shallow and Deep soil, respectively) was explained by differences in nitrate concentration in the drained water, and a smaller effect was associated with differences in drained volume (55% and 48% for Shallow and Deep soil, respectively). Similarly, Díez et al. (2010) could not find an effect of stabilised N-fertilisers on nitrate leaching when the water requirements of maize were adjusted and the drainage was low (71 mm during the crop-growing season). This experiment corroborates that result since the study had a similar volume of drainage for the maize-cropping season (55 mm in Deep soil and 91 mm in Shallow soil from sowing to harvest).

According to the meta-analysis of Yang et al. (2016), the more N fertiliser is applied, the greater reduction in soil N leaching should be expected from using NIs. In this study, N doses were calculated taking into account the potential N uptake and the SMN available at preplanting, and the N rates were low compared to those used by farmers in the region (Jiménez-Aguirre et al., 2014). Maize residues were removed from plots due to the practical difficulty of incorporating maize residues into the soil because of the small size of the lysimeters preventing the use of heavy machinery. This fact could have promoted sub-optimal N conditions during the second and third cropping seasons.

Maize SPAD values were similar to those in other studies at nearby locations (Berenguer et al., 2009); although they tended to be lower in the second growing season. SPAD readings in the wheat crop in this study were higher than the critical value described by Arregui et al. (2006), suggesting acceptable nutritional N-status during the vegetative period, although the low grain N content indicates N-deficit at later stages, affecting grain quality. Grain yields of maize and wheat were in the upper range of the yields normally obtained by growers in the region (Berenguer et al., 2009; Isla et al., 2015), especially the maize during the first growing season.

In a recent paper, Rose et al. (2018) suggest that the fertilisers frequently called enhanced efficiency N fertilisers (EENFs), which include fertilisers with nitrification and urease inhibitors, only allow higher yields compared to standard fertilisers when sub-optimal N rates are used. This makes sense since the agronomic advantage of EENFs compared to conventional fertilisers mainly relies on a significant reduction in N losses with subsequent improvement of the nutritional N-status of crops. In this maize-maize-wheat rotation, no significant advantage of using different stabilised N-fertilisers in terms of yield, total aboveground biomass, or NUE was observed, although the rates of N applied could be considered optimal to sub-optimal (average of 208 and 150 kg N ha^{-1} for maize and wheat, respectively) or at least clearly below the normal rates used by farmers in the region. Other authors have described no differences in grain yield, biomass yield, and aboveground N uptake between fertilisers with and without inhibitors in maize crop. Thus, Guardia et al. (2017) did not see differences between Urea and Urea+NBPT, and Díez-López et al. (2008) did not see differences between Urea and Urea+DMPP. During the wheat season and in Shallow soil, the grain yield was 0.7 Mg ha^{-1} lower in the DMPP than in the Urea treatment, although in both treatments N doses were the same and equally applied in one side-dress application at tillering stage. That contrasts with the results of the meta-analysis of Hu et al. (2014) where NIs did not affect yield at the same number of N fertiliser applications in winter wheat. It can only be hypothesised that an increase in ammonia volatilisation associated with the use of NIs (Pan et al., 2016) may have reduced the N availability in some critical stages inducing yield decrease.

In the case of maize, this study compares not only the effect of the addition of nitrification or urease inhibitors to urea but also the differences in N management: a single application for stabilised N-fertilisers versus two split applications for urea. Due to the higher price of these special fertilisers compared to the price of urea, their adoption by farmers must imply some advantage in practical terms. The main advantage supplied by stabilised N-fertilisers in this study was their ability to provide in maize, using a single side-dress application of N, similar yield, nitrogen uptake, and NUE as the conventional urea treatment in two side-dress applications. However, the exception was the tendency for lower performance of MCDHS in Shallow soil during the second year when N in total aboveground biomass and NUE were significantly different from those of Urea. The results for wheat indicate no significant advantage in terms of yield of using stabilised N-fertilisers compared to conventional urea, although there is a tendency for a higher NUE with NBPT, especially in the Shallow soil.

5. Conclusions

According to the results obtained in this experiment, under optimal irrigation and adjusted N rates, the use of stabilised N-fertilisers do not present advantages in terms of increasing yield and reducing N leaching. However, the use of DMPP or NBPT allows the reduction in the number of side-dress applications in maize, which can be of interest from a practical point of view to simplify fertiliser management. On the other hand, in the Shallow soil, the new urease inhibitor MCDHS, decreased in some cases the yield, N uptake, and NUE compared to the other treatment with a urease inhibitor (NBPT).

References

Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., Vallejo, A., 2014. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. Agric. Ecosyst. Environ. 189, 136–144. doi:10.1016/j.agee.2014.03.036

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. FAO Irrigation and Drainage Paper 56. Food and Agriculture Organization of the United Nations, Italy, 50 pp.

Alonso-Ayuso, M., Gabriel, J.L., Quemada, M., 2016. Nitrogen use efficiency and residual effect of fertilisers with nitrification inhibitors. Eur. J. Agron. 80, 1–8. doi:10.1016/j.eja.2016.06.008

Arregui, L.M., Lasa, B., Lafarga, A., Irañeta, I., Baroja, E., Quemada, M., 2006. Evaluation of chlorophyll meters as tools for N fertilisation in winter wheat under humid Mediterranean conditions. Eur. J. Agron. 24, 140–148. doi:10.1016/j.eja.2005.05.005

Arregui, L.M., Quemada, M., 2006. Drainage and nitrate leaching in a crop rotation under different N-fertiliser strategies: application of capacitance probes. Plant Soil 288, 57–69. doi:10.1007/s11104-006-9064-9

Ayers, R.S., Westcot, D.W., 1985. Water quality for agriculture, in: FAO Irrigation and Drainage Paper 29, Rev. 1, Italy, 97 pp.

Barros, R., Isidoro, D., Aragüés, R., 2012. Irrigation management, nitrogen fertilisation and nitrogen losses in the return flows of La Violada irrigation district (Spain). Agric. Ecosyst. Environ. 155, 161–171. doi:10.1016/j.agee.2012.04.004

Berenguer, P., Santiveri, F., Boixadera, J., Lloveras, J., 2009. Nitrogen fertilisation of irrigated maize under Mediterranean conditions. Eur. J. Agron. 30, 163–171. doi:10.1016/j.eja.2008.09.005

Carrasco, I., Villar, J.M., 2001. Field evaluation of DMPP as a nitrification inhibitor in the area irrigated by the Canal d'Urgell (Northeast Spain), in: Horst, W.J., Schenk, M.K., Bürkert, A., Claassen, N., Flessa, H., Frommer, W.B., Goldbach, H., Olfs, H.-W., Römheld, V., Sattelmacher, B., Schmidhalter, U., Schubert, S., Wirén, N.v., Wittenmayer, L. (Eds.) Plant Nutrition, Food security and sustainability of agro-ecosystems through basic and applied research. Developments in Plant and Soil Sciences, 92. Springer, Netherlands, pp. 764–765.

Cavero, J., Barros, R., Sellam, F., Topcu, S., Isidoro, D., Hartani, T., Lounis, A., Ibrikci, H., Cetin, M., Williams, J.R., Aragüés, R., 2012. APEX simulation of best irrigation and N management strategies for off-site N pollution control in three Mediterranean irrigated watersheds. Agric. Water Manag. 103, 88–99. doi:10.1016/j.agwat.2011.10.021

Díez-López, J.A., Hernaiz-Algarra, P., Arauzo-Sánchez, M., Carrasco-Martín, I., 2008. Effect of a nitrification inhibitor (DMPP) on nitrate leaching and maize yield during two growing seasons. Span. J. Agric. Res. 6, 294–303. doi:10.5424/sjar/2008062-320

Díez, J.A., Arauzo, M., Hernaiz, P.J., Sanz, A., Vallejo, A., 2010. Comparison of nitrification inhibitors to restrict nitrate leaching in a maize crop irrigated under mediterranean conditions. Span. J. Agric. Res. 8, 481–492. doi:10.5424/sjar/2010082-1212

Diez, J.A., Caballero, R., Roman, R., Tarquis, A., Cartagena, M.C., Vallejo, A., 2000. Integrated fertiliser and irrigation management to reduce nitrate leaching in central Spain. J. Environ. Qual., 29, 1539–1547. doi:10.2134/jeq2000.00472425002900050021x

Egea, C., Alarcón, A.L., 2004. Seguimiento y control de la fertirrigación nitrogenada mediante el producto inhibidor de la nitrificación 3,4-dimetilpirazolfosfato, en cultivo de melón tipo verde. Agrochimica XLVIII, 77–88.

Guardia, G., Cangani, M.T., Andreu, G., Sanz-Cobena, A., García-Marco, S., Álvarez, J.M., Recio-Huetos, J., Vallejo, A., 2017. Effect of inhibitors and fertigation strategies on GHG emissions, NO fluxes and yield in irrigated maize. Field Crop. Res. 204, 135–145. doi:10.1016/j.fcr.2017.01.009

Hu, Y., Schraml, M., von Tucher, S., Li, F., Schmidhalter, U., 2014. Influence of nitrification inhibitors on yields of arable crops: A meta-analysis of recent studies in Germany. Int. J. Plant Prod. 8, 33–50. doi:10.22069/ijpp.2014.1371

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. doi:10.1016/j.eja.2014.11.008

Isla, R., Cavero, J., Yagüe, M.R., Quílez, D., 2006. Balances de nitrógeno en cultivo de maíz de regadío en Aragón, in: Ministerio de Educación y Ciencia (Ed.), Monografía INIA, 22. Balance de Nitrógeno En Sistemas de Cultivo de Cereal de Invierno y de Maíz En Varias Regiones Españolas. España, pp. 107–124.

Isla, R., Salmerón, M., Cavero, J., Yagüe, M.R., Quílez, D., 2015. Utility of the end-of-season nitrate test for nitrogen sufficiency of irrigated maize under Mediterranean semi-arid conditions. Spanish J. Agric. Res. 13, 1–12. doi:10.5424/sjar/2015131-6806

Jiménez-Aguirre, M.T., Isidoro, D., Barros-García, R., 2014. Effect of irrigation modernization on water and nitrogen use efficiency, in: Geophysical Research Abstracts. European Geosciences Union General Assembly. Austria.

Martínez-Cob, A., 2008. Use of thermal units to estimate corn crop coefficients under semiarid climatic conditions. Irrig. Sci. 26, 335–345. doi:10.1007/s00271-007-0097-5

Pan, B., Lam, S.K., Mosier, A., Luo, Y., Chen, D., 2016. Ammonia volatilization from synthetic fertilisers and its mitigation strategies: A global synthesis. Agric. Ecosyst. Environ. 232, 283–289. doi:10.1016/j.agee.2016.08.019

Peña-Haro, S., Llopis-Albert, C., Pulido-Velazquez, M., Pulido-Velazquez, D., 2010. Fertiliser standards for controlling groundwater nitrate pollution from agriculture: El Salobral-Los Llanos case study, Spain. J. Hydrol. 392, 174–187. doi:10.1016/j.jhydrol.2010.08.006

Quemada, M., Baranski, M., Nobel-de Lange, M.N.J., Vallejo, A., Cooper, J.M., 2013. Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems and their effects on crop yield. Agric. Ecosyst. Environ. 174, 1–10. doi:10.1016/j.agee.2013.04.018

Rawluk, C.D.L., Grant, C.A., Racz, G.J., 2001. Ammonia volatilization from soils fertilised with urea and varying rates of urease inhibitor NBPT. Can. J. Soil Sci. 81, 239–246. doi:10.4141/S00-052

Ritchie, S.W., Hanway, J.J., Benson, G.O., 1986. How a corn plant develops, in Special report No. 48. Iowa State University of Science and Technology, and Cooperative Extension Service Ames. United States of America, 21 pp

Rose, T.J., Wood, R.H., Rose, M.T., Van Zwieten, L., 2018. A re-evaluation of the agronomic effectiveness of the nitrification inhibitors DCD and DMPP and the urease inhibitor NBPT. Agric. Ecosyst. Environ. 252, 69–73. doi:10.1016/j.agee.2017.10.008

Serna, M.D., Bañuls, J., Quiñones, A., Primo-Millo, E., Legaz, F., Ban, M.D.S.J., Quin, A., 2000. Evaluation of 3,4-dimethylpyrazole phosphate as a nitrification inhibitor in a Citrus-cultivated soil. Biol. Fertil. Soils 32, 41–46. doi:10.1007/s003740000211

Snyder, C.S.S., Bruulsema, T.W.W., Jensen, T.L.L., Fixen, P.E.E., 2009. Review of greenhouse gas emissions from crop production systems and fertiliser management effects. Agric. Ecosyst. Environ. 133, 247–266. doi:10.1016/j.agee.2009.04.021

Tian, L., Cai, Y., Akiyama, H., 2019. A review of indirect N2O emission factors from agricultural nitrogen leaching and runoff to update of the default IPCC values. Environ. Pollut. 245, 300–306. doi:10.1016/j.envpol.2018.11.016

Timilsena, Y.P., Adhikari, R., Casey, P., Muster, T., Gill, H., Adhikari, B., 2015. Enhanced efficiency fertilisers: a review of formulation and nutrient release patterns. J. Sci. Food Agric. 95, 1131–1142. doi:10.1002/jsfa.6812

United Nations, 2015. Sustainable Development Goals. The 2030 Agenda for Sustainable Development. (accessed 24 April 2020).

Yang, M., Fang, Y., Sun, D., Shi, Y., 2016. Efficiency of two nitrification inhibitors (dicyandiamide and 3, 4-dimethypyrazole phosphate) on soil nitrogen transformations and plant productivity: a meta-analysis. Sci. Rep. 6, 22075. doi:10.1038/srep22075

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415–421. doi:10.1111/j.1365-3180.1974.tb01084.x

Zerulla, W., Pasda, G., Hähndel, R., Wissemeier, A.H., 2001. The New Nitrification Inhibitor DMPP (ENTEC®) for Use in Agricultural and Horticultural Crops – An Overview. Plant Nutrition. Springer, Netherlands, pp. 754–755.

Zhang, X., Davidson, E.A., Mauzerall, D.L., Searchinger, T.D., Dumas, P., Shen, Y., 2015. Managing nitrogen for sustainable development. Nature 528, 51–59. doi:10.1038/nature15743

Supplementary material

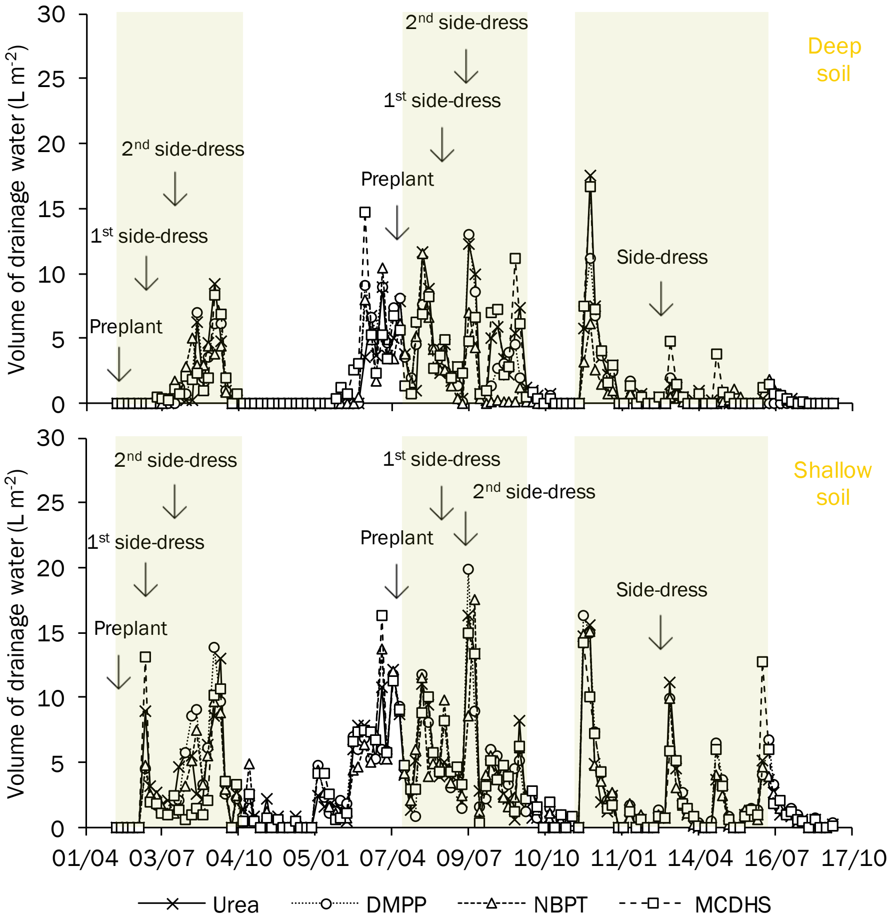


Figure S1. Average weekly volume of drainage water (L m^{-2} $week^{-1}$, n=3) for the different fertiliser treatments (Urea, DMPP, NBPT, and MCDHS). The dynamic is presented for the Deep and Shallow soil. The green areas show the period between seeding and harvest for each crop (maize 1, maize 2, and wheat).

Chapter 4

Feasibility of stabilised nitrogen fertilisers decreasing greenhouse gas emissions under optimal management in sprinkler irrigated conditions

Chapter published in:

Mateo-Marín, N., Quílez, D., Guillén, M., Isla, R., 2020. Feasibility of stabilised nitrogen fertilisers decreasing greenhouse gas emissions under optimal management in sprinkler irrigated conditions. Agric. Ecosyst. Environ. 290, 106725. doi:10.1016/j.agee.2019.106725

Abstract

Stabilised nitrogen (N) fertilisers with nitrification and urease inhibitors have been proposed to abate greenhouse gas (GHG) emissions in agrosystems. Nevertheless, differences in their application and in the management of water and nitrogen rates make it difficult to evaluate their actual utility. The aim of this study is to analyse the possibility for GHG emissions reduction in a 3-year rotation (maize-maize-wheat) by substituting the traditional split-urea application to maize by a single side-dress application of stabilised urea fertiliser. The experiment was performed in 24 drainage lysimeters in two contrasting soil types (Shallow and Deep) under efficient irrigation practices and adjusted N rates under Mediterranean conditions. Nitrous oxide (N_2O) and methane (CH_4) were measured using static closed unvented chambers and the soil mineral N was monitored through periodic soil samplings. CH_4 emissions were generally negligible with occasional tendency the soil acting as a sink more than as a net source. Direct N_2O emissions during the whole rotation showed lower values when a nitrification inhibitor (3,4-dimethylpyrazole phosphate) was added than with conventional urea (Deep soil: 73% lower, $p<0.05$; Shallow soil: 60% lower, ns). Urease inhibitors (N-(n-butyl) thiophosphoric triamide and monocarbamide dihydrogen sulphate) could not abate direct N_2O emissions and their effect depended on the soil type. However, all stabilised fertilisers mitigated N_2O emissions in Deep soil when scaled by grain yield (average 54%). Indirect N_2O emissions associated with nitrate leaching were not affected by the treatments but contributed more to total N_2O emissions in Shallow soil (12%) than in Deep soil (6%). These results suggest that adequate use of nitrification inhibitors could have environmental benefits without lessening agronomic production.

1. Introduction

Agriculture produces direct and indirect greenhouse gas (GHG) emissions: nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) mainly (Tubiello et al., 2015). According to the Intergovernmental Panel on Climate Change (IPCC, 2014), agricultural factors that contribute to GHG release from soils are manure applied to soils, crop residues, synthetic fertilisers, and tillage, among others. Crop nitrogen fertilisation stands out from the rest of the management factors since fertilisation is considered to be responsible for 70% of the worldwide N_2O anthropogenic emissions (Ussiri and Lal, 2013). Nitrous oxide, in addition to standing as the most significant ozone-depleting emissions type, is the third most important GHG (UNEP, 2013) in terms of global warming potential (GWP) due to its long atmospheric lifetime (121 years; Myhre et al., 2013) and its radiative properties (the GWP of 1 kg of N_2O is equivalent to 265 kg of CO_2 when summed over a 100-year period; Myhre et al., 2013).

The large amounts of water and nitrogen applied in irrigated conditions create favourable soil conditions for N_2O emission (Sanz-Cobena et al., 2017) either by nitrification and denitrification processes (Hénault et al., 2012), the two dominant processes of soil N_2O production. In this context of irrigated agriculture, there is a group of irrigation and fertilisation practices with high GHG mitigation potential. In relation to irrigation, adjusting irrigation rates to crop needs and the use of pressure irrigation systems (drip and sprinkler), in comparison to flood or furrow irrigation systems, can decrease N_2O fluxes (Sanz-Cobena et al., 2017). In relation to fertilisation practices, adjustments to N rates to crop needs, N splitting, fertigation, substitution of synthetic fertilisers by manures, injection or immediate incorporation of fertilisers and manure (or slurries) after its application, and use of nitrification and urease inhibitors have been proposed as strategies to reduce N_2O fluxes (Sanz-Cobena et al., 2017).

Nitrification inhibitors (NIs) and urease inhibitors (UIs) as additives to N fertilisers were developed to synchronise the N supply to the N crop demand, avoiding N losses, and thus increasing nitrogen use efficiency (NUE) (Ussiri and Lal, 2013). These fertilisers with inhibitors, frequently called stabilised N-fertilisers, maintain N in less susceptible-to-lose forms. The increase in the duration of N in soils (Huérfano et al., 2015) and the improvement of the NUE (Abalos et al., 2014) could allow a reduction in the N rates or a lessening of the number of fertiliser applications.

Nitrification inhibitors depress the activity of *Nitrosomonas* bacteria in the soil, delaying the first step of the nitrification, which is the oxidation of ammonium to nitrite (Zerulla et al., 2001a, 2001b). NIs contribute to the reduction in N_2O emissions (Cayuela et al., 2017; Recio et al., 2018; Sanz-Cobena et al., 2017) and nitrate leaching losses (Díez-López et al., 2008; Díez et al., 2010; Quemada et al., 2013), but can increase the risk of ammonia (NH_3) volatilisation (Ferguson et al., 1984).

Urease inhibitors delay the conversion of urea to ammonium (enzymatic hydrolysis of urea) by inactivation of the urease enzyme (Ussiri and Lal, 2013). According to several studies, UIs can potentially reduce losses of N by NH_3 volatilisation (Abalos et al., 2012; Cantarella et al., 2018; Sigurdarson et al., 2018), N_2O emissions (Sanz-Cobena et al., 2014, 2012), and nitrate leaching (Abalos et al., 2014; Cameron et al., 2013).

The most commonly used NIs around the world are dicyandiamide (DCD), 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin), and 3,4-dimethylpyrazole phosphate (DMPP) (Trenkel, 2010). Regarding the UIs, the most extensively used is N-(n-butyl) thiophosphoric triamide (NBPT). Another UI, non-'EU fertilising product', monocarbamide dihydrogen sulphate (MCDHS), has been considered by the Spanish Government since 2011 (Orden PRE/630/2011; international patent WO 2007/132032 A1), but no information is available in the scientific literature confirming its potential to stabilise urea-N.

Most studies performed using NIs and UIs to compare their effect to that of conventional fertilisers on yield and N_2O losses do not consider the possibility of reducing the number of N side-dress applications as a strategy and incentive for farmers to use stabilised N fertilisers. Another important factor to elucidate the real impact of stabilised fertilisers on GHG emissions is to assess their effectiveness under limiting N rates (Rose et al., 2018) and efficient irrigation management practices. Therefore, the objective of this study is to evaluate the effect of three different inhibitors in urea (urea with DMPP, NBPT, and MCDHS) applied in a single application in comparison with the traditional urea application on GHG emissions under a 3-year rotation (maize-maize-wheat) and under two soil types in Mediterranean irrigated conditions. The hypothesis was that in comparison to the conventional strategy (split urea in maize), a single application of urea stabilised with inhibitors can reduce N_2O emissions, maintaining crop productivity.

2. Materials and methods

2.1. Site and experimental design

The trial was conducted in the experimental field 'Soto Lezcano', located in the middle Ebro Valley (Zaragoza, Spain), from 2015 to 2017. The area is characterised by a semiarid Mediterranean-continental climate (mean annual maximum and minimum air temperatures of 21.4 °C and 8.3 °C, respectively; yearly average precipitation of 319 mm; and yearly average reference evapotranspiration of 1,239 mm; period 2004–2018).

Table 1. Main physicochemical soil characteristics of Deep and Shallow soil at different depths.

	Deep soil			Shallow soil	
	0-30 cm	30-60 cm	60-125 cm	0-25 cm	25-50 cm
Soil texture	Silt Loam	Silt Loam	Loam	Clay Loam	Clay Loam
Sand (%)	29	31	33	24	30
Silt (%)	52	51	48	40	36
Clay (%)	19	18	19	36	34
Stoniness ($\%_{vol.}$)	3.1	0.9	7.0	11.4	15.2
Available water (mm)	54.5	54.5	114.3	32.1	31.1
P (Olsen) (mg kg^{-1})	30.7	7.8	12.4	14.5	17.5
K (NH_4Ac) (mg kg^{-1})	499	236	72	225	202
Organic matter (%)	1.46	0.94	0.79	2.04	1.24
Soil pH ($1:2.5_{H2O}$)	8.04	7.71	7.65	8.27	8.65

The experiment was carried out in twenty-four drainage lysimeters of 5 m^2 (2.0 × 2.5 m), which had been filled by layers in 2012 with disturbed soil from two different contrasting soil types from the region according to soil depth and stoniness (Supplementary material–Fig. S1). The battery of the 24 lysimeters was located in a 660-m^2 plot (30 × 22 m). The main physicochemical characteristics of the two soils are shown in Table 1. Thus, 12 lysimeters were characterised by deep soil depth and the absence of stones (Deep soil), and 12 lysimeters were characterised by shallow soil depth and frequent stoniness (Shallow soil). Therefore, Deep soil presented a meaningfully higher soil water holding capacity (223 mm) than Shallow soil (63 mm).

The experimental design was a completely randomised block with three replicates for each type of soil. The fertiliser treatments consisted of a) conventional urea (Urea), b) urea with the nitrification inhibitor 3,4-dimethyl pyrazole phosphate at 0.8% (w:w, relative to inhibited N) (DMPP), c) urea with the urease inhibitor N-(n-butyl) thiophosphoric triamide at 0.13% (w:w) (NBPT), and d) urea with the urease inhibitor monocarbamide dihydrogen sulphate at 1.5% (w:w) (MCDHS). These stabilised N-fertilisers were provided by the fertiliser companies allowed to commercialise the inhibitors in Spain. The stabilised fertilisers were solid and were applied by manual broadcast to the soil surface.

A rotation of maize-maize-wheat (*Zea mays* L. hybrid 'Pioneer P1758' and soft wheat *Triticum aestivum* L. cv. 'Rimbaud') was cropped following the management practices described in Table 2.

Table 2. Crop management practices for the whole three-year rotation experiment.

	Maize 1	Maize 2	Wheat
Sowing date	04/05/2015	14/04/2016	10/11/2016
Harvest date	05/10/2015	13/09/2016	03/07/2017
Plant density (plants ha^{-1})	88,083	87,000	286[a]
Date N preplanting	30/04/2015	13/04/2016	-
Date N side-dress 1	15/06/2015	06/06/2016	27/02/2017
Date N side-dress 2	20/07/2015	05/07/2016	-
Total N applied (kg N ha^{-1})			
Deep soil	211	173	150
Shallow soil	236	211	150
Irrigation + Rain (mm)[b]	985	945	609
Crop E.T. (mm)[c]	918	866	578

[a]- kg seed ha^{-1}; [b]- From sowing to harvest; [c]- Obtained from soil water balance.

For the maize crop and in the Urea treatment, the N fertiliser was split into two applications (two-thirds at V6 and one-third at V13 stage), whereas treatments with inhibitors were applied as a single application at V6. The rate of N fertiliser of maize was calculated assuming a total crop uptake of 250 kg N ha^{-1} and discounting the available soil mineral nitrogen at pre-planting for each soil type (0-25 cm in Shallow soil, 0-30 cm in Deep soil) and year (Table 2). In the wheat crop, all treatments received a single N application at the same time (late cereal tillering) at a rate of 150 kg N ha^{-1}. The other macronutrients were also managed to avoid limitations. Thus, conventional fertilisers were applied at pre-planting to maize (50–100–150 kg N–P_2O_5–K_2O ha^{-1}) and wheat (229–154 kg P_2O_5–K_2O ha^{-1}) to avoid P and K limitations.

Weekly irrigation rates were calculated from the reference evapotranspiration (Penman-Monteith equation). Crop coefficients of maize and wheat were estimated according to Martínez-Cob (2008) and FAO procedures (Allen et al., 1998), respectively. The lysimeter area was irrigated using a sprinkler irrigation system, but a drip irrigation network (pluviometry=5 mm h^{-1}) was installed in each lysimeter to compensate for small wind-caused differences in pluviometry among lysimeters. In addition, a 15–20% leaching fraction was included in the calculations to maintain a good soil salt balance due to the moderate salinity of the irrigation water (electrical conductivity average=1.53 dS m^{-1}).

Weeds and pests were controlled using the standard practices of the region, yet no special problems were detected during the rotation.

2.2. Measurements

2.2.1. Greenhouse gas emissions

Static closed unvented chambers (similar to those of Holland et al., 1999) were used to measure N_2O and CH_4 fluxes. One polyvinyl chloride (PVC) collar was inserted 10 cm into the soil in each lysimeter several days before the first sampling. Collars were located between two rows of maize with no plants inside, while in wheat, the collars included plants. Nitrogen fertiliser was applied individually inside each collar to ensure the target rate. PVC chambers coated with a reflective bubble wrap material were fitted into the collars (19.7-cm height, 30.0-cm inner diameter, and 13.9-L volume) at the time of sampling. Fifteen millilitres of air from inside each chamber were taken 0, 30, and 60 minutes after chamber closure using a polypropylene syringe and injected into a 12-mL Exetainer® borosilicate glass vial (Labco Ltd., Lampeter, UK). Air samplings were mostly performed between 10:00 and 11:30 a.m. (Greenwich mean time) considering that soil temperature was the main factor driving diurnal changes in N_2O fluxes (Alves et al., 2012) and that soil temperature at that time was close to the daily average of soil temperature. The frequency of the GHG samplings was higher (every 1–3 days) after fertilisation to capture the expected peak flux of N_2O. There were a total of 37, 25, and 28 sampling dates in each season (maize 1, maize 2, and wheat, respectively), of which 29, 22, and 21 were performed for the period from seeding to harvest.

Air samples were analysed by gas chromatography using an Agilent 7890B chromatograph with an electron-capture (ECD) and flame-ionisation detector (FID). An HP-Plot Q column (15 m of length, 0.32 mm of section, and 0.02 mm of thickness) was used with helium as a carrier gas at 25 mL min^{-1}, and a 5% methane in argon gas mixture at 30 mL min^{-1} was used as a make-up gas for the ECD. The FID, the ECD, and the methaniser were set to 250, 280, and 375 °C, respectively. The injector was set to 50 °C, whereas the oven was set to 35 °C. The obtained detection limits of CH_4 and N_2O were 0.2 and 0.05 ppm (v:v), respectively.

The soil was sampled from 0 to 10 cm to monitor the mineral N concentration in the upper part of the soil profile, one in every two GHG samplings. In these samples, soil water content was obtained by gravimetry (drying at 105 °C until constant weight), and nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were determined in soil extracts (10 g of wet soil + 30 mL of 2 N KCl, shaken for 30 min, and filtered through cellulose filter) by colourimetry using a segmented flow analyser (AutoAnalyser3, Bran + Luebbe, Germany).

Topsoil moisture and temperature at 5-cm depth were also monitored continuously (15' interval) in two lysimeters from each soil type using Hydraprobe sensors (Stevens Water Monitoring Systems Inc., USA). Soil water-filled pore space (WFPS) was estimated according to

Linn and Doran (1984) as the quotient between volumetric soil water content and total soil porosity. Soil calibration curves (R^2=0.72-0.75) were obtained separately for both soil types to convert sensor readings to volumetric soil water content and WFPS values. Total soil porosity (0–5 cm) was calculated considering a particle density of 2.65 Mg m^{-3}, and the soil bulk density was measured *in situ* using the cylinder method (Grossman and Reinsch, 2002) as 1.47 and 1.43 Mg m^{-3} for Deep and Shallow soil, respectively. Daily air temperature and precipitation were registered through an automated weather station located 350 m away from the experimental site.

2.2.2. Nitrate leaching

Weekly drainage from each lysimeter was collected in 50-L graduated tanks set in an underground gallery, and the volume was measured. A 30-mL subsample was collected from each tank to analyse NO_3^- concentrations using a segmented flow analyser (AutoAnalyser3, Bran + Luebbe, Germany). The mass of NO_3^- leached was calculated for each sampling date as the product of the drainage volume by the NO_3^- concentration.

2.2.3. Grain yield

The crops were harvested at maturity (2nd October 2015; 13th September 2016; and 3rd July 2017) to determine grain yield. The results are reported on the basis of 140 g kg^{-1} moisture content for maize and 120 g kg^{-1} moisture content for wheat.

2.3. Data calculations

Fluxes of GHG were calculated fitting a linear regression to gas concentration in the chamber (corrected for air temperature) versus time. Cumulative emissions were estimated for different periods by multiplying the averaged fluxes by the length of the period of two consecutive gas samplings. Fluxes obtained from the static chambers are named as 'direct' emissions.

'Indirect' N_2O emissions are those associated with nitrate leaching which were estimated according to the method established in the 2019 Refinement to the 2006 IPCC Guidelines for

National Greenhouse Gas Inventories (IPCC, 2019). For each lysimeter, the cumulative mass of N lost as nitrate leaching was multiplied by the emission factor (EF_5) of 0.011.

Total N_2O emissions were calculated as the sum of direct and indirect N_2O emissions.

Yield-scaled N_2O emissions (YS_{N2O}; g N Mg^{-1} grain) were calculated as the ratio between the cumulative direct N_2O emissions and the grain yield.

Basal N_2O fluxes were estimated for each lysimeter by removing N_2O peaks to obtain the hypothetical cumulative direct emissions of a control treatment without N fertilisation. A unique treatment-averaged basal N_2O flux was obtained for each soil type and season. Estimated N_2O emission factors (EF, %) were calculated for each lysimeter as the difference between the cumulative direct N_2O emissions measured in each treatment and the estimated basal cumulative N_2O emissions, it was divided by the amount of applied N and multiplied by 100.

2.4. Statistical analysis

Different time periods were considered for the statistical analysis; they were referred to as 'seasons' from sowing to the following sowing, 'crop period' from sowing to harvest, 'intercrop period' from harvest to sowing next year, and 'fertilisation period' from the first side-dress fertiliser application to one month after the second side-dress application.

Variables were transformed (natural logarithm and Box-Cox transformation) when necessary to normalise their distribution and to homogenise the variances, and subjected to two-way (treatment and soil type) analysis of variance. Comparisons among treatments were established with Tukey's test within each soil type since soils are not an eligible variable by the farmer.

A paired *t*-test was used to evaluate differences in daily WFPS and soil temperature between soil types. A one-sample *z*-test was used to check whether cumulative CH_4 emissions were different from zero. The MIXED procedure was used to analyse repeated measurements along time of GHG fluxes and soil N content, according to a first-order autoregressive structure model AR(1). Although significant interaction treatment × sampling times was detected, the global analysis was possible because the interactions were quantitative. Pearson correlation analysis was used to determine the relationship between N_2O fluxes and soil NO_3^- and NH_4^+ concentrations, soil temperature, and WFPS.

In all tests, the level of confidence considered by default was 95%. Statistical analyses were performed using the SAS software University Edition (SAS Institute, Cary, NC).

3. Results

3.1. Soil mineral nitrogen, WFPS, and temperature

The annual pattern of SMN content for the 0 to 10-cm soil depth was closely related to the events of the fertiliser applications (Fig. 1). Noticeable peaks of SMN were observed in the topsoil following N applications that decreased in the subsequent days. The duration of the SMN peaks ranged from 30 to 53 days. SMN content in the stabilised treatments was not directly comparable with that of Urea since the stabilised fertilisers were applied at one time in maize, while Urea was split into two applications. In the one-month period after the single N side-dress application of stabilised fertilisers, in comparison to the other treatments the DMPP treatment always showed the highest values of soil NH_4^+ concentration in this layer and in four of the six cases, it was significantly different from that of the UIs (Table 3). The DMPP treatment presented the largest permanency of ammonium in the soil compared to that of NBPT and MCDHS, being more effective in Shallow soil, e.g., in Shallow soil during the two maize crops, DMPP maintained a N concentration greater than 70 mg N kg^{-1} soil for at least 18 days (Supplementary material–Fig. S2). The behaviour of the NO_3^- concentrations was the opposite of that of NH_4^+, and in general, no significant differences in SMN content were found in the topsoil among the stabilised N-fertilisers in the one-month period that followed side-dress fertilisation.

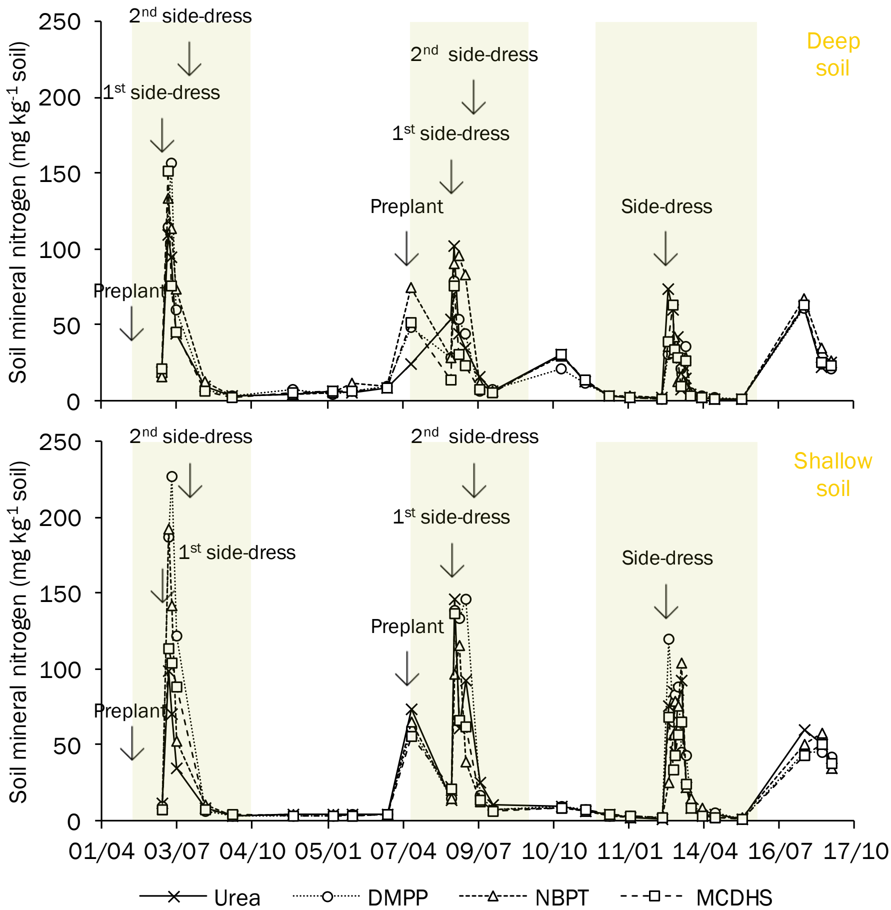


Figure 1. Temporal changes of average soil mineral nitrogen (mg N kg^{-1} soil; n=3) concentration from 0 to 10-cm depth for each fertiliser treatment (Urea, DMPP, NBPT, and MCDHS) and soil type (Deep and Shallow). The three green areas correspond to the period between seeding and harvest of each crop (maize 1, maize 2, and wheat) within the rotation. Arrows indicate fertiliser applications.

WFPS at 5-cm depth throughout the whole rotation ranged from 25% to 90% in Deep soil (average of 56%) and from 24% to 72% in Shallow soil (average of 47%) (Fig. 2a). WFPS was on average 27% higher from seeding to harvest than during the intercrop period (25% higher in Deep soil and 29% higher in Shallow soil) due to the effect of irrigation. Averaged over the whole rotation, Deep soil presented WFPS values 20% higher than those of Shallow soil ($p<0.0001$). Major differences between soils were found during the wheat crop and during the first intercrop period between maize 1 and maize 2.

Table 3. Average topsoil (0-10-cm depth) nitrate, ammonium, and total mineral N concentrations (mg N kg^{-1} soil; n=3) during the one-month period that followed side-dress fertilisation in the stabilised N-fertiliser treatments[a] (DMPP, NBPT, and MCDHS) and for the two soil types (Deep and Shallow). Values followed by the same letter are not significantly different ($p>0.05$; Tukey's test).

	Maize 1			Maize 2			Wheat		
	NO_3^-	NH_4^+	Nmin	NO_3^-	NH_4^+	Nmin	NO_3^-	NH_4^+	Nmin
					Deep soil				
DMPP	31.6 b	39.3 a	70.9	22.8 ab	13.7 a	36.5	9.4 b	21.6 a	31.0
NBPT	69.3 a	0.5 b	69.8	44.9 a	7.6 ab	52.6	16.9 a	10.8 b	27.7
MCDHS	59.4 a	0.6 b	60.1	21.3 b	4.6 b	26.0	14.8 ab	18.4 b	33.1
Treatment	0.001	0.002	0.161	0.042	0.013	0.054	0.014	0.018	0.672
Sampling	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.020	<0.001	0.007
Treat. × Sampl.	0.001	0.003	0.022	0.389	0.004	0.740	0.333	0.092	0.689
					Shallow soil				
DMPP	23.3 b	87.0 a	110.3 a	27.0	49.1 a	76.1	10.7 b	63.3	73.9
NBPT	53.7 a	27.1 b	80.8 ab	32.5	15.3 b	47.8	26.0 a	34.7	60.8
MCDHS	58.2 a	6.0 b	64.2 b	36.6	14.2 b	50.8	17.6 ab	31.1	48.7
Treatment	0.001	0.001	0.040	0.308	0.014	0.070	0.042	0.054	0.191
Sampling	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.165	0.039
Treat. × Sampl.	0.002	<0.001	0.132	0.101	0.016	0.063	0.097	0.416	0.610

[a]- Conventional Urea treatment was not considered in the analysis since it was managed in a different way: splitting application.

Topsoil daily average temperature (5-cm depth) ranged from 0.3 °C to 33.6 °C during the three growing seasons (Fig. 2b). Small but significant differences in soil temperature were found between the two soil types (mean daily temperature of 16.0 °C and 16.8 °C for Deep and Shallow soil, respectively). The largest divergence was found at the end of the rotation, during the wheat crop when the temperature was 9% higher ($p<0.0001$) in Shallow soil than in Deep soil.

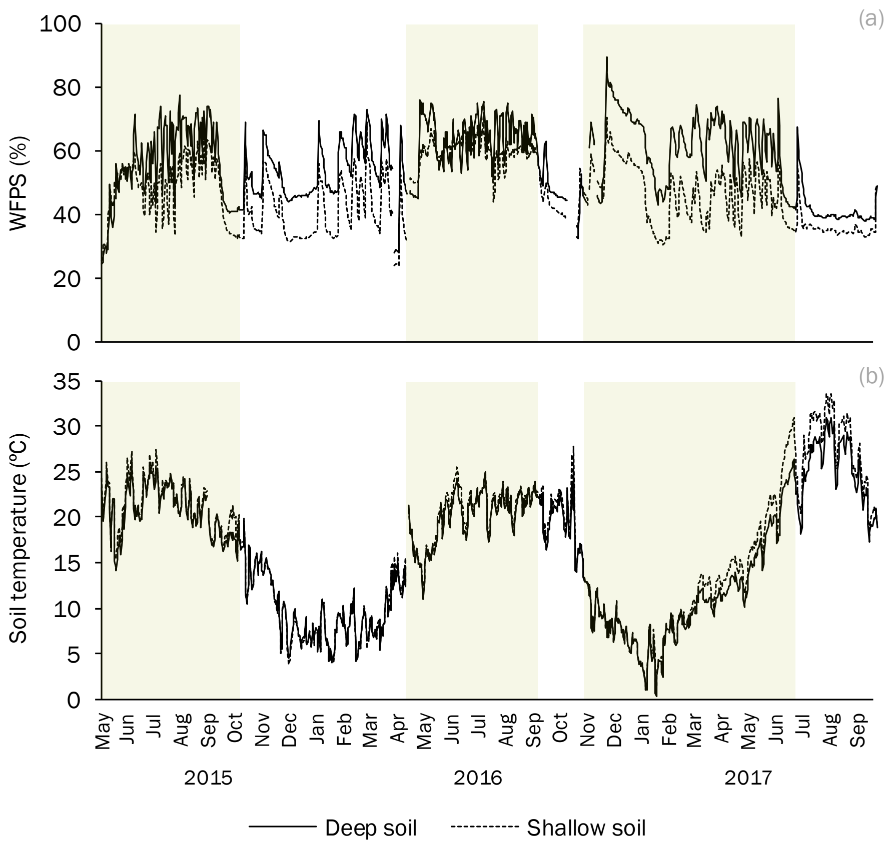


Figure 2. Temporal changes of daily average (n=2) soil water-filled pore space (a) and soil temperature (b) at 5-cm depth for each soil type (Deep and Shallow). The green areas show the period between seeding and harvest of each crop (maize 1, maize 2, and wheat).

3.2. Greenhouse gas emissions

High temporal variability was observed in the N_2O fluxes (Fig. 3), with individual values in the range from −3 to 1,918 g N_2O-N ha^{-1} day^{-1} in Deep soil and from 5 to 2,182 g N_2O-N ha^{-1} day^{-1} in

Shallow soil. Extremely high fluxes were observed after the fertiliser application events (MCDHS reached 1,918 g N_2O-N ha^{-1} day^{-1} in Deep soil and NBPT reached 2,182 g N_2O-N ha^{-1} day^{-1} in Shallow soil, both peaks having a firm performance), and very low fluxes were observed during the rest of the year. Averaging over crops and soils, 97% of N_2O was emitted during the crop periods and the remaining 3% was emitted during the intercrop periods. The accumulated N_2O emissions were highly related to the maximum peak of the N_2O fluxes measured in each lysimeter (maize 1: R^2=0.49; maize 2: R^2=0.92; wheat: R^2=0.81; data not shown).

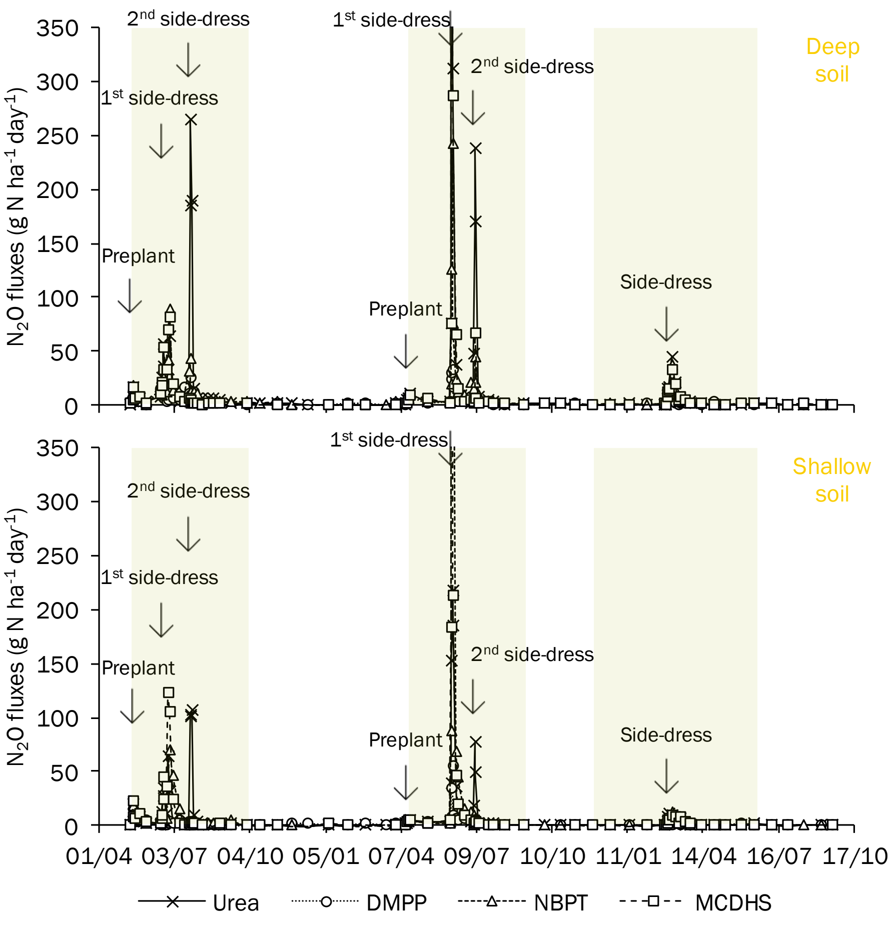


Figure 3. Temporal changes of average N_2O fluxes (g N ha^{-1} day^{-1}; n=3) for each fertiliser treatment (Urea, DMPP, NBPT, and MCDHS) during the three growing seasons (maize 1, maize 2, and wheat) and for the two soil types (Deep and Shallow). The green area shows the period between seeding and harvest of each crop. Arrows show fertiliser applications.

The performance of N_2O emissions peaks did not allow breaking of the Y-axis. Urea and MCDHS reached 656 and 756 g N ha^{-1} day^{-1}, respectively, in Deep soil. NBPT and MCDHS reached 1,014 and 596 g N ha^{-1} day^{-1}, respectively, in Shallow soil, The repeated measure analysis of N_2O fluxes for the 'fertilisation period' showed significant differences among treatments (Fig. 3). DMPP showed the lowest N_2O fluxes for the fertilisation period and was significantly different from Urea (except in maize 1, Shallow soil).

The temporal pattern of the CH_4 fluxes was extremely variable (Supplementary material–Fig. S3) and not related to crop type, period of the year, fertilisation, or irrigation events. The repeated measure analysis did not show differences among the fertiliser treatments regardless of the soil type or season (data not shown).

The soil type significantly affected direct N_2O emissions from the reference Urea treatment: N_2O emissions were more than double in Deep (6.15 kg N_2O-N ha^{-1}) than in Shallow soil (2.92 kg N_2O-N ha^{-1}) (Table 4). However, considering the four treatments, in comparison to soil type, fertiliser treatment had a greater impact on N_2O emissions (Table 4).

Table 4. Average of N_2O emissions (kg N ha^{-1}; n=3) for the different seasons[a], fertiliser treatments (Urea, DMPP, NBPT, and MCDHS), and soil types (Deep and Shallow). Different letters within columns indicate significant differences among treatments (Tukey's test, $p<0.05$) for each soil type.

	Maize 1	Maize 2	Wheat	Maize 1+2	Whole rotation
			Deep soil		
Urea	2.20 a	3.32	0.59 a	5.53 a	6.15 a
DMPP	0.84 b	0.52	0.28 b	1.36 b	1.65 b
NBPT	1.51 ab	1.51	0.56 a	3.04 ab	3.63 ab
MCDHS	1.24 ab	2.68	0.57 a	3.91 ab	4.50 ab
			Shallow soil		
Urea	1.13 ab	1.56 a	0.22	2.69 ab	2.92
DMPP	0.48 b	0.49 b	0.19	0.98 b	1.18
NBPT	1.02 ab	4.12 a	0.18	5.14 a	5.33
MCDHS	1.30 a	2.41 a	0.23	3.71 ab	3.94
Treatment	<0.001	<0.001	<0.001	0.004	0.003
Soil type	0.006	0.964	<0.001	0.632	0.379
Treat. × S. type	0.091	0.047	<0.001	0.043	0.050

[a]- 'Maize 1', 'Maize 2' and 'Wheat' include the period from sowing to the following sowing. 'Maize 1+2' includes from maize 1's sowing to wheat's sowing. 'Whole rotation' includes from maize 1's sowing to end September.

In Deep soil, DMPP significantly reduced cumulative N_2O emissions in comparison to that in Urea in all seasons (with the exception of maize 2). For the whole rotation, DMPP was able to reduce N_2O emissions by 73% (from 6.15 kg N_2O-N ha^{-1} to 1.65 kg N_2O-N ha^{-1}). NBPT and MCDHS were not able to abate N_2O emissions in neither season nor for the whole rotation.

In the Shallow soil, DMPP significantly reduced N_2O emissions in relation to Urea in only the maize 2 season. For the whole rotation, DMPP was able to reduce N_2O emissions by 60% with respect to those in the Urea treatment, although this reduction was significant at p=0.06. UIs (NBPT and MCDHS) quantitatively increased N_2O emissions for the whole rotation; i.e., UIs were not able to reduce emissions significantly in relation to Urea.

Methane emissions were not affected by soil type or fertiliser treatment (Supplementary material–Table S1). Negative emissions were observed in different periods, with the soil acting as a methane sink, although in six out of the eight cases during the whole rotation (4 treatments × 2 soil types), CH_4 emissions were not significantly different from zero (p>0.05).

Estimated indirect N_2O emissions derived from nitrate leaching (Supplementary material–Table S2) did not show differences (p>0.05) among fertiliser treatments for any soil type and considered period. Indirect N_2O emissions presented significant differences between soils. Indirect N_2O emissions for the whole rotation were higher in Shallow soil than in Deep soil for the Urea treatment (136%) and for the average of the four treatments (83%).

For the whole rotation, indirect N_2O emissions in Deep soil were, on average, 0.24 kg N ha^{-1}, whereas direct N_2O emissions were 17 times higher (3.98 kg N ha^{-1}). In Shallow soil, the importance of indirect emissions increased; direct N_2O emissions (3.34 kg N ha^{-1}) were only 8 times higher than indirect N_2O emissions (0.44 kg N ha^{-1}).

In Deep soil, DMPP tended to present lower total N_2O emissions than Urea (Table 5), although the reduction was only significant for wheat. Similarly, DMPP presented lower values compared to Urea in Shallow soil, although differences were not significant. In comparison with conventional fertiliser, urease inhibitors did not significantly affect total N_2O emissions in any of the three seasons in the two soil types. For the whole rotation, DMPP was able to reduce total N_2O emissions by 71% (Deep soil, significant at p=0.053) and 54% (Shallow soil, not significant) in comparison to the conventional Urea treatment.

Table 5. Average total (direct + indirect) N_2O emissions (kg N ha^{-1}; n=3) for the different treatments (Urea, DMPP, NBPT, and MCDHS), seasons[a], and soil types (Deep and Shallow). Different letters within columns indicate significant differences among treatments (Tukey's test, p<0.05) for each soil type.

	Maize 1	Maize 2	Wheat	Maize 1+2	Whole rotation
			Deep soil		
Urea	2.27	3.41	0.62 a	5.70	6.35
DMPP	0.91	0.60	0.32 b	1.52	1.85
NBPT	1.68	1.60	0.57 a	3.30	3.90
MCDHS	1.36	2.80	0.61 a	4.16	4.79
			Shallow soil		
Urea	1.34 ab	1.77	0.27	3.12 ab	3.40 ab
DMPP	0.64 b	0.67	0.24	1.32 b	1.57 b
NBPT	1.30 ab	4.33	0.24	5.64 a	5.88 a
MCDHS	1.46 a	2.56	0.27	4.02 ab	4.29 ab
Treatment	0.005	0.026	0.005	0.007	0.006
Soil type	0.044	0.667	<0.001	0.809	0.485
Treat. × S. type	0.234	0.062	0.016	0.073	0.085

[a]- 'Maize 1', 'Maize 2' and 'Wheat' include the period from sowing to the following sowing. 'Maize 1+2' includes from maize 1's sowing to wheat's sowing. 'Whole rotation' includes from maize 1's sowing to end September.

Treatments with UIs behaved differently depending on the soil type (Table 5). In comparison to Urea, UIs showed lower total N_2O emissions in Deep soil, although higher values occurred in Shallow soil when the whole rotation was considered, yet the differences were not significant in both soil types.

Table 6. Pearson correlation coefficient between N_2O fluxes and soil NO_3^-, soil NH_4^+, soil WFPS, and soil temperature measured in the topsoil (0-10-cm depth). The analysis was performed independently for the different treatments and for the whole dataset.

		Pearson's r			
Treatment	n	NO_3^-	NH_4^+	WFPS	Soil T
Urea	210	0.49	0.21	0.23	0.35
DMPP	210	0.24	0.31	0.21	0.26
NBPT	210	0.47	0.25	0.26	0.34
MCDHS	210	0.53	0.35	ns	0.34
Pooled data	840	0.46	0.33	0.19	0.32

ns: not significant.

Soil NO_3^- content was the variable with the highest correlation to N_2O fluxes (r=0.46) (Table 6), followed by soil NH_4^+ content (r=0.33). When the correlation analysis was performed separately for the different treatments, a different behaviour was observed in the DMPP treatment. Thus, in this treatment, N_2O fluxes presented a higher correlation with soil NH_4^+ (r=0.31) than with soil NO_3^- (r=0.24). WFPS and soil temperature were the variables with weaker correlation to N_2O fluxes when pooled data of the four treatments were considered, even though for some treatments, the correlation was higher for soil temperature than for soil NH_4^+ content (Urea and NBPT). However, the relation between N_2O fluxes and WFPS was non-linear (Fig. 4), maximum N_2O flux values were observed at approximately 60% of soil WFPS, and the highest peaks (>500 g N_2O-N ha^{-1} day^{-1}) of the N_2O fluxes were only observed at approximately 60% WFPS and at extremely high values (>100 kg N ha^{-1}) of topsoil SMN content.

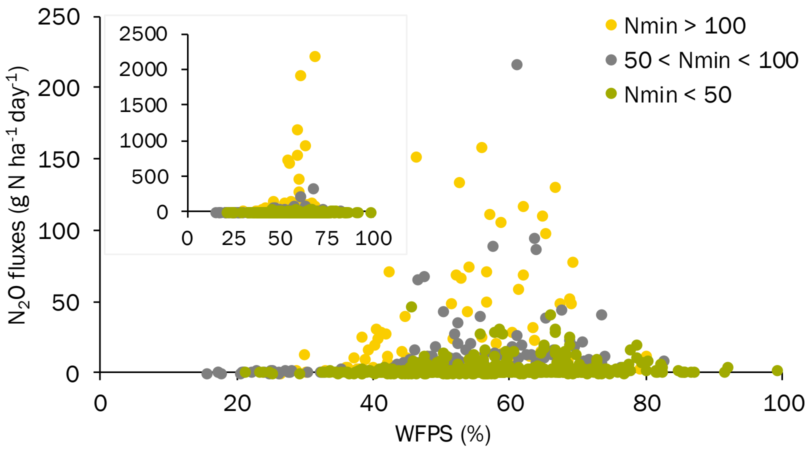


Figure 4. Effect of soil water-filled pore space (WFPS, %) and soil mineral N (Nmin, kg N ha^{-1}) in the topsoil (5-cm depth) on N_2O fluxes (g N ha^{-1} day^{-1}). The whole dataset (n=840) is also presented with a different Y-scale to show the maximum N_2O fluxes observed.

3.3. Yield-scaled N_2O emissions and emission factors

Treatments did not affect yield in the two soil types. The only exception was wheat for Shallow soil since, in comparison to Urea, DMPP presented 10% lower grain production (data not shown).

The fertiliser treatments were more important than the soil type in the YS_{N2O} emissions (Table 7). Yield-scaled N_2O emissions showed differences among treatments depending on the considered period and soil type. DMPP presented the lowest values (except Shallow soil during wheat crop) and was significantly different from Urea in Deep soil for all seasons. Considering the whole rotation, all stabilised N-treatments decreased YS_{N2O} emissions compared to those with Urea in Deep soil, but no effect of inhibitors was detected in Shallow soil. There was a strong relationship (R^2=0.99, n=69) between the N uptake-scaled N_2O emissions (calculated using the aboveground N uptake as the denominator) and the YS_{N2O} emissions (data not shown), and the statistical response to the treatments for the whole rotation was similar for the two variables.

Table 7. Range of the average grain yield (Mg ha^{-1}; n=3) by treatment and average yield-scaled N_2O emissions (g N Mg^{-1} grain; n=3) for the different treatments in different seasons[a] depending on the soil type (Deep and Shallow). Different letters within columns indicate significant differences among treatments (Tukey's test, p<0.05) for each soil type.

	Maize 1	Maize 2	Wheat	Maize 1+2	Whole rotation
			Deep soil		
Yield range	20.1 - 21.1	16.3 - 18.0	8.5 - 8.9	36.3 - 39.1	-
Urea	106 a	192 a	69 a	145 a	131 a
DMPP	40 b	33 b	31 b	37 b	36 b
NBPT	71 ab	84 ab	63 a	78 b	76 b
MCDHS	62 ab	89 ab	67 a	68 b	68 b
			Shallow soil		
Yield range	17.3 - 19.6	12.4 - 15.4	6.0 - 6.7	29.7 - 34.8	-
Urea	64	108 a	33	84 ab	76 ab
DMPP	28	34 b	31	31 b	31 b
NBPT	60	257 a	29	188 a	164 a
MCDHS	75	198 a	37	126 a	110 ab
Treatment	0.007	0.001	0.001	0.001	0.001
Soil type	0.128	<0.001	<0.001	0.141	0.234
Treat. × S. type	0.149	0.005	0.025	0.003	0.004

[a]- 'Maize 1', 'Maize 2' and 'Wheat' include the period from sowing to the following sowing. 'Maize 1+2' includes from maize 1's sowing to wheat's sowing. 'Whole rotation' includes from maize 1's sowing to end September.

Emission factors ranged from 0.03% to 1.91% (Table 8), with an average value of 0.54%. Maize 2 presented the highest values (average of 1.03%), whereas wheat had the lowest values (average of 0.12%). Comparing treatments, the DMPP always presented the lowest EFs, although, considering the whole rotation, DMPP was only different from Urea in the Deep soil.

Table 8. Average emission factor (%, n=3) for the different treatments, seasons[a], and soil types (Deep and Shallow). Different letters within columns indicate significant differences among treatments (Tukey's test, $p<0.05$) for each soil type.

	Maize 1	Maize 2	Wheat	Whole rotation
	Deep soil			
Urea	0.95 a	1.85	0.24 a	1.04 a
DMPP	0.30 b	0.23	0.03 b	0.20 b
NBPT	0.63 ab	0.80	0.22 a	0.57 ab
MCDHS	0.49 ab	1.47	0.23 a	0.73 ab
	Shallow soil			
Urea	0.43 ab	0.69	0.08	0.43
DMPP	0.15 b	0.19	0.06	0.14
NBPT	0.38 ab	1.91	0.05	0.84
MCDHS	0.50 a	1.09	0.08	0.61
Treatment	0.002	0.021	0.002	0.004
Soil type	0.004	0.657	<0.001	0.214
Treat. × S. type	0.071	0.053	0.007	0.052

[a]- 'Maize 1', 'Maize 2' and 'Wheat' include the period from sowing to the following sowing. 'Maize 1+2' includes from maize 1's sowing to wheat's sowing. 'Whole rotation' includes from maize 1's sowing to end September.

4. Discussion

Special care was taken during the experiment to manage the irrigation and the N rates to avoid practices with already well-known negative effects on nitrous oxide emissions. Thus, N fertiliser rates and irrigation management were adjusted to crop needs. Nevertheless, the observed maximum fluxes in N_2O were notably higher than those measured in the same region for a maize crop by Álvaro-Fuentes et al. (2016). For the conventional treatment with urea, emissions peaks higher than 200 g N_2O-N ha^{-1} day^{-1} were measured, while in the previously mentioned study the maximum fluxes were approximately 40 g N_2O-N ha^{-1} day^{-1} for a N application of 300 kg N ha^{-1}, split into three applications of 100 kg N ha^{-1}. This difference is noteworthy considering that the N fertiliser rates of urea used in this study for maize crops were quite similar, between 89 and 148 kg N ha^{-1} (depending on the side-dress application and soil type). The important factor is the type of fertiliser; urea was used in this study as opposed to the ammonium nitrate applied in that of Álvaro-Fuentes et al. (2016). Similarly to this study, Guardia et al. (2017) found maximum fluxes of nitrous oxide of 142 N ha^{-1} day^{-1} with side-dress applications of urea at 180 kg N ha^{-1} in sprinkler-irrigated maize in the central area of Spain. Additionally, N_2O peaks of 200 g N_2O-N ha^{-1} day^{-1} have been described by Martins et al. (2017) with urea rates of 100 kg N ha^{-1} under tropical conditions with air temperatures similar to those found in this study. Also, similar peaks (approximately 200 g N_2O-N ha^{-1} day^{-1}) have been reported by Franco-Luesma et al. (2019) in sprinkler-irrigated maize fertigated with 100 kg N ha^{-1} of N-32 and located on the same experimental farm than this study. The observed variability in the maximum N_2O emissions rates reflects the high number of environmental and management factors that affect N_2O flux. Divergences between the studies could also have been due to the time of day when the N_2O flux was sampled since a diurnal pattern in N_2O has been observed (Xu et al., 2016) under conditions of high mineral N availability (Shurpali et al., 2016); therefore, the selection of sampling time can significantly influence the estimates, especially when fluxes are high.

Treatment with DMPP presented the lowest N_2O emissions for the whole rotation in both soil types. Compiling data from several experiments in Mediterranean areas, Sanz-Cobena et al. (2017) reported reductions in N_2O emissions of 30–50% associated with the use of NIs. Despite the fact that some studies found higher efficiency of NIs to abate N_2O emissions under high fertiliser rates (Yang et al., 2016), in this experiment, DMPP allowed mitigation of 73% (Deep soil) and 60% (Shallow soil, p=0.06) of N_2O emissions in comparison to Urea under adjusted N fertiliser rates. The highest mitigation percentages in comparison with values found in the literature could be related to the intrinsic higher N_2O losses that occur when splitting the N fertiliser compared to a single application (Huérfano et al., 2015). Consequently, the single application of urea with DMPP in this study could have inherently lowered N_2O losses when compared with those in the split application of conventional urea.

In comparison to the conventional Urea treatment, urea stabilised with the two UIs did not significantly reduce N_2O emissions during any of the studied periods. During maize 2, the high emission peaks measured in the MCDHS (Deep soil) and NBPT (Shallow soil) treatments had a noticeable influence on the accumulated values. The absence of differences contrasts with the positive N_2O mitigation effect of UIs (ranging between 30 and 60%) described in the meta-analysis study of Sanz-Cobena et al. (2017) under Mediterranean climate. For instance, urea with NBPT applied to maize crops in Central Spain reduced N_2O emissions by 54% (Sanz-Cobena et al., 2012) and by 50% (Guardia et al., 2017). The main reason for the failure of UIs to inhibit the N_2O emissions might be the non-direct relation between hydrolysis of urea and N_2O emissions (Akiyama et al., 2010).

Maize crops under tropical conditions (Martins et al., 2017) presented higher N_2O emissions when fertilised with urea + NBPT than with conventional urea, a result similar to that observed in this study for Shallow soil. The authors associated this effect with an extension of nitrification period (Smith et al., 2012), favouring the action of nitrifiers (Christianson et al., 1993) leading to an increase in N_2O emissions.

Microbial processes of N_2O production and consumption are mainly driven by soil factors (Ussiri and Lal, 2013). However, in this study, the emission patterns of UI treatments did not seem to respond to the soil water content observed by Sanz-Cobena et al. (2012) in a maize crop under Mediterranean conditions where NBPT led to a loss of effectiveness in the abatement of N_2O fluxes when WFPS was higher than 65%. UIs did not show N_2O mitigation although Shallow soil surpassed the topsoil WFPS of 65% during only 0% and 9% of the days of maize crop in seasons 2015 and 2016, respectively; Deep soil surpassed this threshold more frequently (31% and 48%, respectively), and these conditions were less suitable for NBPT efficiency according to the cited study.

In studies under similar climate conditions where urea + NBPT was applied to maize, yield-scaled N_2O values were in the range of the values obtained in this study. Thus, the study by Guardia et al. (2017) showed values between 37 and 87 g N Mg^{-1} grain, and Sanz-Cobena et al.

(2012) showed YS_{N2O} emissions of 52 g N Mg^{-1} grain (in both cases derived from information in grain yield and N_2O emissions). The exception on similarities is maize 2 in Shallow soil, where YS_{N2O} emissions were extremely high and related to the highest but consistent emission peak measured after fertiliser application. The values obtained for the Urea treatment in the abovementioned studies (85 and 167 g N Mg^{-1} and 130 g N Mg^{-1}, respectively) were in agreement with the results of the present work, which ranged from 64 to 192 g N Mg^{-1}. The single DMPP application in a wheat crop reported lower YS_{N2O} emissions than those derived from Huérfano et al. (2016) (69 and 59 g N Mg^{-1} grain), even though their work was conducted under humid Mediterranean conditions and DMPP was mixed with ammonium sulphate.

In this study, in the one-month period after fertiliser application, urease hydrolysis and nitrification pathways were not affected by the UIs since similar amounts of mineral N (NO_3^- + NH_4^+) were observed in the different treatments. The highest soil NH_4^+ concentrations observed in the DMPP treatment after fertiliser application indicate the expected delay in nitrification, which is consistent with the results of other studies under similar climate conditions; e.g., Díez-López et al. (2008) found a 60-day delay in the nitrification derived from the inhibitory effect of DMPP.

The presence of N in the topsoil governs N_2O emissions because it is the soil factor better explains the variability in N_2O fluxes. Thus, the DMPP treatment showed a different behaviour compared to that of the other treatments, with a higher effect of soil NH_4^+ than NO_3^- content on N_2O fluxes. The delay in nitrification and the SMN content before the fertilisation application could have weakened the NO_3^- contribution compared to that of the other fertiliser treatments. N_2O production is regulated mainly by soil water content and temperature (Barrena et al., 2017). These two factors were positive, although moderately, correlated to N_2O fluxes in this study.

According to Huérfano et al. (2015), the absence of a water table in the root zone and the prevalence of aerobic conditions help soils act as methane sinks. Overall, a zero-balance of CH_4 emissions was observed in this study since in only two treatments (in Deep soil) a significant negative cumulative emission was detected considering the whole 3-year rotation period. The results indicate that no emissions of CH_4 were produced in maize and wheat cropped in sprinkler irrigated fields, that corroborate the results of previous studies (Álvaro-Fuentes et al., 2016; Pareja-Sánchez et al., 2019) under similar climatic and management conditions.

The methodology for N_2O basal emission calculation could have underestimated the EF values since it did not consider residual SMN compared to an actual unfertilised control. Despite that limitation, the EFs estimated for the N fertiliser with DMPP in wheat were 0.03% (Deep soil) and 0.06% (Shallow soil), which were of the same magnitude as those calculated by Huérfano et al. (2015) for the same crop and inhibitor that ranged from 0.03 to 0.07% depending on the season. The EFs obtained for conventional urea for the wheat crop (individual EFs from 0.06% to 0.30%) were within the range of values for cereals (EFMed: 0.26%, 95% confidence interval (CI): ±0.22%, n=53) shown in the meta-analysis of Cayuela et al. (2017). Estimated EFs for Urea in the maize crop had a broader range for both soil types and seasons (individual EFs from 0.31% to 2.50%) in contrast with the interval presented for maize in Cayuela et al. (2017) (EFMed: 0.83%, 95%CI: ±0.26%, n=47). The EF averages for the whole rotation considering all fertiliser treatments were 0.64% (Deep soil) and 0.51% (Shallow soil), which are in agreement with the IPCC Tier I default value for 'all N input in dry climates' (0.5%) (IPCC, 2019). However, it should be remarked the high variability in emission factors found in this study and, therefore, the necessity to progress to more complex models (Tier II and Tier III) for GHG estimation. In fact, the development of mitigation strategies as pointed out by Henault et al. (2012) relays in a better understanding of the determinism of GHG emissions.

Indirect N_2O emissions associated with nitrate lost through leaching and runoff are very complicated to measure, and their values are probably dependent on the specific situation and final fate of water and are, therefore, not evaluated in most studies. Averaging over crops and fertiliser treatments, N_2O emissions associated with nitrate leaching were between 12% (Shallow soil) and 6% (Deep soil) of the total N_2O emissions. The optimal N-fertiliser amounts under conditions of efficient irrigation management in this study must have limited the indirect

N_2O emissions compared to those in other situations with lower irrigation efficiency (e.g., flooded irrigation systems or mismanaged irrigation schedules) and where higher masses of nitrate are leached from cereal fields (Malik et al., 2019). According to that study, and for the worst scenario of low soil water retention, the actual sprinkler irrigation and N management practices in the maize crop led to an estimated mass of nitrate leached of 40 kg N ha^{-1} that will produce estimated indirect N_2O emissions of 0.44 g N ha^{-1}. However, the quantification of indirect N_2O losses from agricultural systems is in initial research stages, and more precise estimations of indirect N_2O emissions are necessary (Tian et al., 2019) to refine the IPCC guidelines and avoid incongruities in the estimations. Accordingly, in the recent IPCC revision, default emission factors have been updated (IPCC, 2019).

5. Conclusions

Nitrous oxide emissions and the effect of the three inhibitors (DMPP, NBPT and MCDHS) on N_2O emission were soil type dependent. The results show that in Deep soil, a single side-dress application of urea with DMPP abated total N_2O emissions in comparison with those in the traditional urea application (split into two applications in maize) at the same N rate. The behaviour of urease inhibitors was completely different in the two soil types, and recommendations should be established in relation to soil characteristics. Thus, in Deep soil, urease inhibitors were able to abate yield-scaled N_2O emissions, while in Shallow soil, UIs increased N_2O and yield-scaled N_2O emissions.

References

Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., Vallejo, A., 2014. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. Agric. Ecosyst. Environ. 189, 136–144. doi:10.1016/j.agee.2014.03.036

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. doi:10.1016/j.chemosphere.2012.04.043

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. doi:10.1111/j.1365-2486.2009.02031.x

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. FAO Irrigation and Drainage Paper 56. Food and Agriculture Organization of the United Nations, Italy, 50 pp.

Álvaro-Fuentes, J., Arrúe, J.L., Cantero-Martínez, C., Isla, R., Plaza-Bonilla, D., Quílez, D., 2016. Fertilization scenarios in Sprinkler-Irrigated corn under mediterranean conditions: effects on greenhouse gas emissions. Soil Sci. Soc. Am. J. 80, 662–671. doi:10.2136/sssaj2015.04.0156

Alves, B.J.R., Smith, K.A., Flores, R.A., Cardoso, A.S., Oliveira, W.R.D., Jantalia, C.P., Urquiaga, S., Boddey, R.M., 2012. Selection of the most suitable sampling time for static chambers for the estimation of daily mean N2O flux from soils. Soil Biol. Biochem. 46, 129–135. doi:10.1016/j.soilbio.2011.11.022

Barrena, I., Menéndez, S., Correa-Galeote, D., Vega-Mas, I., Bedmar, E.J., GonzálezMurua, C., Estavillo, J.M., 2017. Soil water content modulates the effect of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on nitrifying and denitrifying bacteria. Geoderma 303, 1–8. doi:10.1016/j.geoderma.2017.04.022

Cameron, K.C., Di, H.J., Moir, J.L., 2013. Nitrogen losses from the soil/plant system: a review. Ann. Appl. Biol. 162, 145–173. doi:10.1111/aab.12014

Cantarella, H., Otto, R., Soares, J.R., Silva, A.G.B., 2018. Agronomic efficiency of NBPT as a urease inhibitor: a review. J. Adv. Res. 13, 19–27. doi:10.1016/j.jare.2018.05.008

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. doi:10.1016/j.agee.2016.10.006

Christianson, C.B., Baethgen, W.E., Carmona, G., Howard, R.G., 1993. Microsite reactions of urea-nBTPT fertilizer on the soil surface. Soil Biol. Biochem. 25, 1107–1117. doi:10.1016/0038-0717(93)90159-9

Díez-López, J.A., Hernaiz-Algarra, P., Arauzo-Sánchez, M., Carrasco-Martín, I., 2008. Effect of a nitrification inhibitor (DMPP) on nitrate leaching and maize yield during two growing seasons. Span. J. Agric. Res. 6, 294–303. doi:10.5424/sjar/2008062-320

Díez, J.A., Arauzo, M., Hernaiz, P.J., Sanz, A., Vallejo, A., 2010. Comparison of nitrification inhibitors to restrict nitrate leaching in a maize crop irrigated under mediterranean conditions. Span. J. Agric. Res. 8, 481–492. doi:10.5424/sjar/2010082-1212

Ferguson, R.B., Kissel, D.E., Koelliker, J.K., Basel, W., 1984. Ammonia volatilization from surface-applied urea: effect of hydrogen ion buffering capacity1. Soil Sci. Soc. Am. J. 48, 578–582. doi:10.2136/sssaj1984.03615995004800 030022x

Franco-Luesma, S., Álvaro-Fuentes, J., Plaza-Bonilla, D., Arrúe, J.L., Cantero-Martínez, C., Cavero, J., 2019. Influence of irrigation time and frequency on greenhouse gas emissions in a solid-set sprinkler-irrigated maize under Mediterranean conditions. Agric. Water Manag. 221, 303–311. doi:10.1016/j.agwat.2019.03.042

Grossman, R.B., Reinsch, T.G., 2002. 2.1 Bulk density and linear extensibility. In: Dane, J.H., Topp, C.G. (Eds.), Methods of Soil Analysis: Part 4 Physical Methods, pp. 201–228. doi:10.2136/sssabookser5.4.c9

Guardia, G., Cangani, M.T., Andreu, G., Sanz-Cobena, A., García-Marco, S., Álvarez, J.M., Recio-Huetos, J., Vallejo, A., 2017. Effect of inhibitors and fertigation strategies on GHG emissions, NO fluxes and yield in irrigated maize. Field Crop. Res. 204, 135–145. doi:10.1016/j.fcr.2017.01.009

Hénault, C., Grossel, A., Mary, B., Roussel, M., Léonard, J., 2012. Nitrous oxide emission by agricultural soils: a review of spatial and temporal variability for mitigation. Pedosphere 22, 426–433. doi:10.1016/S1002-0160(12)60029-0

Holland, E.A., Robertson, G.P., Greenberg, J., Groffman, P.M., Boone, R.D., Gosz, J.R., 1999. Soil CO2, N2O, and CH4 exchange. In: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins, P. (Eds.), Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, United States of America, pp. 185–201.

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. doi:10.1016/j.eja.2014.11.008

Huérfano, X., Fuertes-Mendizábal, T., Fernández-Diez, K., Estavillo, J.M., GonzálezMurua, 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. doi:10.1016/j.eja.2016.07.001

IPCC, 2019. Chapter 11: N2O Emissions from Managed Soils, and CO2 Emissions From Lime and Urea Application, in: Calvo-Buendia, E., Tanabe, K., Kranjc, A., Baasansuren, J., Fukuda, M., Ngarize S., Osako, A., Pyrozhenko, Y., Shermanau, P., and Federici, S. (Eds.), 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Volume 4: Agriculture, Forestry and Other Land Use. Switzerland, 48 pp.

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. Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Farahani, E., Kadner, S., Seyboth, K., Adler, A., Baum, I., Brunner, S., Eickemeier, P., Kriemann, B., Savolainen, J., Schlömer, S., von Stechow, C., Zwickel, T., and Minx, J.C. (Eds.), Cambridge University Press, United Kingdom and United States of America, 1,435 pp.

Linn, D.M., Doran, J.W., 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48, 1267–1272. doi:10.2136/sssaj1984.03615995004800060013x

Malik, W., Isla, R., Dechmi, F., 2019. DSSAT-CERES-maize modelling to improve irrigation and nitrogen management practices under Mediterranean conditions. Agric. Water Manag. 213, 298–308. doi:10.1016/j.agwat.2018.10.022

Martínez-Cob, A., 2008. Use of thermal units to estimate corn crop coefficients under semiarid climatic conditions. Irrig. Sci. 26, 335–345. doi:10.1007/s00271-007-0097-5

Martins, M.R., Sant'Anna, S.A.C., Zaman, M., Santos, R.C., Monteiro, R.C., Alves, B.J.R., Jantalia, C.P., Boddey, R.M., Urquiaga, S., 2017. Strategies for the use of urease and nitrification inhibitors with urea: impact on N2O and NH3 emissions, fertilizer- 15 N recovery and maize yield in a tropical soil. Agric. Ecosyst. Environ. 247, 54–62. doi:10.1016/j.agee.2017.06.021

Myhre, G., Shindell, D., Bréon, F.M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Stephens, G., Takemura, T., Zhang, H., 2013. Anthropogenic and Natural Radiative Forcing, in: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P.M. (Eds.), Climate Change 2013 - The Physical Science Basis. Cambridge University Press, United Kingdom, pp. 659–740.

Pareja-Sánchez, E., Cantero-Martínez, C., Álvaro-Fuentes, J., Plaza-Bonilla, D., 2019. Tillage and nitrogen fertilization in irrigated maize: key practices to reduce soil CO2 and CH4 emissions. Soil Tillage Res. 191, 29–36. doi:10.1016/j.still.2019.03.007

Quemada, M., Baranski, M., Nobel-de Lange, M.N.J., Vallejo, A., Cooper, J.M., 2013. Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems and their effects on crop yield. Agric. Ecosyst. Environ. 174, 1–10. doi:10.1016/j.agee.2013.04.018

Recio, J., Vallejo, A., Le-Noë, J., Garnier, J., García-Marco, S., Álvarez, J.M., Sanz-Cobena, A., 2018. The effect of nitrification inhibitors on NH3 and N2O emissions in highly N fertilized irrigated Mediterranean cropping systems. Sci. Total Environ. 636, 427–436. doi:10.1016/j.scitotenv.2018.04.294

Rose, T.J., Wood, R.H., Rose, M.T., Van Zwieten, L., 2018. A re-evaluation of the agronomic effectiveness of the nitrification inhibitors DCD and DMPP and the urease inhibitor NBPT. Agric. Ecosyst. Environ. 252, 69–73. doi:10.1016/j.agee.2017.10.008

Sanz-Cobena, A., Abalos, D., Meijide, A., Sanchez-Martin, L., Vallejo, A., 2014. Soil moisture determines the effectiveness of two urease inhibitors to decrease N2O emission. Mitig. Adapt. Strateg. Glob. Change 21, 1131–1144. doi:10.1007/s11027-014-9548-5

Sanz-Cobena, A., Lassaletta, L., Aguilera, E., Prado, Adel, 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. doi:10.1016/j.agee.2016.09.038

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. doi:10.1016/j.agee.2011.12.016

Shurpali, N.J., Rannik, Ü., Jokinen, S., Lind, S., Biasi, C., Mammarella, I., Peltola, O., Pihlatie, M., Hyvönen, N., Räty, M., Haapanala, S., Zahniser, M., Virkajärvi, P., Vesala, T., Martikainen, P.J., 2016. Neglecting diurnal variations leads to uncertainties in terrestrial nitrous oxide emissions. Sci. Rep. 6, 25739. doi:10.1038/srep25739

Sigurdarson, J.J., Svane, S., Karring, H., 2018. The molecular processes of urea hydrolysis in relation to ammonia emissions from agriculture. Rev. Environ. Sci. Biotechnol. 17, 241–258. doi:10.1007/s11157-018-9466-1

Smith, K.A., Dobbie, K.E., Thorman, R., Watson, C.J., Chadwick, D.R., Yamulki, S., Ball, B.C., 2012. The effect of N fertilizer forms on nitrous oxide emissions from UK arable land and grassland. Nutr. Cycl. Agroecosyst. 93, 127–149. doi:10.1007/s10705-012-9505-1

Tian, L., Cai, Y., Akiyama, H., 2019. A review of indirect N2O emission factors from agricultural nitrogen leaching and runoff to update of the default IPCC values. Environ. Pollut. 245, 300–306. doi:10.1016/j.envpol.2018.11.016

Trenkel, M.E., 2010. Slow- and controlled-release and stabilized fertilizers. International Fertilizer Industry Association, France, 160 pp.

Tubiello, F. N., Cóndor-Golec, R. D., Salvatore, M., Piersante, A., Federici, S., Ferrara, A., Rossi, S., Flammini, A., Cardenas, P., Biancalani, R., Jacobs, H., Prasula, P., Prosperi, P., 2015. Estimating Greenhouse Gas Emissions in Agriculture. A Manual to Address Data Requirements for Developing Countries. Food and Agriculture Organization of the United Nations, Italy, 193 pp.

UNEP, 2013. Drawing Down N2O to Protect Climate and the Ozone Layer. A UNEP Synthesis Report. Kenya, 57 pp.

Ussiri, D., Lal, R., 2013. Soil Emission of Nitrous Oxide and its Mitigation. Springer, Netherlands, 378 pp.

Xu, J., Wei, Q., Yang, S., Wang, Y., Lv, Y., 2016. Diurnal pattern of nitrous oxide emissions from soils under different vertical moisture distribution conditions. Chil. J. Agric. Res. 76, 84–92. doi:10.4067/S0718-58392016000100012

Yang, M., Fang, Y., Sun, D., Shi, Y., 2016. Efficiency of two nitrification inhibitors (dicyandiamide and 3, 4-dimethypyrazole phosphate) on soil nitrogen transformations and plant productivity: a meta-analysis. Sci. Rep. 6, 22075. doi:10.1038/srep22075

Zerulla, W., Barth, T., Dressel, J., Erhardt, K., Horchler von Locquenghien, K., Pasda, G., Rädle, M., Wissemeier, A., 2001a. 3,4-Dimethylpyrazole phosphate (DMPP) – a new nitrification inhibitor for agriculture and horticulture. Biol. Fertil. Soils 34, 79–84. doi:10.1007/s003740100380

Zerulla, W., Pasda, G., Hähndel, R., Wissemeier, A.H., 2001b. The New Nitrification Inhibitor DMPP (ENTEC®) for Use in Agricultural and Horticultural Crops – An Overview. Plant Nutrition. Springer, Netherlands, pp. 754–755.

Supplementary material

Table S1. Average cumulative CH_4 emissions (g C ha^{-1}; n=3) for the different seasons[a], fertiliser treatments (Urea, DMPP, NBPT, and MCDHS), and soil types (Deep and Shallow). Different letters within columns indicate significant differences among treatments (Tukey's test, p<0.05) for each soil type.

	Maize 1	Maize 2	Wheat	Maize 1+2	Whole rotation
			Deep soil		
Urea	-422*	-35	-481	-403	-1,021*
DMPP	-544	-242*	-179	-831*	-1,101
NBPT	-349*	-246	765	-594*	191
MCDHS	-708*	41	-502	-676*	-1,074*
			Shallow soil		
Urea	21	-181*	-462	-139	-622
DMPP	-388*	139	-523	-265	-763
NBPT	-130	159	-236	36	-151
MCDHS	-8	-243*	293	-268	84
Treatment	0.542	0.768	0.482	0.754	0.329
Soil type	0.322	0.401	0.774	0.296	0.712
Treat. × S. type	0.823	0.083	0.232	0.835	0.447

[a]- 'Maize 1', 'Maize 2' and 'Wheat' include the period from sowing to the following sowing. 'Maize 1+2' includes from maize 1's sowing to wheat's sowing. 'Whole rotation' includes from maize 1's sowing to end September.

*- Asterisk indicates cumulative CH_4 emissions different from zero.

Table S2. Average estimated indirect N_2O emissions (kg N ha^{-1}; n=3) associated with N leaching for the different treatments (Urea, DMPP, NBPT, and MCDHS), seasons[a], and soil types (Deep and Shallow). Different letters within columns indicate significant differences among treatments (Tukey's test, $p<0.05$) for each soil type.

	Maize 1	Maize 2	Wheat	Maize 1+2	Whole rotation
			Deep soil		
Urea	0.07	0.09	0.04	0.17	0.20
DMPP	0.08	0.08	0.04	0.16	0.20
NBPT	0.17	0.09	0.02	0.25	0.27
MCDHS	0.13	0.13	0.04	0.25	0.29
			Shallow soil		
Urea	0.22	0.21	0.05	0.43	0.48
DMPP	0.16	0.18	0.05	0.34	0.39
NBPT	0.28	0.22	0.05	0.50	0.55
MCDHS	0.16	0.15	0.04	0.31	0.35
Treatment	0.439	0.933	0.739	0.594	0.668
Soil type	0.070	0.002	0.021	0.013	0.010
Treat. × S. type	0.851	0.436	0.387	0.681	0.636

[a]- 'Maize 1', 'Maize 2' and 'Wheat' include the period from sowing to the following sowing. 'Maize 1+2' includes from maize 1's sowing to wheat's sowing. 'Whole rotation' includes from maize 1's sowing to end September.

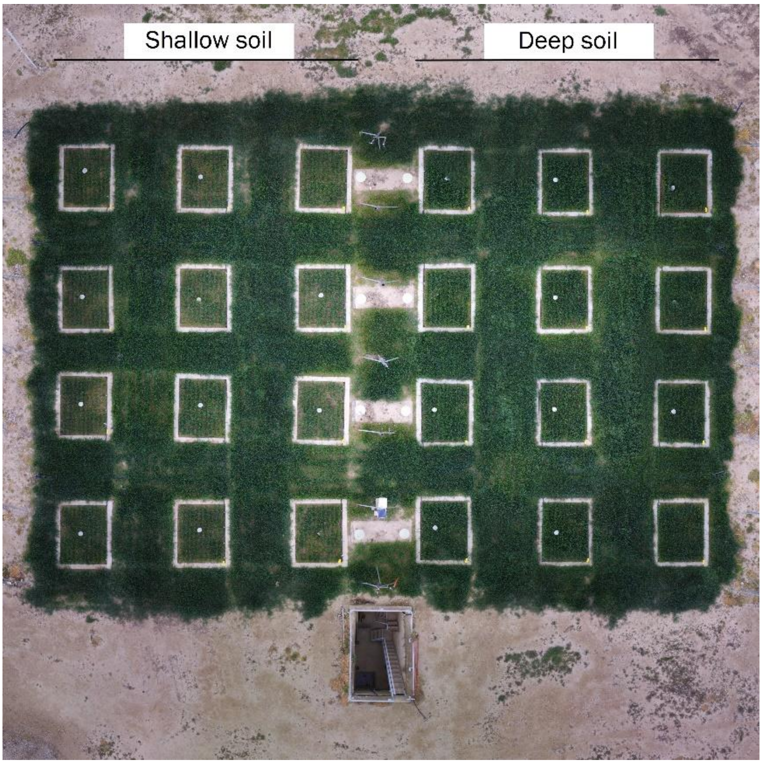


Figure S1. Aerial photography of the lysimeter station. The twelve lysimeters at the right side are those with Deep soil and the twelve lysimeters at the left side are those with Shallow soil.

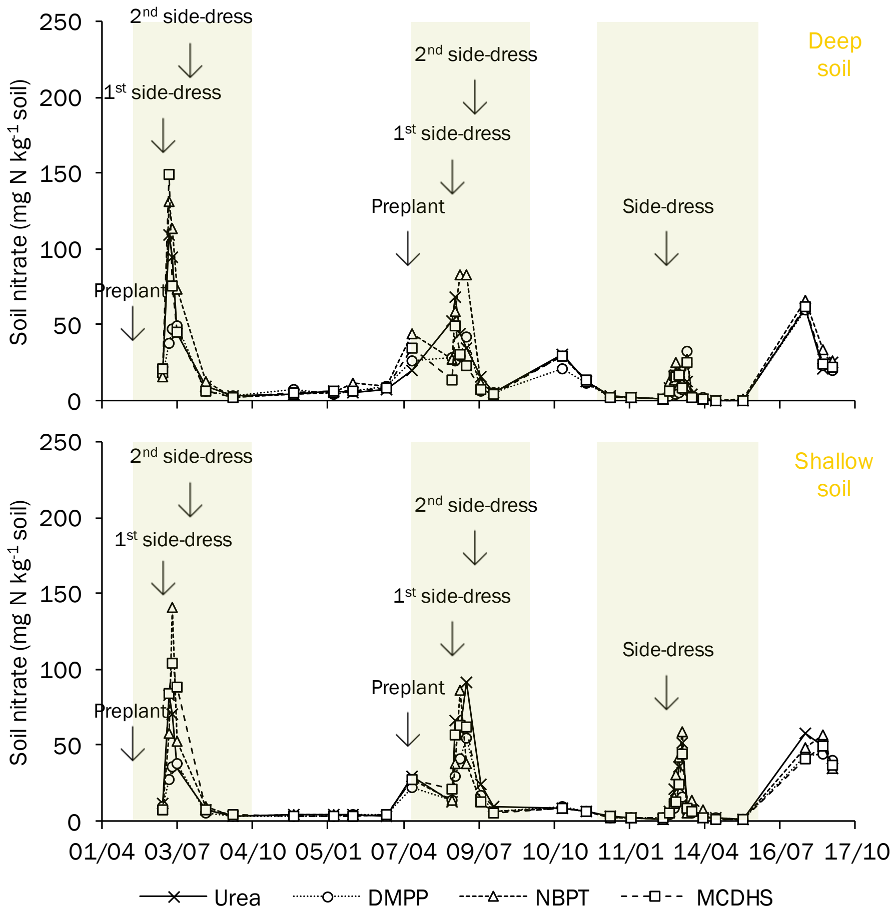


Figure S2a. Temporal changes of average soil nitrate content (mg N kg^{-1} soil; n=3) from 0 to 10-cm depth for each fertiliser treatment (Urea, DMPP, NBPT, and MCDHS) and soil type (Deep and Shallow). The three green areas correspond to the period between seeding and harvest of each crop (maize 1, maize 2, and wheat) within the rotation. Arrows indicate fertiliser applications.

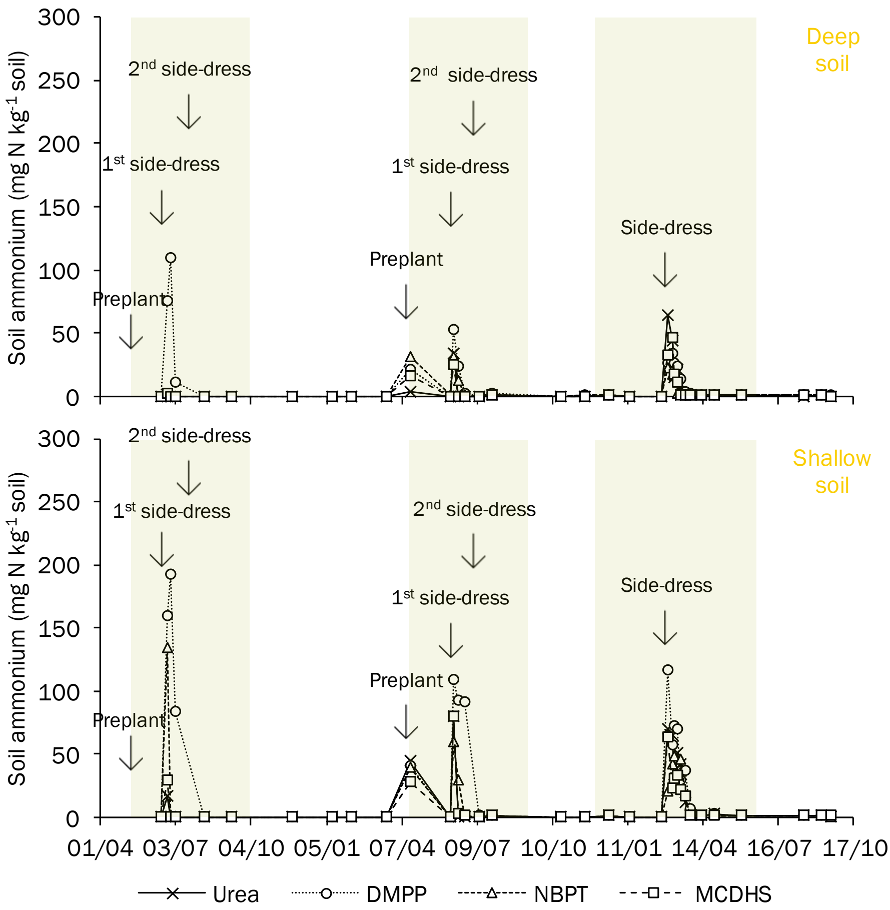


Figure S2b. Temporal changes of average soil ammonium content (mg N kg^{-1} soil; n=3) from 0 to 10-cm depth for each fertiliser treatment (Urea, DMPP, NBPT, and MCDHS) and soil type (Deep and Shallow). The three green areas correspond to the period between seeding and harvest of each crop (maize 1, maize 2, wheat) within the rotation. Arrows indicate fertiliser applications.

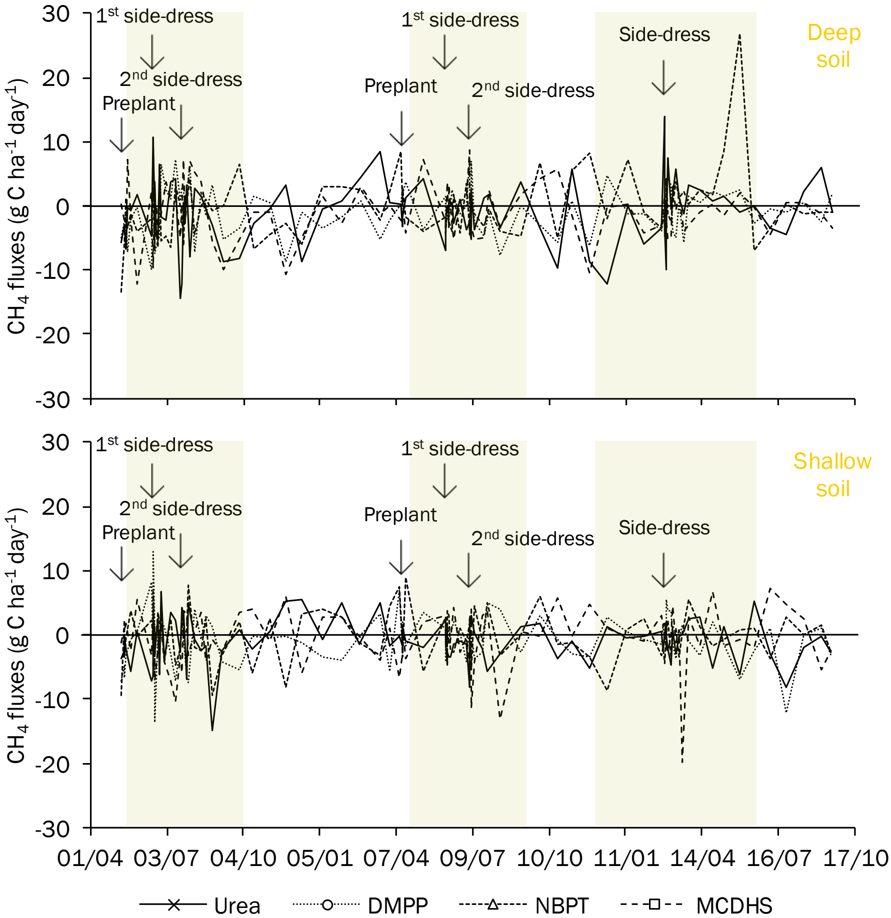


Figure S3. Temporal changes of average CH_4 fluxes (g C ha^{-1} day^{-1}; n=3) for each fertiliser treatment along the three growing seasons (maize 1, maize 2, wheat) and for the two soil types (Deep and Shallow). The shadow area shows the period between seeding and harvest of each crop. Arrows show fertiliser applications.

Chapter 5

Agronomic and environmental implications of substituting synthetic nitrogen for pig slurry in a wheat crop

Abstract

Using animal manures and slurries as fertilisers is an encouraging strategy to reuse and recycle nutrients and to strive towards a circular economy at regional scale. However, the environmental risks associated with the management and use of these sources of nutrients should be avoided. In this context, the objectives of the study are to assess crop productivity and environmental effects of (1) the use of pig slurry (PS) as substitute for a synthetic fertiliser (urea, U) and (2) the addition of a urease inhibitor (monocarbamide dihydrogen sulphate, MCDHS) to pig slurry (PSI). Agronomic parameters (grain yield, grain protein, nitrogen uptake, and nitrogen use efficiency indexes) and greenhouse gas (nitrous oxide - N_2O, methane - CH_4) emissions were evaluated in a three-year wheat crop. A target rate of 120 kg NH_4^+-N ha^{-1} as U, PS, or PSI (main factor) was applied at tillering and it was supplemented at stem elongation (secondary factor) with 0, 30, 60, or 90 kg N ha^{-1} in the form of ammonium nitrate. Nitrous oxide emissions did not depend ($p>0.05$) on the nitrogen (N) source, synthetic urea or pig slurry. Grain yield and nitrogen use efficiency indexes did not show differences ($p>0.05$) among U and PS treatments. Nitrogen application at stem elongation did not influence yield but affected grain protein content in the three main treatments. Higher unaccounted N was obtained from a soil N balance in PS treatments (PS and PSI) compared to U fertilisation, which could be due to higher ammonia volatilisation, although further studies should be conducted to confirm this hypothesis. In conclusion, pig slurry can replace synthetic fertiliser without loss of productivity and with similar greenhouse gas emissions. The addition of the urease inhibitor MCDHS to pig slurry was not able to show agronomic or environmental benefits under the agro-environmental evaluated conditions.

1. Introduction

Wheat is one of the main cultivated cereals around the world with 214.8 million hectares (FAOStat, 2020). In Spain, wheat production represents 34% of grain cereal productions (MAPA, 2020) since the Mediterranean climate, despite their higher unpredictability, allows producing high-quality wheat with adequate management practices (Borghi et al., 1997), especially bread wheat with more demanding quality standards.

Spain is the European country with the highest porcine livestock population, reaching 30.8 million heads in 2018 (EUROSTAT, 2020). Pig slurry (PS) applied as fertiliser to winter cereal and maize crops is the most common recycling method for this product (Maris et al., 2016). Pig slurry application to crops at the same nitrogen (N) rate than that used for synthetic fertilisers can result in similar crop yields (Goss et al., 2013). However, high rates of nutrients on farmland, usually desynchronised with crop demand, increase notably the risk of environmental pollution (Daudén and Quílez, 2004). Pollution associated with PS application includes nitrate (NO_3^-) leaching, an increase in soil heavy metal concentration (mainly zinc and copper), and ammonia (NH_3) and nitrous oxide (N_2O) emissions (Aguilera et al., 2013; Gómez-Garrido et al., 2018; Jensen et al., 2016; Sanz-Cobena et al., 2019). Accordingly, different management practices can be applied to reduce these environmental risks; e.g., PS applied at adequate rates leads to minimising N losses by drainage (Diez et al., 2001) and early slurry incorporation with tillage after its application is recommended to control NH_3 volatilisation (Yagüe et al., 2019). Although the traditional PS application is before cereal sowing, application at cereal tillering stage expands its application time window and improves its usability (Bosch-Serra et al., 2015), but the slurry remains over the soil surface (Jiménez-de-Santiago et al., 2019) increasing the risk of noteworthy gaseous N losses. In this situation, managing practices such as incorporating immediately the manure after its application or injecting slurries into the soil, which abate NH_3 and indirect N_2O emissions (Sanz-Cobena et al., 2017), are unfeasible, with exception of irrigated areas where PS can be incorporated by irrigation. Moreover, the control of N_2O emissions poses a challenge as liquid organic fertilisers have relatively high release levels compared to other manures due to the high proportion of ammonium-N (Aguilera et al., 2013). For these reasons, the addition of inhibitors to N fertilisers could provide the opportunity to reduce greenhouse gas (GHG) emissions up to 55% in irrigated Mediterranean agriculture as was pointed out by Sanz-Cobena et al. (2017).

Inhibitors are considered a mitigation tool by the Intergovernmental Panel on Climate Change (IPCC) since they avoid N losses through the synchronisation of N supply to crop demand. Nitrification inhibitors delay the transformation of ammonium (NH_4^+) to nitrite (NO_2^-) during the first step of the nitrification process, carried out by soil *Nitrosomonas* bacteria (Zerulla et al., 2001a, 2001b), reducing N_2O fluxes (Cayuela et al., 2017; Mateo-Marín et al., 2020; Recio et al., 2018), and NO_3^- leaching (Díez-López et al., 2008; Díez et al., 2010; Quemada et al., 2013), but increasing the risk of NH_3 losses by volatilisation (Ferguson et al., 1984; Sanz-Cobena et al., 2017). Urease inhibitors delay the transformation of urea-N to ammonium-N by inactivation of the urease enzyme (Snyder et al., 2009), reducing NH_3 volatilisation (Cantarella et al., 2018; Menéndez et al., 2009; Sanz-Cobena et al., 2014), N_2O emissions (Sanz-Cobena et al., 2017, 2012), and NO_3^- leaching (Abalos et al., 2014; Cameron et al., 2013).

Although many compounds might have potential as inhibitors (Kiss and Simihăian, 2002) and some of them have been patented (Chien et al., 2009), their evaluation under different agro-environmental conditions is critical. Monocarbamide dihydrogen sulphate (MCDHS; international patent WO 2007/132032 A1) has been classified as a urease inhibitor and the Spanish Government has accepted its use since 2011 (Orden PRE/630/2011). However, the product has not been widely assessed and no information can be found in the scientific literature relative to its effectivity inhibiting the urease enzyme and its effect on the soil N dynamics.

In this context, the first objective of this study is to evaluate in a bread wheat crop under semiarid irrigated conditions the effect of substituting synthetic N fertiliser for pig slurry on crop productivity and greenhouse gas emissions. The second objective is to assess the effect of adding the urease inhibitor monocarbamide dihydrogen sulphate to pig slurry on crop productivity, soil nitrogen dynamics, and greenhouse gas emissions. It was hypothesised that even when the pig slurry urea has been transformed to ammonium, the urease inhibitor could have effect since the micro-acidification due to hydrolysis of the MCDHS molecule can reduce ammonia volatilisation, showing effects on the studied parameters.

2. Materials and methods

2.1. Site and experimental design

The study was conducted in the experimental field 'Soto Lezcano' (41° 43' 49" N, 0° 49' 2" O) in the middle Ebro river basin (Zaragoza, Spain) in a Typic Xerofluvent soil (Soil Survey Staff, 2014). The physicochemical characteristics of the soil are shown in Table 1. The climate of the region is semiarid Mediterranean-continental (mean annual air temperature of 14.6 °C; mean annual precipitation of 318 mm; mean annual reference evapotranspiration of 1,243 mm; period 2004-2019).

Table 1. Main physicochemical soil characteristics at the beginning of the experiment (2015).

	0-30 cm	30-60 cm	60-90 cm
Soil texture	Silt Loam	Silt Loam	Loam
Sand (%)	32.5	31.1	38.2
Silt (%)	50.5	51.9	49.5
Clay (%)	17.0	17.0	12.3
Stoniness ($\%_{vol.}$)	1	1	1
Equivalent calcium carbonate (g kg^{-1})	40	41	39
Total nitrogen (Kjeldahl) (mg kg^{-1})	1,350	940	620
Phosphorous (Olsen) (mg kg^{-1})	43	12.1	<5.0
Potassium (NH_4Ac) (mg kg^{-1})	408	231	101
Organic matter (%)	1.84	0.92	0.50
pH ($1{:}2.5_{H2O}$)	8.36	8.36	8.28
Electrical conductivity ($1{:}5_{H2O}$) (dS m^{-1})	0.265	0.261	0.307

Bread wheat (*Triticum aestivum* L. cv. 'Rimbaud') was cultivated under sprinkler irrigation during three growing seasons (2015/16, 2016/17, and 2017/18) according to the management characteristics presented in Table 2. Irrigation requirements were calculated weekly from the reference evapotranspiration estimated with the Penman-Monteith equation and the wheat crop coefficients according to the FAO procedure (Allen et al., 1998).

Table 2. General crop management characteristic of wheat seasons.

	2015/16	2016/17	2017/18
Sowing date	26/11/2015	30/12/2016	16/11/2017
Harvest date	07/07/2016	04/07/2017	06/07/2018
Plant density (kg seed ha^{-1})	170	200	175
Date N side-dress at tillering	24/02/2016	21/03/2017	22/03/2018
Date N side-dress at stem elongation	05/04/2016	18/04/2017	24/04/2018
Irrigation + Rain (mm)*	380	435	428
Crop E.T. (mm)	416	429	383

*- From sowing to harvest.

The experimental design was a split-plot with four replications. The main factor included three different fertilisation strategies at tillering (Table 3): a) urea at the rate of 120 kg N ha^{-1} (U120); b) pig slurry at the target rate of 120 kg NH_4^+-N ha^{-1} (PS120); and c) pig slurry mixed with the urease inhibitor monocarbamide dihydrogen sulphate at the target rate of 120 kg NH_4^+-N ha^{-1} (PSI120). The second factor of the split-plot consisted in four rates of synthetic N at stem elongation: 0 (AN0), 30 (AN30), 60 (AN60), and 90 (AN90) kg N ha^{-1} in form of ammonium nitrate (Table 3).

Table 3. Fertiliser treatments depending on the target N rate and timing of side-dress application.

Pig slurry treatments			Synthetic treatments		
	Tillering	Stem elongation		Tillering	Stem elongation
	PS	NH_4NO_3		Urea	NH_4NO_3
	kg NH_4^+-N ha^{-1}	kg N ha^{-1}		kg N ha^{-1}	kg N ha^{-1}
PS120-AN0	120	0	U120-AN0	120	0
PS120-AN30	120	30	U120-AN30	120	30
PS120-AN60	120	60	U120-AN60	120	60
PS120-AN90	120	90	U120-AN90	120	90
PSI120-AN0	120	0	Control	0	0
PSI120-AN30	120	30	U60-AN0	60	0
PSI120-AN60	120	60	U90-AN0	90	0
PSI120-AN90	120	90	U150-AN0	150	0

Besides, four supplementary treatments were added with different rates of N in form of urea at tillering: 0 (Control), 60 (U60-AN0), 90 (U90-AN0), and 150 (U150-AN0) kg N ha^{-1}, with no N application at stem elongation (Table 3). These treatments were included to calculate the nitrogen fertiliser replacement value of the pig slurry in PS120 and PSI120.

The size of the experimental plots was 6.0 × 7.0 m for pig slurry treatments and 6.0 × 3.5 m for urea treatments. Pig slurry was applied using trail hoses and the dose was calculated according to its ammonium-N content, which was measured *in situ* using Quantofix N-volumeter and by conductimetry (Yagüe and Quílez, 2012). Slurry samples were collected for further analysis in the laboratory (Table 4). Before the PS application to experimental units, the tractor plus tank was calibrated in the same field to assess the relationship between velocity and dose of PS applied (weighing the tank before and after the application). Despite that, applying appropriate amounts of PS to reach target N rates was a challenge and the actual N applied is shown in Table 4. The urease inhibitor MCDHS was added and mixed in the slurry tank according to the rate recommended by the manufacturing company (2.5 L per 1 Mg of pig slurry).

Table 4. Physicochemical characteristics of the slurry from fattening pigs and amount of pig slurry and nitrogen applied each season.

	2015/16	2016/17	2017/18
Density (kg m^{-3})	1,016	1,020	1,012
pH	-	7.6	8.4
Electrical conductivity at 25 °C (dS m^{-1})	386	316	203
Dry matter (kg DM m^{-3})	22.8	37.2	13.3
Organic matter (kg OM m^{-3})	9.6	23.0	6.5
Ammonium nitrogen (kg N m^{-3})	2.7	3.1	2.1
Organic nitrogen (kg N m^{-3})	0.7	0.8	0.8
Phosphorous (kg P_2O_5 m^{-3})	0.1	0.4	0.5
Potassium (kg K_2O m^{-3})	2.4	3.1	3.0
PS (m^3 ha^{-1})	27.2	37.1	56.6
PS (kg NH_4^+-N ha^{-1})	74	114	118
PSI (m^3 ha^{-1})	28.6	34.5	57.5
PSI (kg NH_4^+-N ha^{-1})	85	110	129

To analyse the effects of MCDHS in the soil N dynamic an additional experiment (miniplots) was installed in the same field with the treatments Control, PS120-AN0, and PSI120-AN0 in a randomised block design with four replications. The size of each experimental plot was 3.6 × 2.0 m. Pig slurry was applied manually the same day in both experiments.

A short irrigation event (2 mm) was applied to incorporate N fertilisers into the soil. At presowing, 70 kg P_2O_5 ha^{-1} and 150 kg K_2O ha^{-1} were applied to avoid limitations of these nutrients. One month before seeding, previous season's straw was incorporated into the soil using a harrow. The control of weeds, diseases, and pests was performed according to local management practices.

2.2. Measurements and determinations

2.2.1. Soil sampling

The soil of each plot was sampled before fertiliser application (15th February 2016, 20th February 2017, and 7th February 2018) and after harvest (14th July 2016, 25th September 2017, and 31st July 2018) at depths 0-30 cm, 30-60 cm, and 60-90 cm.

In the miniplots experiment, the soil was sampled 39 times at depths 0-15 cm and 15-30 cm from pig slurry application to harvest. The time interval between samplings increased from 1 day after the application to 15 days at the end of the season.

Soil samples were sieved through a 3-mm mesh. One subsample was used to determine the soil water content by gravimetry (drying at 105 °C until constant weight). Another subsample (10 g of fresh soil) was extracted with 30 mL of 2 N KCl, shaken for 30 min, and filtered through cellulose filter. NO_3^- and NH_4^+ concentrations in the extracts were determined by colourimetry using a segmented flow analyser (AutoAnalyser 3, Bran+Luebbe, Germany).

2.2.2. Crop sampling

Two subareas of 0.54 m^2 were randomly selected in each plot and hand-harvested to determine the harvest index and obtain the total biomass of the whole plot. An area of 1.65 m wide and the length of the experimental plot (3.5 m for U treatment and 7.0 m for PS and PSI treatments) was mechanically harvested to determine grain yield (reported on the basis of 120 g kg^{-1} moisture content). Grain and straw N contents were analysed by dry combustion (TruSpec CN, LECO, St. Joseph, MI, USA) in samples previously dried at 65 ºC and ground.

2.2.3. Greenhouse gas emissions

Four treatments were selected to measure N_2O and CH_4 fluxes to the atmosphere. These treatments were U120-AN30, PS120-AN30, and PSI120-AN30, which were considered *a priori* the treatments that theoretically better would cover wheat N requirements, and the Control treatment that was necessary to calculate N_2O emission factors. Fluxes were measured using static closed unvented chambers (Holland et al., 1999) of 18.5-cm height and 30.0-cm inner diameter and made in polyvinyl chloride. They were constituted by a collar inserted 10 cm into the soil and an upper part wrapped by a reflective insulation film. Plants inside the collars were cut periodically to the ground level. Gas samples were taken 0, 20, 40, and 60 min after chamber closure in 2015/16 and 2016/17, and 0 and 60 min after chamber closure in 2017/18. Fifteen millilitres of air from chamber headspace were injected into a 12-mL exetainer borosilicate glass vial (Model 038W, Labco) using a polypropylene syringe. Air samplings were started roughly at the hour with the mean temperature of the day (between 9:30h and 11:00h GMT; Alves et al., 2012). Samples were analysed by gas chromatography using the same equipment and technique that were described in detail by Franco-Luesma et al. (2019), an Agilent 7890B gas chromatography system with HP-Plot Q column and electron-capture, flame-ionisation and methaniser detectors.

Greenhouse gas fluxes were calculated as the linear increment in gas concentration within the chamber corrected by the air temperature and multiplied by the ratio between the chamber headspace and the soil area occupied by the chamber (MacKenzie et al., 1998).

Samples were taken from 17/02/2016 to 27/11/2018. The frequency of GHG samplings was daily just after fertilisation events and then measurements were performed at longer intervals with a total of 18, 32, and 36 samplings for 2015/16, 2016/17, and 2017/18 seasons, respectively. At each sampling date, soil moisture and soil temperature were measured at 5-cm depth using portable sensors (HH2 Moisture Meter Delta-T ML3 and ML2 ThetaProbe, and TME MM2000 Single Input Thermocouple Thermometer).

2.3. Data and statistical analysis

The grain yield response to N rates in synthetic fertiliser treatments was adjusted using the linear-plateau model (Cerrato and Blackmer, 1990) for each cropping season (Eq. 1):

$$\text{If } F < C;\quad Y = a + b \cdot F$$

$$\text{If } F \geq C;\quad Y = Y_{max} = a + b \cdot C \qquad \text{[Eq. 1]}$$

where *Y* is the grain yield; *F* is the applied N rate; *a* (intercept) is the yield at 0 kg N ha^{-1}; *b* is the increase in yield per unit increase in *F*; and *C* is the critical N rate or N rate above which the maximum yield (Y_{max}) is obtained.

The nitrogen fertiliser replacement value (NFRV) of pig slurry treatments was calculated as the rate of synthetic N that produces the same yield than the PS120-AN0 and PSI120-AN0 treatments and it was estimated from the response curve of the urea treatments for each season (Fig. 1).

The efficiency in the use of nitrogen was evaluated using two indexes. The mineral N contained in the applied slurry (i.e., NH_4^+-N) was used for the calculations since it was considered that the contribution of pig slurry organic N and its residual effect was not substantial during the period of the experiment. The nitrogen use efficiency (NUE) is the ratio between the total N uptake by the aboveground biomass (kg ha^{-1}) and the ammonium-N applied by fertilisation (kg ha^{-1}). The apparent recovery efficiency of N applied (RE_N) in total aboveground biomass is the increment in the aboveground N uptake due to the N application per mineral-N applied rate (Eq. 2):

$$RE_N = \frac{U_T - U_0}{F_T} \qquad \text{[Eq. 2]}$$

where U_T is the N uptake by aboveground biomass; U_0 is the N uptake by aboveground biomass in the unfertilised control plot; and F_T is the applied mineral-N in the *T* treatment.

The efficiency indicators were calculated and analysed for two fertiliser strategies: i) fertiliser treatments that showed the best agronomic response to the N application (120 kg N ha^{-1} at tillering and 0 kg N ha^{-1} at stem elongation), and ii) treatments that, during the experimental design, were considered that would suit better to crop necessities (120 kg N ha^{-1} at tillering and 30 kg N ha^{-1} at stem elongation).

The grain protein content was calculated multiplying the total N content of the grain by the factor 5.7 (Wrigley and Batey, 2012).

The N_2O emission factor (EF, %) was calculated as the difference between N_2O emissions in fertilised and unfertilised (Control) plots, divided by the total N applied in the fertilised plots, and multiplied by 100. The yield-scaled N_2O emission (YS_{N2O}; g N kg^{-1} grain) was determined as the ratio between the N_2O emissions and the grain yield of the plot.

The unaccounted N (kg N ha^{-1} yr^{-1}) was calculated from a soil N balance considering the soil depth 0-90 cm and including the three growing seasons (from February 2016 to July 2018). Thus, the mean annual unaccounted N (N_{unac}; kg N ha^{-1} yr^{-1}) was calculated as the difference between N inputs and N outputs and divided by three years (Eq. 3):

$$N_{unac} = N_{input} - N_{output} = (N_{is} + N_f + N_i + N_m) - (N_{fs} + N_u + N_{N2O}) \qquad [Eq. 3]$$

where N_{is} is the initial soil mineral N; N_f is the N applied with fertilisers; N_i is the N applied with irrigation water; N_m is the net N mineralisation; N_{fs} is the final soil mineral N; N_u is the N uptake by aboveground biomass; and N_{N2O} is the N_2O emission (if applicable). The net N mineralisation was estimated from the Control plots (non-N fertilised treatment), as the difference between N inputs and N outputs, assuming that N losses by ammonia volatilisation and nitrate leaching were unimportant in this treatment compared to the other terms of the balance.

To estimate the risk of nitrate leaching, the drainage was calculated for each season at 0.9-m depth using the simplified one-dimensional water balance described in Eq. 4:

$$D = P + I - ETc \pm \Delta SW \qquad [Eq. 4]$$

being D the drainage; P the precipitation; I the irrigation; ETc the estimated crop evapotranspiration; and ΔSW the variation of soil water content. There were no visible signs of surface runoff and it was considered negligible.

The normal data distribution and uniformity of variance were verified using Shapiro-Wilk and Levene's test, respectively. Data were transformed when was necessary (Box-cox transformation). Normalised data were subjected to analysis of variance (MIXED procedure) and differences of means were compared with the Tukey's test. Student's t-test was used to compare paired samples (soil N concentration). Comparison of regression lines of grain N content vs. unaccounted N was performed using F-test. One-sample z-test was used to check whether cumulative CH_4 emissions were different from zero. Repeated measure analysis along time, according to a first-order autoregressive structure model AR(1), was performed to compare N_2O and CH_4 fluxes among treatments for the period between the first fertiliser application and one month after the second application.

In all tests, the default level of significance considered was 0.05. Statistical software used was SAS® University Edition (SAS Institute, Cary, NC).

3. Results

3.1. Productive parameters

The maximum grain yield (Y_{max}, Fig. 1) was higher in the first season (8,357 kg ha^{-1}) than in the second and third seasons (5,491 and 5,543 kg ha^{-1}, respectively). No response of grain yield to N rates of synthetic urea applied at tillering was observed in the first year (2015/16). In seasons 2016/17 and 2017/18, there was a positive response of yield to N application and critical N rates were established at 34 and 59 kg N ha^{-1}, respectively (Fig. 1); much lower than the expected critical rates in the range 120-150 kg N ha^{-1}. NFRV could not be quantified due to the low or lack of response of yield to N application. The grain yield was affected by the N source ($p<0.01$) for in the first season (2015/16) since PS120-AN0 showed 21% higher grain yield than U120-AN0 (Fig. 2). In the three years, yield in PS120-AN0 and PSI120-AN0 was not lower than in U120-AN0, indicating that the fertiliser value of PS120 and PSI120 was at least similar to that of U120.

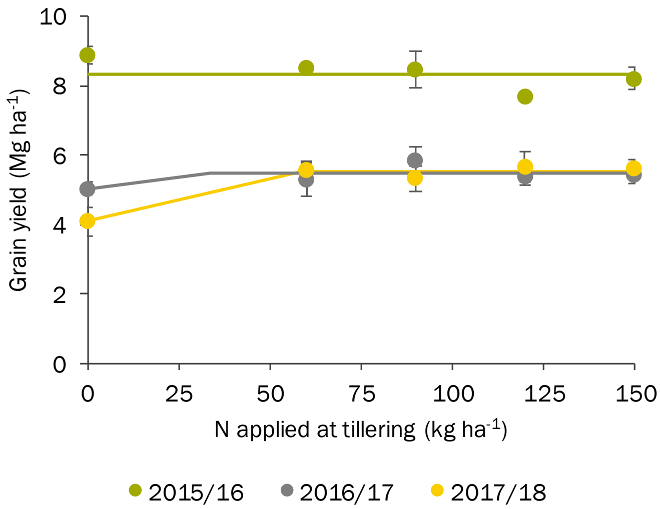


Figure 1. Grain yield response curves to total nitrogen application at tillering in the urea treatments in 2015/16, 2016/17 and 2017/18 seasons. Vertical bars indicate standard error (n=4).

The grain yield was not affected by a second N side-dress application at stem elongation in any of the three seasons (Fig. 2).

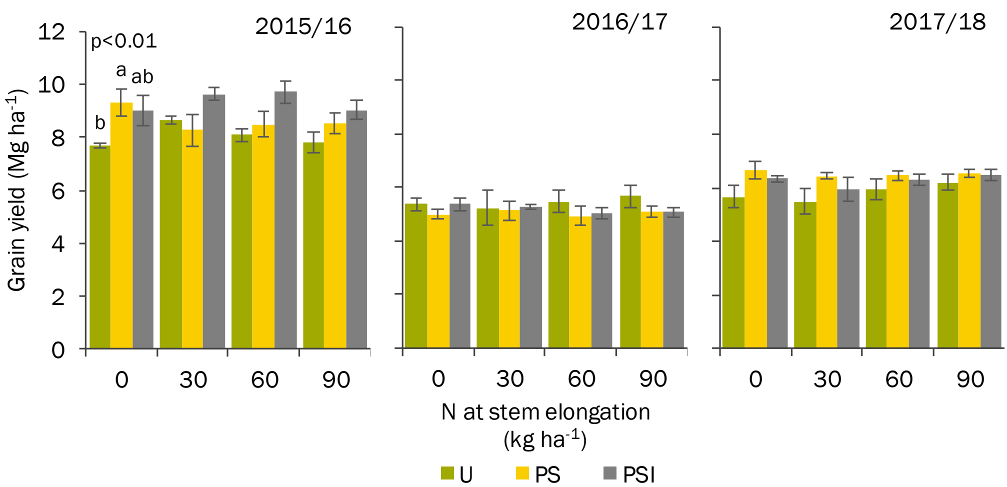


Figure 2. Grain yield response to total nitrogen application at stem elongation in 2015/16, 2016/17, and 2017/18 seasons depending on the N source at tillering. Vertical bars indicate standard error (n=4).

However, the second N application increased the grain protein content with a linear response as the N applied increases (Fig. 3a).

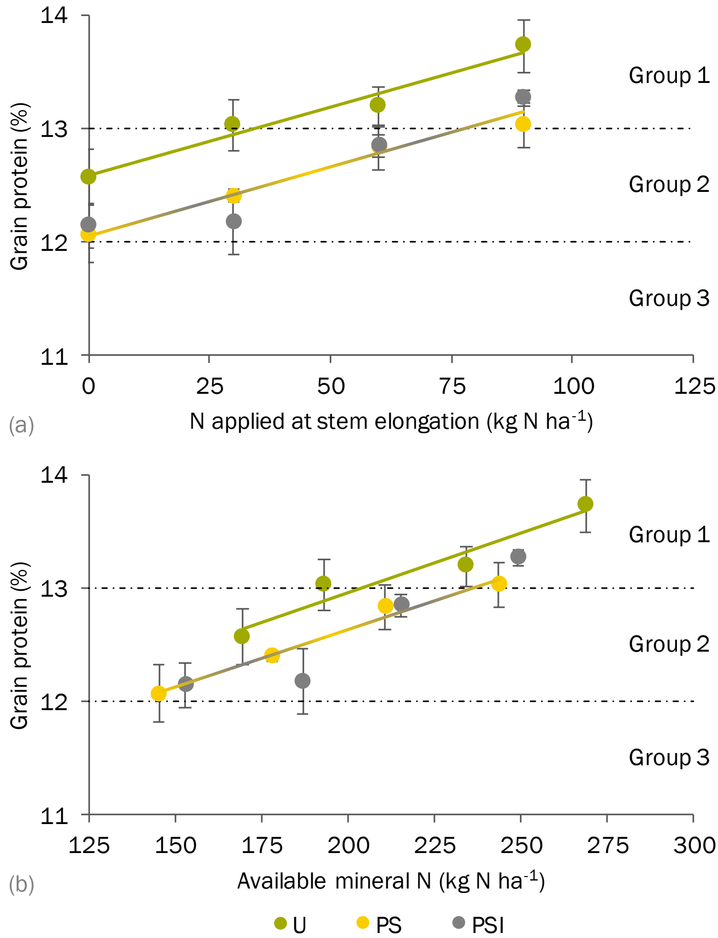


Figure 3. Relationship between the 3-year average grain protein (%) and the rate of N applied at stem elongation (kg N ha^{-1}) (Fig. a) and between the 3-year average grain protein (%) and the available N (soil N from 0 to 30 cm in February + mineral-N applied; kg N ha^{-1}) (Fig. b) for the different fertiliser strategies (U, PS, or PSI)[1]. Vertical bars indicate the standard error (n=4). Horizontal lines separate grain protein groups (Real Decreto 190/2013) for industrial use.

[1]- Fertiliser treatments represented are U: U120-AN0, U120-AN30, U120-AN60, U120-AN90; PS: PS120-AN0, PS120-AN30, PS120-AN60, PS120-AN90; PSI: PSI120-AN0, PSI120-AN30, PSI120-AN60, PSI120-AN90.

For a given N application at stem elongation, U treatments presented higher ($p<0.05$) grain protein content than treatments with pig slurry (PS and PSI), i.e., N source at tillering application determined differences in grain protein. This difference was also observed when grain protein was related to available N (Fig. 3b), defined as soil mineral N (SMN) content from 0 to 30 cm in February plus mineral-N applied. There were no differences between N sources in SMN content (residual effect) from 0 to 30 cm in February for any season (data not shown), so the observed differences in grain protein were associated to the N source at tillering. The difference in available N between U and PS treatments to reach the average grain protein content (12.8%) was 28.8 kg N ha^{-1}.

It was no effect of treatments without a second N application on NUE and RE_N (Table 5). Besides, no significant differences in these N efficiency indexes were observed among the treatments for the strategy with the second application of 30 kg N ha^{-1} (data not shown).

Table 5. Average nitrogen use efficiency indexes (NUE and RE_N; n=4) for the fertilised treatments U120-AN0, PS120-AN0, and PSI120-AN0 in the three cropping seasons (2015/16, 2016/17, and 2017/18).

	U120-AN0	PS120-AN0	PSI120-AN0	p-value
NUE				
2015/16	2.79	4.97	4.56	0.105
2016/17	1.85	1.71	1.91	0.345
2017/18	1.48	1.38	1.35	0.737
RE_N				
2015/16	-0.05	0.12	0.31	0.836
2016/17	0.48	0.26	0.39	0.367
2017/18	0.65	0.52	0.56	0.657

3.2. Greenhouse gas emissions

Individual values of methane fluxes ranged from -23.9 to 34.4 g C ha^{-1} day^{-1} (data not shown) with a random distribution without any apparent relation with fertilisation events, fertiliser type, or crop phenology. This apparently chaotic behaviour derived in no significant differences among N sources neither in fluxes nor in cumulative emissions. Only in the control treatment during the 2016/17 season the cumulative CH_4 emissions was different ($p<0.05$) from zero (Table 6).

Individual values of N_2O fluxes ranged from -2.3 to 94.6 g N ha^{-1} day^{-1} in 2015/16, from -1.5 to 434.16 g N ha^{-1} day^{-1} in 2016/17, and from -2.5 to 197.5 g N ha^{-1} day^{-1} in 2017/18.

Table 6. Average (n=4) of N_2O and CH_4 emissions (g N ha^{-1} and g C ha^{-1}) in the four treatments (Control, U120-AN30, PS120-AN30, and PSI120-AN30) for the three cropping seasons (from sowing to the following sowing in 2015/16, 2016/17, and 2017/18) and the whole experiment (2015/18). Different letters within rows indicate significant differences among treatments (Tukey's test; p<0.05).

	Control	U120-AN30	PS120-AN30	PSI120-AN30	p-value
N_2O (g N ha^{-1})					
2015/16	233 b	1,624 a	1,314 a	1,428 a	<0.001
2016/17	576 b	2,101 ab	2,427 a	2,638 a	0.009
2017/18	519 b	2,129 ab	3,094 a	2,538 a	0.008
2015/18	1,532 b	6,140 a	7,262 a	7,086 a	0.007
CH_4 (g C ha^{-1})					
2015/16	22	-53	-281	-89	0.752
2016/17	-45*	-226	-204	-281	0.783
2017/18	-346	16	16	-323	0.053
2015/18	46	-681	43	-534	0.708

* indicates cumulative CH_4 emissions different from zero (p<0.05).

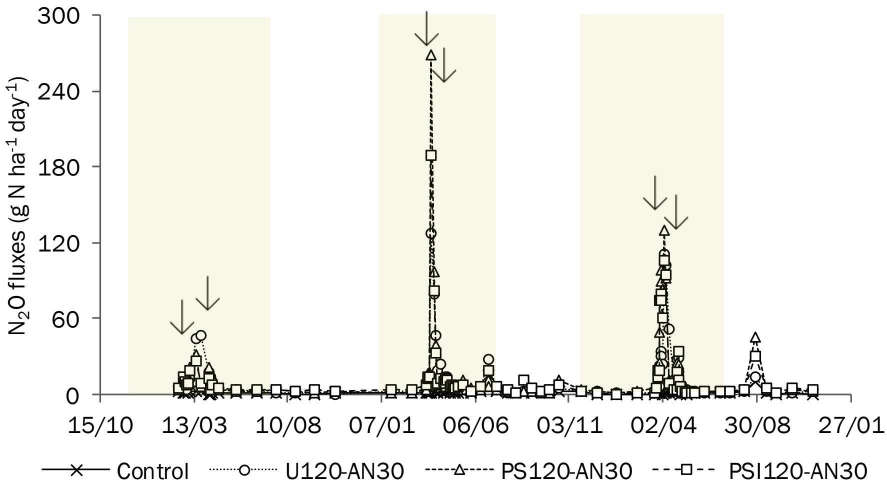


Figure 4. Temporal changes of average N_2O fluxes (g N ha^{-1} day^{-1}; n=4) for each fertiliser treatment (Control, U120-AN30, PS120-AN30, and PSI120-AN30) along the three growing seasons (2015/16, 2016/17, and 2017/18). The green area shows the period between seeding and harvest of each season. Arrows show fertiliser applications.

Peaks of N_2O emissions (Fig. 4) were observed mainly after the first side-dress fertiliser application when soil temperature ranged from 12 ºC to 21 ºC (data not shown) and soil WFPS ranged from 45% to 70% (Fig. 5).

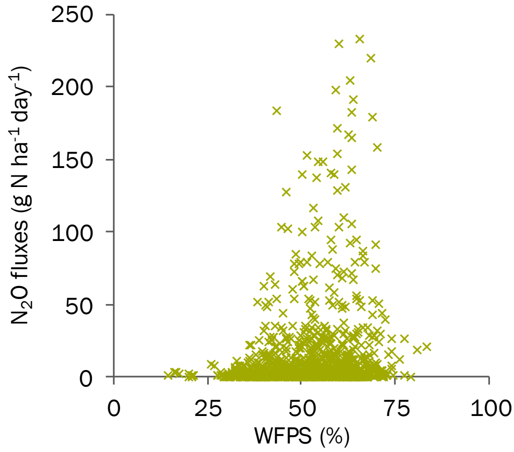


Figure 5. Effect of soil water-filled pore space (WFPS, %) in the topsoil (5-cm depth) on N_2O fluxes (g N ha^{-1} day^{-1}).

On average, 75% of N_2O was emitted during the cropping season (from sowing to harvest). The repeated measure analysis of N_2O fluxes from the first fertiliser application to one month after the second application did not show significant differences among the three fertiliser treatments in any of the three seasons. Significant differences in N_2O emissions were observed for the whole experiment among the Control and the fertilised treatments (Table 6). These differences were also observed in each season, although during the second and third season, the differences between Control and U treatment were not significant due to the high variability between replicates. There were no significant differences related to the substitution of synthetic fertiliser (U120-AN30) for pig slurry (PS120-AN30) or to the addition of the urease inhibitor to the pig slurry (PSI120-AN30).

The N_2O EFs ranged between 0.91% and 1.42% (Table 7) and, averaging over treatments, values were 21% and 27% higher in the second and third season, respectively, compared to the first season. EFs did not present significant differences among the three fertilised treatments for any of the cropping periods. Yield-scaled N_2O emissions were lower for the first season (average 0.16 g N kg^{-1} grain) than for the other two seasons (Table 7). Mean YS_{N2O} emission in 2016/17 (0.46 g N kg^{-1} grain) and in 2017/18 (0.42 g N kg^{-1} grain) were 184% and 159%

higher than in 2015/16, respectively. Differences among the fertilised treatments were not detected in this parameter for any of the seasons ($p>0.05$).

Table 7. Average (n=4) N_2O EF (%) and YS_{N2O} emission (g N_2O-N kg^{-1} grain) in the different fertilised treatments (U120-AN30, PS120-AN30, and PSI120-AN30) for the three cropping seasons (2015/16, 2016/17, and 2017/18) and the whole experiment (2015/18). The data include the crop period (sowing to harvest) and the intercrop period (harvest to the following crop sowing).

	U120-AN30	PS120-AN30	PSI120-AN30	p-value
EF (%)				
2015/16	0.93	0.91	0.92	0.999
2016/17	1.02	1.06	1.26	0.929
2017/18	1.07	1.42	1.01	0.510
2015/18	1.02	1.18	1.12	0.469
YS_{N2O} (g N kg^{-1} grain)				
2015/16	0.19	0.15	0.15	0.919
2016/17	0.41	0.49	0.49	0.964
2017/18	0.38	0.48	0.41	0.763
2015/18	0.31	0.36	0.33	0.893

3.3. Dynamic of nitrogen in the soil

Soil mineral nitrogen responded to N applications, reaching peaks of ~20 mg NO_3^--N kg^{-1} soil and 20-35 mg NH_4^+-N kg^{-1} soil at 0-15-cm depth the day after the fertilisation (Fig. 6). These SMN values went down until reaching, in two months, the same amounts than in the Control treatment. At the end of the first and third season, soil nitrate rose in the 0-15-cm depth (Fig. 6), although the increase was not such noticeable at 15-30-cm depth (data not shown). The reduction of SMN concentration with depth was a trend during the whole experiment since, averaging over dates, nitrate and ammonium concentrations were 66% and 176% higher, respectively, in the first sampled depth (0-15 cm) than in the second one (15-30 cm) (data not shown).

In the two months after the fertilisation, the dynamic of soil mineral N did not differ significantly between PS and PSI treatments for all seasons, independent of the N form and soil depth (Table 8). The exception was the 2015/16 season when treatment with inhibitor presented 10% higher NO_3^- concentration than treatment without inhibitor. This difference could not be appreciated in total mineral N concentration (data not shown).

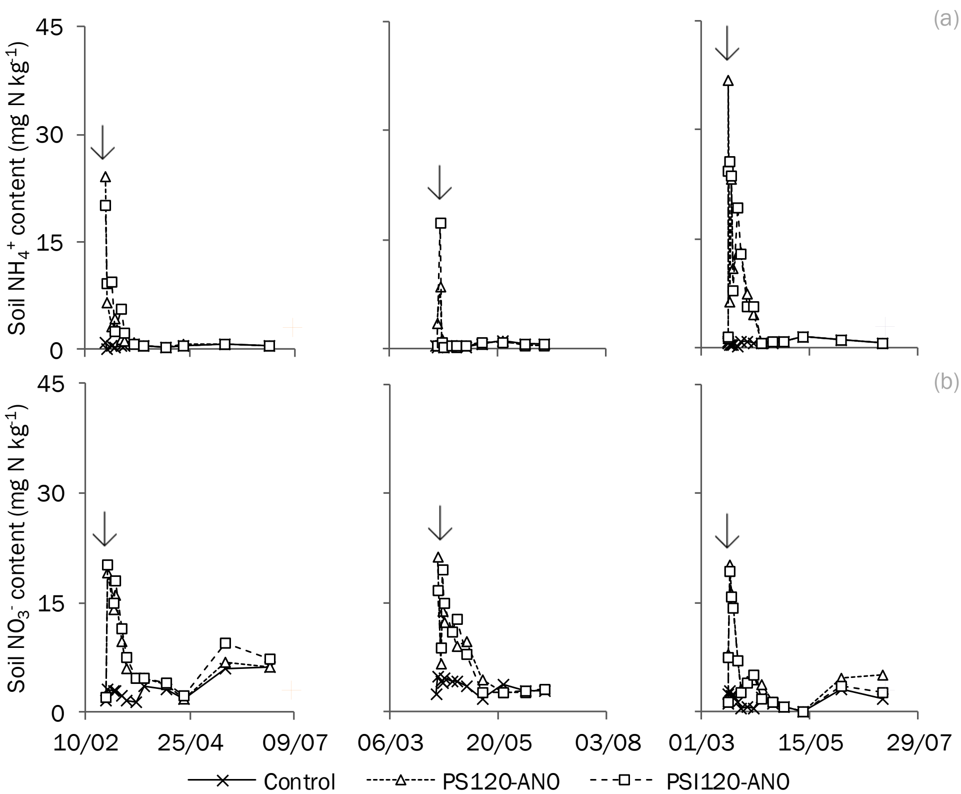


Figure 6. Soil ammonium content (Fig. a) and soil nitrate content (Fig. b) from 0 to 15-cm depth of miniplots experiment. Arrows indicate N applications.

Table 8. Average soil inorganic nitrogen concentrations (mg NO_3^--N kg^{-1} soil and mg NH_4^+-N kg^{-1} soil; n=4) for treatments with a single PS application at tillering of 120 kg N ha^{-1}. Data are shown at different depths (0-15 cm and 15-30 cm) for each cropping season (2015/16, 2016/17, and 2017/18). Each period includes data from a day before fertilisation until two months later. Different letters within rows indicate significant differences among treatments (Student's t-test, $p<0.05$).

	NO_3^- (mg N kg^{-1} soil)			NH_4^+ (mg N kg^{-1} soil)		
	PS	PSI	p-value	PS	PSI	p-value
0-15 cm						
2015/16	8.1 b	8.9 a	0.010	4.3	5.1	0.202
2016/17	9.4	10.0	0.552	1.8	2.3	0.566
2017/18	6.6	6.3	0.139	9.4	10.1	0.578
15-30 cm						
2015/16	3.7	3.7	0.850	0.4	0.5	0.125
2016/17	7.1	6.9	0.853	1.0	1.1	0.794
2017/18	3.5	3.4	0.573	1.8	2.3	0.943

3.4. Nitrogen balance

The soil mineralisation was estimated equal to the amount of unaccounted N in the Control treatment (129 kg N ha^{-1} yr^{-1} from 0 to 90-cm depth). For the three experimental years, unaccounted N was linearly related to the total N applied by fertilisation (Fig. 7), but the relationship changed depending on the fertiliser source. The effect of the inhibitor applied to the slurry did not affect the observed relationship and a pooled regression (PS+PSI) was considered. However, the urea treatments behaved differently ($p<0.05$) than the PS treatments, with lower unaccounted N across the different ranges of applied N. Averaging across the different N rates, urea treatments presented about 41 kg N ha^{-1} yr^{-1} less unaccounted N than the slurry treatments.

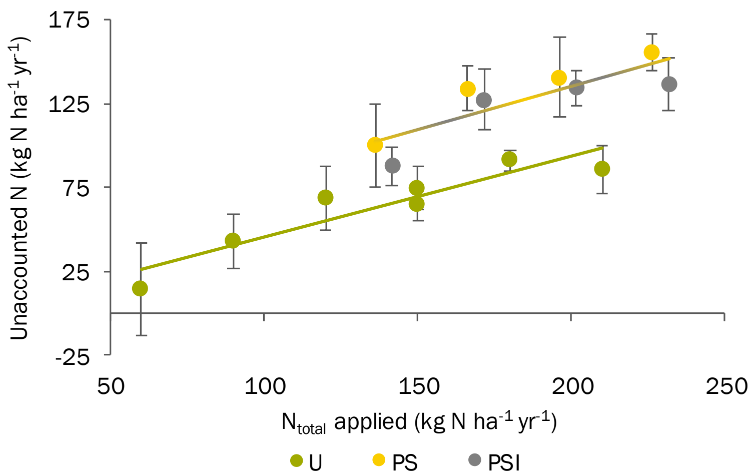


Figure 7. Relationship between the unaccounted N (kg N ha^{-1} yr^{-1}) obtained from the 0-90 cm soil N balance and the total N applied (kg N ha^{-1} yr^{-1}) for the whole experimental period. Vertical bars indicate the experimental standard error (n=4).

According to the soil water balance, the risk of nitrate drainage was low during the experiment. The volume of water drained below 0.9-m depth (from February to July) was zero for the first two seasons and only in the third season (2017/18) the drainage was estimated to be 73 mm. In this case, 95% of this volume was concentrated after an unusually high rainfall period (83 mm) occurred between days 07/04/2018 and 11/04/2018, twenty days after the first side-dress N fertilisation.

4. Discussion

Pig slurry application allowed reaching similar productive values than those obtained by synthetic urea fertilisation since grain yield was not affected by the N source at tillering application. No grain yield reduction associated to the use of pig slurry substituting synthetic fertilisers have been either reported in other studies under different crops (Hernández et al., 2013; Moreno-García et al., 2017; Plaza-Bonilla et al., 2017). A second N application as ammonium nitrate at stem elongation did not increase grain yield compared to a unique side-dress N application, which is in agreement with the inconsistent response of grain yield to variations in the timing and splitting of N fertiliser reported by López-Bellido et al., (2005). However, the second side-dress N application at stem elongation allowed an increase in grain protein, which corroborates previous studies like Debaeke et al. (1996) that suggested that the split and late application of N guarantees a better distribution of N in the kernel. This increase in grain protein was observed when the N rates increased in the application at stem elongation even though they exceeded the critical N rate above which the maximum yield was obtained. Under similar irrigated Mediterranean conditions, Lloveras et al. (2001) also reported higher N rates required to achieve high bread-making quality than to obtain the highest grain yield. Higher grain protein values allowed classifying the wheat in group 1, according to the current Spanish legislation (Real Decreto 190/2013), indicating an optimal industrial use of this product, although other additional variables are normally evaluated to categorise the wheat. Further, grain protein was influenced in this study by the N source applied at tillering. Lower N rates of ammonium-N at stem elongation were necessary to reach the protein threshold for belonging to the maximum group of the above-cited regulation when the tillering side-dress application was previously performed with urea compared with those treatments that received pig slurry. It can be speculated lower NH_3 volatilisation losses in urea treatments compared to slurry treatments, favouring higher N availability and a subsequent increase in grain protein.

Unintentionally, the experiment took place under relatively high N availability conditions, which led to a low grain yield response to N application for the three cropping seasons. Thus, the soil mineral N (0-30-cm depth) in the Control treatment was high before the first side-dress application, especially for the first and second year (2015/16: 59 kg N ha^{-1}; 2016/17: 52 kg N ha^{-1}; 2017/18: 27 kg N ha^{-1}). In this context, N use efficiency indexes presented small values and reached similar figures to the literature with time, especially in the last season. NUE values during the whole experiment exceeded the threshold of 0.9 proposed as an indicator of soil nutrient mining (EU Nitrogen Expert Panel, 2015). However, as only grain was exported from the plot, the depletion of soil N was not so important. Thus, recalculating NUE index using N grain uptake instead of total aboveground N uptake and averaging over treatments the values of 0.66 (2015/16), 0.42 (2016/17), and 0.47 (2017/18) were obtained for the three growing seasons. Soil N mining can also be accelerated by high RE_N (Ladha et al., 2005); however, in this study, RE_N values were lower than the mean value of 0.57 in the analysis of Ladha et al. (2005). Just in the third cropping season, the average RE_N reached values within the normal range (0.50-0.80) in well-N-managed systems for cereal crops (Dobermann, 2007). The lower values obtained for the first season can be explained by the absence of response to the N application.

In this study, MCDHS did not affect N_2O emissions as would be expected for the application of a urease inhibitor to pig slurry that has transformed the urea-N to ammonium-N before the mixture. Nonetheless, the experiment allows discarding other potential effects associated with the presence of dihydrogen sulphate in the molecule like decreasing soil pH nearby the soil-fertiliser interphase, with a subsequent effect on N dynamics. The slurry treated with MCDHS did not reduce the pH of PS compared to the non-treated slurry (data not shown), which is in agreement with the absence of significant differences in soil mineral N content between both treatments (PI vs. PSI). The only difference in soil nitrate concentrations found (2015/16) between organic fertilisers with and without MCDHS was not consistent through soil depths and seasons, and ammonium-N and mineral-N were not affected. This absence of differences between treatments means no effect of the inhibitor on the soil N dynamic; in fact, soil nitrate and ammonium concentrations followed the same evolution with time (amount and pattern) in both fertiliser treatments.

Nitrous oxide emissions responded to fertiliser application independently of the N source and happened under soil WFPS conditions (40-70%) that favoured the nitrification (Dalal et al., 2003). Absence of differences in N_2O emissions between urea and pig slurry might be attributed to the similar mineralised nature of the N forms they contain (urea-N in urea fertiliser and ammonium-N in pig slurry). Noticeable differences in the maximum N_2O flux peaks were observed among the three seasons, with a lower peak during the first year. This fact might be attributed to a rainfall event (24.5 L) which happened three days after the first fertiliser application of season 2015/16, displacing the mineral-N to deeper layers compared to the other seasons. The importance of the location of fertiliser on N_2O emissions was demonstrated by Liu et al. (2006), who reported between 40-70% higher fluxes when fertiliser was at 0-5-cm depth compared to fertiliser located at 10-15 cm.

In agreement with the study of Guardia et al. (2017) under similar environmental condtions, methane fluxes had a small contribution to the total GHG budget. This emission depends on the presence of soil anaerobic conditions and the incorporation of organic matter (Sanz-Cobena et al., 2017). In the present study, the soil was mostly maintained under aerobic conditions and the amount of organic matter contained into the pig slurry was of relatively minor importance (261-853 kg ha^{-1}). Therefore, the substitution of synthetic fertiliser for pig slurry did not increase CH_4 emissions, in the same way that those results obtained by Guardia et al. (2017) when urea was substituted by the liquid fraction of the pig slurry.

Liu and Powers (2012) indicated that N_2O EF for swine slurry application was similar to the default value (EF_1=1%) indicated by the IPCC (2006). According to Cayuela et al. (2017), the EFs of organic-liquid fertilisers do not differ from 1% significantly and they are the fertiliser type with highest EF (0.85% ± 0.30, n=30), whereas synthetic fertiliser EFs were lower than 1%. However, the 2019 Refinement to the 2006 IPCC Guidelines for National Gas Inventories (IPCC, 2019) changed the default EF of 'All N inputs in dry climates' to 0.5%. The present study did not show consistent differences in N_2O EFs between synthetic and slurry treatments, with values close to 1% in a high N availability scenario and two N applications (120 kg N ha^{-1} and 30 kg N ha^{-1}). The EFs calculated applying N at the right rate according to crop necessities and using N_2O emissions measured in chambers with plants would be essential to assess the real impact of pig slurry on the emissions.

The water balance shows that N losses by nitrate leaching were relatively low in the whole experiment, although the drainage produced in the third season, twenty days after the first side-dress N application, could have been produced in a critical moment according to Gómez-Garrido et al. (2018). However, the low SMN content (13.6 kg N ha^{-1} from 0 to 30 cm the day before the rainfall event) rejects a high potential risk of nitrate leaching. In this regard, Gómez-Garrido et al. (2018) claimed to avoid N losses by a proper irrigation calendar considering foreseen heavy rains.

Unaccounted N was strongly related to N applied. The main components of unaccounted N were NH_3 volatilisation, N_2 emissions, and immobilised NH_4^+. Irrigation practices used in the experiment could have helped to reduce N losses by nitrate leaching since the irrigation was adjusted to crop necessities. The slurry was applied in strips using trail hoses that reduces NH_3 volatilisation in comparison to the traditional splashing over a plate (Yagüe et al., 2019). Moreover, N was incorporated into the soil after fertilisation with 2-mm irrigation event. The tendency of higher unaccounted N for slurry treatments compared to synthetic-N treatments agrees with lower grain N values in pig slurry than in synthetic treatments. It is hypothesised that higher NH_3 losses could have happened in pig slurry treatments because of the effect of the canopy; i.e., pig slurry could have remained in the leaves, despite the 2-mm irrigation event, increasing the contact surface area and the ammonia volatilisation.

5. Conclusions

Pig slurry can replace the N necessary for bread wheat production under irrigated conditions without yield penalties and with similar nitrogen use efficiency compared to the synthetic urea fertiliser. Besides, the use of pig slurry does not increase the N_2O losses compared to the use of the urea, one of the most popular synthetic fertilisers. However, higher uncertainties probably associated with volatilisation losses can jeopardise grain protein when slurry rates are not properly adjusted.

MCDHS added to pig slurry does not seem to have any agronomic or environmental benefit under the agro-environmental conditions of the study; thus, grain yield, GHG emissions, EF, and YS_{N2O} were not different depending on the inhibitor addition.

References

Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., Vallejo, A., 2014. 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

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. doi:10.1016/j.agee.2012.09.006

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. FAO Irrigation and Drainage Paper 56. Food and Agriculture Organization of the United Nations, Italy, 50 pp.

Alves, B.J.R., Smith, K.A., Flores, R.A., Cardoso, A.S., Oliveira, W.R.D., Jantalia, C.P., Urquiaga, S., Boddey, R.M., 2012. Selection of the most suitable sampling time for static chambers for the estimation of daily mean N2O flux from soils. Soil Biol. Biochem. 46, 129–135. doi:10.1016/j.soilbio.2011.11.022

Borghi, B., Corbellini, M., Minoia, C., Palumbo, M., Di Fonzo, N., Perenzin, M., 1997. Effects of Mediterranean climate on wheat bread-making quality. Eur. J. Agron. 6, 145–154. doi:10.1016/S1161-0301(96)02040-0

Bosch-Serra, À.D., Ortiz, C., Yagüe, M.R., Boixadera, J., 2015. Strategies to optimize nitrogen efficiency when fertilizing with pig slurries in dryland agricultural systems. Eur. J. Agron. 67, 27–36. doi:10.1016/j.eja.2015.03.003

Cameron, K.C., Di, H.J., Moir, J.L., 2013. Nitrogen losses from the soil/plant system: a review. Ann. Appl. Biol. 162, 145–173. doi:10.1111/aab.12014

Cantarella, H., Otto, R., Soares, J.R., Silva, A.G.B., 2018. Agronomic efficiency of NBPT as a urease inhibitor: a review. J. Adv. Res. 13, 19–27. doi:10.1016/j.jare.2018.05.008

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. doi:10.1016/j.agee.2016.10.006

Cerrato, M.E., Blackmer, A.M., 1990. Comparison of models for describing corn yield response to nitrogen fertilizer. Agron. J. 82, 138–143. doi:10.2134/agronj1990.00021962008200010030x

Chien, S.H., Prochnow, L.I., Cantarella, H., 2009. Chapter 8. Recent developments of fertilizer production and use to improve nutrient efficiency and minimize environmental impacts. Adv. Agron. 102, 267–322. doi:10.1016/S0065-2113(09)01008-6

Dalal, R.C., Wang, W., Robertson, G.P., Parton, W.J., 2003. Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. Aust. J. Soil Res. 41, 165–195. doi:10.1071/SR02064

Daudén, A., Quílez, D., 2004. Pig slurry versus mineral fertilization on corn yield and nitrate leaching in a Mediterranean irrigated environment. Eur. J. Agron. 21, 7–19. doi:10.1016/S1161-0301(03)00056-X

Debaeke, P., Aussenac, T., Fabre, J.L., Hilaire, A., Pujol, B., Thuries, L., 1996. Grain nitrogen content of winter bread wheat (Triticum aestivum L.) as related to crop management and to the previous crop. Eur. J. Agron. 5, 273–286. doi:10.1016/S1161-0301(96)02038-2

Díez-López, J.A., Hernaiz-Algarra, P., Arauzo-Sánchez, M., Carrasco-Martín, I., 2008. Effect of a nitrification inhibitor (DMPP) [3,4-dimethylpyrazole phosphate] on nitrate leaching and maize yield during two growing seasons. Spanish J. Agric. Res. 6, 294–303. doi:10.5424/sjar/2008062-320

Díez, J.A., Arauzo, M., Hernaiz, P.J.J., Sanz, A., Vallejo, A., 2010. Comparison of nitrification inhibitors to restrict nitrate leaching in a maize crop irrigated under Mediterranean conditions. Spanish J. Agric. Res. 8, 481–492. doi:10.5424/sjar/2010082-1212

Diez, J.A., de la Torre, A.I., Cartagena, M.C., Carballo, M., Vallejo, A., Muñoz, M.J., 2001. Evaluation of the Application of Pig Slurry to an Experimental Crop Using Agronomic and Ecotoxicological Approaches. J. Environ. Qual. 30, 2165–2172. doi:10.2134/jeq2001.2165

Dobermann, A., 2007. Nutrient use efficiency – measurement and management, in: Fertilizer Best Management Practices. General Principles, Strategy for Their Adoption and Voluntary Initiatives vs Regulations. Papers presented at the IFA International Workshop on Fertilizer Best Management Practices 7-9 March 2007, Brussels, Belgium, pp. 1–28.

EU Nitrogen Expert Panel, 2015. Nitrogen Use Efficiency (NUE) - an indicator for the utilization of nitrogen in agriculture and food systems. Wageningen University, Netherlands, 47 pp.

EUROSTAT, 2020. Pig population - annual data. (accessed 24 April 2020).

FAOStat, 2020. FAOSTAT database collections: Crop production. (accessed 24 April 2020).

Ferguson, R.B., Kissel, D.E., Koelliker, J.K., Basel, W., 1984. Ammonia volatilization from surface-applied Urea: effect of hydrogen ion buffering capacity. Soil Sci. Soc. Am. J. 48, 578–582. doi:10.2136/sssaj1984.03615995004800030022x

Franco-Luesma, S., Álvaro-Fuentes, J., Plaza-Bonilla, D., Arrúe, J.L., Cantero-Martínez, C., Cavero, J., 2019. Influence of irrigation time and frequency on greenhouse gas emissions in a solid-set sprinkler-irrigated maize under Mediterranean conditions. Agric. Water Manag. 221, 303–311. doi:10.1016/j.agwat.2019.03.042

Gómez-Garrido, M., Faz-Cano, Á., Martínez-Martínez, S., Carmona-Garcés, D.M., Büyükkılıç-Yanardag, A., 2018. Chapter 12 - Nitrogen dynamic and leaching in calcareous soils amended with pig slurry, in: Muñoz, M.A. and Zornoza, R. (Eds.) Soil Management and Climate Change, Effects on Organic Carbon, Nitrogen Dynamics, and Greenhouse Gas Emissions. Academic Press, pp. 171–187.

Goss, M.J., Tubeileh, A., Goorahoo, D., 2013. Chapter Five - A Review of the Use of Organic Amendments and the Risk to Human Health, in: Advances in Agronomy, 120. Elsevier, pp. 275–379.

Guardia, G., Cangani, M.T.M.T., Sanz-Cobena, A., Junior, J.L.L., Vallejo, A., 2017. Management of pig manure to mitigate NO and yield-scaled N2O emissions in an irrigated Mediterranean crop. Agric. Ecosyst. Environ. 238, 55–66. doi:10.1016/j.agee.2016.09.022

Hernández, D., Polo, A., Plaza, C., 2013. Long-term effects of pig slurry on barley yield and N use efficiency under semiarid Mediterranean conditions. Eur. J. Agron. 44, 78–86. doi:10.1016/j.eja.2012.09.001

Holland, E.A., Robertson, G.P., Greenberg, J., Groffman, P.M., Boone, R.D., Gosz, J.R., 1999. Soil CO2, N2O, and CH4 exchange. In: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins, P. (Eds.), Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, United States of America, pp. 185–201.

IPCC, 2006. Chapter 11: N2O Emissions from Managed Soils, and CO2 Emissions From Lime and Urea Application, in Prepared by the National Greenhouse Gas Inventories Programme, Eggleston H.S., Buendia L., Miwa K., Ngara T. and Tanabe K. (Eds.). 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Volume 4: Agriculture, Forestry and Other Land Use, IGES, Japan, 54 pp.

IPCC, 2019. Chapter 11: N2O Emissions from Managed Soils, and CO2 Emissions From Lime and Urea Application, in: Calvo-Buendia, E., Tanabe, K., Kranjc, A., Baasansuren, J., Fukuda, M., Ngarize S., Osako, A., Pyrozhenko, Y., Shermanau, P., and Federici, S. (Eds.), 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Volume 4: Agriculture, Forestry and Other Land Use. Switzerland, 48 pp.

Jensen, J., Larsen, M.M., Bak, J., 2016. National monitoring study in Denmark finds increased and critical levels of copper and zinc in arable soils fertilized with pig slurry. Environ. Pollut. 214, 334–340. doi:10.1016/j.envpol.2016.03.034

Jiménez-de-Santiago, D.E., Yagüe, M.R., Bosch-Serra, À.D., 2019. Soil water repellency after slurry fertilization in a dryland agricultural system. Catena 174, 536–545. doi:10.1016/j.catena.2018.11.040

Kiss, S., Simihăian, M., 2002. Improving efficiency of urea fertilizers by inhibition of soil urease activity. Springer, Netherlands, 417 pp.

Ladha, J.K., Pathak, H., J. Krupnik, T., Six, J., van Kessel, C., 2005. Efficiency of Fertilizer Nitrogen in Cereal Production: Retrospects and Prospects, in: Advances in Agronomy, 87. Elsevier, pp. 85–156.

Liu, X.J., Mosier, A.R., Halvorson, A.D., Zhang, F.S., 2006. The impact of nitrogen placement and tillage on NO, N2O, CH4 and CO2 fluxes from a clay loam soil. Plant Soil 280, 177–188. doi:10.1007/s11104-005-2950-8

Liu, Z., Powers, W., 2012. Meta-Analysis of Greenhouse Gas Emissions from Swine Manure Land Application, in: 2012 ASABE Annual International Meeting. American Society of Agricultural and Biological Engineers, United States of America, 5 pp.

Lloveras, J., Lopez, A., Ferran, J., Espachs, S., Solsona, J., 2001. Bread-making wheat and soil nitrate as affected by nitrogen fertilization in irrigated Mediterranean conditions. Agron. J. 93, 1183–1190. doi:10.2134/agronj2001.1183

López-Bellido, L., López-Bellido, R.J., Redondo, R., 2005. Nitrogen efficiency in wheat under rainfed Mediterranean conditions as affected by split nitrogen application. F. Crop. Res. 94, 86–97. doi:10.1016/j.fcr.2004.11.004

MacKenzie, A.F., Fan, M.X., Cadrin, F., 1998. Nitrous Oxide Emission in Three Years as Affected by Tillage, Corn-Soybean-Alfalfa Rotations, and Nitrogen Fertilization. J. Environ. Qual. 27, 698. doi:10.2134/jeq1998.00472425002700030029x

Maris, S.C., Teira-Esmatges, M.R., Bosch-Serra, A.D., Moreno-García, B., Català, M.M., 2016. Effect of fertilising with pig slurry and chicken manure on GHG emissions from Mediterranean paddies. Sci. Total Environ. 569–570, 306–320. doi:10.1016/j.scitotenv.2016.06.040

Mateo-Marín, N., Quílez, D., Guillén, M., Isla, R., 2020. Feasibility of stabilised nitrogen fertilisers decreasing greenhouse gas emissions under optimal management in sprinkler irrigated conditions. Agric. Ecosyst. Environ. 290, 106725. doi:10.1016/j.agee.2019.106725

Menéndez, S., Merino, P., Pinto, M., González-Murua, C., Estavillo, J.M., 2009. Effect of N-(n-butyl) thiophosphoric triamide and 3,4 dimethylpyrazole phosphate on gaseous emissions from grasslands under different soil water contents. J. Environ. Qual. 38, 27–35. doi:10.2134/jeq2008.0034

MAPA, 2020. Superficies y producciones anuales de cultivos. Datos avances de cereales, año 2018. (accessed 24 April 2020).

Moreno-García, B., Guillén, M., Quílez, D., 2017. Response of paddy rice to fertilisation with pig slurry in northeast Spain: Strategies to optimise nitrogen use efficiency. F. Crop. Res. 208, 44–54. doi:10.1016/j.fcr.2017.01.023

Plaza-Bonilla, D., Cantero-Martínez, C., Bareche, J., Arrúe, J.L., Lampurlanés, J., Álvaro-Fuentes, J., 2017. Do no-till and pig slurry application improve barley yield and water and nitrogen use efficiencies in rainfed Mediterranean conditions? F. Crop. Res. 203, 74–85. doi:10.1016/j.fcr.2016.12.008

Quemada, M., Baranski, M., Nobel-de Lange, M.N.J., Vallejo, A., Cooper, J.M., 2013. Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems and their effects on crop yield. Agric. Ecosyst. Environ. 174, 1–10. doi:10.1016/j.agee.2013.04.018

Recio, J., Vallejo, A., Le-Noë, J., Garnier, J., García-Marco, S., Álvarez, J.M., Sanz-Cobena, A., 2018. The effect of nitrification inhibitors on NH3 and N2O emissions in highly N fertilized irrigated Mediterranean cropping systems. Sci. Total Environ. 636, 427–436. doi:10.1016/j.scitotenv.2018.04.294

Sanz-Cobena, A., Abalos, D., Meijide, A., Sanchez-Martin, L., Vallejo, A., 2014. Soil moisture determines the effectiveness of two urease inhibitors to decrease N2O emission. Mitig. Adapt. Strateg. Glob. Chang. 21, 1131–1144. doi:10.1007/s11027-014-9548-5

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. doi:10.1016/j.agee.2016.09.038

Sanz-Cobena, A., Misselbrook, T.H., Hernáiz, P., Vallejo, A., 2019. Impact of rainfall to the effectiveness of pig slurry shallow injection method for NH3 mitigation in a Mediterranean soil. Atmos. Environ. 216, 116913. doi:10.1016/j.atmosenv.2019.116913

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. doi:10.1016/j.agee.2011.12.016

Snyder, C.S., Bruulsema, T.W., Jensen, T.L., Fixen, P.E., 2009. Review of greenhouse gas emissions from crop production systems and fertilizer management effects. Agric. Ecosyst. Environ. 133, 247–266. doi:10.1016/j.agee.2009.04.021

Soil Survey Staff, 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, United States of America, 360 pp.

Ussiri, D., Lal, R., 2013. Soil Emission of Nitrous Oxide and its Mitigation. Springer, Netherlands, 378 pp.

Wrigley, C.W., Batey, I.L., 2012. Assessing grain quality, in: Breadmaking (Second edition) Improving Quality. Woodhead Publishing Series in Food Science, Technology and Nutrition, pp. 149–187.

Yagüe, M.R., Quílez, D., 2012. On-farm measurement of electrical conductivity for the estimation of ammonium nitrogen concentration in pig slurry. J. Environ. Qual. 41, 893. doi:10.2134/jeq2011.0352

Yagüe, M.R., Valdez, A.S., Bosch-Serra, À.D., Ortiz, C., Castellví, F., 2019. A short-term study to compare field strategies for ammonia emission mitigation. J. Environ. Qual. 48, 179–184. doi:10.2134/jeq2018.05.0218

Zerulla, W., Barth, T., Dressel, J., Erhardt, K., Horchler von Locquenghien, K., Pasda, G., Rädle, M., Wissemeier, A., 2001a. 3,4-Dimethylpyrazole phosphate (DMPP) – a new nitrification inhibitor for agriculture and horticulture. Biol. Fertil. Soils 34, 79–84. doi:10.1007/s003740100380

Zerulla, W., Pasda, G., Hähndel, R., Wissemeier, A.H., 2001b. The New Nitrification Inhibitor DMPP (ENTEC®) for Use in Agricultural and Horticultural Crops – An Overview. Plant Nutrition. Springer, Netherlands, pp. 754–755.

Chapter 6

Gaseous nitrogen losses derived from pig slurry fertilisation: can they be reduced with additives?

Abstract

The use of pig slurry as fertiliser is associated with gaseous nitrogen (N) losses, especially ammonia (NH_3) and nitrous oxide (N_2O), leading to environmental problems and a reduction of its fertiliser value. The objective of this study is to evaluate the effect of three compounds added to the pig slurry aimed to decrease NH_3 and N_2O losses while maintaining grain yield. The experiment was conducted in an irrigated wheat crop and the treatments were: i) non-N-fertilised control, ii) pig slurry (PS), iii) pig slurry with the urease inhibitor monocarbamide dihydrogen sulphate (PS-UI), iv) pig slurry with a microbial activator in development (PS-A), and v) pig slurry with the nitrification inhibitor 3,4-dimethylpyrazole phosphate (PS-NI). Pig slurry was applied at a target rate of 120 kg NH_4^+-N ha^{-1}. Ammonia volatilisation was measured using semi-opened free static chambers after presowing and tillering PS application in season 2016/17. Greenhouse gas emissions (nitrous oxide and methane) were measured using static closed unvented chambers after tillering PS application in seasons 2016/17 and 2017/18. Soil mineral N concentration was determined for both seasons through periodic soil samplings at 0-15-cm and 15-30-cm depth. Ammonia volatilisation was estimated to be 7-9% and 19-23% of NH_4^+-N applied after presowing and tillering applications, respectively. Additives were not able to reduce NH_3 emissions in any of the application moments. PS-NI was the only treatment effective in reducing N_2O emissions by 70% respect to those in PS treatment. Productive parameters were not affected by the application of the additives because of the lack of effect controlling NH_3 losses and the low contribution of N_2O losses to the N balance (<1 kg N_2O-N ha^{-1}). Thus, the use of 3,4-dimethylpyrazole phosphate would be recommended from an environmental perspective, although without grain yield benefits.

1. Introduction

Ammonia (NH_3) is an important anthropogenic contaminant in the atmosphere based on its total emission respect the rest of air pollutants (EEA, 2019a). Ammonia reacts with atmospheric nitric and sulphuric acids to form fine particulate matter ($PM_{2.5}$), considered a major environmental risk to human health (Hristov, 2011) since it is responsible for more than 410,000 extra premature deaths a year in Europe (EEA, 2019a). Besides, NH_3 emissions induce substantial environmental damages due to its effect on air pollution, soil acidification, water eutrophication, and loss of biodiversity (Ti et al., 2019; Vitousek et al., 1997).

Agriculture is responsible for the 92% of NH_3 emissions in Europe (EEA, 2019a), 80% of these emissions are attributable to livestock production systems and the remaining 20% is associated to inorganic fertilisers (EEA, 2016). In particular, spreading of manures and slurries for crop fertilisation causes 25% of these NH_3 emissions (EEA, 2019b). North-eastern Spain is a hotspot of NH_3 (Guevara et al., 2019) since this area gathers more than 15 million head of pigs (MAPA, 2020) comprising the 8% of total European pig livestock population (FAOStat, 2020).

The use of slurries as fertilisers also implies nitrous oxide (N_2O) emissions that might be influenced by the highly mineralised nature of the nitrogen (N) contained in this product (Aguilera et al., 2013) in comparison to other organic fertilisers. Nitrous oxide is the largest ozone-depleting substance (UNEP, 2013) and the third most contributing emission to the greenhouse gas effect because of its atmospheric lifetime (121 years) and its radiative properties (GWP-100 yr. of 265) (Myhre et al., 2013).

Gaseous N losses associated with slurry management can be controlled through physical and chemical procedures. Ammonia volatilisation can be decreased using trail hoses for the slurry application instead of the splash plate (Yagüe et al., 2019), using slurries with low dry matter content (Bosch-Serra et al., 2014), and acidifying slurries (Fangueiro et al., 2015). Sanz-Cobena et al. (2017) compiled a set of practices applicable to organic fertilisation to mitigate N_2O emissions, some of them also reduce NH_3 volatilisation. These practices include slurry injection into the soil or immediate incorporation of slurries into the soil after their application, adjusting N rates to cover crop nutritional needs, and the use of inhibitors (nitrification inhibitors or urease inhibitors) as additives to fertilisers.

Nitrification inhibitors (NIs) are applied on ammonium-based fertilisers to delay the conversion of ammonium to nitrite by the depression of the *Nitrosomonas* activity (Zerulla et al., 2001a, 2001b). Urease inhibitors (UIs) are considered for urea-based fertilisers since these substances delay the conversion of urea to ammonium (NH_4^+) by the inhibition of the urease enzyme activity (Ussiri and Lal, 2013).

3,4-dimethylpyrazole phosphate (DMPP), one of the most extensively used NI (Abalos et al., 2014), has been traditionally blended into mineral fertilisers. However, a novel formulation based on DMPP (Vizura®) has been developed for liquid manure and biogas digestate. Monocarbamide dihydrogen sulphate (MCDHS; international patent WO 2007/132032 A1) is another substance marketed as urease inhibitor (Orden PRE/630/2011) but there is no available information in the scientific literature to support its potential under field conditions. The manufacturing company also claims the protection of ammonium-N controlling pH levels and decreasing NH_3 volatilisation due to the micro-acidification produced in the hydrolysis of the MCDHS molecule, releasing protons H^+.

In this context, the objective of this study is to evaluate in a wheat crop and under Mediterranean irrigated conditions, the effect of MCDHS, a soil microbial activator (in development in the project CDTI IDI-20170513), and Vizura® added to pig slurry on NH_3 volatilisation and greenhouse gas (GHG) emissions.

2. Materials and methods

2.1. Site and experimental design

The trial was conducted at the experimental field ‘Soto Lezcano’ (middle Ebro Valley, Spain) during two wheat-growing seasons (2016/17 and 2017/18) under semiarid Mediterranean-continental irrigated conditions. The climate is characterised by mean annual air temperature of 14.6 °C and mean annual precipitation and reference evapotranspiration of 318 mm and 1,243 mm, respectively (period 2004-2019). The trial was established on a Typic Xerofluvent soil (Soil Survey Staff, 2014; Table 1) where bread wheat (*Triticum aestivum* L. cv. 'Rimbaud') was cultivated under sprinkler irrigation. Crop water needs were calculated weekly from the reference evapotranspiration (ET) estimated with the Penman-Monteith equation and the locally adapted crop coefficients (Kc) according to FAO procedures (Allen et al., 1998). Thereby, the crop received a total of 435 mm and 428 mm of water (rain plus irrigation) to cover the estimated crop ET of 429 mm and 383 mm, respectively, during the two cropping seasons.

Table 1. Physicochemical characteristics of the soil before establishing the trial.

	0-30 cm	30-60 cm
Soil texture	Silt Loam	Silt Loam
Sand (%)	32.5	31.1
Silt (%)	50.5	51.9
Clay (%)	17.0	17.0
Stoniness ($\%_{vol.}$)	1	1
Total nitrogen (Kjeldahl) (mg kg^{-1})	1,350	940
Phosphorous (Olsen) (mg kg^{-1})	43	12.1
Potassium (NH_4Ac) (mg kg^{-1})	408	231
Organic matter (%)	1.84	0.92
pH ($1{:}2.5_{H2O}$)	8.36	8.36
Electrical conductivity ($1{:}5_{H2O}$) (dS m^{-1})	0.265	0.261

The experiment had a randomised block design with four replicates and four treatments with a plot size of 2.0 × 3.6 m. The experiment was replicated at three times: autumn 2016, spring 2017 (both in the same crop cycle), and spring 2018. Slurry from fattening pigs was used in the three trials (Table 2) and the four evaluated additives were provided by the manufacturing companies. The slurry was applied by hand to the experimental plots at a target rate of 120 kg NH_4^+-N ha^{-1} in all treatments (actual rates in Table 2) except in Control treatment. Fertiliser treatments were: a) non-N application (Control); b) pig slurry (PS); c) pig slurry mixed with the urease inhibitor monocarbamide dihydrogen sulphate (PS-UI); d) pig slurry mixed with a soil microbial activator (PS-A) or with nitrification inhibitor 3,4-dimethylpyrazole phosphate (Vizura®; PS-NI). In spring 2018, the PS-A was substituted by the PS-NI treatment. The additives were applied according to the rate recommended by the manufacturing company: 2.5 L of MCDHS in 1 Mg of pig slurry, 2.5 kg of soil microbial activator in 1 Mg of pig slurry, and 3 L of Vizura® per hectare. Pig slurry was applied in autumn 2016 (14th November 2016) at presowing and in spring 2017 and 2018 (7th April 2017 and 19th March 2018, respectively) at tillering. At presowing in the two cropping seasons, 70 kg P_2O_5 ha^{-1} and 150 kg K_2O ha^{-1} were applied to avoid limitations of these two nutrients.

Table 2. Physicochemical characteristics of the pig slurry and nitrogen applied for each moment.

	2016/17		2017/18
	Presowing Autumn 2016	Tillering Spring 2017	Tillering Spring 2018
Density (kg m^{-3})	1,030	1,034	1,022
pH	-	7.6	7.8
Electrical conductivity at 25 °C (dS m^{-1})	306	302	354
Dry matter (kg DM m^{-3})	41.4	73.4	31.6
Organic matter (kg OM m^{-3})	26.1	53.7	17.1
Ammonium nitrogen (kg N m^{-3})	3.2	4.5	4.0
Organic nitrogen (kg N m^{-3})	0.3	0.9	0.9
Phosphorous (kg P_2O_5 m^{-3})	0.6	0.3	0.6
Potassium (kg K_2O m^{-3})	4.0	4.3	4.5
Ammonium-N (kg NH_4^+-N ha^{-1})	141	157	158
Total-N (kg N ha^{-1})	155	208	191

The crop was managed according to standard practices in the region. Wheat was sown on 30th December 2016 and 16th November 2017 at a plant density of 200 and 175 kg seed ha^{-1}, respectively. The crop was harvested at wheat maturity (4th July 2017 and 6th July 2018). Straw was hashed and incorporated to the soil before subsequent wheat seeding. Weeds, diseases and pests were controlled and no special problems were detected during the experimental period.

2.2. Ammonia volatilisation

Ammonia volatilisation was measured using semi-opened free static chambers (SOC), similar to those of Araújo et al. (2009). Two polyethylene terephthalate chambers per plot (31.0-cm height and 10.3-cm inner diameter, 2-L volume bottle with the bottom cut) were located 2 cm above the soil surface and with a 2-cm diameter upper hole that ensured airflow (Fig. 1). The removed bottom was situated 2 cm above the upper hole to avoid the entrance of rain and irrigation water into the chamber. Inside the chamber, there was an absorbent Spontex® Origin foam strip (Mapa Spontex Ibérica SA, Sant Cugat del Vallès, Spain) of 25.0 × 2.5 × 0.5 cm (length × wide × thickness) and 0.087 g cm^{-3} (density) previously impregnated in acid solution (60 mL H_2SO_4, 1 mol dm^{-3} + glycerine (2% v/v)). The bottom end of the strip was immersed in a 100-mL plastic jar with 50 mL of the acid solution in permanent contact with the strip. At each sampling, the trapped ammonia in the foam strip was extracted with 250 mL of 2 M KCl. The extracts were analysed to determine ammonium concentration by colourimetry using a segmented flow analyser (AutoAnalyser 3, Bran+Luebbe, Germany).

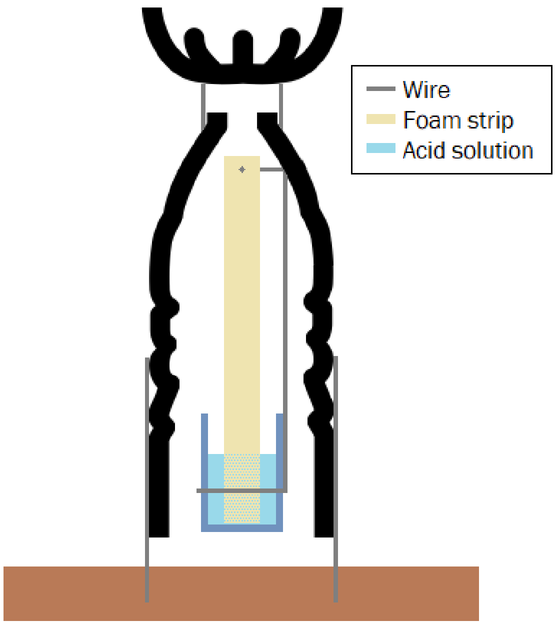


Figure 1. Sketch of semi-opened free static chamber components.

Samplings started the day of slurry application with a frequency from twice measures a day during the first two days to once a week during the last weeks. Samplings were performed until 28 days after fertilisation in autumn 2016 and 14 days after fertilisation in spring 2017 since spring weather conditions and irrigation management promoted that volatilisation stooped in a shorter timespan than in autumn. Ammonia volatilisation was not measured in spring 2018.

The efficiency of the SOC trapping ammonia system was determined in the laboratory using triplicate solutions with known concentrations of ammonium (16.2 mg NH_4^+ L^{-1}, 10.8 mg NH_4^+ L^{-1}, and 8.5 mg NH_4^+ L^{-1}). After 24 hours, the NH_3 trapped by foam strips and the remained NH_4^+ in the solution were quantified to determine through the difference the NH_3 volatilised. SOC efficiency was defined as the ratio between NH_3 trapped by the foam and NH_3 volatilised. Efficiency in the field could not be determined because the solution with the known concentration was systematically polluted.

2.3. Greenhouse gas emissions

The closed-chamber technique and the N_2O flux measurement procedure were similar to those described by Mateo-Marín et al. (2020). Shortly, polyvinyl chloride upper cover of chambers (18.5-cm height and 30.0-cm inner diameter) wrapped in reflective insulation film were set on collars inserted 10 cm into the soil, creating a 13.1-L chamber headspace. At each sampling, 15 mL of inner air were taken using a polypropylene syringe at 0 and 60 min after chamber closure. The samples were injected into 12-mL Exetainer borosilicate glass vials (Model 038W, Labco). Samplings started roughly at the hour with the mean temperature of the day (between 9:30h and 11:00h GMT; Alves et al., 2012). Samples were analysed by gas chromatography using the equipment and technique described in detail by Franco-Luesma et al. (2019), an Agilent 7890B gas chromatography system with HP-Plot Q column and electron-capture, flame-ionisation and methaniser detectors.

Greenhouse gas emission rates were calculated as the linear increment in gas concentration (corrected for the air temperature) in the chamber headspace and multiplied by the ratio between the chamber volume and the soil area covered by the chamber (MacKenzie et al., 1998).

In both seasons, GHG measurements started just before the pig slurry application at tillering and ended at harvest. The sampling frequency was higher after fertilisation (once a day), and then measurements were spreading from once a week to once every two weeks, with a total of 12 and 17 sampling dates in spring 2017 and 2018, respectively.

The N_2O emission factor (EF, %) was calculated as the ratio between the difference of cumulative N_2O emissions in fertilised and unfertilised N plots, and the amount of the N applied in the fertilised plots and multiplied by 100. The yield-scaled N_2O emission (YS_{N2O}; g N kg^{-1} grain) is the ratio between the cumulative N_2O emissions and the grain yield.

2.4. Soil mineral nitrogen

Two soil core samples at two depths (0-15 cm and 15-30 cm) were taken per plot with daily frequency the first 5 days after fertilisation, and decreasing the frequency later to reach once a week at the end of the sampling period (total of 11, 12, and 15 sampling dates in autumn 2016, spring 2017, and spring 2018, respectively). Samples were sieved (3 mm) and two subsamples were obtained. A subsample was dried at 105 °C until constant weight to determine gravimetric soil water content. Another subsample of 10 g of fresh soil was extracted with 30 mL of 2 N KCl, shaken for 30 min, and filtered through cellulose filter. Nitrate (NO_3^-) and ammonium concentration in extracts were analysed by colourimetry using a segmented flow analyser (AutoAnalyser 3, Bran+Luebbe, Germany).

2.5. Productive parameters and efficiency in the use of N

At wheat maturity, each plot was hand-harvested in two random areas of 0.54 m^2 to obtain the grain (adjusted to 120 g kg^{-1}) and aboveground biomass yield. Subsamples of grain and straw were dried at 65 ºC and milled to obtain the grain and straw N concentration by dry combustion (TruSpec CN, LECO, St. Joseph, MI, USA).

Two parameters were used to compare the efficiency in the use of N between treatments. The mineral N contained in the applied slurry (i.e., NH_4^+-N) was used for the calculations since it was considered that the contribution of pig slurry organic N and its residual effect was not substantial during the period of the experiment. The nitrogen use efficiency (NUE) is the relation between the total aboveground N uptake and the ammonium-N applied by fertilisation. The apparent N recovery efficiency (RE_N) is the increment in the aboveground N uptake due to the N application per unit of mineral-N applied (Eq. 1):

$$RE_N = \frac{U_T - U_0}{F_T} \qquad [Eq. 1]$$

where U_T is the N uptake by aboveground biomass in the T treatment; U_0 is the N uptake by aboveground biomass in the unfertilised control plot (deprived of N application); and F_T is the amount of mineral-N applied in the T treatment.

2.6. Statistical analysis

Statistical analyses were performed using SAS® software (University Edition, SAS Institute Inc., Cary, NC, USA). Normal distribution and homogeneity of variance were checked by Shapiro-Wilk and Levene's test, respectively, and variables were transformed when necessary (Box-cox transformation). Analysis of variance (MIXED procedure) was used to assess the existence of treatment effects and differences in treatment means were established with the Tukey's test at the 0.05 significance level. In cases with measurements over time (ammonia, greenhouse gases, and soil mineral nitrogen), repeated measure analysis was used according to a first-order autoregressive structure model AR(1).

3. Results

3.1. Ammonia volatilisation

Semi-opened free static chambers efficiency determined in the laboratory was 24.6% ± 0.7% (mean ± standard error).

During the field experiment, there were substantial differences in the environmental conditions after PS applications in autumn and spring (Fig. 2a and Fig. 3a). Thus, the average temperature was 7.2 and 15.0 °C after PS applications in autumn and spring, respectively. Similarly, the average wind speed was 1.1 and 2.0 m s^{-1} after PS applications in autumn and spring, respectively.

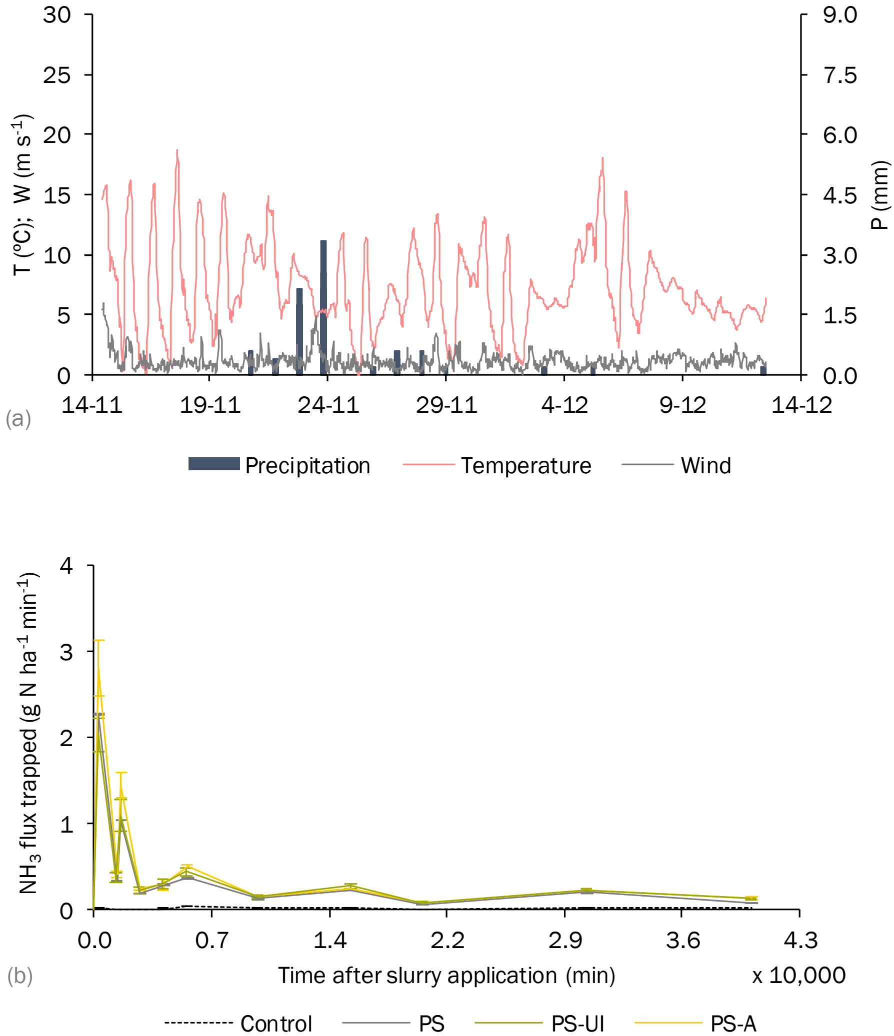
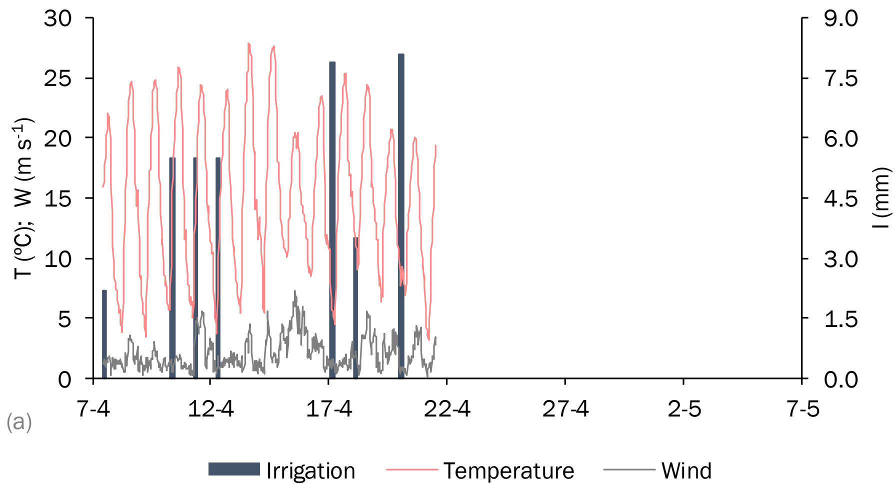


Figure 2. Half-hourly changes in temperature (T; °C) and wind speed (W; m s^{-1}), and daily changes in precipitation (P; mm) (Fig. a). Temporal changes of average ammonia fluxes (g N ha^{-1} min^{-1}) trapped by the semi-opened free static chamber for each treatment (Control, PS: pig slurry, PS-UI: pig slurry + MCDHS, and PS-A: pig slurry + microbial activator) after presowing application (Fig. b). Vertical bars indicate standard error (n=4).



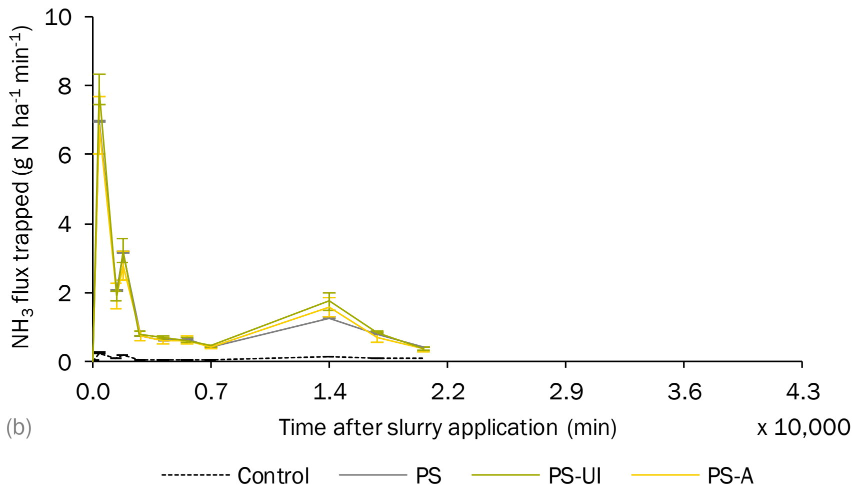


Figure 3. Half-hourly changes in temperature (T; ºC) and wind speed (W; m s^{-1}), and daily changes in irrigation (I; mm) (Fig. a). Temporal changes of average ammonia fluxes (g N ha^{-1} min^{-1}) trapped by the semi-opened free static chamber for each treatment (Control, PS: pig slurry, PS-UI: pig slurry + MCDHS, and PS-A: pig slurry + microbial activator) after side-dress application (Fig. b). Vertical bars indicate standard error (n=4).

The highest NH_3 peak was trapped in the first sampling after pig slurry application, next 4.5 h in autumn 2016 (Fig. 2b) and 6.5 h in spring 2017 (Fig. 3b). Ammonia trapped in fertilised treatments were 2.40 ± 0.21 g N ha^{-1} min^{-1} in autumn 2016 and 7.24 ± 0.43 g N ha^{-1} min^{-1} in spring 2017 (mean ± standard error). At the following sampling (23 h and 24 h after pig slurry application), NH_3 decreased to 0.38 ± 0.03 g N ha^{-1} min^{-1} after the presowing application (autumn 2016) and 1.95 ± 0.16 g N ha^{-1} min^{-1} after the tillering application (spring 2017). At the third sampling (27 h and 31 h after pig slurry application), NH_3 increased again to 1.22 ± 0.11 g N ha^{-1} min^{-1} and 3.05 ± 0.19 g N ha^{-1} min^{-1} in autumn 2016 and spring 2017, respectively. The following sampling dates showed declining NH_3 emissions (Fig. 2b and 3b), although an additional emission peak was trapped 10 days after slurry application in spring 2017. This peak is thought to be related to an error in the sampling caused by the degradation of the foam strips during the 5-day exposition period between 12th and 17th April.

No significant differences (repeated measure analysis) in NH_3 fluxes among the three pig slurry treatments were observed; however, all three treatments presented significantly higher fluxes than the non-fertilised control. The NH_3 fluxes were 280% and 479% higher in the fertilised treatments than in the Control for autumn 2016 and spring 2017, respectively.

Table 3. Average cumulative ammonia volatilised* (kg N ha^{-1}; n=4) and percentage respect ammonium nitrogen applied with the pig slurry in the different treatments (PS: pig slurry, PS-UI: pig slurry + MCDHS, and PS-A: pig slurry + microbial activator). The results are presented separately by application moment (presowing and side-dress application) and timespan considered (3, 14, and 28 days) after application.

	PS	PS-UI	PS-A	p-value
Autumn 2016				
3 days	5.4 (3.8%)	5.3 (3.8%)	6.6 (4.6%)	0.197
14 days	9.0 (6.4%)	9.6 (6.8%)	11.2 (7.9%)	0.181
28 days	9.7 (6.9%)	10.5 (7.4%)	12.1 (8.6%)	0.158
Spring 2017				
3 days	17.9 (13.0%)	19.0 (13.8%)	20.8 (15.2%)	0.695
14 days	26.4 (19.3%)	28.9 (21.1%)	31.6 (23.0%)	0.609

*- Considering 24.6% as the efficiency of the method to trap the ammonia volatilised and discounting the amount of ammonia trapped in the control (background) treatment.

Cumulative ammonia volatilised after three, fourteen, and twenty-eight days of pig slurry application are presented in Table 3. During the first three days, NH_3 losses reached more than 50% of the total NH_3 emitted during the whole measurement period (51% for autumn 2016 and 65% for spring 2017) and fourteen days after fertilisation, NH_3 losses represented 91% of the total losses of the period. No differences (p>0.05) were found in the cumulative NH_3 emissions among fertiliser treatments for any period (3, 14, or 28 days, whatever it was the application moment, presowing or side-dress). Ammonia volatilisation losses in the period of fourteen days after pig slurry application were, on average, 34% larger ($p<0.0001$) during spring (side-dress at tillering) than during autumn (presowing application).

3.2. Greenhouse gas emissions

Mean CH_4 fluxes ranged from -8.0 g C ha^{-1} day^{-1} to 9.1 g C ha^{-1} day^{-1} in both cropping seasons (Fig. 4c and 5c). No differences in CH_4 fluxes were observed among treatments (repeated measure analysis; $p>0.05$). Cumulative CH_4 emissions were not affected by slurry application; moreover, the use of additives with pig slurry did not show an effect on CH_4 emissions (Table 4). These emissions were negative and significantly different from zero in some treatments, although without a consistent pattern of methane sinks through the seasons.

Table 4. Average cumulative GHG emissions (g N_2O-N ha^{-1} and g CH_4-C ha^{-1}; n=4) in the different fertiliser treatments (Control, PS: pig slurry, PS-UI: pig slurry + MCDHS, PS-A: pig slurry + microbial activator, and PS-NI: pig slurry + DMPP). The results are presented separately by growing seasons with fertiliser application at side-dress (2016/17 and 2017/18). Values followed by the same letter were not significantly different ($p>0.05$, Tukey's test).

	Control	PS	PS-UI	PS-A	p-value
Spring 2017					
N_2O	229.9 b	610.6 a	685.3 a	661.5 a	<0.001
CH_4	-37.8	-36.3	-119.3*	4.3	0.590

	Control	PS	PS-UI	PS-NI	p-value
Spring 2018					
N_2O	111.8 b	460.9 a	1014.4 a	139.5 b	<0.001
CH_4	-241.8*	-137.8*	-13.2	-211.8*	0.241

*- CH_4 emissions are different from zero.

Mean N_2O fluxes ranged from -0.5 g N ha^{-1} day^{-1} to 66.9 g N ha^{-1} day^{-1} for both cropping seasons (Fig. 4b and 5b).

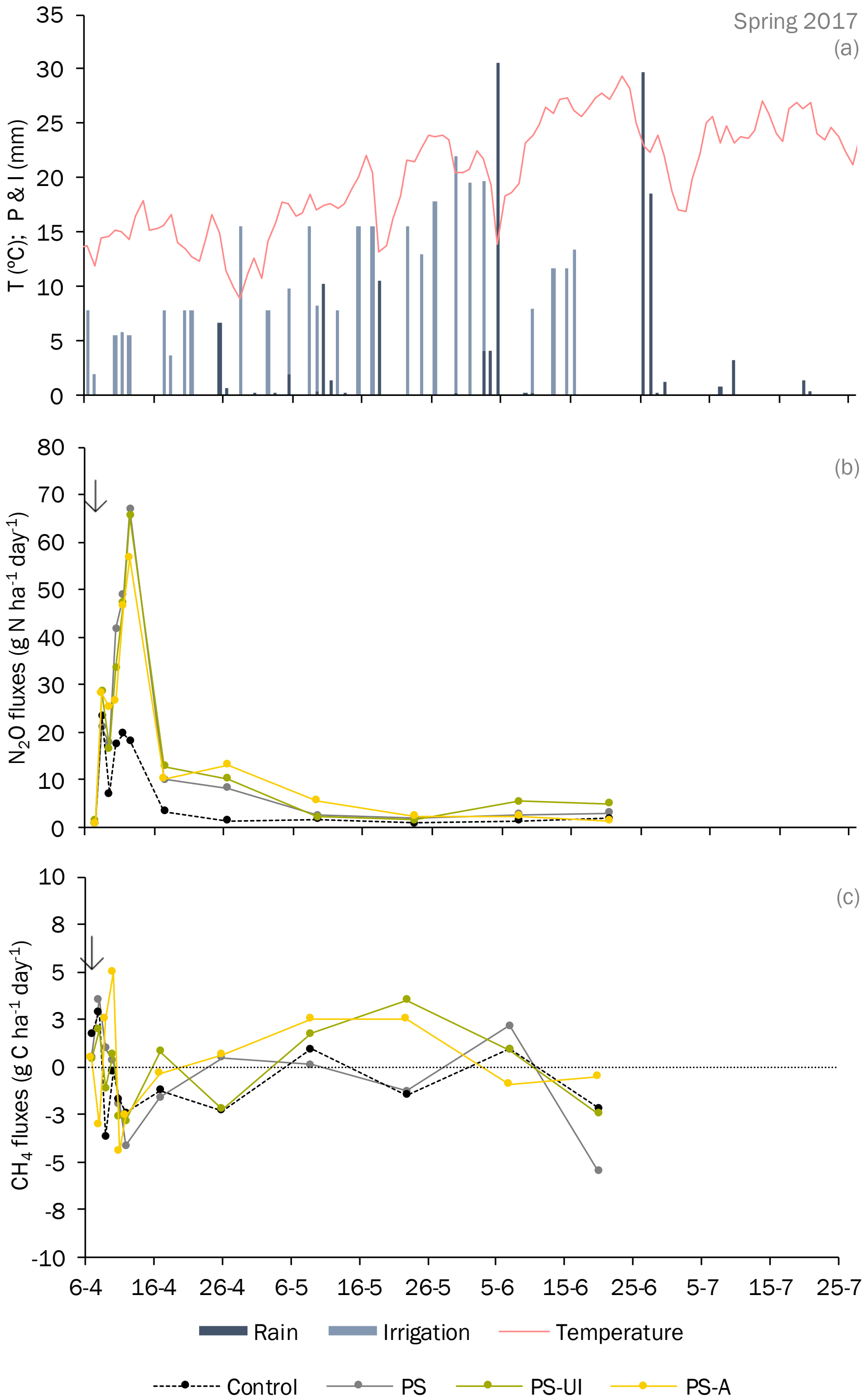


Figure 4. Mean daily air temperature (ºC), precipitation and irrigation water (mm) (Fig. a) and temporal N_2O fluxes (g N ha^{-1} day^{-1}) (Fig. b) and temporal CH_4 fluxes (g C ha^{-1} day^{-1}) (Fig. c) for each treatment (Control, PS: pig slurry, PS-UI; pig slurry + MCDHS, PS-A: pig slurry + microbial activator) after pig slurry application in spring 2017. Arrows indicate fertilisation day.

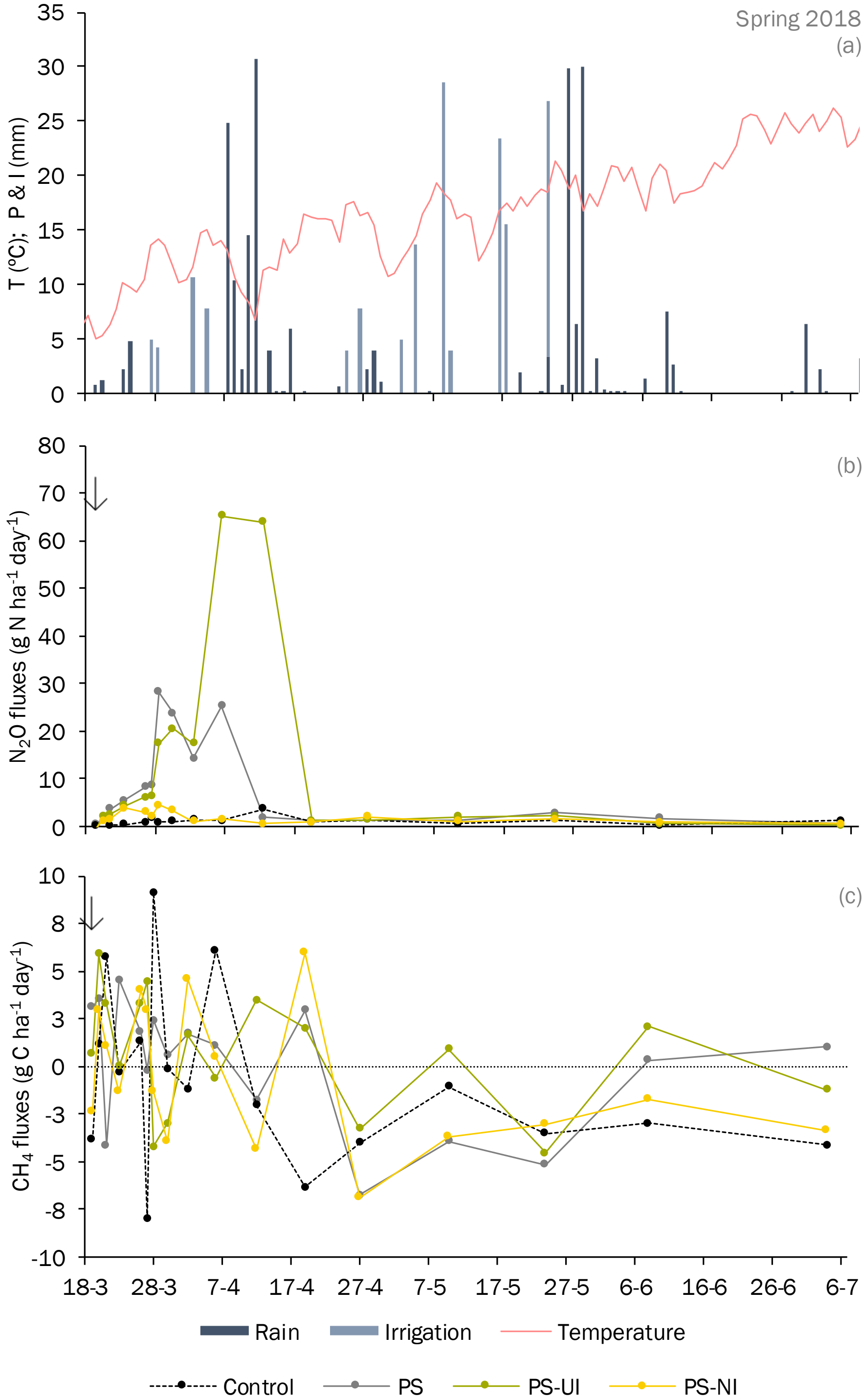


Figure 5. Mean daily air temperature (ºC), precipitation and irrigation water (mm) (Fig. a) and temporal N_2O fluxes (g N ha^{-1} day^{-1}) (Fig. b) and temporal CH_4 fluxes (g C ha^{-1} day^{-1}) (Fig. c) for each treatment (Control, PS: pig slurry, PS-UI; pig slurry + MCDHS, PS-NI: pig slurry + DMPP) after pig slurry application in spring 2018. Arrows indicate fertilisation day.

Significant differences were observed between treatments: Control showed lower N_2O fluxes in comparison to the fertilised treatments in spring 2017, and Control and PS-NI showed lower N_2O fluxes than PS and PS-A in spring 2018 (repeated measure analysis; $p<0.05$). When cumulative N_2O emissions were analysed (Table 4), significant differences attributed to the lower emissions in the Control were also observed, but no differences were found among the other three PS treatments in spring 2017. The N_2O emissions were on average 2.8 times higher in the PS treatments than in the non-fertilised control. However, in spring 2018, the PS-NI treatment decreased the N_2O emissions at the same level that the Control, with lower emissions than the PS and PS-UI treatments ($p<0.05$). Although a large N_2O emission was observed in the PS-UI (1,014 g N ha^{-1}) during spring 2018, it was not significantly different from that in the PS.

Nitrous oxide EF and YS_{N2O} emission did not show differences between treatments in spring 2017, but in spring 2018 the treatment with NI presented the lowest values (Table 5) for both variables. EF was 92% and 97% significantly lower in PS-NI than in PS and PS-UI, respectively; and YS_{N2O} was 71% and 87% significantly lower in PS-NI than in PS and PS-UI, respectively.

Table 5. Average (n=4) N_2O EF (%) and YS_{N2O} (g N_2O-N kg^{-1} grain) for the different fertilised treatments (PS: pig slurry, PS-UI: pig slurry + MCDHS, PS-A: pig slurry + microbial activator, and PS-NI: pig slurry + DMPP). The results are presented separately for the two PS applications (spring 2017 and spring 2018). Values followed by the same letter were not significantly different (p>0.05, Tukey test).

	PS	PS-UI	PS-A	p-value
Spring 2017				
EF	0.19	0.22	0.21	0.483
YS_{N2O}	0.11	0.13	0.13	0.312
	PS	**PS-UI**	**PS-NI**	**p-value**
Spring 2018				
EF	0.24 ab*	0.60 a	0.02 b*	0.033
YS_{N2O}	0.07 a	0.15 a	0.02 b	0.001

*- Differences were found when just the two treatments were analysed using Tukey's test. This test was used since the huge values of N_2O emissions in PS-UI hid differences.

3.3. Soil mineral nitrogen

In autumn PS application, soil nitrate concentration was affected by treatments in the two soil layers and soil mineral N concentration was affected by treatments in the top layer (Table 6).

Table 6. Average (n=4) nitrate (NO_3^-, mg N kg^{-1} soil), ammonium (NH_4^{+}-, mg N kg^{-1} soil) and mineral N (Nmin; mg N kg^{-1} soil) concentration from 0 to 15-cm and 15 to 30-cm depths in the one month after PS application in the fertiliser treatments (PS: pig slurry, PS-UI: pig slurry + MCDHS, PS-A: pig slurry + microbial activator, and PS-NI: pig slurry + DMPP). The results are presented separately by periods (autumn 2016, spring 2017, and spring 2018). Values followed by the same letter were not significantly different (p>0.05, Tukey's test).

	PS	PS-UI	PS-A	Treat.[1]	Sampl.[1]	Treat.×Sampl.[1]
Autumn 2016 - Presowing						
0-15 cm						
NO_3^-	15.5 ab	12.6 b	18.2 a	<0.001	<0.001	1.000
NH_4^+	35.1	34.1	41.0	0.654	<0.001	0.534
Nmin	50.6 ab	46.8 b	59.2 a	0.001	<0.001	0.628
15-30 cm						
NO_3^-	13.0 ab	12.0 b	14.4 a	0.007	0.241	1.000
NH_4^+	9.9	8.5	8.8	0.992	<0.001	0.733
Nmin	22.9	20.5	23.2	0.214	<0.001	0.901
Spring 2017 - Side-dress						
0-15 cm						
NO_3^-	11.1	11.8	14.8	0.053	<0.001	0.131
NH_4^+	2.0	2.7	2.0	0.276	<0.001	0.646
Nmin	13.1	14.5	16.8	0.124	<0.001	0.093
15-30 cm						
NO_3^-	8.3	8.1	8.5	0.737	<0.001	0.822
NH_4^+	1.1	1.2	0.7	0.320	<0.001	0.185
Nmin	9.4	9.3	9.2	0.896	<0.001	0.674

	PS	PS-UI	PS-NI	Treat.[1]	Sampl.[1]	Treat.×Sampl.[1]
Spring 2018 - Side-dress						
0-15 cm						
NO_3^-	8.4 a	7.9 a	5.7 b	<0.001	<0.001	0.574
NH_4^+	11.8	12.7	15.6	0.301	<0.001	0.621
Nmin	20.1	20.7	21.4	0.948	<0.001	0.707
15-30 cm						
NO_3^-	4.4	4.2	3.5	0.054	<0.001	0.148
NH_4^+	2.0	2.7	4.9	0.079	<0.001	0.286
Nmin	6.5	6.9	8.4	0.433	<0.001	0.545

[1]Repeated measure analysis considering the fertiliser treatment, sampling date, and their interaction.

Differences were only significant between PS-UI and PS-A, and no effects of the additives mixed with the PS were detected. However, these differences were not noticed in the side-dress application (spring 2017) whatever it was the considered depth (0-15 cm or 15-30 cm). During the next season (spring 2018), soil nitrate concentration (0-15 cm) in PS-NI was 32% and 28%

lower than in PS and PS-UI ($p<0.05$), respectively; and soil ammonium concentration (0-15 cm) in PS-NI was 32% and 23% higher than in PS and PS-UI, respectively, but not significantly different. The opposite behaviour of soil nitrate and ammonium concentrations generated no differences among treatments in soil mineral N content.

3.4. Productive parameters and efficiency in the use of N

No differences in grain yield ($p>0.05$) were found among treatments for any of the three analysed periods (Table 7).

Table 7. Average (n=4) of productive parameters and N efficiency indexes in the different fertiliser treatments (Control, PS: pig slurry, PS-UI: pig slurry + MCDHS, PS-A: pig slurry + microbial activator, and PS-NI: pig slurry + DMPP). The results are presented separately by periods (2016/17 with presowing and side-dress application, and 2017/18). Values followed by the same letter were not significantly different ($p>0.05$, Tukey's test).

	Control	PS	PS-UI	PS-A	p-value
Autumn 2016 - Presowing					
Grain (kg ha^{-1})	7,237	6,139	5,695	6,410	0.083
Aboveground biomass (kg ha^{-1})	14,454	13,542	13,549	14,037	0.719
Grain N (kg ha^{-1})	153.9	122.4	118.8	134.4	0.048*
Total aboveground N (kg ha^{-1})	204.8	181.5	192.9	204.1	0.466
NUE	-	1.29	1.37	1.45	0.525
RE_{NT}	-	-0.16	-0.08	0.00	0.525
Spring 2017 - Side-dress					
Grain (kg ha^{-1})	6,098	5,591	5,186	5,123	0.087
Aboveground biomass (kg ha^{-1})	13,954	13,831	13,141	12,760	0.529
Grain N (kg ha^{-1})	129.7 a	119.9 ab	110.3 ab	107.2 b	0.042
Total aboveground N (kg ha^{-1})	195.5	202.0	187.0	178.9	0.384
NUE	-	1.19	1.13	1.31	0.241
RE_N	-	0.04	-0.05	-0.12	0.292

	Control	PS	PS-UI	PS-A	p-value
Spring 2018 - Side-dress					
Grain (kg ha^{-1})	5,938	6,837	6,471	6,245	0.393
Aboveground biomass (kg ha^{-1})	14,263 b	17,041 a	16,473 a	15,857 ab	0.007
Grain N (kg ha^{-1})	99.2	122.2	108.3	107.7	0.245
Total aboveground N (kg ha^{-1})	153.0	196.3	168.9	173.3	0.136
NUE	-	1.65	1.33	1.43	0.104
RE_N	-	0.36	0.13	0.17	0.210

*- Significant effects of fertiliser treatments ($p<0.05$) from the analysis of variance procedure, but Tukey's test did not show differences.

Aboveground biomass did not show effect of the treatments for the two periods of season 2016/17; however, in 2017/18 growing season, aboveground biomass in the Control treatment was 16% and 13% lower ($p<0.05$) than in PS and PS-UI treatments, respectively (Table 7). Total aboveground N was not affected in the three periods by the fertiliser strategy, but grain N was influenced ($p=0.04$) by the treatments when fertiliser was applied at tillering in spring 2017 (Table 7). Nitrogen use efficiency and recovery N efficiency did not show differences among treatments, independently of the season and the moment of N application (Table 7).

4. Discussion

The hours that follow PS application were critical for NH_3 losses. In this regard, the Directive (EU) 2016/2284 rightly suggests incorporating manures and slurries into the soil within four hours of spreading to reduce ammonia emissions from livestock manure. In this study, 2% and 7% of applied ammonium-N were volatilised as NH_3 within the first 4.5 h and 6.5 h after the presowing and side-dress application, respectively. These values contrast with NH_3 losses of 0.9% of total ammonium-N applied at presowing reported by Yagüe et al. (2019) in a bordering region the first 3.5 h after the spreading with trail-hose. According to the authors, soil moisture and pig slurry characteristics (dry matter) influence NH_3 losses: high soil WFPS and high slurry dry matter boost NH_3 volatilisation. These variables in conjunction with others as meteorological conditions, soil pH, soil management, and measurement method (Hafner et al., 2018) could explain the differences between both studies.

Weather conditions determined the NH_3 evolution. Ammonia volatilisation was higher during diurnal hours (first and third samplings) than at the nighttime hours (second sampling) even when exposure times at night (average 18.5 hours) were longer than diurnal exposures (average 5.5 hours). Higher temperature and wind speed during diurnal hours increased the volatilisation (Fig. 2 and 3; mean thermal amplitude of 8.2 ºC and mean wind speed amplitude of 2.3 m s^{-1}). The effect of the daily pattern of air temperature and wind speed on NH_3 emissions was already observed by Li et al. (2018). Similarly, differences in weather conditions between application moments might have been one of the factors responsible for higher emissions at tillering application than at presowing since at side-dress application the temperature and wind speed were higher than at presowing.

Slurries with low dry matter promote the infiltration on the soil, reducing NH_3 volatilisation compared to slurries with a high dry matter which flavour crust formation and lower infiltration rates (Bosch-Serra et al., 2014). In the present study, two contrasting slurries in term of dry matter (DM), 41.4 kg DM m^{-3} (autumn 2016) and 73.4 kg DM m^{-3} (spring 2017), had to be used. This fact could also affect the comparison of NH_3 volatilisation between application moments.

Irrigation is another factor that could be relevant to compare presowing and side-dress applications. A short irrigation event of 2 mm was applied immediately after PS tillering application to wash up the slurry placed on the canopy to avoid negative effects on leaves. Three days after the N application, irrigation was resumed to satisfy crop water requirements. This practice could have reduced the potential for NH_3 volatilisation in April.

Apart from the cited variables, additives could alter NH_3 volatilisation. UIs are considered a strategy to reduce NH_3 emissions when they are added to urea-based fertilisers or manures since they delay the transformation of urea into ammonium (Sigurdarson et al., 2018). However, the addition of UI to pig slurry is a questionable strategy because of the high probability of fast transformation of urea into ammonium after excretion. MCDHS was evaluated in the study due to the possibility that micro-acidification, through the hydrolysis of the MCDHS molecule, could reduce ammonia volatilisation or have potential effects over N dynamics (e.g., acting as NI). Nevertheless, the presence of dihydrogen sulphate in the molecule could not reduce the pH of the slurry (data not shown). Moreover, no changes were observed in soil mineral N concentrations, ammonia losses, GHG emissions, or yield in PS-UI treatment in comparison to PS treatment, which rejects effects due to MCDHS addition.

The microbial activator, PS-A, was able to maintain higher levels of nitrate in the soil than the urease inhibitor, PS-UI, but it was not able to show differences with PS values. Besides, despite the higher soil NO_3^- concentration, it did not affect N_2O emissions.

A nitrification inhibitor added to slurry can increase yields and N uptake, and reduce NO_3^- leaching and N_2O emission, improving its fertiliser value (Ruser and Schulz, 2015). Although the reduction of N_2O emissions is very effective, the NH_3 volatilisation might increase if slurries are not incorporated into the soil (Ruser and Schulz, 2015). Few studies have evaluated the use of NI mixed with pig slurry to mitigate N_2O losses in the Mediterranean climate (Guardia et al., 2017). Under this climatic condition, Recio et al. (2018) assessed the use of the nitrification inhibitor DMPP when was added to pig slurry on both N_2O and NH_3 emissions, obtaining significant abating effect on direct N_2O emissions and no significant affection of cumulative NH_3 emissions. The present work corroborates the effect of DMPP mitigating N_2O emissions and its associated reduction in EF and YS_{N2O} compared to the standard PS treatment. The *Nitrosomonas* activity inhibition could be sensed from the lower soil NO_3^- and higher soil NH_4^+ concentrations, although significant differences were only observed for nitrate in the 0-15-cm depth. In this regard, topsoil N processes are the most influential on N_2O emissions since N_2O produced in this layer can escape to the atmosphere (Yoh et al., 1997), whereas N_2O produced at deeper layers might not reach the troposphere (Neftel et al., 2000).

In this study, avoiding N_2O losses did not turn into a significant increment in N efficiency as could be expected: the less N_2O losses, the more N availability, and the more N absorption and N efficiency by plants. However, this fact was unnoticed in the efficiency indicators because of the low contribution of N_2O emissions to the N balance (<1 kg N_2O-N ha^{-1}) and the non-limiting soil N conditions proven by the high yields in unfertilised treatments during the two growing seasons and.

5. Conclusions

Important N losses due to ammonia volatilisation were observed after pig slurry fertilisation, lower in autumn (7-9% of NH_4^+-N applied) than in spring application (19-23% of NH_4^+-N applied) but none of the three additives evaluated in this work was effective to reduce them independently of the PS application moment, at presowing or at tillering. Nitrous oxide emissions were a minor component of the N balance (averaging 0.4% of NH_4^+-N applied), although they have a high global warming potential. DMPP showed a good performance to reduce N_2O fluxes (roughly 70%) and, accordingly, yield-scaled N_2O emissions and N_2O emission factors. The use of pig slurry with additives had neither advantages nor disadvantages in terms of agronomic productivity and N use efficiency.

References

Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., Vallejo, A., 2014. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. Agric. Ecosyst. Environ. 189, 136–144. doi:10.1016/j.agee.2014.03.036

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. doi:10.1016/j.agee.2012.09.006

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. FAO Irrigation and Drainage Paper 56. Food and Agriculture Organization of the United Nations, Italy, 50 pp.

Alves, B.J.R., Smith, K.A., Flores, R.A., Cardoso, A.S., Oliveira, W.R.D., Jantalia, C.P., Urquiaga, S., Boddey, R.M., 2012. Selection of the most suitable sampling time for static chambers for the estimation of daily mean N2O flux from soils. Soil Biol. Biochem. 46, 129–135. doi:10.1016/j.soilbio.2011.11.022

Araújo, E.S.E. da S., Marsola, T., Miyazawa, M., Soares, L.H. de B.L.H.B., Urquiaga, S., Boddey, R.M.R.M., Alves, B.J.R.B.J.R., 2009. Calibração de câmara semiaberta estática para quantificação de amônia volatilizada do solo. Pesqui. Agropecuária Bras. 44, 769–776. doi:10.1590/S0100-204X2009000700018

Bosch-Serra, À.D., Yagüe, M.R., Teira-Esmatges, M.R., 2014. Ammonia emissions from different fertilizing strategies in Mediterranean rainfed winter cereals. Atmos. Environ. 84, 204–212. doi:10.1016/j.atmosenv.2013.11.044

EEA, 2019a. Air quality in Europe - 2019 report. Publications Office of the European Union, Luxembourg, 99 pp.

EEA, 2019b. European Union emission inventory report 1990-2017 under the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP). Publications Office of the European Union, Luxembourg, 143 pp.

EEA, 2016. European Union emission inventory report 1990–2013 under the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP). Publications Office of the European Union, Luxembourg, 125 pp.

Fangueiro, D., Hjorth, M., Gioelli, F., 2015. Acidification of animal slurry– a review. J. Environ. Manage. 149, 46–56. doi:10.1016/j.jenvman.2014.10.001

FAOStat, 2020. FAOSTAT database collections: Pig livestock population. (accessed 24 April 2020).

Franco-Luesma, S., Álvaro-Fuentes, J., Plaza-Bonilla, D., Arrúe, J.L., Cantero-Martínez, C., Cavero, J., 2019. Influence of irrigation time and frequency on greenhouse gas emissions in a solid-set sprinkler-irrigated maize under Mediterranean conditions. Agric. Water Manag. 221, 303–311. doi:10.1016/j.agwat.2019.03.042

Guardia, G., Cangani, M.T.M.T., Sanz-Cobena, A., Junior, J.L.L., Vallejo, A., 2017. Management of pig manure to mitigate NO and yield-scaled N2O emissions in an irrigated Mediterranean crop. Agric. Ecosyst. Environ. 238, 55–66. doi:10.1016/j.agee.2016.09.022

Guevara, M., Tena, C., Porquet, M., Jorba, O., Pérez García-Pando, C., 2019. HERMESv3, a stand-alone multiscale atmospheric emission modelling framework – Part 2: bottom-up module. Geosci. Model Dev. Discuss. 1–51. doi:10.5194/gmd-2019-295

Hafner, S.D., Pacholski, A., Bittman, S., Burchill, W., Bussink, W., Chantigny, M., Carozzi, M., Génermont, S., Häni, C., Hansen, M.N., Huijsmans, J., Hunt, D., Kupper, T., Lanigan, G., Loubet, B., Misselbrook, T., Meisinger, J.J., Neftel, A., Nyord, T., Pedersen, S.V., Sintermann, J., Thompson, R.B., Vermeulen, B., Vestergaard, A.V., Voylokov, P., Williams, J.R., Sommer, S.G., 2018. The ALFAM2 database on ammonia emission from field-applied manure: Description and illustrative analysis. Agric. For. Meteorol. 258, 66–79. doi:10.1016/j.agrformet.2017.11.027

Hristov, A.N., 2011. Technical note: Contribution of ammonia emitted from livestock to atmospheric fine particulate matter (PM2.5) in the United States. J. Dairy Sci. 94, 3130–3136. doi:10.3168/jds.2010-3681

Li, M., Wang, Y., Adeli, A., Yan, H., 2018. Effects of application methods and urea rates on ammonia volatilization, yields and fine root biomass of alfalfa. F. Crop. Res. 218, 115–125. doi:10.1016/j.fcr.2018.01.011

MacKenzie, A.F., Fan, M.X., Cadrin, F., 1998. Nitrous Oxide Emission in Three Years as Affected by Tillage, Corn-Soybean-Alfalfa Rotations, and Nitrogen Fertilization. J. Environ. Qual. 27, 698. doi:10.2134/jeq1998.00472425002700030029x

Mateo-Marín, N., Quílez, D., Guillén, M., Isla, R., 2020. Feasibility of stabilised nitrogen fertilisers decreasing greenhouse gas emissions under optimal management in sprinkler irrigated conditions. Agric. Ecosyst. Environ. 290, 106725. doi:10.1016/j.agee.2019.106725

MAPA, 2020. Encuestas ganaderas. Análisis del número de animales por tipos: Resultados de porcino, año 2018. (accessed 24 April 2020).

Myhre, G., Shindell, D., Bréon, F.M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Stephens, G., Takemura, T., Zhang, H., 2013. Anthropogenic and Natural Radiative Forcing, in: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P.M. (Eds.), Climate Change 2013 - The Physical Science Basis. Cambridge University Press, United Kingdom, pp. 659–740.

Neftel, A., Blatter, A., Schmid, M., Lehmann, B., Tarakanov, S.V., 2000. An experimental determination of the scale length of N2O in the soil of a grassland. J. Geophys. Res. Atmos. 105, 12095–12103. doi:10.1029/2000JD900088

Recio, J., Vallejo, A., Le-Noë, J., Garnier, J., García-Marco, S., Álvarez, J.M., Sanz-Cobena, A., 2018. The effect of nitrification inhibitors on NH3 and N2O emissions in highly N fertilized irrigated Mediterranean cropping systems. Sci. Total Environ. 636, 427–436. doi:10.1016/j.scitotenv.2018.04.294

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. doi:10.1002/jpln.201400251

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. doi:10.1016/j.agee.2016.09.038

Sigurdarson, J.J., Svane, S., Karring, H., 2018. The molecular processes of urea hydrolysis in relation to ammonia emissions from agriculture. Rev. Environ. Sci. Biotechnol. 17, 241–258. doi:10.1007/s11157-018-9466-1

Soil Survey Staff, 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, United States of America, 360 pp.

Ti, C., Xia, L., Chang, S., Yan, X., 2019. Potential for mitigating global agricultural ammonia emission: A meta-analysis. Environ. Pollut. 245, 141–148. doi:10.1016/j.envpol.2018.10.124

UNEP, 2013. Drawing Down N2O to Protect Climate and the Ozone Layer. A UNEP Synthesis Report. Kenya, 57 pp.

Ussiri, D., Lal, R., 2013. Soil Emission of Nitrous Oxide and its Mitigation. Springer, Netherlands, 378 pp.

Vitousek, P.M., Aber, J., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, G.D., 1997. Human alteration of the global nitrogen cycle: causes and consequences issues in ecology. Issues Ecol. 1, 1–17. doi:10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2

Yagüe, M.R., Valdez, A.S., Bosch-Serra, À.D., Ortiz, C., Castellví, F., 2019. A short-term study to compare field strategies for ammonia emission mitigation. J. Environ. Qual. 48, 179–184. doi:10.2134/jeq2018.05.0218

Yoh, M., Toda, H., Kanda, K., Tsuruta, H., 1997. Diffusion analysis of N2O cycling in a fertilized soil. Nutr. Cycl. Agroecosystems 49, 29–33. doi:10.1023/A:1009757829417

Zerulla, W., Barth, T., Dressel, J., Erhardt, K., Horchler von Locquenghien, K., Pasda, G., Rädle, M., Wissemeier, A., 2001a. 3,4-Dimethylpyrazole phosphate (DMPP) – a new nitrification inhibitor for agriculture and horticulture. Biol. Fertil. Soils 34, 79–84. doi:10.1007/s003740100380

Zerulla, W., Pasda, G., Hähndel, R., Wissemeier, A.H., 2001b. The New Nitrification Inhibitor DMPP (ENTEC®) for Use in Agricultural and Horticultural Crops – An Overview. Plant Nutrition. Springer, Netherlands, pp. 754–755.

Chapter 7

Reviewing methodological aspects to estimate nitrous oxide emissions from agrosystems

Part of this chapter published in:

Mateo-Marín, N., R. Isla, and D. Quílez. 2020. A simple methodology to estimate plant volume in nitrous oxide emission studies. J. Environ. Qual. 49, 769-773. doi: 10.1002/jeq2.20077.

Abstract

Differences in methodologies for greenhouse gas (GHG) measurements and emission estimation using closed-chamber techniques can complicate the comparison of studies. Aspects such as the presence or absence of plants inside measurement chambers or the model selected to describe the variation of gas concentration inside chambers might influence emission estimation. This chapter attempt to assess the importance of both effects on nitrous oxide (N_2O) fluxes using data sets from three wheat field trials. Sixteen twin pairs of static closed unvented chambers, one with presence of plants and another with absence, were used to determine the effect of plants on N_2O fluxes. Fifty-one static closed unvented chambers with sampling times at 0-20-40-60 minutes were used to compare N_2O emissions estimated with the non-linear revised Hutchinson/Mosier model (HMR) and the linear model (LR), and the effect of reducing the number of sampling times from four to two. A robust and practical image analysis-based procedure was developed to estimate biomass volume inside the chambers. The volume of chamber displaced by wheat plants was small, with a maximum of 2.2% of the chamber volume at anthesis. N_2O emissions were 35% lower in chambers with plants in comparison to chambers where plants were cut, and this was accompanied by lower soil mineral nitrogen concentration (37%) and soil temperature (1 ºC at 5-cm depth) under plant presence. Although gas exchange gradient did not decrease with time inside the chambers, linear regression N_2O estimates were 18% lower than HMR estimates due to erroneous behaviour of the HMR model at low fluxes. The use of the HMR package seems not to be justified under our experimental conditions. In similar conditions to that of these experiments, sampling times could be reduced from four to two times (0 and 60 minutes) without losing accuracy (<0.3%) in N_2O emission estimation.

1. Introduction

Due to climate change concerns, the number of scientific publications related to greenhouse gas (GHG) emissions from agricultural systems has increased exponentially in recent years (Parkin et al., 2012). Although a variety of techniques are available for GHG measurement (Holland et al., 1999) and several recent reviews have made methodological recommendations (Global Reserch Alliance on Agricultural Greenhouse Gases, 2015; Olfs et al., 2018; Pavelka et al., 2018), there is no standard methodology for flux measurements. The estimation of surface-atmosphere GHG exchange is associated with several sources of error that may lead to inaccuracies in instantaneous and cumulative emissions (Livingston et al., 1995) that can compromise their reliability (Rochette and Eriksen-Hamel, 2008).

Most flux measurement studies utilise chamber-based techniques whereby gas samples are collected and subjected to infrared or gas chromatograph analysis (Eugster and Merbold, 2015). Static chambers are the most commonly used method for being relatively inexpensive, portable, compact, and easy to replicate and to operate *in situ* (Collier et al., 2016; Tallec et al., 2019). When using static chambers, special attention should be given to chamber and sampling design, deployment conditions and times, and flux calculation methodologies (Livingston et al., 1995) to avoid or minimise systematic errors. Two aspects that are not frequently considered during the estimation of nitrous oxide (N_2O) fluxes are (1) the direct and indirect effects of plants inside the chamber and (2) the choice of the most suitable model to describe the observed gas variation inside the chambers. Regarding the first issue, trimming or folding plants to facilitate lid closure are common strategies (Collier et al., 2016). However, Bruhn et al. (2014) reported that leaf surfaces of several plant species made a noticeable contribution to N_2O release. Besides, cutting plants can modify the soil moisture, temperature and nitrate concentration that are variables that affect N_2O emissions (Ussiri and Lal, 2013). Therefore, the presence of undisturbed plants during chamber measurements seems important for a correct N_2O emission estimation. The inclusion of plants inside static chambers has the drawback that, as plants grow, it is often necessary to increase chamber height with a concomitant reduction in the GHG detection sensitivity (Collier et al., 2016). Another objection is that the presence of plants may endanger the achievement of gas homogeneity within the headspace (Clough, 2015), hindering the gas sampling. Further, plant volume inside the chamber is rarely, if ever, measured and discounted from chamber headspace in the GHG flux calculation (Morton and Heinemeyer, 2018), even though plant volume reduces the effective chamber headspace and leads to inaccurate flux estimation (Livingston et al., 1995). As a consequence of disregarding plant volume, an overestimation of the fluxes is expected (Morton and Heinemeyer, 2018).

Concerning the second issue, the variation of gas concentration within static chambers with time has been described by both linear and non-linear models (Livingston et al., 1995). The linear model is appropriate when the net rate of gas exchange is constant over the measurement period; otherwise, a non-linear model of concentration change over time should be employed (Livingston et al., 1995). Hutchinson and Mosier (1981) formulated an exponential equation (HM model) to quantify emissions when the gas exchange gradient decreases with time as the gas accumulates in the chamber. Based on the HM model, Pedersen et al. (2010) developed a procedure (HMR) for soil-atmosphere trace-gas flux estimation with static chambers. The HMR procedure requires a minimum of four measurement times per chamber and recommends the non-linear exponential model if it can be fitted to the data, and in cases where the parameter estimation fails, for example when the concentration change does not decline over time, linear regression or no flux is recommended (Pedersen et al., 2010). This procedure was implemented as a free add-on package into the RStudio software.

The static chamber methodology is labour-demanding owing to the necessity of personnel to manage the chambers, draw air samples at different times, and perform gas concentration analyses (Tallec et al., 2019). Thus, to reduce experimental costs, in many studies (Abalos et al., 2017; Franco-Luesma et al., 2019; Guardia et al., 2018; Maris et al., 2016; Recio et al., 2018) fluxes are estimated with two or at most three sampling times and, as a consequence, using the linear regression approach.

In this context, the objectives of this study are to assess in a winter cereal crop i) how N_2O emissions are affected by the presence or absence of plants inside the collars of static chambers; ii) a new image analysis-based procedure proposed to estimate plant volume inside closed chambers; iii) the differences in N_2O flux estimates by linear regression (LR) and HMR procedure; and iv) the magnitude of the error in flux estimation if sampling times are reduced from four to two to diminish labour and gas determination costs.

2. Materials and methods

2.1. Site and experimental design

The study was performed with data gathered in three field trials cropped to irrigated wheat (*Triticum aestivum* L. cv. 'Rimbaud' in trial 1 and 2, cv. 'Guadalupe' in trial 3) in semiarid Mediterranean-continental climate in Spain. Trials 1 and 2 were located in Montañana (mean annual air temperature (Tm) of 14.6 ºC; mean annual precipitation (Pm) of 319 mm; mean annual reference evapotranspiration (ETo) of 1,239 mm; period 2003-2018) and trial 3 in Almudévar (Tm=13.7 ºC; Pm=374 mm; ETo=1,271 mm; period 2006-2018). The effect of presence or absence of plants on the estimation of N_2O fluxes from the system was evaluated in trial 1. Data collected in trials 2 and 3 were used to compare N_2O fluxes estimated by the HMR and LR models, and to assess the potential error in flux estimates due to the reduction in the number of sampling times per chamber from four to two.

Trial 1

Trial 1 was located in a 1.2-ha field of the experimental farm of CITA in a deep (>1.2 m) silty-loam textured Typic Xerofluvent soil (Soil Survey Staff, 2014) during two crop seasons (2016/17 and 2017/18). The experiment had a complete randomised block design with four replicates and four treatments designed to compare the effect of three additives mixed with pig slurry: a urease inhibitor, a nitrification inhibitor, and a compound designed to improve the soil microbial activity. In each of the 16 experimental plots, the collars of two static unvented chambers (more details below) were installed at 0.2-m distance (twin chambers); in one of the collars, plants were cut periodically to the ground level whereas in the other plants were allowed to grow without disturbance inside the collar. Wheat plants surrounding chambers were allowed to grow normally. Air inside the chambers was sampled 29 times between 07/04/2016 and 04/07/2018 (Table 1) at two times (0 and 60 minutes) after chamber closure.

Trial 2

Trial 2 was located in the same field as trial 1 (2015/16 and 2016/17). The experiment had a split-plot design with 16 treatments and four replicates that compared mineral and pig slurry fertilisation. One static unvented chamber was installed in each of the four replicates of four selected treatments. The treatments included a non-N fertilised control and three treatments with, respectively, urea, pig slurry, and pig slurry with a urease inhibitor at the theoretical optimum N rate (Table 1). Air samples were drawn from the chambers on 40 sampling dates in the period from 17/02/2016 to 27/06/2017 at four times (0, 20, 40, and 60 minutes) after chamber closure.

Trial 3

Trial 3 was located in a 5.8-ha field at Almudévar. The soil is a Typic Xerofluvent (Soil Survey Staff, 2014) with a depth >0.9 m and with a silty-clay-loam texture. Twenty static unvented chambers were installed at different locations in the plot to obtain information about the spatial variability of N_2O fluxes. Chambers were sampled from 21/01/2015 to 23/06/2015 (Table 1) at four times (0, 20, 40, and 60 minutes) after chamber closure.

The three trials were sprinkler-irrigated satisfying crop necessities according to the reference evapotranspiration (Penman-Monteith equation) and the Kc crop coefficients proposed by FAO (Allen et al., 1998). The phosphorus and potassium were managed to avoid growth limitations, and weeds and pests were controlled by local management practices.

Table 1. General experimental characteristics and key dates in the three trials.

	Trial 1	Trial 2	Trial 3
Site	Montañana 41° 43' N, 0° 49' W 221 masl	Montañana 41° 43' N, 0° 49' W 221 masl	Almudévar 42° 1' N, 0° 35' W 389 masl
Sowing date	30/12/2016 16/11/2017	26/11/2015 30/12/2016	26/12/2014
Harvest date	04/07/2017 06/07/2018	07/07/2016 04/07/2017	02/07/2015
N fertilisation dates and rates	07/04/2017 & 19/03/2018: 120 kg N ha^{-1} (pig slurry)	24/02/2016 & 21/03/2017: 120 kg N ha^{-1} (pig slurry) 01/04/2016 & 18/04/2017: 30 kg N ha^{-1} (NH_4NO_3)	18/12/2014: 30 kg N ha^{-1} (NH_4-N) 09/03/2015: 92 kg N ha^{-1} (Urea)
Chambers	32	16	20
Sampling dates (period)	12 (Apr. 2017 - Jun. 2017) 17 (Mar. 2018 - Jul. 2018)	18 (Feb. 2016 - Oct. 2016) 22 (Jan. 2017 - Jun. 2017)	17 (Jan. 2015 - Jun. 2015)

2.2. Estimation of plant volume

A novel non-destructive procedure is proposed in this work to estimate the volume displaced by the plants inside the chambers. The approach is based on the relationship between canopy image area (derived from zenithal images) and plant volume. Wheat plants located inside the collars (experimental plots of trial 1) were described periodically according to their phenological stage (Zadoks et al., 1974) and photographed. At the same time, in an area adjacent to the experimental plots, a secondary chamber collar was established to photograph wheat plants encompassed by it at the same phenological stage. All plants inside this secondary collar (0.071 m^2) were cut, frozen (-30 ºC), and placed into a glass test tube to determine their volume by water displacement. Three differently sized test tubes (500 mL, 1,000 mL, and 2,000 mL) were used throughout the study, with sequentially larger tubes used as plant volumes expanded due to growth. Between two and six measurements were used at each phenological stage to determine canopy image area and plant volume.

Zenithal photographs were managed according to the orthoimage technique for canopy image analysis described by Lordan et al. (2015) to obtain the area projected by the canopy. Photographs (2.3×10^3 pixels cm^{-2}) were taken with a compact camera (Canon PowerShot SX210 IS) at 1.20-m height over the soil surface. Plants outside the collar were covered (hidden) by a piece of cardboard to isolate all the canopy area projected outside the vertical projection of the collar. A ruler was added on the piece of cardboard to scale the image. The photographed green area was isolated (Photoshop CS5; Adobe Systems, San Jose, CA) and processed (ImageJ, National Institutes of Health, Bethesda, MD) to select all the wheat canopy pixels, obtaining the canopy image area (Fig. 1), which was corrected by the image scale. The relation between plant volume and canopy image area (in the secondary collars) was established using a linear regression model that pooled data from all phenological stages. Then, the volume of the plants within each collar located in the experimental plots was estimated from their canopy image area by using the linear model and solving for plant volume.

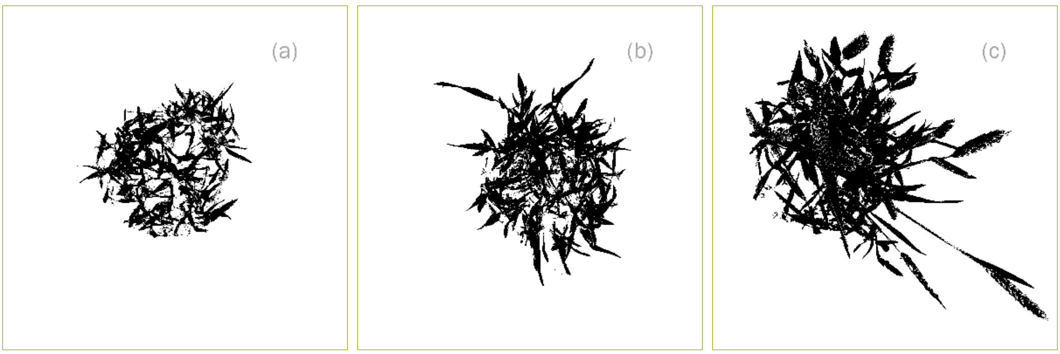


Figure 1. Isolation and selection of green area corresponding to wheat located within a chamber at Zadoks scale stage 32 (2nd node detectable, Fig. 1a), 45 (Boots swollen, Fig. 1b), and 65 (Anthesis half-way, Fig. 1c).

2.3. Soil parameters

Soil mineral nitrogen (Nmin), soil water-filled pore space (WFPS), and soil temperature were measured in trial 1 during season 2017/18 to assess the influence of presence or absence of plants on these variables that affect N_2O emissions. Two areas were delimited within each plot, in one of them, plants were removed in several circular surfaces identical to that of the chamber collar; whereas in the other area, plants let them grew. One in every two GHG samplings, three soil cores (2.5-cm diameter) were taken at 0 to 0.1-m depth in each delimited area. The composite fresh soil sample was sieved through a 3-mm mesh and a subsample of 10 g fresh soil was extracted with 30 mL of 2 N KCl, shaken for 30 min, and filtered through cellulose filters. The nitrate (NO_3^-) and ammonium (NH_4^+) concentration in the extracts were analysed by colourimetry with a segmented flow analyser (AutoAnalyser 3, Bran+Luebbe, Germany).

At the same time as GHG samplings, soil temperature (TME MM2000, West Sussex, UK) and soil moisture content (HH2 Delta-T with ML2 and ML3 probes, London, UK) were measured at 5-cm depth in the delimited areas with plants presence and absence. Delta-T probes were previously calibrated to determine gravimetric soil water content for this specific soil (R^2=0.79***; n=320). Soil WFPS was calculated according to Linn and Doran (1984) as the quotient between the volumetric soil moisture content and the total soil porosity. Total soil porosity was estimated considering a particle density of 2.65 Mg m^{-3} and the soil bulk density from 0 to 5 cm, determined *in situ* with the cylinder method (Grossman and Reinsch, 2002) as 1.39 Mg m^{-3}.

2.4. Greenhouse gas emissions

The closed-chamber technique was used in the three trials to measure N_2O fluxes. The chambers were similar to those static closed unvented chambers described by Holland et al. (1999). They were constructed with polyvinyl chloride water pipes of 0.30-m of inner diameter and consisted of two parts: a collar of 0.12-m height inserted into the soil 0.10 m, and an upper part of 0.165-m height (0.177-m in trial 3) closed by a polyvinyl chloride tap and wrapped by reflective insulation film to reduce heating of the air inside the chambers during the closure time. Thus, the chamber volume was 13.1 L for trials 1 and 2, and 13.9 L for trial 3. In trial 1, the height of the upper cover did not change during the course of the study; plants were folded when necessary to facilitate chamber closure. This strategy did not affect plants' growth because of their flexibility, although some stems were damaged on the last sampling date just before harvest. In trials 2 and 3, plants inside the chambers were cut and therefore only fluxes from the soil were measured. Gas samples were taken starting roughly at the hour when the air temperature equals the mean temperature of the day (between 9:30h and 11:00h GMT) (Alves et al., 2012). At each sampling time, 15-mL air samples were injected into 12-mL Exetainer borosilicate glass vials (model 038W, Labco Ltd., UK). To capture the N_2O peaks, the frequency of GHG samplings was higher in the 30 days following fertilisation and then the time interval between measurements was increased. The number of samplings and the sampling period depended on the trial and season (Table 1). An Agilent 7890B gas chromatograph with electron-capture (ECD) and flame-ionisation detector (FID) was used to analyse air samples by gas chromatography. The procedure was the same used by Franco-Luesma et al. (2019). In short, an HP-Plot Q column (length of 15 m, section of 320 μm, and thickness of 20 μm) was used with helium as a carrier gas at 25 mL min^{-1} and a 5% methane in argon gas mixture at 30 mL min^{-1} was used as a make-up gas for the ECD. The FID, the ECD, and the methaniser were set to 250, 280, and 375 ºC, respectively. The injector was set to 50 ºC, whereas the oven to 35 ºC. The obtained detection limit of N_2O was 0.05 ppm (v:v).

Gas concentration in the chambers was corrected for the air temperature. The N_2O flux calculation considered the ratio between the chamber volume and the soil area (MacKenzie et al., 1998).

In trial 1, the air was sampled at the same time in chambers with presence and absence of plants. The N_2O fluxes were estimated as the difference in N_2O concentration divided by the time interval between the two sampling times (linear model).

In trials 2 and 3, two approaches were considered to estimate the N_2O flux using the four sampling times (0, 20, 40, and 60 minutes): i) linear regression, and ii) HMR procedure (Pedersen et al., 2010). The HMR procedure was carried out using the HMR package (version 1.0.0) of the RStudio software. The HMR package fits an exponential model if possible, but when there is a lack of fitting, the HMR package recommends 'Linear regression' or 'No flux'

(Pedersen et al., 2010). When the HMR package was used, the adopted criterion was the default recommendation, except when the recommendation was 'No flux' in which case LR was chosen (HMR-N). A visual inspection of the results was conducted to verify the selection.

The N_2O fluxes estimated using HMR-N were compared to those estimated by LR, using in both cases the information of the four sampling times (0, 20, 40, and 60 minutes). For the evaluation of errors in the reduction of sampling times, the N_2O fluxes estimated using three timespans (0-20 minutes, 0-40 minutes, and 0-60 minutes) were compared to those estimated by LR at 0-20-40-60 minutes.

An observation with extremely large cumulative emission (21 kg N ha^{-1}, trial 3) was not included in the two above-cited analyses.

2.5. Statistical analysis

Normal distribution was verified by the Shapiro-Wilk test for data series of the three trials. In most cases, distribution was not normal and, thus, the non-parametric Wilcoxon signed-rank test was used to compare paired samples of these data series. Previously to perform the paired test and when several trials and/or seasons were considered in the analysis, a comparison of regression lines (presence vs. absence of plants, LR vs. HMR-N) was conducted. If no differences between regression lines were found, data from different trials and/or seasons were pooled before the statistical analysis. The default confidence level considered was 95%. All the statistical analysis were performed with SAS® University Edition.

3. Results

3.1. Methodology for estimation of plant volume inside chambers

Wheat plant volume can be precisely estimated through canopy image analysis since a strong relation (R^2=0.96, p<0.001, RMSE=18.2 mL) was found between the two variables (Fig. 2a). The measured volume of the plants located inside the collar ranged from 0.6% to 2.2% of the chamber volume (CV from 1 to 11%), depending on the phenological stage. The maximum plant volume (2.2%) was measured at anthesis (stage 65 according to the Zadoks scale; Fig. 2b).

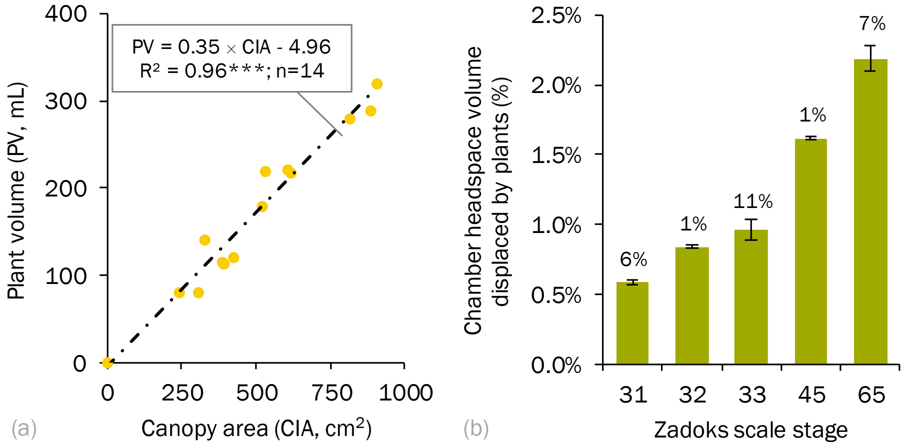


Figure 2. Relationship between wheat canopy image area (cm²) and plant volume (PV, mL) at five phenological stages (Fig. a). Mean volume (%) of the chamber displaced by wheat plants at different growth stages* (Fig. b). Vertical lines show the standard error and numbers above the bars indicate the coefficient of variation.

*Zadoks scale stage: 31- 1st node detectable, 32- 2nd node detectable, 33- 3rd node detectable, 45- Boots swollen, and 65- Anthesis half-way.

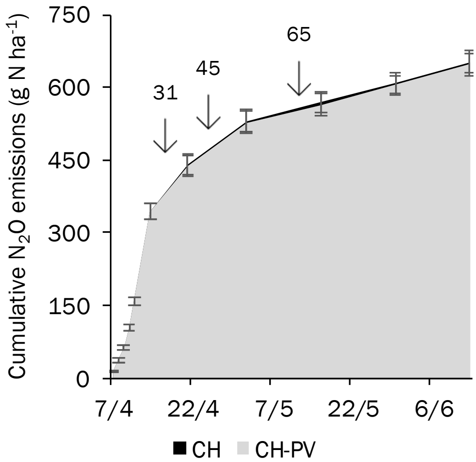


Figure 3. Cumulative N_2O emissions with time (g N ha^{-1}) whether plant volume was not discounted from the chamber headspace (CH) and whether plant volume was discounted (CH–PV) for the calculation of the emissions. Arrows indicate the Zadoks scale stage (31- 1st node detectable, 45- Boots swollen, and 65- Anthesis half-way) at three moments. Vertical lines show the standard error.

When the N_2O emissions (Fig. 3) were calculated by adjusting for the proportion of the chamber displaced by wheat plants (thereby changing the chamber headspace volume), the cumulative N_2O emissions (season 2016/17) were 0.9% lower (646.7 g N ha^{-1} vs. 652.5 g N ha^{-1}; mean difference 5.8 ± 0.5 g N ha^{-1}) than when plant volume was disregarded from the calculations.

3.2. Effect of presence or absence of plants inside chambers

Average N_2O fluxes were 17% ($p<0.01$) and 44% ($p<0.001$) lower in chambers with presence of plants than in chamber with absence of plants for seasons 2016/17 and 2017/18, respectively (Fig. 4a). The lower N_2O fluxes measured with presence of plants inside the chambers lead to significant 35% lower cumulative N_2O emissions compared with those estimated in chambers with absence of plants (Fig. 4b). The volume of plants was not considered in the calculation of fluxes in chambers with presence of plants since the volume of plants inside the chambers was not estimated during the second season.

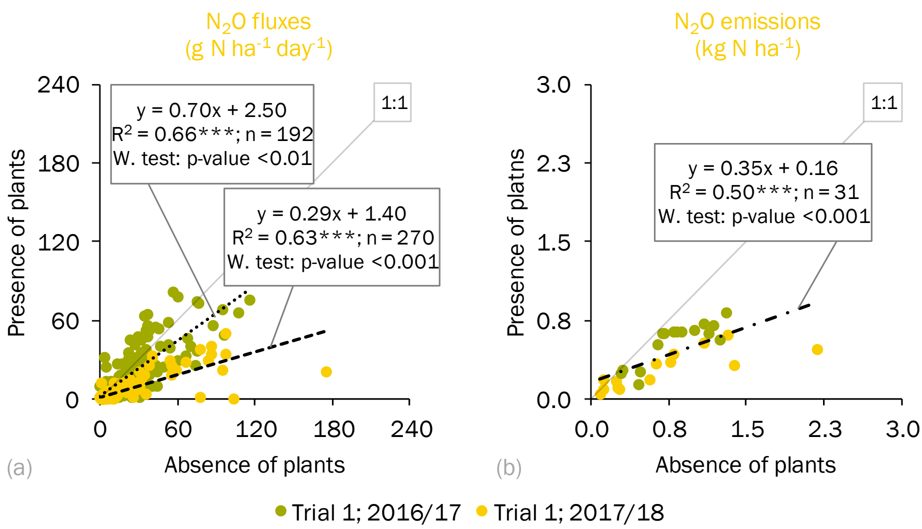


Figure 4. Relationship between N_2O fluxes (Fig. a) and N_2O emissions (Fig. b) obtained in chambers with presence and absence of plants in two different years. The p-values correspond to the comparison of the Wilcoxon signed-rank test.

Soil mineral N content (0-0.1-m depth) was significantly higher in absence of plants than with presence of plants (Fig. 5). Nitrate concentration showed significant differences on six of the ten sampling dates (data not shown) and it was, on average across all samplings, 86% higher ($p<0.001$) in areas with absence of plants than in areas with presence of plants (Fig. 5a). The differences in soil ammonium concentration were less frequent than for nitrate concentration, since in only one of the ten sampling dates the difference was significant (data not shown). Averaging over all sampling dates, ammonium content in areas with absence of plants was 47% higher ($p<0.01$) than in areas with presence of plants (Fig. 5b).

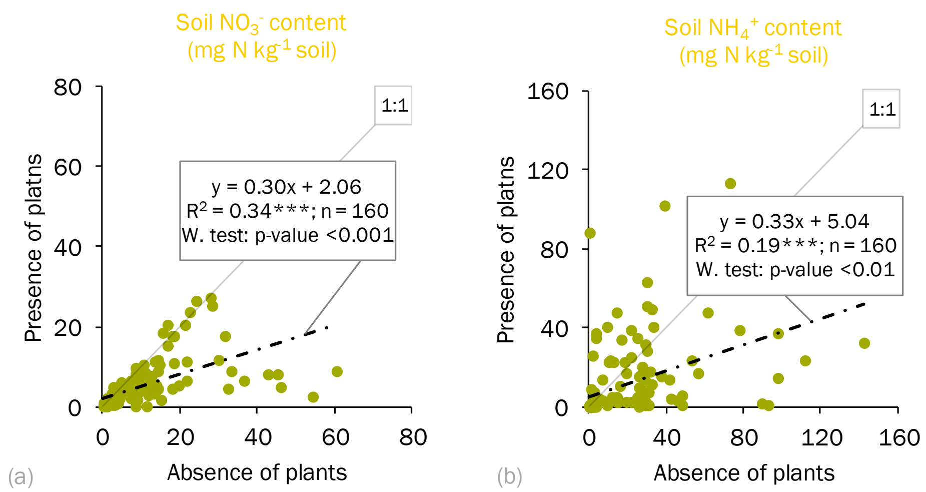


Figure 5. Relationship between soil nitrate (Fig. a) and soil ammonium (Fig. b) concentrations in areas with presence and absence of plants.

Average soil temperature at 0.05-m depth was 22.3 °C in absence of plants and 21.3 °C in presence; thus, topsoil areas with absence of plants were 1 °C warmer than areas with plants ($p<0.001$). Differences were significant in three of the five sampling dates (Fig. 6a).

Averaging all sampling dates, small but significant differences in soil WFPS between areas with presence (55.0%) and absence (53.8%) of plants were observed. Thus, on average the WFPS was 2% higher ($p<0.05$) in areas with presence of plants than in areas with absence of them, even though significant difference were only detected on the first sampling date (Fig. 6b).

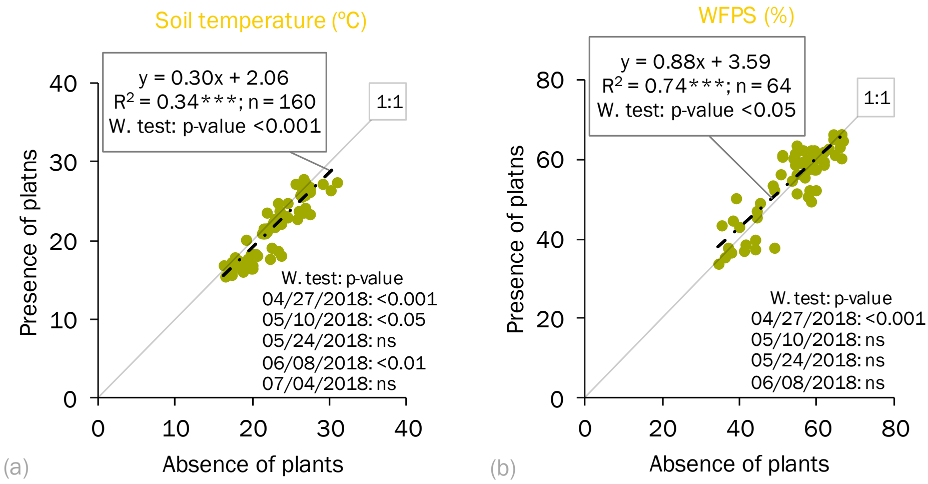


Figure 6. Relationship between soil temperature (Fig. a) and soil water-filled pore space (Fig. b) in areas with presence and absence of plants. Dates indicate the analysis separately by sampling dates.

3.3. Linear vs. HMR model for N_2O flux estimation

The HMR package recommended the exponential model in 51% (trial 2, season 2015/16), 44% (trial 2, season 2016/17), and 34% (trial 3) of the flux calculations. The linear regression was recommended in 34% (trial 2, season 2015/16), 51% (trial 2, season 2016/17), and 50% (trial 3) of the total number of fluxes, and the 'No flux' option in 15% (trial 2, season 2015/16), 6% (trial 2, season 2016/17), and 17% (trial 3) of the total cases.

Considering all the observations, N_2O flux estimates were 15% lower ($p<0.001$) using LR than HMR-N (Fig. 7a). Differences were consistent across the three trials (between 11% and 24%), although the largest differences were observed at the trial that had lowest fluxes (trial 2, season 2015/16). Average cumulative N_2O emissions (Fig. 7b) were 18% lower ($p<0.001$) when using LR compared to HMR-N.

At low N_2O fluxes (<15.4 g N ha^{-1} day^{-1}, 75% of the database) and for the HMR-N procedure, the exponential model was selected in 36% of the cases, whereas 64% of them were obtained using LR. Differences between LR and HMR-N estimations at low N_2O fluxes (Fig. 8) influenced cumulative N_2O emissions.

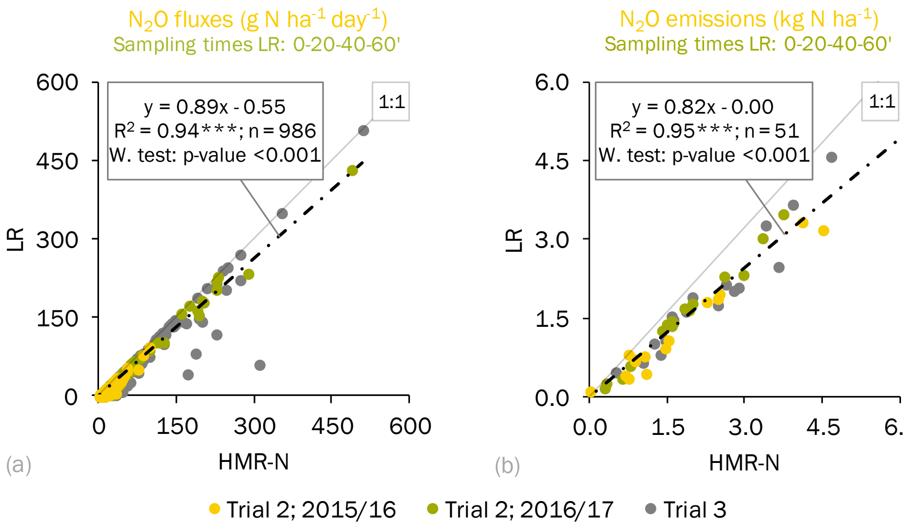


Figure 7. Relationship between N_2O fluxes (g N ha^{-1} day^{-1}; Fig. a) and N_2O emissions (kg N ha^{-1}; Fig. b) obtained by the LR model and the HMR-N procedure for several trials and seasons.

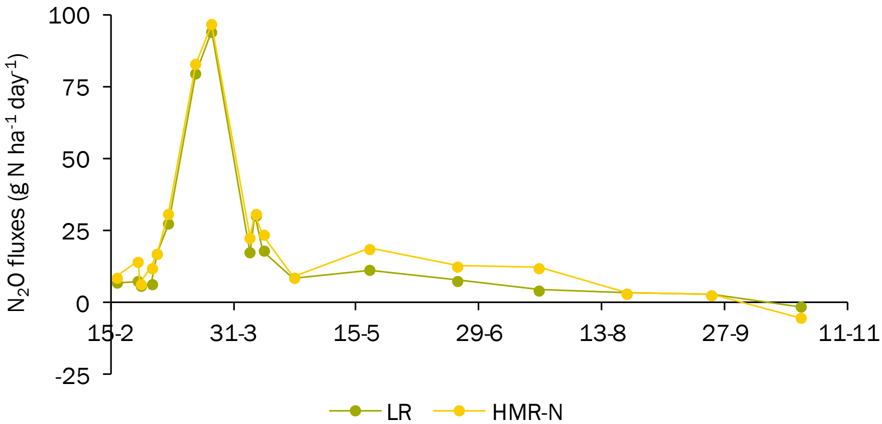


Figure 8. Temporal changes of N_2O fluxes (g N ha^{-1} day^{-1}) estimated with HMR-N and LR procedures for a chamber with mineral fertilisation in season 2015/16.

3.4. Effect of reducing the number of sampling times

There was a strong relation (Fig. 9, left) between N_2O fluxes estimated with four sampling times (LR for 0-20-40-60 min) and with two sampling times (0-20 min, 0-40 min, and 0-60 min). The Wilcoxon signed-rank test detected significant differences between calculations with 40- and

60-min closure times and with four sampling times; those were 1.4% and 0.1% higher, respectively, than four-point estimates.

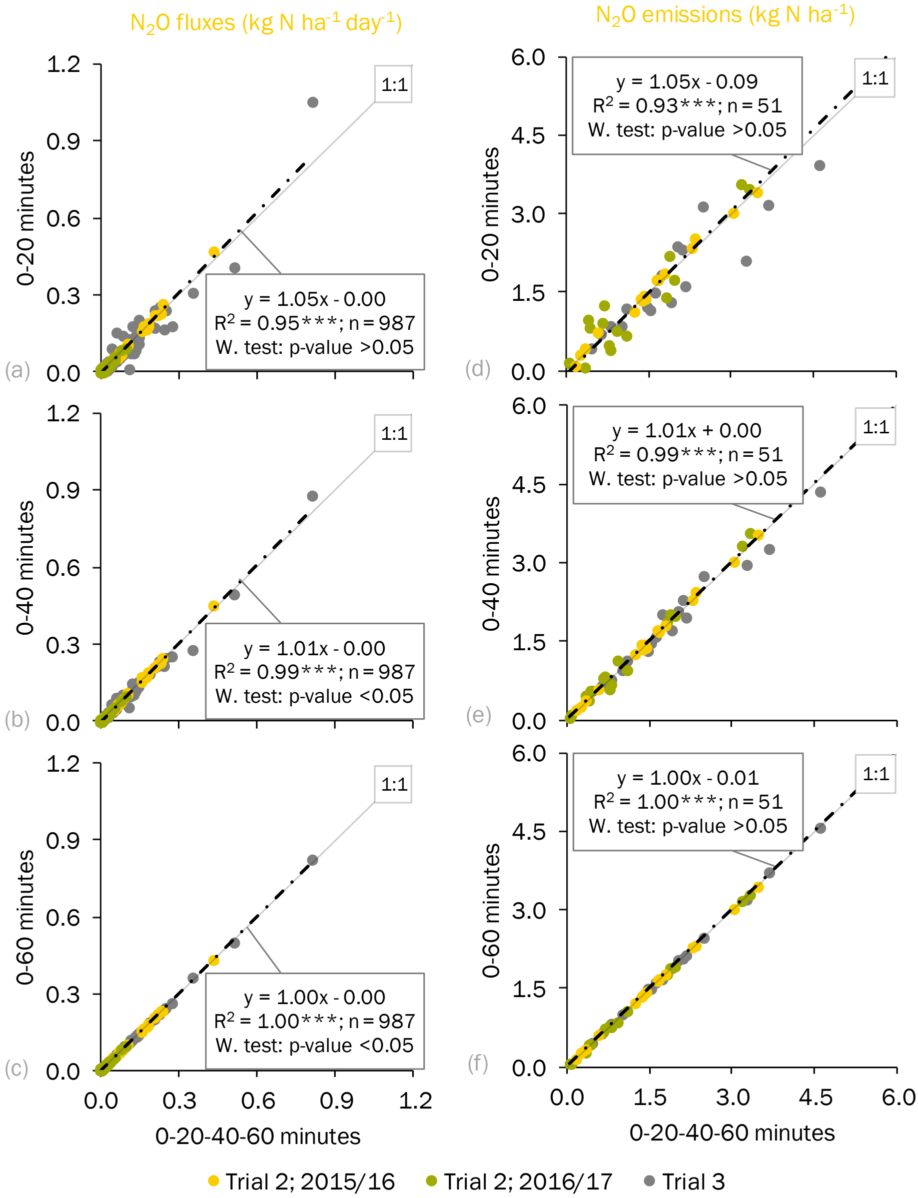


Figure 9. N_2O fluxes (kg N ha^{-1} day^{-1}, left) and N_2O emissions (kg N ha^{-1}, right) obtained using four (0-20-40-60 min) and two sampling times (Fig. a and Fig. d: 0-20 min; Fig. b and Fig. e: 0-40 min; Fig. c and f: 0-60 min) for several trials and seasons.

It was observed a greater number of negative fluxes for estimation at the shortest (20-min) time interval; negative fluxes were reduced 38% and 50% for 40-min and 60-min intervals, respectively (Table 2). This effect could be associated with low gas concentration inside the chamber, close to the analytical quantification limit (0.15 ppm_v).

It was not observed smaller average fluxes for longer closure time (Table 2). It neither was observed (Fig. 10) for high N_2O fluxes (>200 g N $ha^{-1}day^{-1}$; p>0.05 Wilcoxon signed-rank test), except for the highest flux since the gas exchange gradient decreased with time (0-20 min: 1,053.2 g ha^{-1} day^{-1}; 0-40 min: 880.4 g ha^{-1} day^{-1}; 0-60 min: 822.8 g ha^{-1} day^{-1}). Thus, for N_2O fluxes lower than this data point (<500 g N ha^{-1} day^{-1}), a decrease in the gas exchange gradient as the gas accumulated inside the chambers was not observed.

Table 2. Statistical characteristic of N_2O flux estimates using different closure times.

	0-20 min	0-40 min	0-60 min
N_2O fluxes (g ha^{-1} day^{-1})			
Mean	18.1	18.2	18.9
Minimum	-10.5	-5.6	-3.2
Maximum	1,053.2	880.4	822.8
Number of negative fluxes	164	102	83

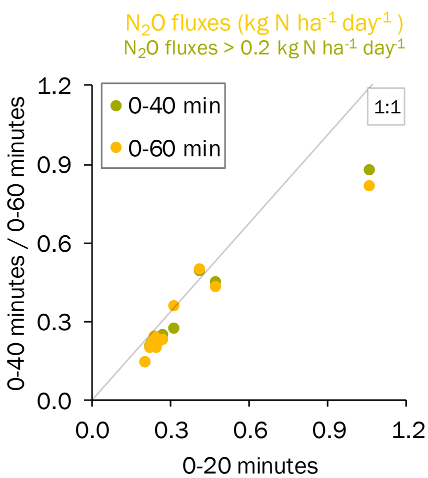


Figure 10. Relationship between fluxes estimated for 20 minutes closure chamber and those estimated for 40 and 60 minutes closure chamber when N_2O fluxes were higher than 200 g N ha^{-1} day^{-1}.

For cumulative N_2O emissions (Fig. 9, right) a strong relationship was also observed between estimates with four and two sampling points. In this case, the Wilcoxon signed-rank test did not detect significant differences between four and two sampling times, independently of the closure time.

4. Discussion

4.1. Effect of presence or absence of plants inside chambers

Higher N_2O fluxes were estimated with absence of plants than with presence of plants inside the chambers. The opposite result might be expected according to the different effects observed in other studies. Bruhn et al. (2014) found that the effect of natural sunlight (ultraviolet radiation) on a grassland ecosystem increased N_2O emissions by 30% compared to plants located in darkened chambers. In this regard, with the use of opaque chambers in this study, the overall effect of ultraviolet radiation on plant N_2O fluxes could not have been fully elucidated. Other studies conducted in soybean and maize (Chen et al., 2002), canola and barley (Chang et al., 1998), and wheat (Baruah et al., 2012) have observed higher N_2O fluxes through the canopy because of the conveyance of N_2O produced in the soil. However, the complete interruption of N_2O transmission through the stem after cutting plants cannot be claimed in this study. The observed differences in N_2O fluxes between chambers with presence and absence of plants could be mainly attributed to the detected dissimilarities in soil nitrate and ammonium concentrations and soil temperature. Soil mineral nitrogen content has a high positive correlation to nitrification and denitrification rates (Ussiri and Lal, 2013) and it is probably the main factor driving N_2O emissions. Thus, the 60% higher cumulative N_2O emissions observed in chambers with plants absence could be, at least in part, explained by 86% higher nitrate concentration and 47% higher ammonium concentration.

Soil temperature affects N_2O emissions from the soil and some studies have revealed a positive relation according to a non-linear model (Schaufler et al., 2010). The higher soil temperature, the greater N_2O emissions, at least up to 40 °C (Castaldi, 2000). The small differences observed in this study between areas with plant presence (21.3 ºC) and plant absence (22.3 ºC), probably associated with the higher soil shading by plant presence, could have also contributed to higher N_2O fluxes in absence of plants since the maximum temperature measured in these areas did not reach the above-cited threshold of 40ºC.

The observed lower soil WFPS in areas with absence of plants compared to areas with plants might be explained by an increase in direct soil evaporation due to the higher solar radiation that reaches the soil surface. The opposite effect, areas with plants having lower WFPS than areas with absence of plants was observed by López-Fernández et al. (2007) in maize crop at the 0-10-cm depth; however, differences were attributed to water consumption by the crop. It is well-known the increase of N_2O fluxes when the soil WFPS increases in the range of 40 to 68% (Dalal et al., 2003). That was the WFPS interval that encompassed the 87% of the soil samples in this study. Nevertheless, this positive effect on N_2O fluxes seemed to be counterbalanced by the opposite effects of soil mineral nitrogen content and soil temperature.

Another important consideration would be the effect of cutting plants on soil microbiota since N losses by nitrification and denitrification reactions are catalysed by soil microorganisms (predominantly bacteria and archaea) (Coskun et al., 2017). The microenvironment created by plant roots and its microbial associations at the soil-root interface, characterised by distinct physical, chemical and biological conditions (Koo et al., 2005) would have been modified. However, microbial abundance was not measured and differences of N_2O emissions cannot be empirically attributed to this cause.

4.2. Methodology for estimation of plant volume inside chambers

The image analysis proposed here is a viable methodology to adjust for changes in headspace volume due to plant growth inside chambers, as there was a high correlation between the estimated canopy image area and the measured volume of plants, and a small error in plant volume estimation. This image-based method fulfils the premises of Morton and Heinemeyer (2018) regarding the necessity of a simple, effective, and non-destructive method for assessing plant volume in chamber-based techniques for GHG measurements. In addition, it is a more objective methodology than the visual assessment of two observers proposed by Morton and

Heinemeyer (2018). It is advisable to establish a relationship between plant volume and canopy image area for each experiment, even for crops similar to the one in this study, because differences in plant architecture are expected among cultivars with different growth habits. The determination of plant volumes by the water displacement method using test tubes could present challenges when whole plants do not fit into test tubes, but it could be solved by breaking up the plants prior to freezing.

According to the results, cumulative N_2O emissions were slightly overestimated when disregarding plant volume in the calculations, which was a negligible but systematic error. The smaller contribution of plant volume to differences in cumulative N_2O emissions (0.9%) compared with the volume of chamber displaced by plants (0.6-2.2%) was a result of plant volume being low when emissions were at their greatest. Similar results were observed by Collier et al. (2016), who detected small but significant effects on calculated fluxes after adjusting for 1.4-2.2% the within-chamber alfalfa volume (variation of 0.7-1.7% in the flux rate). Disregarding plant volume may be more relevant for long-term experiments and for emission factor estimation since plant volume is lower in unfertilised than in fertilised plots. Therefore, in agreement with Collier et al. (2016), estimating plant volumes whenever possible is recommended. Nonetheless, researchers' objectives (e.g., to obtain emission factors, compare different treatments, quantify absolute emission values) will dictate the relevance of considering the plant volume into the calculations.

4.3. Linear vs. HMR model for N_2O flux estimation and effect of reducing the number of sampling times

The HMR procedure is useful to correct the underestimations of fluxes caused by long enclosure time (Kandel et al., 2016). This study has not detected that 60 minutes can be considered a long closure time in the conditions of the experiments since for fluxes below 500 g N ha^{-1} day^{-1} (99.9% of the database), the gas exchange gradient did not decrease with time. A reduction in the closure time would be justified whether the number of sampling times is reduced to two when expecting high N_2O fluxes (e.g., after nitrogen fertilisation events).

At low fluxes, non-linear estimates were significantly higher than linear estimates even though gas saturation inside the chamber was not expected. A possible explanation is that the HMR package recommended the non-linear model for small fluxes because of the uncertainty in gas concentration determination at low concentrations, i.e., at a concentration close to the quantification limit of the equipment. Pedersen et al. (2010) also stated that 'data with a small signal-to-noise ratio can pose a dilemma because the different models may estimate dramatically different fluxes'. Despite the likely erroneous recommendation, the most noticeable outliers (Fig. 7a), found at medium-high N_2O fluxes, did not mean an extreme difference in the cumulative emission.

Based on both above-cited premises, the no saturation of chambers and the erroneous election of the HMR model at low fluxes, the use of the HMR package seems not to be justified under our experimental conditions.

Sampling times can be reduced from four to two sampling times without losing much accuracy. For N_2O fluxes below 500 g N ha^{-1} day^{-1} and a chamber with ratio area:volume of 5.1-5.4, a time interval of 60 minutes is recommended with an associated error due to the reduction in the number of sampling times less than 0.30%. The sampling at times 0 and 20 minutes is not recommended in similar experimental conditions for the risk associated with high uncertainty in gas concentration determination at sampling dates with low fluxes, habitual in Mediterranean conditions.

5. Conclusions

The image-based method proposed to estimate plant volume was a reliable, simple, and non-destructive technique to provide canopy volume estimates. Nevertheless, the small plant volume observed for a wheat crop compared to the chamber volume implies a small although systematic effect on cumulative N_2O emissions.

The removal of plants inside the chambers can lead to a large overestimation (35%) of cumulative N_2O emissions in a wheat crop due to differences in soil mineral nitrogen concentrations and soil temperature.

The use of the HMR model was not justified in the conditions of the experiments because of the non-observed saturation of the chambers in 60 minutes with fluxes below 500 g N ha^{-1} day^{-1} and the recommendation of the non-linear model at low fluxes using the HMR package. The LR approach with two sampling times could be a reasonable solution but the election of closure times should be made considering the expected N_2O fluxes depending on the crop and environmental conditions. For fluxes below 500 g N ha^{-1} day^{-1} and a chamber with ratio area:volume of 5.1-5.4, a 60 minutes interval will provide accurate estimates.

To facilitate the comparison among different studies, it is advisable to report properly about measurement conditions, knowing the effect of plant volume on GHG emissions, comprehending flux trends to select the best regression, and sampling and selecting enclosure times based on the economic and labour possibilities.

References

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. 238, 36–45. doi:10.1016/j.agee.2016.02.003

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. FAO Irrigation and Drainage Paper 56. Food and Agriculture Organization of the United Nations, Italy, 50 pp.

Alves, B.J.R., Smith, K.A., Flores, R.A., Cardoso, A.S., Oliveira, W.R.D., Jantalia, C.P., Urquiaga, S., Boddey, R.M., 2012. Selection of the most suitable sampling time for static chambers for the estimation of daily mean N2O flux from soils. Soil Biol. Biochem. 46, 129–135. doi:10.1016/j.soilbio.2011.11.022

Baruah, K.K., Gogoi, B., Borah, L., Gogoi, M., Boruah, R., 2012. Plant morphophysiological and anatomical factors associated with nitrous oxide flux from wheat (Triticum aestivum). J. Plant Res. 125, 507–516. doi:10.1007/s10265-011-0464-4

Bruhn, D., Albert, K.R., Mikkelsen, T.N., Ambus, P., 2014. UV-induced N2O emission from plants. Atmos. Environ. 99, 206–214. doi:10.1016/j.atmosenv.2014.09.077

Castaldi, S., 2000. Responses of nitrous oxide, dinitrogen and carbon dioxide production and oxygen consumption to temperature in forest and agricultural light-textured soils determined by model experiment. Biol. Fertil. Soils 32, 67–72. doi:10.1007/s003740000218

Chang, C., Janzen, H.H., Cho, C.M., Nakonechny, E.M., 1998. Nitrous oxide emission through plants. Soil Sci. Soc. Am. J. 62, 35–38. doi:10.2136/sssaj1998.03615995006200010005x

Chen, X., Cabrera, M.L., Zhang, L., Wu, J., Shi, Y., Yu, W.T., Shen, S.M., 2002. Nitrous oxide emission from upland crops and crop-soil systems in northeastern China. Nutr. Cycl. Agroecosystems 62, 241–247. doi:10.1023/A:1021202114354

Clough, T., 2015. Chamber design, in: De Klein, C., Harvey, M. (Eds.), Nitrous Oxide Chamber Methodology Guidelines. Ministry for Primary Industries, New Zealand, pp. 19–33.

Collier, S.M., Dean, A.P., Oates, L.G., Ruark, M.D., Jackson, R.D., 2016. Does Plant Biomass Manipulation in Static Chambers Affect Nitrous Oxide Emissions from Soils? J. Environ. Qual. 45, 751. doi:10.2134/jeq2015.07.0377

Coskun, D., Britto, D.T., Shi, W., Kronzucker, H.J., 2017. Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. Nat. Plants 3, 17074. doi:10.1038/nplants.2017.74

Dalal, R.C., Wang, W., Robertson, G.P., Parton, W.J., 2003. Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. Aust. J. Soil Res. 41, 165–195. doi:10.1071/SR02064

Eugster, W., Merbold, L., 2015. Eddy covariance for quantifying trace gas fluxes from soils. SOIL 1, 187–205. doi:10.5194/soil-1-187-2015

Franco-Luesma, S., Álvaro-Fuentes, J., Plaza-Bonilla, D., Arrúe, J.L., Cantero-Martínez, C., Cavero, J., 2019. Influence of irrigation time and frequency on greenhouse gas emissions in a solid-set sprinkler-irrigated maize under Mediterranean conditions. Agric. Water Manag. 221, 303–311. doi:10.1016/j.agwat.2019.03.042

Global Reserch Alliance on Agricultural Greenhouse Gases, 2015. Nitrous Oxide Chamber Methodology Guidelines. De Klein, C., Harvey, M. (Eds.) Ministry for Primary Industries, New Zealand, 146 pp.

Grossman, R.B., Reinsch, T.G., 2002. 2.1 Bulk density and linear extensibility. In: Dane, J.H., Topp, C.G. (Eds.), Methods of Soil Analysis: Part 4 Physical Methods, Soil Science Society of America, United States of America, pp. 201–228.

Guardia, G., Sanz-Cobena, A., Sanchez-Martín, L., Fuertes-Mendizábal, T., González-Murua, C., Álvarez, J.M., Chadwick, D., Vallejo, A., 2018. 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. doi:10.1016/j.agee.2018.06.033

Holland, E.A., Robertson, G.P., Greenberg, J., Groffman, P.M., Boone, R.D., Gosz, J.R., 1999. Soil CO2, N2O, and CH4 exchange. In: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins, P. (Eds.), Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, United States of America, pp. 185–201.

Hutchinson, G.L., Mosier, A.R., 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci. Soc. Am. J. 45, 311. doi:10.2136/sssaj1981.03615995004500020017x

Kandel, T.P., Lærke, P.E., Elsgaard, L., 2016. Effect of chamber enclosure time on soil respiration flux: A comparison of linear and non-linear flux calculation methods. Atmos. Environ. 141, 245–254. doi:10.1016/j.atmosenv.2016.06.062

Koo, B.-. J., Adriano, D.C., Bolan, N.S., Barton, C.D., 2005. Root exudates and microorganisms. Encycl. Soils Environ. 421–428. doi:10.1016/B0-12-348530-4/00461-6

Linn, D.M., Doran, J.W., 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48, 1267–1272. doi:10.2136/sssaj1984.03615995004800060013x

Livingston, G., Hutchinson, G., Matson, P.A., Harriss, R., 1995. Enclosure-based measurement of trace gas exchange: Applications and sources of error, in: Matson, P.A., Harriss, R.C. (Eds.), Biogenic Trace Gases: Measuring Emissions from Soil and Water. United Kingdom, pp. 14–51.

López-Fernández, S., Díez, J.A., Hernáiz, P., Arce, A., García-Torres, L., Vallejo, A., 2007. Effects of fertiliser type and the presence or absence of plants on nitrous oxide emissions from irrigated soils. Nutr. Cycl. Agroecosystems 78, 279–289. doi:10.1007/s10705-007-9091-9

Lordan, J., Pascual, M., Fonseca, F., Montilla, V., Papió, J., Rufat, J., Villar, J.M., 2015. An image-based method to study the fruit tree canopy and the pruning biomass production in a peach orchard. HortScience 50, 1809–1817. doi:10.21273/HORTSCI.50.12.1809

MacKenzie, A.F., Fan, M.X., Cadrin, F., 1998. Nitrous Oxide Emission in Three Years as Affected by Tillage, Corn-Soybean-Alfalfa Rotations, and Nitrogen Fertilization. J. Environ. Qual. 27, 698. doi:10.2134/jeq1998.00472425002700030029x

Maris, S.C., Teira-Esmatges, M.R., Bosch-Serra, A.D., Moreno-García, B., Català, M.M., 2016. Effect of fertilising with pig slurry and chicken manure on GHG emissions from Mediterranean paddies. Sci. Total Environ. 569–570, 306–320. doi:10.1016/j.scitotenv.2016.06.040

Morton, P.A., Heinemeyer, A., 2018. Vegetation matters: Correcting chamber carbon flux measurements using plant volumes. Sci. Total Environ. 639, 769–772. doi:10.1016/j.scitotenv.2018.05.192

Olfs, H.-W., Westerschulte, M., Ruoss, N., Federolf, C.-P., Zurheide, T., Vergara Hernandez, M.E., Neddermann, N., Trautz, D., Pralle, H., Fuß, R., Well, R., 2018. A new chamber design for measuring nitrous oxide emissions in maize crops. J. Plant Nutr. Soil Sci. 181, 69–77. doi:10.1002/jpln.201700008

Parkin, T.B., Venterea, R.T., Hargreaves, S.K., 2012. Calculating the detection limits of chamber-based soil greenhouse gas flux measurements. J. Environ. Qual. 41, 705. doi:10.2134/jeq2011.0394

Pavelka, M., Acosta, M., Kiese, R., Altimir, N., Brümmer, C., Crill, P., Darenova, E., Fuß, R., Gielen, B., Graf, A., Klemedtsson, L., Lohila, A., Longdoz, B., Lindroth, A., Nilsson, M., Jiménez, S.M., Merbold, L., Montagnani, L., Peichl, M., Pihlatie, M., Pumpanen, J., Ortiz, P.S., Silvennoinen, H., Skiba, U., Vestin, P., Weslien, P., Janous, D., Kutsch, W., 2018. Standardisation of chamber technique for CO2, N2O and CH4 fluxes measurements from terrestrial ecosystems. Int. Agrophysics 32, 569–587. doi:10.1515/intag-2017-0045

Pedersen, A.R., Petersen, S.O., Schelde, K., 2010. A comprehensive approach to soil-atmosphere trace-gas flux estimation with static chambers. Eur. J. Soil Sci. 61, 888–902. doi:10.1111/j.1365-2389.2010.01291.x

Recio, J., Vallejo, A., Le-Noë, J., Garnier, J., García-Marco, S., Álvarez, J.M., Sanz-Cobena, A., 2018. The effect of nitrification inhibitors on NH3 and N2O emissions in highly N fertilized irrigated Mediterranean cropping systems. Sci. Total Environ. 636, 427–436. doi:10.1016/j.scitotenv.2018.04.294

Rochette, P., Eriksen-Hamel, N.S., 2008. Chamber Measurements of Soil Nitrous Oxide Flux: Are Absolute Values Reliable? Soil Sci. Soc. Am. J. 72, 331. doi:10.2136/sssaj2007.0215

Schaufler, G., Kitzler, B., Schindlbacher, A., Skiba, U., Sutton, M.A.A., Zechmeister-Boltenstern, S., 2010. Greenhouse gas emissions from European soils under different land use: effects of soil moisture and temperature. Eur. J. Soil Sci. 61, 683–696. doi:10.1111/j.1365-2389.2010.01277.x

Soil Survey Staff, 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, United States of America, 360 pp.

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. doi:10.1016/j.agrformet.2018.09.017

Ussiri, D., Lal, R., 2013. Soil Emission of Nitrous Oxide and its Mitigation. Springer, Netherlands, 378 pp.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415–421. doi:10.1111/j.1365-3180.1974.tb01084.x

Chapter 8

General discussion

This Doctoral Thesis has assessed the potential of nitrification and urease inhibitors to reduce nitrogen (N) losses and, thus, to enhance nitrogen use efficiency (NUE) in different crops and N-fertiliser strategies. The importance of the topic lies in the fact that improvements in NUE in crop production are critical for addressing the global challenges of food security, environmental degradation, and climate change (Zhang et al., 2015). Besides, the number of factors (soil moisture, soil temperature, soil texture, among others; Ussiri and Lal, 2013) that controls the action of inhibitors makes essential their study under different agro-environmental conditions to evaluate their utility in commercial fields at the same time that other advantages are considered (reduction in the N dose or lessening in the number of N applications).

In this Thesis, the use of nitrification and urease inhibitors was combined with good irrigation and N management practices according to crop necessities to evaluate the increase in NUE and decrease in reactive N losses in the field trials. The considered approach deserves attention since several studies have questioned the assessment of inhibitors effectivity under N rates not adjusted to crop demand, reporting higher benefits reducing N losses (Rose et al., 2018; Yang et al., 2016) than could be obtained under optimal management practices. In trial 1, nitrification and urease inhibitors mixed with urea were tested in a sprinkler irrigated maize-maize-wheat rotation in two contrasting soil types in drainage lysimeters. In trial 2, a urease inhibitor mixed with pig slurry was evaluated during three years in a sprinkler irrigated wheat crop. However, the low response of wheat yield to N application in trial 2 indicated an unexpected high N rate scenario. Hereafter, the influence of inhibitors under these management situations on N losses and NUE is discussed.

Nitrate leaching

In the three-year maize-maize-wheat rotation and in the two contrasting soil types (Deep and Shallow), the good adjustment of irrigation water to crop needs produced very small N losses by nitrate leaching, especially in the Deep soil. Although the mass of nitrate leaching strongly depends on the amount of drainage (Diez et al., 2000); in this study, a high percentage of nitrate leaching variability (97% and 72% in Shallow and Deep soil, respectively) was explained by differences in nitrate concentration, whereas a smaller effect was associated with differences in drained volume (R^2 of 55% and 48% for Shallow and Deep soil, respectively). The low drainage volume could cause the absence of differences associated with the use of nitrification and urease inhibitors, in the same way than in the study of Díez et al. (2010), also with good irrigation management and similar drainage volumes. Besides, the good adjustment of N fertilisation to crop needs facilitated no effect of the nitrification and urease inhibitors reducing N leaching in the two soil types. In this sense, the meta-analysis of Yang et al. (2016) shows that the higher N-fertiliser rate is applied, the greater N-leaching reduction is expected when NIs are used.

In the three years wheat crop experiment, the volume of water drained, estimated by a daily water balance, was relatively low (73 mm for the whole 3 year period) because of the good adjustment of irrigation to crop necessities and the soil depth, and most (95%) of the drained volume was associated with a heavy rainfall event. In this trial, the mass of N leaching could not be calculated; however, the low soil mineral nitrogen (SMN) content (13.6 kg N ha^{-1} from 0 to 30 cm the day before the heavy rainfall event) rejects a high potential risk of nitrate leaching.

Soil mineral nitrogen

The good adjustment of N-fertiliser rates was responsible for non-significant differences in SMN content among treatments at the end of the three-year rotation, in contrast to the effect of inhibitors on residual SMN reported by other studies, such as Alonso-Ayuso et al. (2016).

After three consecutive years of wheat crop (trial 2), significant differences were found in SMN content (0-90 cm) between Urea treatments (106 kg N ha^{-1}) and pig slurry (64 kg N ha^{-1}) and pig slurry with MCDHS (73 kg N ha^{-1}) treatments. These differences may be associated with higher ammonia losses in pig slurry than in urea application at tillering. In the same way that was observed in trial 1, no significant effect of MCDHS on final SMN content was detected.

Differences in soil nitrate content at 0-15-cm depth were observed in the miniplot wheat experiment between the pig slurry and the pig slurry with Vizura® (DMPP) treatments: 23% of reduction of soil NO_3^- content in the treatment with the nitrification inhibitor compared to the treatment without NI laid bare the effect of *Nitrosomonas* delaying the oxidation of NH_4^+ to NO_2^-. No consistent differences in SMN content were observed when the urease inhibitor (MCDHS) was added to the pig slurry; thus, it is hypothesised that soil N dynamic was not affected by this new inhibitor, which is in agreement with the non-significant effect of MCDHS on SMN content at the end of trial 2, after the three consecutive years of wheat crop.

Greenhouse gas emissions

Under the studied experimental conditions, methane emissions were negligible without significant differences among fertiliser treatments whatever it was the N source. Occasionally, emissions had a sink effect although it lacked consistency. These results corroborate previous studies under similar climatic and management conditions (Álvaro-Fuentes et al., 2016; Guardia et al., 2017; Pareja-Sánchez et al., 2019).

Because of the above-cited context of food security, the efficiency of inhibitors controlling N_2O losses is discussed in terms of absolute emissions and yield-scaled N_2O emissions (YS_{N2O}). The comparison of yield-scaled N_2O emissions among different agricultural practices allows balancing food security with mitigating N_2O emissions (Sainju, 2016). The addition of DMPP was able to reduce significantly the YS_{N2O} by 73% in Deep soil and 59% in Shallow soil with respect to the traditional Urea treatment, and by 71% with respect to the pig slurry treatment. These results were mainly obtained because of an important reduction in N_2O emissions (73%, 60%, and 70%, respectively) with no consistent effects on grain yield. The results agree with those presented in a meta-analysis for Mediterranean conditions of Sanz-Cobena et al. (2017) which reports mitigation of N_2O emissions but without yield improvement associated with DMPP.

The addition of urease inhibitors to urea and pig slurry did not affect ($p>0.05$) N_2O emissions in any experiment. The unsuccessful action of UIs reducing N_2O emissions in the three-year rotation might be explained owing to the non-direct relation between hydrolysis of urea and N_2O emissions (Akiyama et al., 2010). The non-existent effect of MCDHS on N_2O emissions in the three-year wheat crop might be justified due to the transformation of urea-N to ammonium-N form previously to the addition of the urease inhibitor since this type of inhibitor acts delaying the enzymatic hydrolysis of urea (Ussiri and Lal, 2013).

Nevertheless, some significant effects were detected decreasing YS_{N2O} depending on the soil type and the type of N source because of the joint action of both parameters (N_2O emissions and grain yield). When UIs (MCDHS and NBPT) were added to urea, they reduced significantly YS_{N2O} respect the traditional fertilisation in the Deep soil but did not in the Shallow soil. However, no effect on YS_{N2O} was observed when MCDHS was added to pig slurry compared to urea or pig slurry application: neither N_2O emissions nor grain yield was affected by the UI addition ($p>0.05$). Neither the substitution of urea for pig slurry affected yield, N_2O and YS_{N2O} emissions.

Regarding absolute N_2O emissions during three-cropping seasons and in soils with a similar depth, values were in the same order of magnitude in the traditional fertiliser treatment of the rotation (6.15 kg N ha^{-1}, Deep soil) than in the synthetic fertilisation of the wheat crop (6.14 kg N ha^{-1}), despite the higher N necessities of maize in comparison to wheat and the warmer temperatures during maize fertilisation. Nitrous oxide emissions may have been overestimated by 35% in the three-year wheat crop because of cutting plants inside the chambers. The lack of plants inside the collars, even with plants surrounding the chambers, leaded significant changes in soil mineral nitrogen content and soil temperature, which have a noteworthy effect on N_2O emissions.

The effect of considering the volume displaced by plants into the chambers was evaluated and the results indicated that it had a minor effect on N_2O emissions (647 g N ha^{-1} considering plant volume vs. 653 g N ha^{-1} disregarding plant volume in the calculations) since the percentage of chamber occupied by plants reached a maximum of 2.2%. The method to estimate plant volume developed in this Thesis using an image analysis-based procedure was reliable, simple, and non-destructive.

In this Thesis, N_2O losses were estimated by the closed-chamber technique using a linear model, assuming non-saturation effect during chamber enclosure. The comparison with the HMR-N (selection of non-linear revised Hutchinson/Mosier model when was suggested by the HMR package, otherwise linear model selection) could suggest that the linear model was underestimating emissions. However, a deeper study of the N_2O fluxes at lower sampling times (0-20') indicated that no effect of saturation is happening under the experimental conditions presented in this Thesis.

Gaseous losses and nitrogen balance

Nitrous oxide loss is environmentally important in terms of global warming potential and ozone depletion (UNEP, 2013), but in agronomical terms, this N loss has a minor relevance and are negligible compared to other N losses. Under the near-optimal N rates used in the field trials presented in this Thesis, N_2O losses were a small percentage of N available for crops (0.9% in the three-year rotation and 1.0% in the three-year wheat crop). N losses associated with N_2O emissions accounted only the 0.4% of the ammonium-N applied with the pig slurry in the wheat crop in the miniplots. However, those N losses associated with ammonia volatilisation were twenty times more (7-9% of NH_4^+-N applied) after presowing application and fifty times more (19-23% of NH_4^+-N applied) after tillering application. The vulnerability to NH_3 losses was higher during the first hours after N application and diurnal hours in agreement with Yagüe et al. (2019) and Li et al. (2018), respectively. However, the two evaluated additives (MCDHS and microbial activator) did not affect NH_3 volatilisation.

The ammonia volatilisation losses might have been responsible for the lower wheat grain protein and higher unaccounted N observed in the pig slurry treatments compared to urea treatments. Further research should be conducted to confirm this hypothesis since studies in the region under different crops have not shown these contrasting trends between N sources (Bosch-Serra et al., 2015; Moreno-García et al., 2017).

Agronomic parameters

In agronomic terms, the control of N_2O emissions has been proposed to increase NUE (Ladha et al., 2005). However, in this Thesis, the reduction of N_2O emissions through DMPP addition did not result in a significant increase in NUE, whatever it was the N source (urea or pig slurry). The above-cited small differences in N losses associated with the application of inhibitors did not affect any nitrogen use efficiency indicators or agronomic parameters.

Overall, agronomic parameters (grain yield, aboveground biomass, and N uptake) were not affected by the use of inhibitors in any experiment, although interestingly, they allowed reaching the same values saving one side-dress N application compared to the traditional fertilisation in maize. The reduction in the number of applications, assuming good management of water irrigation, could decrease fuel needs and operation times, which would also mitigate the CO_2 emissions and prevent soil compaction associated with agricultural tasks (Huérfano et al., 2015; van den Akker and Soane, 2005). These additional advantages might encourage the use of inhibitors by farmers.

In summary, this Thesis has proved that, in the irrigated semiarid conditions of the experiments, the different urease and nitrification inhibitors evaluated mixed with urea or with pig slurry at the dose recommended by the manufacturing companies did not have an effect on crop agronomic parameters. Moreover, urease and nitrification inhibitors were not able to reduce nitrate leaching and only DMPP, mixed with both urea and pig slurry, was able to reduce N_2O and yield-scaled N_2O emissions consistently in the scenario of good irrigation and fertilisation practices evaluated in the field experiments.

References

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. doi:10.1111/j.1365-2486.2009.02031.x

Alonso-Ayuso, M., Gabriel, J.L., Quemada, M., 2016. Nitrogen use efficiency and residual effect of fertilisers with nitrification inhibitors. Eur. J. Agron. 80, 1–8. doi:10.1016/j.eja.2016.06.008

Álvaro-Fuentes, J., Arrúe, J.L., Cantero-Martínez, C., Isla, R., Plaza-Bonilla, D., Quílez, D., 2016. Fertilization scenarios in Sprinkler-Irrigated corn under mediterranean conditions: effects on greenhouse gas emissions. Soil Sci. Soc. Am. J. 80, 662–671. doi:10.2136/sssaj2015.04.0156

Bosch-Serra, À.D., Ortiz, C., Yagüe, M.R., Boixadera, J., 2015. Strategies to optimize nitrogen efficiency when fertilizing with pig slurries in dryland agricultural systems. Eur. J. Agron. 67, 27–36. doi:10.1016/j.eja.2015.03.003

Díez, J.A., Arauzo, M., Hernaiz, P.J.J., Sanz, A., Vallejo, A., 2010. Comparison of nitrification inhibitors to restrict nitrate leaching in a maize crop irrigated under Mediterranean conditions. Spanish J. Agric. Res. 8, 481–492. doi:10.5424/sjar/2010082-1212

Diez, J.A., Caballero, R., Roman, R., Tarquis, A., Cartagena, M.C., Vallejo, A., 2000. Integrated fertiliser and irrigation management to reduce nitrate leaching in central Spain. J. Environ. Qual., 29, 1539–1547. doi:10.2134/jeq2000.00472425002900050021x

Guardia, G., Cangani, M.T.M.T., Sanz-Cobena, A., Junior, J.L.L., Vallejo, A., 2017. Management of pig manure to mitigate NO and yield-scaled N2O emissions in an irrigated Mediterranean crop. Agric. Ecosyst. Environ. 238, 55–66. doi:10.1016/j.agee.2016.09.022

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. doi:10.1016/j.eja.2014.11.008

Ladha, J.K., Pathak, H., J. Krupnik, T., Six, J., van Kessel, C., 2005. Efficiency of Fertilizer Nitrogen in Cereal Production: Retrospects and Prospects, in: Advances in Agronomy, 87. Elsevier, pp. 85–156.

Li, M., Wang, Y., Adeli, A., Yan, H., 2018. Effects of application methods and urea rates on ammonia volatilization, yields and fine root biomass of alfalfa. F. Crop. Res. 218, 115–125. doi:10.1016/j.fcr.2018.01.011

Moreno-García, B., Guillén, M., Quílez, D., 2017. Response of paddy rice to fertilisation with pig slurry in northeast Spain: Strategies to optimise nitrogen use efficiency. F. Crop. Res. 208, 44–54. doi:10.1016/j.fcr.2017.01.023

Pareja-Sánchez, E., Cantero-Martínez, C., Álvaro-Fuentes, J., Plaza-Bonilla, D., 2019. Tillage and nitrogen fertilization in irrigated maize: key practices to reduce soil CO2 and CH4 emissions. Soil Tillage Res. 191, 29–36. doi:10.1016/j.still.2019.03.007

Rose, T.J., Wood, R.H., Rose, M.T., Van Zwieten, L., 2018. A re-evaluation of the agronomic effectiveness of the nitrification inhibitors DCD and DMPP and the urease inhibitor NBPT. Agric. Ecosyst. Environ. 252, 69–73. doi:10.1016/j.agee.2017.10.008

Sainju, U.M., 2016. A Global Meta-Analysis on the Impact of Management Practices on Net Global Warming Potential and Greenhouse Gas Intensity from Cropland Soils. PLoS One 11, e0148527. doi:10.1371/journal.pone.0148527

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. doi:10.1016/j.agee.2016.09.038

UNEP, 2013. Drawing Down N2O to Protect Climate and the Ozone Layer. A UNEP Synthesis Report. Kenya, 57 pp.

Ussiri, D., Lal, R., 2013. Soil Emission of Nitrous Oxide and its Mitigation. Springer, Netherlands, 378 pp.

van den Akker, J.J.H., Soane, B., 2005. Compaction. Encycl. Soils Environ. 285–293. doi:10.1016/B0-12-348530-4/00248-4

Yagüe, M.R., Valdez, A.S., Bosch-Serra, À.D., Ortiz, C., Castellví, F., 2019. A short-term study to compare field strategies for ammonia emission mitigation. J. Environ. Qual. 48, 179–184. doi:10.2134/jeq2018.05.0218

Yang, M., Fang, Y., Sun, D., Shi, Y., 2016. Efficiency of two nitrification inhibitors (dicyandiamide and 3, 4-dimethypyrazole phosphate) on soil nitrogen transformations and plant productivity: a meta-analysis. Sci. Rep. 6, 22075. doi:10.1038/srep22075

Zhang, X., Davidson, E.A., Mauzerall, D.L., Searchinger, T.D., Dumas, P., Shen, Y., 2015. Managing nitrogen for sustainable development. Nature 528, 51–59. doi:10.1038/nature15743

Chapter 9

Conclusiones generales

1. Bajo prácticas óptimas de manejo del riego y del nitrógeno en una rotación maíz-maíz-trigo, el uso de fertilizantes nitrogenados estabilizados con inhibidores no presentó ventajas en términos de aumento del rendimiento en grano, mejora de la eficiencia del uso de nitrógeno o reducción de la cantidad de nitrato drenada.

2. El uso del inhibidor de la nitrificación DMPP y del inhibidor de la ureasa NBPT permitió la reducción del número de aplicaciones de nitrógeno en la cobertera del maíz manteniendo la producción de grano.

3. El inhibidor de la nitrificación DMPP fue el único producto evaluado capaz de disminuir significativamente las emisiones directas de óxido nitroso en comparación con el tratamiento estándar de urea en la rotación maíz-maíz-trigo, siendo la reducción del 60% en el suelo Profundo y del 73% en el suelo Somero.

4. En la rotación maíz-maíz-trigo, el DMPP logró reducir las emisiones directas de óxido nitroso por unidad de rendimiento en los dos tipos de suelo respecto a una aplicación tradicional de urea; sin embargo, el efecto de los inhibidores de la ureasa NBPT y MCDHS sobre las mismas dependió del tipo de suelo, ya que solamente en el suelo Profundo las emisiones por unidad de rendimiento disminuyeron significativamente.

5. La fertilización nitrogenada con purín porcino permitió alcanzar una producción de trigo y una eficiencia en el uso del nitrógeno similar a la fertilización con urea, sin observarse diferencias significativas en las emisiones directas de gases de efecto invernadero (óxido nitroso y metano). Sin embargo, se detectó un mayor contenido de proteína en grano con urea, posiblemente debido a unas menores pérdidas de nitrógeno tal como queda reflejado en el balance de nitrógeno, que se suponen asociadas a mayores pérdidas por volatilización de amoniaco con la aplicación de purín porcino.

6. La adición al purín porcino del inhibidor de la ureasa MCDHS o del potenciador de la biomasa microbiana evaluado no presentó ventajas agronómicas (producción de grano, proteína en grano, eficiencia en el uso del nitrógeno) ni medioambientales (volatilización de amoniaco, emisiones directas de óxido nitroso y metano) en un cultivo de trigo.

7. La adición de Vizura® (DMPP) al purín porcino consiguió mitigar en un 70% las emisiones directas de óxido nitroso en un cultivo de trigo, sin comprometer el rendimiento y la proteína en grano o la eficiencia en el uso del nitrógeno.

8. La metodología propuesta basada en análisis de imagen para la estimación del volumen de las plantas de trigo dentro de las cámaras estáticas cerradas para corregir la medida de gases de efecto invernadero resultó precisa y sencilla.

9. La no consideración del volumen de las plantas dentro de las cámaras produce una sobrestimación pequeña, aunque sistemática, en las estimaciones de emisión de óxido nitroso. La medición e inclusión de esta variable en el cálculo de las emisiones dependerá de los objetivos del estudio.

10. El corte periódico de las plantas localizadas en el interior de las cámaras para facilitar las medidas de emisiones de gases de efecto invernadero aumentó significativamente las emisiones directas de óxido nitroso debido al incremento del contenido de nitrógeno mineral y de la temperatura en los primeros centímetros del suelo, por lo que es una práctica totalmente desaconsejable.

11. La utilización del paquete HMR para el cálculo de los flujos de óxido nitroso proporcionó estimas en promedio 18% mayores que la regresión lineal. Sin embargo, el hecho de que el paquete HMR recomiende mayoritariamente el uso del modelo exponencial con flujos de emisión bajos, con nula probabilidad de una reducción de los

flujos de emisión debida a la acumulación de óxido nitroso dentro de las cámaras, indica que el uso del modelo lineal es más adecuado en las condiciones estudiadas.

12. Bajo condiciones de emisión directa de óxido nitroso similares a las presentadas en este estudio, la reducción de los tiempos de muestreo de cuatro (0, 20, 40 y 60 minutos) a dos (0 y 60 minutos) parece una estrategia útil para rebajar los costes asociados al muestreo y análisis de los gases, con un impacto reducido en la precisión de las estimas de las emisiones (0,3%).