Universitat de Lleida

Effects of organic and mineral nitrogen fertilization of maize in irrigated Mediterranean environments

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TESI DOCTORAL

Effects of organic and mineral nitrogen fertilization of maize in irrigated Mediterranean environments

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"La vida no es la que uno vivió,

sino la que recuerda

y cómo la recuerda para contarla"

Gabriel García Márquez, Vivir para contarla

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GENERAL INDEX

ABSTRACT

Maize (*Zea mays* L.) is one of the most important crops in the irrigated areas of the Ebro valley (north-eastern Spain), and is part of the traditional rotation of crops. Intensive farming is carried out in many areas of the Ebro valley, where significant amounts of nitrogen fertilizer are used as well as manures because of extensive livestock farming, particularly in pig production.

Surveys conducted in the Ebro valley area have shown that about half of the area under maize is fertilized with mineral fertilizer, while the other half receives organic applications, mostly pig slurry manure supplemented with mineral fertilizer. It is very common in maize cultivation to apply N rates much higher than the crop uptake, a practice that can aggravate the problem of contamination of aquifers with nitrates and also affect the economic profitability of the crop. In addition to possible contamination by nitrates, the continuous use of slurry as fertilizer in a given plot can cause contamination of the soil by accumulation of heavy metals such as Cu and/or Zn. Therefore, optimization of mineral N and organic fertilizer applications is very important to minimize contamination and increase profitability.

To improve and assist in adjusting the nitrogen fertilization of maize, two field trials were conducted between 2002 and 2015. In the first trial, the effects were evaluated of the application of seven rates of mineral N (0, 100, 150, 200, 250, 300 and 400 kg N ha^{-1} $year^{-1}$), and in the second three doses of fattening pig slurry (0, 30 and 50 m^3 ha^{-1} $year^{-1}$) combined with three rates of mineral N (0, 100 and 200 kg N ha^{-1} $year^{-1}$) were evaluated for twelve years.

In both trials, the response to nitrogen fertilization varied from year to year, and was influenced by initial mineral N content in the soil and by the rate of N fertilization (both organic and mineral). Soil sampling to a 30 cm depth proved to be sufficient in our soils and maize growing conditions, where 203 kg N ha^{-1} of available N (soil mineral N + mineral N applied) before sowing were required in the first 30 cm to obtain maximum yields of grain (~14 Mg ha^{-1}). The initial soil N content is a factor that must be taken into account when N recommendations are given in maize.

In the mineral N fertilization trial, the rate of 200 kg N ha^{-1} was the one which allowed average maximum grain yields (~14 Mg ha^{-1}), presented an N use efficiency greater than 80%, and also moderate residual N content. This N rate, in turn, coincides with the maximum allowed in the study area, an area designated as vulnerable to nitrate pollution according to current legislation.

In the trial involving pig slurry applications, it was possible to obtain good grain yields (~14 Mg ha^{-1}) by fertilizing only with pig slurry at the highest dose used (50 m^3 ha^{-1}) during all years, or also by fertilizing with 30 m^3 ha^{-1} of slurry combined with 100 kg ha^{-1} of N mineral at sidedress, the latter being very close to the maximum value permitted by current legislation.

After twelve years of continuous applications of pig slurry, at rates almost twice those allowed by current legislation, an increase in soil Cu and Zn content was detected, although the concentrations were well below levels considered phytotoxic or not permitted by current legislation. Soil contamination by Cu and Zn applied with pig slurry does not appear to represent, in our conditions, a risk in the near future given current rates of soil accumulation. However, we believe that further studies are required to determine long-term risks of Cu and Zn soil contamination.

In our study, optimal N rates for maize coincided, in general, with minimal effects on the environment (reduced soil residual N), so it appears to be possible to obtain good maize yields while minimizing environmental impact.
Fertilization with mineral N and pig slurry, accompanied by soil incorporation of crop residues, resulted in increases in soil organic carbon levels in the first 30 cm soil layer, indicating that they contribute to the maintenance or improvement of soil quality.

RESUMEN

El maíz (Zea mays L.) es uno de los cultivos más importantes en los regadíos del valle de Ebro (noreste de España), y forma parte de las rotaciones tradicionales de cultivos extensivos. En muchas zonas del valle del Ebro se lleva a cabo una agricultura intensiva, donde se utilizan grandes cantidades de fertilizante nitrogenado, y también se aplican estiércoles en abundancia, ya que hay una gran actividad ganadera intensiva, donde predomina la producción porcina.

Encuestas llevadas a cabo en la zona del valle del Ebro han mostrado que alrededor de la mitad de la superficie cultivada de maíz se fertiliza con abono mineral, y la otra mitad recibe aplicaciones orgánicas, en su mayoría purín de cerdo complementado con abono mineral. Es muy frecuente que el maíz reciba dosis de N muy superiores a las que el cultivo pueda extraer, práctica que puede agravar la problemática de la contaminación de los acuíferos con nitratos, y también afectar a la rentabilidad económica del cultivo. Además de la posible contaminación por nitratos, el continuo uso de purines como fertilizante en una determinada parcela puede causar contaminación del suelo por acumulación de metales pesados, como el Cu y/o Zn. Por lo tanto, la optimización del abonado del maíz con N mineral y orgánico es muy importante para minimizar la contaminación e incrementar la rentabilidad.

Para mejorar y ayudar a ajustar la fertilización nitrogenada del maíz, se realizaron dos ensayos de campo, entre 2002 y 2015. En el primer ensayo se evaluaron los efectos de la aplicación de siete dosis de N mineral (0, 100, 150, 200, 250, 300 y 400 kg N ha^{-1} $año^{-1}$), y en el segundo se evaluaron tres dosis de purín de cerdo de cebo (0, 30 y 60 m^3 ha^{-1} $año^{-1}$) combinadas con tres dosis de N mineral (0, 100 y 200 kg N ha^{-1} $año^{-1}$), durante doce años.

La respuesta a la fertilización nitrogenada varió de un año a otro y estuvo influenciada por el contenido inicial de N mineral en el suelo y por la dosis de fertilización con N (tanto orgánica como mineral). El muestreo del suelo hasta 30 cm de profundidad demostró ser suficiente en nuestras condiciones de cultivo de maíz, donde 203 kg N disponible (N mineral del suelo + N mineral aplicado) ha^{-1} antes de la siembra fueron necesarios en los primeros 30 cm para obtener máximos rendimientos de grano (~14 Mg

ha^{-1}). El contenido inicial de N en el suelo es un factor que debe tenerse en cuenta cuando se dan recomendaciones de abonado N en maíz.

En el ensayo de fertilización con N mineral, la dosis de 200 kg N mineral ha^{-1}, fue la que a largo plazo permitió máximos rendimientos de grano (~14 Mg ha^{-1}), una eficiencia del uso del N mayor del 80%, y unos contenidos de N residual moderados. Esta dosis a su vez coincide con las aplicaciones máximas de N permitidas en la zona de estudio, una zona designada como vulnerable a la contaminación por nitratos según la legislación vigente.

En el ensayo de fertilización con purín, fue posible obtener buenos rendimientos de grano (~14 Mg ha^{-1}) durante todos los años, fertilizando solo con purín a la dosis más alta (50 m^3 ha^{-1}), y también fertilizando con 30 m^3 ha^{-1} de purín combinado con 100 kg ha^{-1} de N mineral en cobertera, siendo esta última dosis muy parecida al máximo permitido por la legislación vigente.

Después de doce años de aplicaciones continuadas de purín de cerdo, a una dosis de prácticamente el doble de la permitida según la legislación vigente, se detectó un incremento del contenido de Cu y Zn del suelo, aunque las concentraciones estuvieron muy por debajo de las consideradas fitotóxicas o las no autorizadas por la legislación vigente. La contaminación del suelo por Cu y Zn aplicado con el purín no parece representar, en nuestros suelos, un riesgo en un futuro próximo teniendo en cuenta las tasas actuales de acumulación en el suelo, sin embargo, creemos que deberían tenerse en cuenta en un futuro más lejano.

En nuestro estudio, las dosis óptimas de N para el maíz coincidieron con los mínimos efectos sobre el medioambiente (reducción del N residual), de esta forma parece ser posible obtener buenos rendimientos de maíz minimizando a su vez el impacto ambiental.

Tanto la fertilización con N mineral como con purín de cerdo, acompañada de una incorporación al suelo de los residuos de cosecha, resultó en incrementos de los niveles de carbón orgánico del suelo en los primeros 30 cm, indicando que contribuyen al mantenimiento o mejora de la calidad del suelo.

RESUM

El panís (*Zea mays* L.) és un dels cultius més importants als regadius de la vall de l'Ebre (nord-est d'Espanya), i forma part de les rotacions tradicionals de cultius extensius. En moltes zones de la vall de l'Ebre es porta a terme una agricultura intensiva, on s'utilitzen grans quantitats de fertilitzant nitrogenat, i també s'apliquen grans quantitats de fems, ja que hi ha una gran activitat ramadera intensiva, on predomina la producció porcina.

Enquestes dutes a terme a la zona de la Vall de l'Ebre han mostrat que al voltant de la meitat de la superfície conreada de panís es fertilitza amb adob mineral, i l'altra meitat rep aplicacions orgàniques, majoritàriament purins de porc complementat amb adob mineral. És molt freqüent que el panís rebi dosis de N molt superiors a les que el conreu pot extreure, pràctica que pot agreujar la problemàtica de la contaminació dels aqüífers amb nitrats, i també afectar la rendibilitat econòmica del conreu. A més de la possible contaminació per nitrats, el ús continu de purins com a fertilitzant en una determinada parcel·la pot causar contaminació del sòl per acumulació de metalls pesants, com el Cu i/o Zn. Per tant, l'optimització de l'abonat del panís amb N mineral i orgànic és molt important per minimitzar la contaminació i incrementar la rendibilitat.

Per millorar i ajudar a ajustar la fertilització nitrogenada del panís, es va realitzar dos assaigs de camp entre 2002 i 2015. A un assaig, es van avaluar els efectes de l'aplicació de set dosis de N mineral (0, 100, 150, 200, 250, 300 i 400 kg N ha^{-1} any^{-1}), i a l'altre es van evaluar tres dosis de purins de porc d'engreix (0, 30 i 50 m^3 ha^{-1} any^{-1}) combinades amb tres dosis de N mineral (0, 100 i 200 kg N ha^{-1} any^{-1}), durant dotze anys.

En tots dos assajos la resposta a la fertilització nitrogenada va variar d'un any a un altre, i va estar influenciada pel contingut inicial de N mineral al sòl i per la dosi de fertilització amb N (tant orgànica com mineral). El mostreig del sòl fins a 30 cm de profunditat va demostrar ser suficient en les nostres condicions de cultiu de panís, on 203 kg N disponible ha^{-1} (N mineral del sòl + N mineral aplicat) abans de la sembra van ser necessaris en els primers 30 cm per obtenir màxims rendiments de gra (~14 Mg ha^{-1}). El contingut inicial de N al sòl és un factor que s'ha de tenir en compte quan es donen recomanacions d'abonat N en blat de moro.

En l'assaig de fertilització amb N mineral, la dosi de 200 kg N mineral ha^{-1} va esser la que a llarg termini va permetre obtenir màxims rendiments de gra (~14 Mg ha^{-1}), una eficiència de l'ús del N major del 80%, i uns continguts de N residual moderats. Aquesta dosi al seu torn coincideix amb el màxim permès a la zona d'estudi, una zona designada com a vulnerable a la contaminació per nitrats segons la legislació vigent.

En l'assaig de fertilització amb purins, va ser possible obtenir bons rendiments de gra (~14 Mg ha^{-1}) durant tots els anys fertilitzant només amb purins a la dosi més alta (50 m^3 ha^{-1}), i també fertilitzant amb 30 m^3 ha^{-1} de purins combinat amb 100 kg ha^{-1} addicionals de N mineral en cobertora, sent aquesta última dosi molt semblant al màxim permès per la legislació vigent.

Després de dotze anys d'aplicacions continuades de purins de porc, a una dosi de pràcticament el doble de la permesa per la legislació vigent, es va detectar un increment del contingut de Cu i Zn del sòl, encara que les concentracions van estar molt per sota de les considerades fitotòxiques o les no autoritzades per la legislació vigent. La contaminació del sòl per Cu i Zn aplicat amb el purí no sembla representar un risc sota les nostres condicions en un futur pròxim tenint en compte les taxes actuals d'acumulació al sòl, però, creiem que s'haurien de tenir en compte un futur més llunyà.

Al nostre estudi, les dosis òptimes de N per al panís van coincidir amb els mínims efectes per al medi ambient (disminució de N residual), d'aquesta manera sembla ser possible obtenir bons rendiments de panís minimitzant al seu torn l'impacte ambiental.

Tant la fertilització amb N mineral com amb purí de porc acompanyada d'una incorporació al sòl dels residus de collita, va resultar en increments dels nivells de carbó orgànic del sòl en els primers 30 cm, indicant que contribueixen al manteniment o millora de la qualitat del sòl.

GENERAL INTRODUCTION

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The Ebro Valley

The Ebro Valley or Ebro depression is a vast region of the northeastern Iberian Peninsula through which the Ebro river flows. It is characterized by a semiarid climate with average annual rainfalls ranging from 200 to 400 mm (Creus, 1996). Despite its semiarid environment, this valley is one of the most important agricultural areas of Spain due to the presence of numerous farms and irrigation infrastructures. In the Ebro Valley there are 906,000 ha of crops under irrigation. The irrigation surface is divided into flood-irrigation (55%), sprinkler irrigation (25%), and drip irrigation (20%) (CHE, 2017). In general, flood-irrigation systems are associated with low irrigation uniformity and efficiency whereas sprinkler and drip irrigation systems are more modern and associated with high irrigation efficiencies (Cavero et al., 2003; Lecina et al., 2005).

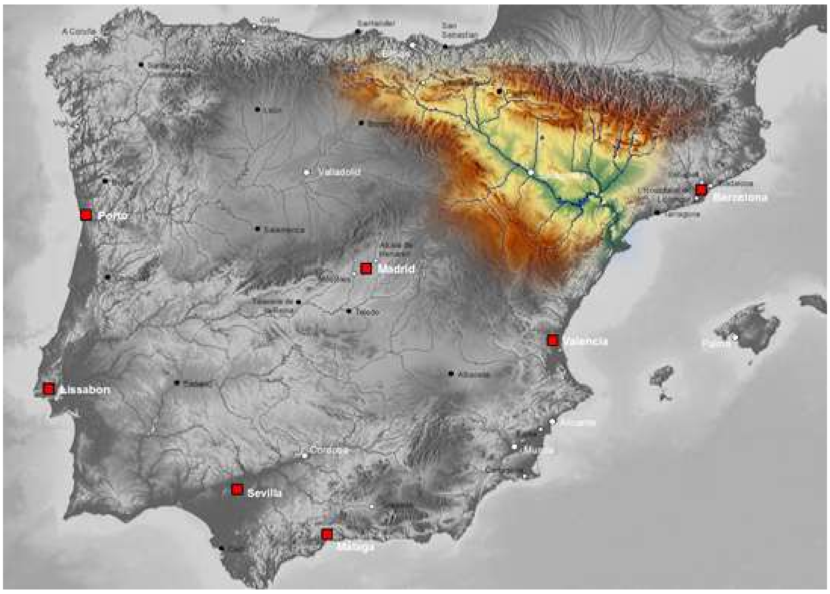

Fig. 1. Ebro river basis (MAPA). Adapted from: Confederación Hidrográfica del Ebro (2017).

Maize in the Ebro Valley

Maize (*Zea Mays* L.) is grown in about 130.000 ha in the Ebro Valley, representing 15% of the irrigated area and 36% of Spanish maize production (MAPAMA, 2016). Maize grain yields range from 8 to 18 Mg ha^{-1} (14% moisture) depending, among other factors, on the area, soil type, irrigation system and year (Cela et al., 2013), with some of these yields considered high in comparison with other rainfed production areas of the world. This variability is often not taken into account when recommending fertilization rates and can generate environmental issues.

Livestock production in the Ebro Valley

Intensive pig farming is a very important activity in several countries of Europe, and since 2015 Spain has been the leading European country producer with 29 million head in 2016 (Eurostat, 2017), with the Ebro River Valley comprising 49% of Spanish production (MAGRAMA, 2016). In this area pig slurry (PS) is often (90% of cases) applied to agricultural land because it is the most economical and quickest form of management (Sisquella et al., 2004). The application of PS has several benefits because of its fertilizer value, organic matter input and the improvement of soil quality properties (Zebarth et al., 1999; Biau et al., 2012), but it is also well known that it can lead to environmental problems, such as nitrate leaching to underground water, gaseous losses to the atmosphere or heavy metal soil accumulation (Nicholson et al., 1999). The destiny and dynamics of N applied with PS is complex. PS contains about 70 % inorganic N, in NH_4^+-N form, which rapidly transforms into nitrate and is mainly available for the crop the year of application (Chantigny et al., 2004), but it is susceptible to be lost by volatilization, with incorporation into the soil and the application method used playing a significant role (Schröder, 2005).

Nitrogen importance in maize production

Nitrogen is the most important crop-yield limiting factor in the world, together with water, and is a key element in maize production (Mueller et al., 2012). In this respect, crop optimal N inputs translate directly into yield and economic profits for maize farmers, whereas insufficient N leads to poor yields, and excess N applications can increase environmental contamination due to N volatilization, runoff and leaching (Pikul et al., 2005; Sánchez and Swaminathan, 2005). Data from surveys in the Ebro Valley about N fertilization in maize indicate that farmers apply rates higher than 300 kg N ha^{-1} in >86 % cases (Sisquella et al., 2004; Isidoro et al., 2006). As total plant N uptake is normally between 250 to 300 kg ha^{-1} (Berenguer et al., 2008; Cela et al., 2011), there is a high risk of not using N efficiently.

The nitrate leaching problem

At present, one of the most important problems affecting intensive agriculture in different parts of Europe is underground water pollution due to N leaching. For this reason, an EU Nitrate Directive (European Union, 1991) has limited the amount of N that can be applied to agricultural land. In many irrigated areas of the Ebro Valley, underground waters are frequently polluted with nitrate, often with concentrations above 50 mg NO^-_3 L^{-1} (Ferrer et al., 1997), the maximum concentration allowed by the European Union (1991). This contamination affects 41% of aquifers in Catalonia (García, 2016). Consequently, as in many other regions of the EU, some areas of the Ebro Valley have been declared zones vulnerable to nitrate leaching. In these areas, no more than 170 kg N ha^{-1} yr^{-1} from organic materials can be applied to agricultural land, and in most of these areas no more than 200 kg N ha^{-1} yr^{-1} of mineral fertilizer can be applied to maize fields (DOGC, 2009).

Improving nitrogen use efficiency

Indicators play a key role in management and policy. Indicators need to have an analytically sound basis and sound underpinning, because managers and policy makers need reliable data and information as well as robust tools to be able to make the right analyses, decisions and actions. Nitrogen use efficiency (NUE) is a key indicator in agriculture, but until very recently there was no uniform and robust methodology and protocol for its estimation and use. Most studies have been undertaken in crop production (Ladha et al., 2005; Fixen et al., 2014). Improving the NUE of crops is one of the most effective means of increasing productivity and profitability while reducing environmental damage (Cassman et al., 2003; Davidson et al., 2015). NUE affects many of the recently proposed/defined Sustainable Development Goals (SDGs) for the post-2015 era, recently accepted by 193 countries of the United Nations General Assembly (SDSN, 2015). A common definition of NUE in crop production is the ratio of yield to the amount of available soil N (Moll et al 1982; López-Bellido et al., 2005). However, an alternative easy-to-use indicator for NUE has recently been reported and approved by the European Nitrogen Expert Panel (EUNEUP, 2015), namely NUE = N output/N input.

Some assessment methods have been implemented for the mitigation of N losses in agriculture (Cuttle et al., 2007), with the most important of these being land use soil management, livestock management, farm infrastructure and fertilizer management (including manure). With respect to fertilizer management, there are six main strategies that can be used to limit loss of nitrate-N (Shcarf, 2015):

1. Apply N at rates that do not exceed crop needs
2. Manage irrigation systems to reduce loss potential
3. Apply N at a time when loss before crop uptake is unlikely
4. Use a nitrogen source that converts more slowly to nitrate
5. Use nitrification inhibitors with nitrogen fertilizer applications
6. Trap unused or escaping nitrate-N

In this thesis, different rates and combinations of mineral N and organic fertilizer in maize production will be assessed in order to provide farmers with decision support tools to avoid overfertilization or yield penalties, while optimizing NUE and minimizing possible nitrate leaching. Determination of soil N levels before fertilizer applications and after harvesting, in-season or end-of-season monitoring of N status in plants and determination of plant N uptakes need to be studied in the irrigated conditions of the Ebro Valley.

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GENERAL OBJECTIVES

GENERAL OBJECTIVES

The main objective of this thesis is to contribute to the optimization of N fertilization of high yielding irrigated maize. The aim is to improve nitrogen use efficiency (NUE) and thereby reduce soil and environmental impacts. Two field experiments were conducted growing maize under Mediterranean conditions in the Ebro Valley (northeast Spain). Both experiments began in 2002 and finished in in 2015.

The field experiments consisted of assessing the long-term effects mineral N fertilization and combinations of pig slurry and mineral N applications in sprinkler irrigation conditions.

The main objectives of the study were:

1. To assess the effects over the time course of the study of different rates of mineral N fertilizer and of pig slurry, on maize yield, NUE and soil N and organic C.

2. To determine the amount of N needed (from organic and mineral source) to obtain satisfactory yields in irrigated conditions, and the implications of complying with the current legislation about N fertilization in the study area.

3. To determine the potential risk of Cu and Zn soil accumulation as a result of long-term pig slurry applications.

This document consists of three independent chapters presented in the format of a journal article. For this reason, some parts, such as the Materials and Methods sections, may contain some repetitions.

Some of these chapters have already been accepted for publication in scientific journals while others are currently under revision.

CHAPTER I

LONG-TERM EFFECTS OF MINERAL NITROGEN FERTILIZER ON IRRIGATED MAIZE AND SOIL PROPERTIES

Long-term effects of mineral nitrogen fertilizer on irrigated maize and soil properties

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Abstract

Nitrogen (N) is a key determinant of growth and grain yield in maize (*Zea mays* L.) and is therefore economically and environmentally important. We investigated the performance of maize crops in a 12-year experiment (2002-2007, 2010-2015) under sprinkler irrigation in a petrocalcic calcixerept soil in northeast Spain, with controlled mineral N application rates (0, 100, 150, 200, 250, 300 and 400 kg N ha^{-1} yr^{-1}). The application rate affected maize GY, biomass, N uptake, SPAD units, soil N levels, N efficiencies and soil organic carbon (SOC). Average maximum GY's (~15 Mg ha^{-1}) required 203 kg N ha^{-1} of available N (defined as initial soil NO_3^- plus N fertilizer) in the 0–30 cm horizon, confirming the importance of the soil N content. Nitrate levels in the 0–30 cm horizon for maximum yields achieved a R^2 value in the plateau fitting model similar to the 0–60 and 0–90 cm horizons. GY's increased at a rate of 192 kg ha^{-1} yr^{-1}, suggesting a combination of genetic improvement in the hybrids and also an improvement in agronomic management. The 200 kg N ha^{-1} fertilizer treatment achieved almost the highest GY's (~14 Mg ha^{-1}) with simultaneous high nitrogen use efficiency (0.83 kg kg^{-1}). Mineral N fertilization also increased the stock of SOC in the 0–30 cm horizon.

1. Introduction

Maize is the second most important crop globally in terms of cultivation area and revenue, and it is therefore important to understand in detail how this crop responds to production inputs (Desjardins, 2014). Nitrogen (N) is the most intensively studied fertilizer component because it is essential for plant growth and has a major impact on yield (Cardwell, 1982). Optimal N inputs translate directly into profits for maize farmers (Pikul et al., 2005) whereas insufficient N leads to poor yields, poor human nutrition, and soil degradation (Sánchez and Swaminathan, 2005).

N fertilization represents ~20% of overall maize production costs in high yielding areas under sprinkler irrigation (Lloveras and Cabases, 2014). However, excess N applications lead to environmental contamination due to N volatilization, runoff and leaching. Nitrates are one of the most common forms of groundwater contamination in Europe. To reduce nitrate pollution, the Nitrates Directive limits the amount of N that can be applied to agricultural land in European vulnerable zones (Directive 91/676/CEE).

Maize crops show variable responses to the application of N fertilizer due to differences in genotype, soil characteristics and climate in different regions and seasons (Hanway, 1962; Karlen et al., 1987; Cerrato and Blackmer, 1990; Berenguer et al., 2009). Most crops incorporate less than 50% of N inputs (Fageria and Baligar, 2005) so the improvement of N utilization is an important agronomic, economic and environmental goal (Zhang et al., 2015). This is facilitated by the assessment of long-term responses to N in different maize agricultural systems, particularly by investigating N availability and N use efficiency (NUE) in order to optimize the grain yield (GY). NUE is usually defined as the ratio of N in the harvested product to the amount of N supplied as fertilizer (Quemada and Gabriel, 2016) or the ratio of GY to the amount of available soil N (Moll et al 1982; López-Bellido et al., 2005). NUE affects many of the recently proposed/defined Sustainable Development Goals (SDGs) for the post-2015 era, recently accepted by 193 countries of the United Nations General Assembly (SDSN, 2015). Improving the NUE of crops is one of the most effective means of increasing productivity and profitability while reducing environmental damage (Cassman et al., 2003; Davidson et al., 2015).

Modern maize hybrids have become more productive but the grain N concentration (GNC) has generally declined (Duvick, 2005; Ciampitti and Vyn, 2013). N availability affects both these factors, so the development of strategies for the simultaneous optimization of GY and GNC will have an important impact on future global food security and nutritional quality (Onimisi et al., 2009). The combination of maize stover and N fertilizer can also influence soil organic carbon (SOC) levels (Biau et al., 2012; Karlen et al., 2011). The removal of stover causes a progressive decline in yields by reducing the level of SOC (Wilhelm et al., 2004).

In the Ebro Valley (northeast Spain) maize is grown under Mediterranean conditions with irrigation and the range of average GY is 9–17 Mg ha^{-1} (Cela et al., 2013). The Ebro Valley is characterized by semi-arid Mediterranean conditions with inceptisols and entisols soils. Some literature has already been published concerning the effects of N on maize GY under irrigated conditions in the area (Villar-Mir et al., 2002; Berenguer et al. 2009; Isla and Quílez, 2011; Cela et al. 2013). Cela et al. (2013) reported a soil test calibration study of 23 site-years and a plateau fitting, showing that maize GY was maximized with 208 kg N ha^{-1} of soil-available N content (defined as initial soil NO_3^- plus N fertilizer) (0–90 cm), in sprinkler-irrigated maize preceded by a cereal crop, with an average GY of 13 Mg ha^{-1}. Berenguer et al. (2009) carried out a 4-year study in the same area, and found that the application of ~160 kg N ha^{-1} was necessary to achieve maximum GY. Similar studies have been carried out in other regions. For example, Blackmer et al. (1989) reported that in Iowa (USA) 20–25 ppm NO_3^- (0–30 cm) was necessary in late spring to achieve the highest GY. Similarly, Cui et al. (2008) studied 1883 fields in the North China Plain and found by plateau fitting that 180 kg Nha^{-1} of soil-available N content (0–90 cm) was necessary to achieve the maximum GY of 8.4 Mg ha^{-1}. Adjusting soil nitrate tests for maize under irrigated conditions could improve NUE and profits while minimizing nitrate leaching caused by excess application (Blackmer et al., 1989). Previous research has also shown that chlorophyll meters can reliably indicate N stress in maize, allowing the adjustment of N application rates (Scharf et al., 2006).

The adoption of optimal N application rates is both economically and environmentally beneficial. In the Ebro Valley, agronomic advisors recommend to apply more than 300 kg N ha^{-1} to maize fields with grain yield potential of about 15 Mg ha^{-1} (Villar et al., 2016a) , and we hypothesize that less amount of

N is required to obtain satisfactory yields. In irrigated Mediterranean agrosystems, very few long-term studies have been carried out to determine optimal N inputs in terms of GY, NUE, soil N concentration and the impact on soil quality indicators, to assess the sustainability of mineral N fertilization. Furthermore, these studies should address the medium-term and long-term residual effects of N fertilizers and also allow to make N recommendations based on soil tests. With the purpose of providing information about it, we carried out a 12-year experiment to assess the long-term effects of continuous mineral N fertilization on mono-cropped irrigated maize. At each N level, we measured GY, biomass yield, grain and plant N content and uptake rates, SPAD units, soil N levels (before planting, in late spring and after harvesting) and SOC stocks. Our data will help to optimize maize productivity in the Ebro Valley while minimizing the environmental impact of N fertilizer application.

2. Materials and Methods

2.1. Location and experimental design

A field experiment was conducted over a 12─year period (2002─2007, 2010─2015) at the IRTA research station in Gimenells, northeast Spain (41°65'N, 0°39'E). Its historic (1989─2015) average temperature is 13.9°C (19.4°C during the maize growing season) and its average annual precipitation is 369 mm (184 mm during the maize growing season) (Figure 1) (Generalitat de Catalunya, 2016). The soil is well drained with no salinity problems and is classified as petrocalcic calcixerept (Soil Survey Staff, 2014), with a petrocalcic horizon at 90–100 cm depth (Table 1).

The statistical design was a complete randomized block with four replications. The N treatments were randomized during the first year (2002) and applied to the same plots every year thereafter. The experimental plot size was 165 m^2 (15 x 11 m). Maize was grown throughout the study period (2002–2015), although wheat and maize were grown in 2008 and 2009, respectively, without N fertilization, and these 2 years were not considered in the analysis. The fertilization treatments consisted on seven N rates: 0, 100, 150, 200, 250, 300 and 400 kg N ha^{-1} (hereafter N0, N100, N150, N200, N250, N300 and N400, respectively). The N400 treatment was applied only in the last 2 years, when the maize yields were likely to respond to applications higher than 300 kg N ha^{-1}. N was applied as ammonium nitrate (34.5% N). During the first 6 years, 50 kg N ha^{-1} was applied before planting and the rest was applied as two equal side dressings. Thereafter, all N input was applied as two equal side dressings, first at the V3–V4 stage and second at the V5–V7 stage (Ritchie and Hanway, 1982). Phosphate and/or potassium oxide were applied before planting every 1-3 years, at rates of 150 kg P_2O_5 ha^{-1} and 250 kg K_2O ha^{-1}. Levels of P and K were always kept above 25 mg kg^{-1} (Olsen method) and 200 mg kg^{-1} (Ammonium acetate method), respectively, assuring there was not a lack of these elements for maize production (Villar-Mir and Villar-Mir, 2016b). In the previous 10 years of experiment (1991−2001) wheat was grown in the experimental field, except in 2000, in which vetch was grown and incorporated into the soil.

2.2. Maize management

Maize was planted during the first or second week of April (depending on the year) at a density of 8 plants per m2 in 2002, increasing to 9.5 plants per m2 in 2015, with a row width of 0.72 m. Different maize cultivars for grain production were used during the 12 years of the experiment, with a new cultivar introduced every 2 years according to the commercial maize catalogues of the seed companies. All cultivars represented FAO cycles 600–700. The hybrid 'Dracma' (Funk's) was planted in 2002 and 2003, 'Sele' (Monsanto) in 2004 and 2005, 'Helen' (Limagrain) in 2006 and 2007, DKC6666 (Dekalb) in 2010 and 2011, Lerma (Fitó) in 2012 and 2013, and PR33Y72 (Pioneer) in 2014 and 2015. Hybrids were chosen according to regional technical reports presenting the Official Variety Trials results (Betbesé i Lucas et al., 2006; Serra et al., 2007; López et al., 2009; Serra et al., 2010). Harvesting took place during September/October after the plants reached physiological maturity. Maize stover was chopped and removed after harvest in the first 4 years of the experiment and incorporated into the soil in the remaining 8 years. A pre-emergence herbicide (1 L ha^{-1} 96% metolachlor and 3 L ha^{-1} 47.5% atrazine) was applied to control weeds for the first 6 years of the experiment, whereas 3.3 L ha^{-1} Trophy® (40% acetoclor plus 6% diclormid) was applied for the last 6 years. We also applied 1 L ha^{-1}

Banvel® (20% fluoxypyr) as a post-emergence herbicide to control *Abutilon theophrasti* Medik, if necessary.

Maize was sprinkler-irrigated with approximately 6000 m^3 ha^{-1} yr^{-1} of water, depending on the climatic conditions of the growing period in each year. Given rain and irrigation, the maize received approximately 6500–7500 m^3 ha^{-1} of water during the growing period (April to September). The irrigation water was of good quality and did not contain significant amounts of nitrates.

2.3. Measurement of performance parameters

GY was measured by harvesting two central rows (1.4 × 15 m) of each plot (15 x 11 m) with an experimental plot combine. Grain moisture was determined from a 300-g sample taken from each plot with a GAC II (Dickey-John, Auburn, IL, USA) and the GY was adjusted to 14% moisture. Subsamples were taken and GNC values were determined by near infrared (NIR) spectroscopy, using a previously calibrated 500 Infrared Analyser (Bran+Luebbe, Norderstedt, Germany). Total grain N uptake was calculated by multiplying the GNC and GY values. The aboveground biomass yield was determined at physiological maturity by harvesting plants from two central rows (1.4 x 4 m) at ground level. Subsamples were chopped and dried to determine the dry matter (DM) content of the biomass and whole-plant N concentrations (by NIR spectroscopy as above). The total plant N uptake (N_{plant}) was calculated by multiplying plant N concentration by the biomass production. SPAD units for 10 plants per plot were measured on the ear leaf at silking stage R1 using a Minolta SPAD-502 manual leaf chlorophyll meter.

Soil samples for the determination of NO_3^--N levels were taken before planting (N_{ini}) and after harvesting (N_{res}) throughout the experimental period, at a depth of 0–90 cm from three consecutive horizons (30 cm each), and at pre side-dress at a depth of 0-30cm. Soil samples from each plot contained a mixture of five cores per plot. Soil nitrates were extracted with deionised water and measured using test strips with a Nitrachek® device calibrated according to the standard procedure (Bischoff et al., 1996). NH_4^+−N levels were not taken into account because the quantity of NH_4^+ in the soil is negligible compared to NO_3^- levels, based on our previous research and other studies of the same region (Villar-

Mir et al., 2002; Berenguer et al., 2009). Subsamples were used to measure the SOC content at the beginning of the experiment in 2002 and at the end in 2015. SOC was measured by dichromate oxidation (Walkley-Black method; Allison, 1965) and consisted of a mix of five soil samples per plot from the 0–30 cm soil horizon.

The inclusion of a control treatment without N fertilization (N0) allowed to estimate the amount of N supplied by the soil (N_{min}) in the absence of N fertilization. The N_{min} value includes mineralized N, N provided by biological fixation, N provided by atmospheric deposition, and ammonia released from the interlaminar spaces within the clay. N_{min} was calculated for the control plots (N0) using the following equation (Jarvis et al., 1996): $N_{min} = N_{res} + N_{plant} - N_{ini}$. NUE, NUE for grain yield (NUE_{gy}), the apparent nitrogen recovery fraction (ANR) and the agronomic nitrogen efficiency (ANE) were calculated for each plot from 2003 to 2015 (Moll et al., 1982; Fageria and Baligar, 2005; López-Bellido et al., 2005; Zhang et al., 2015; EUNEP, 2015). NUE (kg kg^{-1}) was defined as the ratio between N output (in the grain) and N input (fertilizer). NUE_{gy} (kg kg^{-1}) was defined as the ratio of GY (14%) to N supply, where the N supply was the sum of the soil inorganic N at planting (N_{ini}) (0–90 cm), the mineralized soil N (N_{min}) and the total amount of N applied as mineral fertilizer. ANR (kg kg^{-1}) was defined as the ratio of plant N uptake at Nx – plant N uptake at N0, to the amount of N applied with fertilizer at Nx. ANE (kg kg^{-1}) was defined as the ratio of biomass at Nx − biomass at N0, to N applied with fertilizer at Nx.

Soil nitrogen availability was determined from the soil pre-planting nitrogen (PPNT) content and also from pre-side dressing nitrogen content (PSNT) (maize at the V3 stage) plus the N fertilization rate (N_{fert}) (Schröder, 2000). The relative grain yield (RY) was calculated every year as the ratio between $yield_{plot}$ and $yield_{max}$, where $yield_{plot}$ is the yield of each plot and year, and $yield_{max}$ is the highest yield of all plots in a given year (Blackmer et al., 1989).

2.4. Statistical analysis

The response models used in this study were the linear with plateau (LP) model (Cerrato and Blackmer, 1990) and the quadratic model (Chen et al., 2010). Other fitting models were tested but these models fitted the data best (had the highest determination coefficients). LP models were fitted to model the response of relative yield, and grain and plant N content to available N using the data of individual plots of the 12 years. Quadratic model was sued to establish the relation between residual N and available N. Significant difference between treatments was determined by analysis of variance using the Mixed Model in the Statistical Analysis System (JMP 12, SAS Institute 2012). N rate and year were set as fixed factors and the replication as a random effect. Means were separated by LSMeans Tukey's HSD test ($p < 0.05$) where levels not connected by the same letter are significantly different.

3. Results and discussion

3.1. Grain and biomass yields

The GY during the 12-yr experimental period for each N fertilization treatment is presented in Figure 2. The GY varied greatly among the different N treatments, and also depended on the year. The average yields during the entire study period ranged from 6.8 Mg ha^{-1} for the N0 treatment to 17.4 Mg ha^{-1} for the N400 treatment. The lowest N fertilization rate sufficient to achieve optimal yields was 200 kg N ha^{-1} (Table 2, Figure 2) which differs from the 150 kg N ha^{-1} reported by Berenguer et al. (2009) for the first 4 years of their experiment. The N200, N250 and N300 treatments achieved statistically similar GY values throughout the study period (Figure 2).

In the first year of the experiment there were no significant differences among the N fertilization treatments because the initial soil N content was high. After 3 years (2002–2005), the GY in the N0 treatment plots decreased from 13.5 to 5.6 Mg ha^{-1} due to the lack of N fertilization and the consequent decrease in soil mineral N. Thereafter, the GY of the N0 treatment oscillated between 3.9 and 6.4 Mg ha^{-1} depending on the year (Figure 2), with an average of 6.75 Mg ha^{-1} throughout the 12-yr experimental period (Table 2). The N400 treatment achieved a GY of 17.7 Mg ha^{-1} in 2014, statistically different than N300 (Figure 2). In 2015, there was no statistically significant difference in GY between the N400 and N300 treatment plots (17.12 and 16.76 Mg ha^{-1}, respectively). The N300 treatment increased the average 12 year GY by 112% compared to the N0 (control). These maximum yields obtained are higher than those reported in the same region by Villar-Mir (2002) and Berenguer et al. (2009), but similar to those reported by Yagüe and Quílez (2010).

Biomass yields followed a similar profile to the GY, increasing at higher N input rates up to 200 kg N ha^{-1} (Table 2). The N300 treatment produced an average biomass yield of 29 Mg ha^{-1} of DM, compared to 17 Mg ha^{-1} of DM for the N0 treatment. These values are normal for the Ebro Valley region, and similar to those reported by Berenguer et al. (2009). Maize grain and biomass yields varied among treatments from year to year, as indicated by the significant interaction Nitrogen x Year (Table 2 and Figure 2). This variability is higher in a continuous maize cropping systems than in systems featuring crop rotation (Grover et al., 2009).

3.2 Maize yields

In order to estimate the rate of yield gain (grain and biomass) during the 12-yr experimental period, we used N300 as a non-limiting N treatment that achieved the best production. The yield trend was characterized by significant linear slopes (yield change per year), producing values of 192 and 177 kg ha^{-1} yr^{-1} for grain and biomass, respectively (Figure 3). The average GY for the N300 treatment in 2015 was 16.8 Mg ha^{-1}, whereas the initial GY in 2012 was 14.5 Mg ha^{-1}. These results suggest an increase of 2.3 Mg ha^{-1} or 16% between 2002 and 2015, which probably reflects both genetic and agronomic improvement. We changed the hybrids every 2 years, increased the density of cultivation (Lobell et al., 2014), and potentially achieved better fertilization management because the fertilization rates (N300) were the same in all 12 years. The biomass yield also increased throughout the study period albeit with a shallower gradient compared to the GY slope. The harvesting index, defined as the ratio of dry matter GY to dry matter biomass (including grain), increased slightly during the experimental period from 0.40 in 2002 to 0.45 in 2015 (data not shown). GY increases have occasionally been associated with a slight increase in the harvesting index (Lorenz et al., 2010). Lobell et al. (2014) reported a maize yield increase from 1995 to 2012 in the US Midwest due to genetic improvement and increasing plant densities. Duvick (2005a) also reported a linear gain in GY of 75 kg ha^{-1} yr^{-1} from 1935 to 2005 in the US. In the

Ebro Valley, an annual gain of 225 kg ha^{-1} was reported from 1983 to 2006 (Serra et al., 2007). This is similar to the gain we observed during our experimental period.

3.3 Critical soil N concentrations

The average PPNT at 0–30 cm was 50 ± 35 kg N ha^{-1}, whereas the PPNT at 0–60 cm was 100 ± 77 kg N ha^{-1}, the PPNT at 0–90 cm was 129 ± 97 kg N ha^{-1}, and the PSNT at 0–30cm was 34 ± 22 kg N ha^{-1}. The LP fitting models between RY and the soil available N tests were calculated taking these four parameters into account. The R^2 values were very similar, ranging from 0.74 to 0.79 (Figure 4). The four soil N test were useful and reliable under our growing conditions. The data also indicate that sampling the soil to a depth of 60 or 90 cm is unnecessary in our growing conditions because there are little or no improvement in the R^2 value compared to soil samples taken at 30 cm. This phenomenon was also reported by Sims et al. (1995) and Schröder et al. (1998), reflecting the close relationship between the soil mineral N supply in the upper 30 cm and deeper horizons, and the fact that most N is taken up by maize from the upper 30 cm horizon. Nevertheless, Sims et al. (1995) and Schröder et al. (1998) proposed the inclusion of deeper horizons in the sample under certain conditions (not relevant in our experiment) such as lightly textured soils in which precipitation may have moved mineral N to deeper horizons.

The GY increased linearly with increasing N availability until a plateau was reached (Figure 4) and the N availability at this point was defined as the critical nitrogen concentration (CNC). The CNC required to achieve the maximum GY was very similar for the PPNT (0–30 cm) and PSNT (0−30 cm), i.e. 203 and 201 kg N ha^{-1}, respectively, reflecting the low level of N uptake until the V3 stage, i.e. less than 0.5 kg N ha^{-1} per day (Schröeder, 1999). Similar values were also reported by Cela et al. (2013) and Schröder et al. (2000). The CNC required to achieve the maximum GY for the PPNT (0–90 cm) was 269 kg N ha^{-1}. In the same region, Cela et al. (2013) observed a R^2 of 65% between the RY and the available N at 0–90 cm before planting, and the maximum GY was achieved at a CNC value of 208 kg N ha^{-1}, which is lower than our observed result. Isla and Quílez (2011) reported that 244 kg N ha^{-1} was needed at 0–90 cm for maximum GY in their area of the Ebro Valley. The average yields of our maize plots (4–17 Mg ha^{-1}) were higher than in several maize fields in which soil nitrate calibration tests were carried out (Sainz Rozas et al., 2000; Cela et al., 2013). This remarkable outcome indicates that the soil N concentration that separates responsive from non-responsive fields can vary with the observed yield potentials (Fox et al., 1989), thus explaining why we obtained higher CNC values than other authors under irrigated conditions (Schröder et al., 2002, Ferrer et al., 2003). Perhaps the varieties, agronomic practices and more favorable growing conditions in our experiment achieved a higher yield potential, explaining why we observed higher values for the required amount of available N in the soil.

The average N mineralization in the N0 control plots (0–90 cm) was 107 kg N ha^{-1} average of the entire 12-yr experimental period and 84 kg N ha^{-1} if only last 5 years were taken into account. A N fertilization response curve including the soil N mineralization into the available N (soil NO_3^-–N + N fertilizer) did not show an increase in correlation compared to curves that ignored N mineralization.

3.4 Effect of soil nitrogen availability on the N content of the grain and whole plant

The relationships between available N in the soil and the N content of the grain and plant (including stover and grain) are represented in Figure 5. A strong relation was observed for the grain N content but not the plant N content, with R^2 values of 0.49 and 0.17, respectively. Both regressions were significant at $p < 0.01$. The critical available N to achieve maximum grain and plant N contents were 340 and 274 kg N ha^{-1}, respectively. Maximum grain and plant N concentrations were 1.40% and 1.04%, respectively, similar to the values reported by Berenguer et al. (2009) in the same area, and those reported by Chen et al. (2010) in China. In the latter case, a linear plateau fitting model was also used and the relations were marginally better. In Germany, under rain-fed conditions, Herrmann and Taube (2005) reported a highest plant N concentration at silage maturity of 1.05%, which is also similar to our results.

3.5 Grain and whole plant N uptake

The uptake of N into the grain and whole plant increased with increasing N application rates and varied from year to year (Table 2), in agreement with previous studies under similar and dissimilar conditions (Cox and Cherney, 2001; Derby et al., 2005, Halvorson et al., 2006, Daudén and Quílez, 2004). The average N uptake into the grain in our experiments ranged from 69 to 178 kg N ha^{-1} for the N0 and N300 N fertilization treatments, respectively. The highest N uptake into the plant was 304 kg N ha^{-1} under the N300 treatment, which may reflect the larger amount of aboveground biomass in these plots (Table 2). Similar types of response were observed for grain and plant N uptake (Table 2). Increases in N concentration or plant N uptake was not necessarily related to increased yields (e.g. in the N200 treatment). These results suggest some degree of luxury N consumption. The N uptake by the plant required to produce 1 Mg ha^{-1} of dry grain differed among the treatments, ranging from 21.3 kg N Mg^{-1} in the N0 control to 23.3 and 24.7 kg N Mg^{-1} in the N200 and N300 treatments, respectively (data not shown). These results are similar to previous reports, with values ranging from 21 to 30 kg N Mg^{-1} (Olson and Sander, 1988; Roberts, 2007). The N concentrations in our grain ranged from 1.07% in the N0 control to 1.35% and 1.38% in the N200 and N300 treatments, respectively, similar to the values reported by Berenguer et al. (2009).

3.6 Profile of residual N levels in the soil

The average soil NO_3^-–N levels in the N0 and N300 treatments were 76 and 179 kg N ha^{-1} respectively before planting, and 70 and 270 kg N ha^{-1} respectively after harvesting (Table 2). These values indicate that significant amounts of NO_3^-–N can be found in the soil at the beginning of the maize growing season (given the normal precipitation in winter conditions) and at the end. The dynamic profiles of residual (after harvest) soil NO_3^-–N levels in the 0–90 cm horizon are presented in Figure 6. Both the initial and residual soils N levels varied from year to year and were influenced by the different treatments (Table 2 and Figure 6). There was a quadratic relation between the amount of N available before planting and the residual N content (Figure 7). In the first year of experiment, the initial N content was very high in all plots and all treatments resulted in similar values of residual N (400–600 kg N ha^{-}

^{1}) except the N0 control, with residual N levels of 184 kg N ha^{-1} (Figure 6). This high initial value can be explained by the incorporation of a vetch crop into the soil in the year 2000, and the cultivation of a wheat crop the following year. From 2002 onwards, the residual N content declined in all plots, especially those treated with 150 kg N ha^{-1} or lower. The N300 treatment always resulted in the highest residual N values, except for the last 2 years of the experiment, when the N400 treatment was included. The N400 treatment resulted in residual N values of 335 and 382 kg N ha^{-1} in 2014 and 2015, respectively. Similar values have been reported in the same area by others (Villar-Mir et al., 2002; Vázquez et al., 2006, Berenguer et al., 2009; Yagüe and Quílez, 2013), and also in the US (Halvorson, 2005). The N0, N100 and N150 treatments resulted in similar residual N values after 3 years so the risk of nitrate leaching under these three conditions in winter would be very comparable.

As stated earlier, the N200 treatment was the lowest input to achieve optimal GY (Table 2). Because this treatment also resulted in moderate initial and residual soil N levels, it could be the most suitable treatment to meet yield requirements while also protecting the environment, especially in our N vulnerable zones (Generalitat de Catalunya, 2009).

The average loss of N during winter (i.e. the difference between the initial NO_3^-–N content before sowing and the residual content after harvesting the previous year) ranged from –9 to –131 kg N ha^{-1} in the N0 and N300 treatments, respectively (the first year of the experiment was excluded).

3.7 Efficiency of nitrogen utilization

The NUE, NUE_{gy}, ANR and ANE values were dynamic at the beginning of the experiment but reached effectively constant levels after the first 2 years (data not shown). The NUE and NUE_{gy} values declined during the first 2 years and then stabilized. In contrast, the ANR and ANE values initially low due to the high initial soil N content, promoting N uptake and biomass production even under the control treatment (N0). The first year the ANR ranged from –0.03 to 0.29 kg kg^{-1} and the ANE from –3.9 to 22.4 kg kg^{-1} (Berenguer et al., 2009). The average NUE ranged from 0.59 to 0.83 kg kg^{-1} in the treatments achieving the highest GY's (N200, N250 and N300), meaning that 59–83% of the nitrogen added to the system is present in harvested products (EUNEP, 2015). Quemada and Gabriel (2016) reported a very similar range of NUE values representing irrigated maize in central Spain. Our NUE values are higher than the typical 50% or lower recovery of N reported by Fageria and Baligar (2005) based on global crops. In our field trials, the highest NUE_{gy} value was 39.9 kg kg^{-1} (N0) but the N200 treatment achieved the highest GY (13.62 Mg ha^{-1}) with a relatively high NUE_{gy} of 31.9 kg kg^{-1}. These NUE_{gy} values are similar to those observed in other experiments. For example, Di Paolo and Rinaldi (2007) reported a NUE_{gy} value of 18.6 kg kg^{-1} at 300 kg N ha^{-1} for a maize crop in Italy, but their GY's were lower than ours (10 Mg ha^{-1}). If we consider crops with a similar yield to ours, Barbieri et al. (2008) reported a NUE_{gy} value of 40.7 kg kg^{-1} for a treatment without N fertilization in Argentina, which is highly similar to our observations. It is notable that our agronomic conditions (sprinkler irrigation and all N applied as side dressing in two applications in most years) promote efficiency N utilization. We observed differences in the average ANR value between treatments but the differences were not statistically significant. The ANE decreased at higher N application rates and ranged from 41 to 61 kg kg^{-1} in the N100 and N300 treatments, respectively, similar to the values reported by Vanlauwe et al. (2011). Few studies report the NUE, NUE_{gy}, ANR and ANE values of maize under our conditions, with GY's of up to 12 Mg ha^{-1}. The Catalan Decree 136/2009 (Generalitat de Catalunya, 2009) controlling the fulfillment of the EU Nitrates Directive allows the application of 200 kg N ha^{-1} yr^{-1} to maize fields in the study area, which coincides with the application rate required for relatively high NUE.

3.8 Soil organic carbon

SOC levels increased across all fertilization treatments (Table 3). The initial SOC content (0–30 cm horizon) in 2002 was 53.6 Mg ha^{-1}. After 12 years, this had increased by 5.2 Mg ha^{-1} in the N0 treatment, by 7.31 Mg ha^{-1} in the N100 treatment and by 12.7 Mg ha^{-1} in the N300 treatment. The treatments ranked as follows in terms of their ability to increase the SOC: N200 > N250 > N300 > N150 > N100 > N0. However, there were no significant differences between applications of 150 kg N ha^{-1} or higher, and all these treatments resulted in SOC sequestration rates of ~1 Mg ha^{-1} yr^{-1} (Table 3) showing that N fertilization together with stover incorporation could be used to maintain or even improve the SOC content. Purakayastha et al. (2008) also reported increases in SOC levels in their N0 control treatment which may reflect the better root and aboveground biomass production compared to the years preceding the experiment (Izaurralde et al., 2000).

SOC stocks in Mediterranean areas are rarely reported at the regional scale, in our case the Ebro Valley. Álvaro-Fuentes et al. (2011) modeled the SOC stocks and changes in agricultural soils in the Ebro Valley, reporting a value of 46 Mg ha^{-1} in the 0–30 cm horizon for irrigated arable land. Martínez et al. (2016) reported a SOC sequestration rate of 0.54 Mg ha^{-1} yr^{-1} for maize crops in irrigated sandy soils in the Ebro Valley supplied with mineral fertilizer. Appropriate N application rates of 200 kg N ha^{-1} or higher (Table 2) not only improve GY but also contribute to the sequestration of atmospheric CO_2 into SOC by promoting plant growth.

3.9 SPAD units

There is a close relationship between SPAD units and relative grain yields (Piekielek et al., 1995; Schröder et al., 2000; Rostami et al., 2008) and SPAD meter readings correlate strongly with leaf N levels as well as leaf chlorophyll levels (Wood et al., 1992; Waskom et al., 1996). Our SPAD meter readings were able to detect N deficiency but no N excess, probably because not all N is converted into chlorophyll when large amounts of N are available (Dwyer et al., 1995; Varvel et al., 1997a). The lineal plateau model appeared to be reliable in our experiment (Figure 8). Statistical differences in SPAD units between treatments at the silking R1 stage were not observed between N rates equal or higher than 150 kg N ha^{-1} or higher (Table 2). We observed a linear increase in relative yield as SPAD-units increased until reaching 59.3 SPAD units, at which point the RY reached the plateau. Our measurements were higher than the optimum of 52–56 SPAD units initially reported by Piekielek et al. (1995), and fall within the optimum 50–61 SPAD units range as reported by Berenguer et al. (2009) and Malek (2015) for applications of 150 kg N ha^{-1} or higher. This is also very close to the plateau point of 58 SPAD units reported by Dwyer et al. (1995). These differences between reports may reflect differences in the experimental parameters such as different maize hybrids (Schepers et al., 1996; Sunderman et al., 1997), planting times (Jemison and Lyttle, 1996), GY's and site year effects (Blackmer and Schepers, 1995;

Varvel et al., 1997b). The portable chlorophyll meter can be used to diagnose N sufficiency in irrigated maize, but soil tests are essential to determine the precise amount of N required and thus to optimize GY.

4. Conclusions

During the 12-yr experiment, the amount of available N (soil NO_3^-−N + N fertilization) required before planting or side dressing to achieve the maximum GY of ~14 Mg ha^{-1} was an average of ~200 kg N ha^{-1} in the 0–30 cm horizon. The measurement of N availability before planting or side-dressing in the 0–30 cm horizon therefore appears suitable and very important for the specification of optimal N fertilization application rates for maize cultivated with sprinkler irrigation under Mediterranean conditions, and is just as accurate as sampling to 60 or 90 cm depth.

The initial and residual N levels in the soil were depleted during the experimental period when low levels of N fertilizer were applied. This reduction was less significant in plots receiving more than 200 kg N ha^{-1}, where the initial and residual N levels in the soil increased with increasing N application rates, showing the importance of adjusting the N rates if for reduction of residual N at harvesting. Soil mineralization in the 0–90 cm horizon was 107 kg N ha^{-1} over the whole 12 year period, and 84 kg N ha^{-1} yr^{-1} when considering only the last 5 years of experiment.

The SOC increased in all plots but the levels increased more when mineral N fertilizer was applied. An application rate of 150 kg N ha^{-1} or more resulted in a SOC sequestration rate of ~1 Mg C ha^{-1} yr^{-1}. Mineral N fertilization combined with stover incorporation should therefore be considered as a strategy to improve or maintain soil quality.

The treatment of 200 kg N ha^{-1} was sufficient to produce optimal GY while also achieving relatively high NUE (0.83). This N rate is similar to the maximum N application rate allowed under current local legislation (Generalitat de Catalunya, 2009) and is therefore sufficient to produce high maize GY's in our irrigated conditions.

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Table 1. Main soil properties at the beginning of the experiment (2002).

	Horizon depth (cm)		
	Ap	Bw	Bk
	0-23	23-69	69-117
Sand, g kg^{-1}	390	380	450
Silt, g kg^{-1}	400	420	380
Clay, g kg^{-1}	210	200	170
pH*	8.3	8.3	83
Ec, dS m^{-1}	0.2	0.34	0.59
CEC, cmolc kg^{-1}	13	-	-
Organic carbon, g kg^{-1} **	13	8	4
Bulk density, g cm^{-3}	1.40	1.56	1.63
Available water holding capacity, mm	29	67	54
P, mg kg^{-1} ***	31	-	-
K, mg kg^{-1} ****	217	-	-

* Water (1:2.5)

** Walkey-Black method

*** Olsen method

**** Ammonium acetate method

Table 2. Effect of N fertilization rates on average (2002-2007, 2010-2015) grain yield, biomass yield, grain N uptake (up), plant N uptake (up), chlorophyll SPAD-Units, soil NO_3^--N before planting and after harvesting, nitrogen use efficiency (NUE), grain yield NUE (NUE_{gy}), apparent nitrogen recovery fraction (ANR) and agronomic nitrogen efficiency (ANE).

N rate (kg N ha^{-1})	Grain Yield (Mg ha^{-1})	Biomass Yield (Mg ha^{-1})	Grain N uptake (kg N ha^{-1})	Plant N uptake (kg N ha^{-1})	SPAD-Units	Soil initial N (kg N ha^{-1})	Soil residual N (kg N ha^{-1})	NUE (kg kg^{-1})	NUE_{gy} (kg kg^{-1})	ANR (kg kg^{-1})	ANE (kg kg^{-1})
0	6.8 d	16.6 d	69 d	124 e	40.3 c	76 c	70 d	-	39.9 a	-	-
100	11.1 c	22.7 c	120 c	189 d	52.9 b	102 bc	112 cd	1.20 a	37.6 ab	0.63 a	61 a
150	12.9 b	25.6 b	152 b	239 c	55.7 ab	107 bc	121 cd	1.01 b	37.2 ab	0.75 a	60 a
200	13.6 ab	26.9 ab	166 ab	273 b	57.0 a	145 ab	165 bc	0.83 c	31.9 b	0.74 a	52 ab
250	13.9 ab	27.8 a	170 a	283 ab	56.6 a	163 a	213 ab	0.68 d	28.0 c	0.63 a	45 b
300	14.3 a	28.9 a	178 a	304 a	57.7 a	179 a	270 a	0.59 d	25.6 d	0.60 a	41 b
Nitrogen	**	**	**	**	**	**	**	**	**	**	**
Error A	-	-	-	-	-	-	-	-	-	-	-
Year	**	**	**	**	**	**	**	**	**	**	**
Nitrogen*Year	**	**	**	**	**	**	**	**	**	**	**

** Significant at the 0.01 level. Letters show LSMeans separation by Tukey HSD Test.

Table 3. Soil organic carbon (SOC) stock and sequestration rate in the 0-30cm soil horizon at the end of the experiment (2015).

N rate (kg N ha^{-1})	SOC (Mg C ha^{-1}) (0-30cm)	
	Stock (2015)	Sequestration rate [a] (yr^{-1})
0	58.83 c	0.43 c
100	60.90 bc	0.61 bc
150	65.94 ab	1.03 ab
200	68.05 a	1.21 a
250	66.78 ab	1.10 ab
300	66.28 ab	1.05 ab
Nitrogen	**	**
Block	**	**
Nitrogen x Block	**	**

[a] Calculated according to an initial soil organic carbon stock of 53.59 Mg ha^{-1} measured in 2002.

Figure 1. Annual precipitation and mean annual temperature for the experimental period (2002−2007, 2010−2015) with the mean values for the experimental period and for the historical period (1989-2015). Maize Growing Period Precipitation (GP P), Winter Period Precipitation (WP P), Maize Growing Period Temperature (GP T) and Winter Period Temperature (WP T). Growing Period is from April to September and Winter Period from October to March.

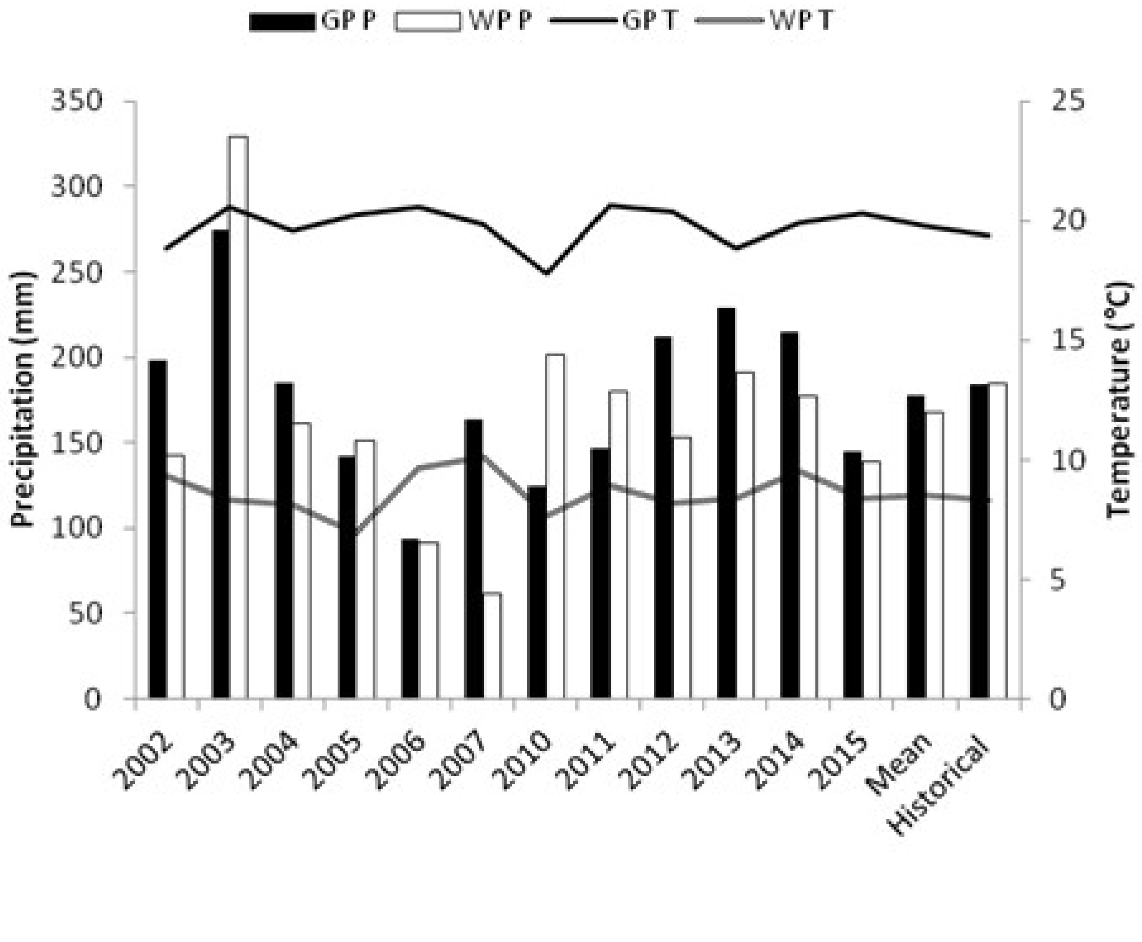


Figure 2. Effect of N rate on grain yield through the experimental period (2002−2007, 2010−2015).

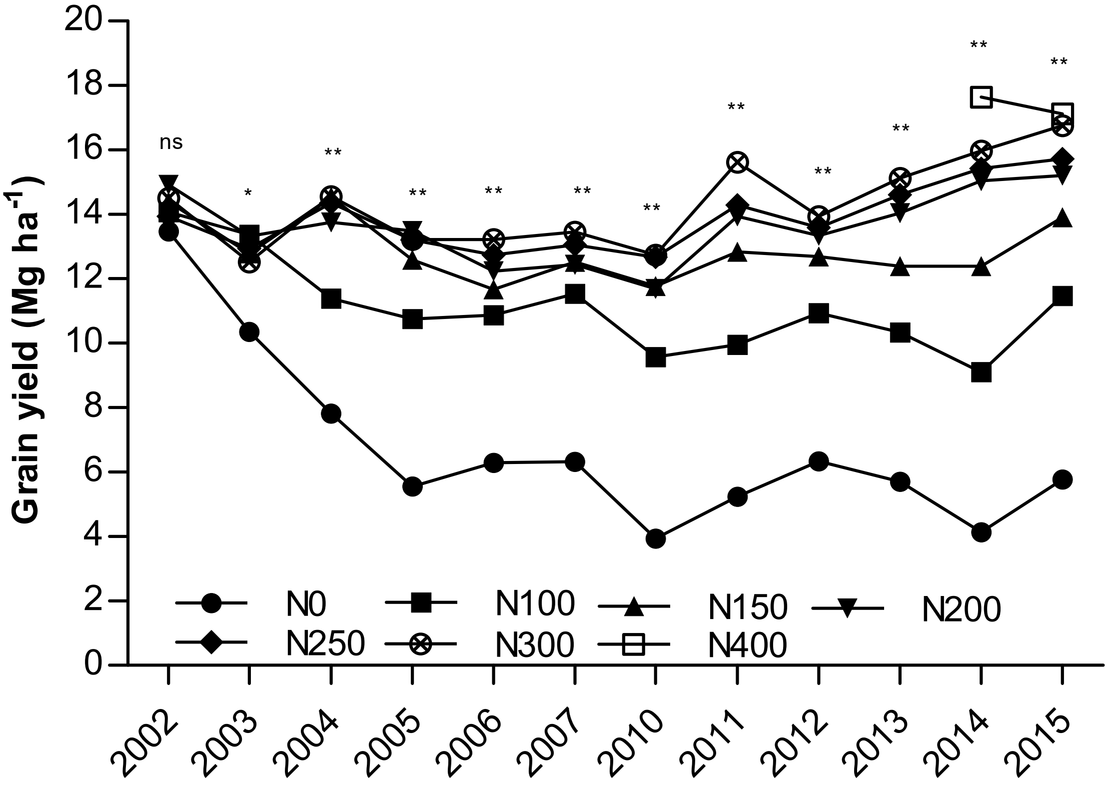


Figure 3. Grain yield (GY) (14%moisture) and dry matter biomass yield (BY) (0% moisture) of the N300 treatment (300 kg N ha^{-1} yr^{-1}) through the experimental period (2002−2007, 2010−2015). * Significant at the $p<0.05$ level.

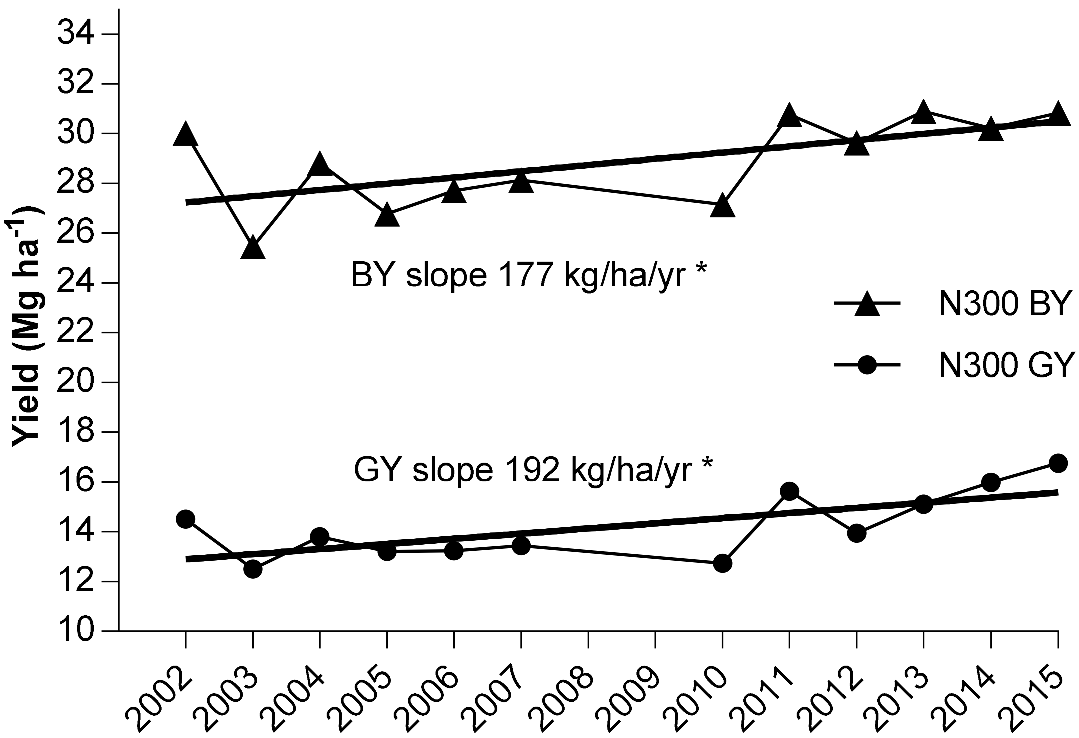


Figure 4. A, B, C, D. Relationship between relative maize grain yields and soil available-N (soil N + N fertilizer) calculated for four data sets. All regressions were significant at $p < 0.001$. CNC: Critical nitrate content for maximum relative maize yields ± standard error. PPNT: Pre Planting Nitrogen Test. PSNT: Pre Sidedress Nitrogen Test.

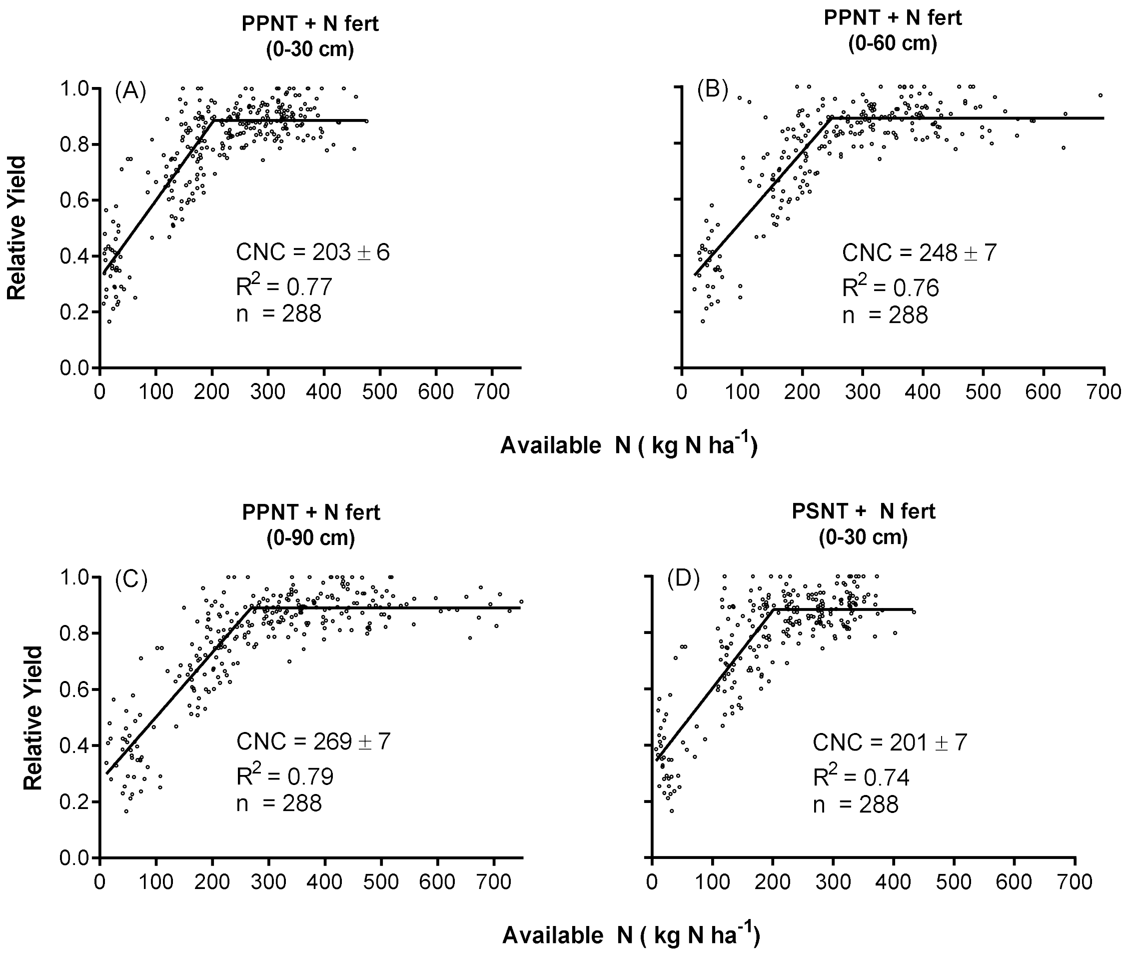


Figure 5. A, B. Relationship between grain and plant N content and soil available-N calculated with soil N before planting in the first 90 cm plus N fertilizer. CNC: critical nitrate content for maximum N concentrations ± standard error.

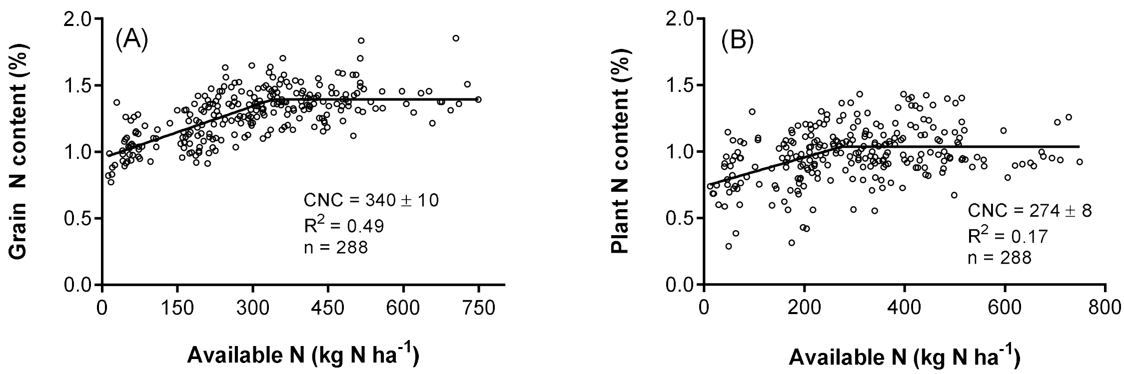


Figure 6. Effect of N fertilization rates on residual soil N content (after harvesting 0-90cm) through the experimental period (2002−2007, 2010−2015).

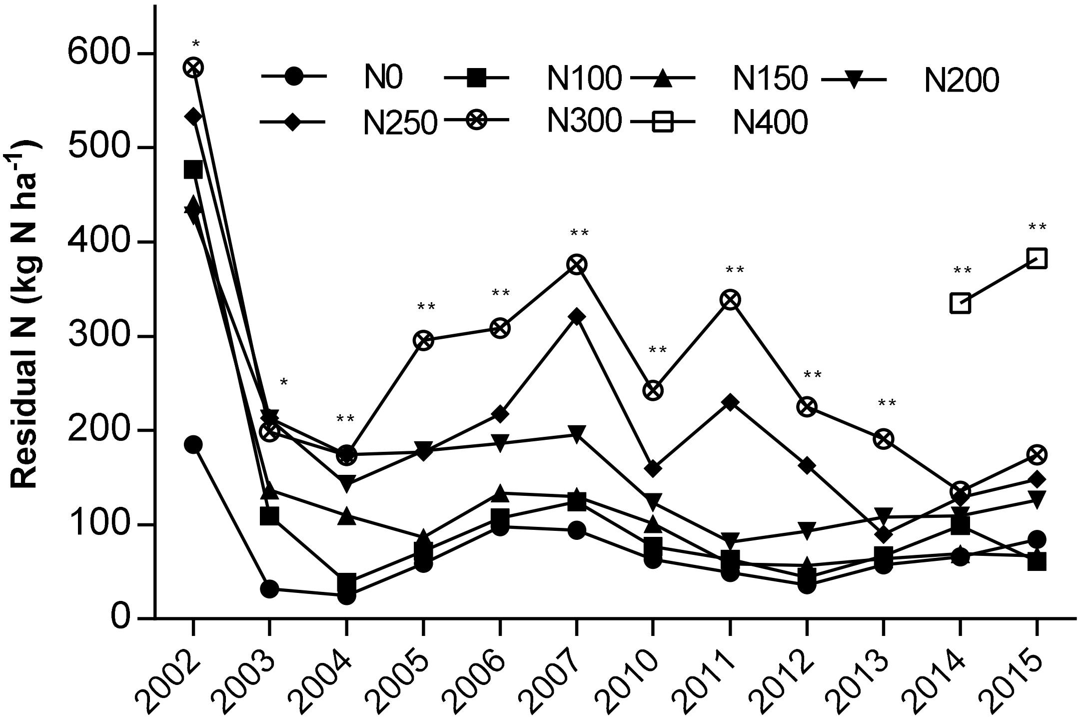


Figure 7. Relationship between residual N content (after harvesting 0-90cm) and pre planting available N content (0-90cm) during the experimental period.

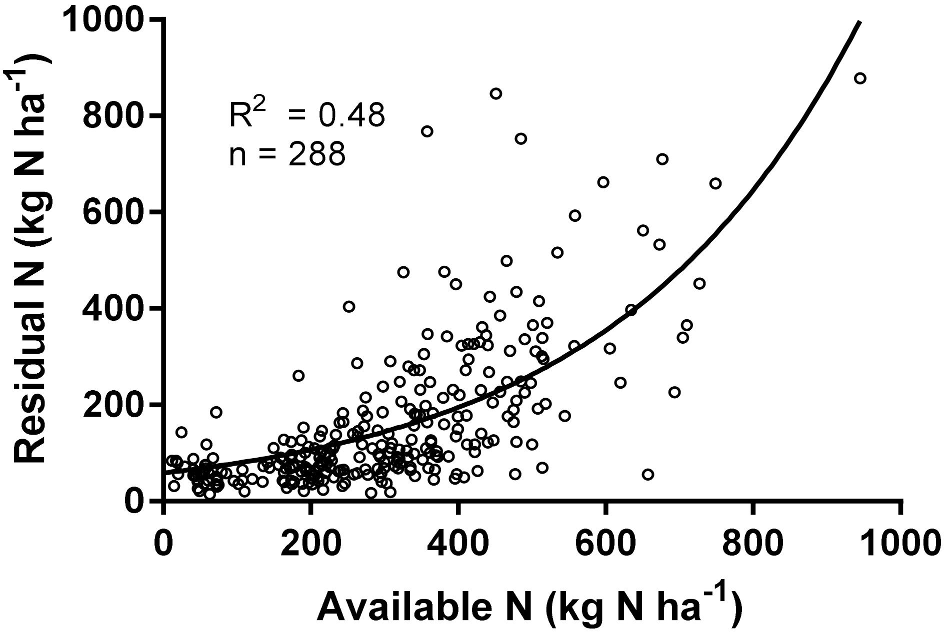
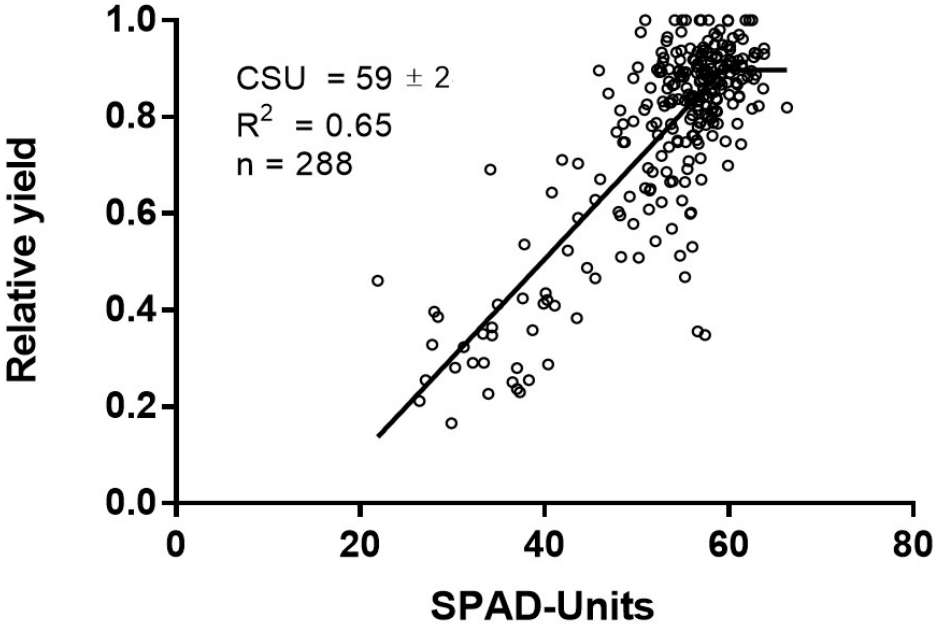


Figure 8. Relationship between relative grain yield and SPAD-Units at R1 stage. CSU = critical SPAD units.



CHAPTER II

LONG-TERM EFFECTS OF PIG SLURRY COMBINED WITH MINERAL NITROGEN ON MAIZE IN A MEDITERRANEAN ENVIRONMENT

Long-term effects of pig slurry combined with mineral nitrogen on maize in a Mediterranean irrigated environment

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Keywords: nitrogen fertilizer, organic fertilizer, grain yield, soil nitrogen, stalk nitrate, nitrogen fertilizer value

Under review

Abstract

Intensive pig farming generates large amounts of manure which is applied to agricultural fields as slurry. It is therefore important to know the nitrogen (N) fertilizer values and long-term environmental effects of different pig slurry (PS) application rates. We investigated the performance of sprinkler-irrigated maize crops in a Mediterranean environment with different fertilizer treatments as part of a 12-year experiment (2002–2007, 2010–2015). We compared PS applied using the surface splash plate method at rates of 0, 30 and 50 m^3 ha^{-1} yr^{-1} combined with mineral N fertilizer application rates of 0, 100 and 200 kg N ha^{-1} yr^{-1} as a side-dressing. The treatments affected maize grain and biomass yields, N uptake, chlorophyll levels, soil N levels, N use efficiency (NUE) and basal stalk nitrate levels. Satisfactory average grain yields (~13.5 Mg ha^{-1}) were achieved at application rates of ~200–300 kg N ha^{-1} with average residual N levels below 180 kg N ha^{-1} (0–90 cm depth) and a NUE greater than 50%. However, the maximum average grain yields (~14.5 Mg ha^{-1}) required treatments providing more than 400 kg N ha^{-1} yr^{-1} but resulted in residual N levels greater than 300 kg N ha^{-1} and the NUE fell below 50%. The relative N fertilizer value of PS applied at 30 and 50 m^3 ha^{-1} yr^{-1} was 51% of the mineral N value, suggesting that more efficient application methods are required. The end of season basal stalk nitrate test was useful for the quantification of excess nitrogen, and 2.5 g NO_3^- kg^{-1} was considered the maximum optimal concentration for our cropping conditions and grain yields.

1. Introduction

Intensive pig farming is an economically important industry in several European countries. Spain is the leading European producer with 29 million head in 2016 (Eurostat, 2017), 49% of which were farmed in the Ebro River Valley (MAPAMA, 2016a). This provides social and economic benefits in rural areas, but the disposal of manure is a problem. In 90% of the intensive production facilities in Spain, the manure slurry is applied to agricultural land using the rapid and inexpensive splash plate method (Sisquella et al., 2004). The benefits of soil application include the fertilizer value of pig slurry, the provision of organic matter and the improvement of soil quality (Breching and McDonald, 1994; Zebarth et al., 1999; Biau et al., 2012). However, there are also negative environmental consequences such as nitrate leaching to groundwater and the release of gases into the atmosphere (Nicholson et al., 1999).

Nitrate is one of the most common chemical contaminants found in water. Many areas in Europe have been designated as vulnerable to nitrate leaching, and 22% of springs in the USA have nitrate levels exceeding federal limits (USEPA, 1990; World Resources Institute, 1998). Most of these polluted zones are irrigated areas that are used to grow crops such as maize with a high nitrogen (N) demand, but others are areas used for livestock production (Strebel et al., 1989). In Spain, 36% of all cultivated land (~6.4 million ha) is considered vulnerable to nitrate leaching (Fernandez, 2008). In these areas, the European Nitrates Directive 91/676 and corresponding local regulations establish maximum amounts of N from organic materials and/or mineral fertilizer that can be applied to agricultural land annually depending on the crop. In Mediterranean areas, the N limits for manures and slurries are often determined using information which is inappropriate for these conditions (Yagüe and Quílez, 2010a).

The fate and dynamic behaviour of N applied as pig slurry is complex. Pig slurry contains ~70% inorganic N (in the form of NH_4^+) which rapidly transforms into nitrate and is predominantly available to the crop during the year of application (Chantigny et al., 2004). This source of N is susceptible to volatilization, but the amount lost is influenced by the application method which determines how the slurry is incorporated into the soil (Huijsmans and De Mol, 1999; Schils and Kok, 2003; Scröder, 2005). Splash plate application results in the loss of 20% of the NH_4^+ in the first 3–4 h after application before the remainder is incorporated into the soil (Yagüe and Quílez, 2013). Pig slurry also contains organic N, although a lower proportion than other manures (Schröder, 2005). This must be mineralized before it is available to crops (Bernal et al., 1993; Sørensen and Amato, 2002). Some of the organic N is immobilized by microbes, and NH_4^+ may be fixed by minerals in clay soils (Chantigny et al., 2004). This generates a residual effect that can last for several years after application.

Long-term pig slurry applications have a cumulative effect on the availability of N and other nutrients (Yagüe and Quílez, 2010b; Cela et al., 2011). The nitrogen fertilizer value (NFV) and residual effect have been tested in the laboratory, and medium-term field studies have also been reported in some areas of Europe (Giola et al., 2012; Sieling et al., 2014). Maize is important in this regard because it is the second most widely cultivated crop in the EU (Eurostat, 2016) and the principal crop in the irrigated areas of the Ebro River Valley (MAPAMA, 2016b), and because N uptake is higher in maize than in other common crops (Chen et al., 2014). Maize monoculture is often treated with pig slurry complemented with a side-dressing of inorganic N fertilizer (Sisquella et al., 2004; Schröder et al., 2005). The slurry can partially or completely replace inorganic N fertilizer but repeated or large applications run the risk of nitrate pollution (Berenguer et al., 2008; Yagüe and Quílez, 2010a). The NFV of pig slurry has been determined in the absence of mineral N fertilizer to evaluate the residual effects of repeated applications (Yagüe and Quílez, 2013a) but the NFV of pig slurry in the year of application has not been reported. Excess N accumulates in the lower portion of maize stalks and this effect can be used as a guide for N management strategies in subsequent years. The end-of-season basal stalk nitrate (BSN) test measures nitrate concentrations in the lower portion of the stalks at physiological maturity, and can highlight when "luxury consumption" of N has occurred (Binford et al., 1992b; Fox et al., 2001).

Nitrogen use efficiency (NUE) is the ratio of the N removed by harvesting and the N input required during cultivation. It is one of the most common and effective indicators of increasing crop productivity and profitability with limited environmental degradation (EUNEP, 2015). NUE has influenced many of the Sustainable Development Goals for the post-2015 era, recently accepted by 193 countries of the United Nations General Assembly (SDSN, 2015). The NUE of pig slurry is difficult to determine due to variations in slurry composition, time of application, application method, application rate, soil and climatic conditions, and N mineralization rates (Schröder et al. 2005; Lalor et al., 2011). Some studies have assessed the short and medium term effects of pig slurry on irrigated maize (Daudén and Quílez, 2004; Berenguer et al., 2008; Yagüe and Quílez, 2010a) but effects spanning more than 4 years have not been reported.

Here we compared the effects of three pig slurry application rates before sowing (0, 30 and 50 m^3 ha^{-1}) combined with three rates of mineral N side-dressing (0, 100 and 200 kg N ha^{-1}) to determine the NFV of pig slurry compared with mineral N fertilizer and its potential environmental effects. These application rates approximate the maximum N rate allowed by the European Directive 91/676 for organic N sources (170 kg N ha^{-1}) and about twice this rate. We hypothesized that satisfactory but not maximum grain yields could be achieved at these rates and therefore sought to determine the environmental effects of higher rates. We measured maize grain and biomass yields, soil N concentrations at the beginning and end of the growing season, plant and grain N uptake, NUE, chlorophyll levels and BSN levels during a 12-year period in which monoculture maize was cultivated under sprinkler-irrigated Mediterranean conditions.

2. Materials and methods

2.1 Field experiment

The maize field experiment was conducted at the IRTA research station in Gimenells, north-east Spain (41°65'N, 0°39'E) over 12 years (2002–2007 and 2010–2015). We used the same plots throughout the experiment, comprising a well-drained petrocalcic calcixerept soil (Soil Survey Staff, 2014) with a petrocalcic horizon at 90–100 cm soil depth and no salinity (Table 1). The climate in the area is semi-arid Mediterranean, with high temperatures (historical mean 19.4°C) and low precipitation (historical mean 184 mm) during the growing season (historical 1989–2015) (Figure 1). The trial was conducted with sprinkler irrigation, providing approximately 650 mm water depending on the climatic conditions during the growing period in each year. The irrigation water was good quality and did not contain significant amounts of nitrates.

The experimental design was a split-plot, with three pig slurry rates as the main plots (0, 30 and 50 m^3 ha^{-1} yr^{-1}, described hereafter as PS0, PS30 and PS50, respectively). As stated above, the PS30 treatment represents approximately 170 kg N ha^{-1}, which is the maximum N from organic source allowed by European Directive 91/676, and the PS50 treatment is about twice this rate. The pig slurry application rates were combined with three mineral N fertilization rates as subplots (0, 100, and 200 kg N ha^{-1} yr^{-1}, described hereafter as N0, N100 and N200, respectively). In 2002, the plots were randomized, and thereafter each treatment was applied to the same plot. To minimize year-to-year variations in nutrient concentration, the pig slurry was always obtained from the same neighbouring farm and was applied in March using the same commercial splash plate spreader machine and tractor driver. It was incorporated into the soil to a depth of 20 cm by cultivation 3–4 h after application and approximately 3 weeks before sowing. The composition of the pig slurry is summarized in Table 2, and was within the normal range for slurries produced in the area (Moral et al., 2008). Specific pig slurry application rates meeting pre-defined N targets can sometimes be difficult to achieve with commercial machinery (Daudén and Quílez, 2004). In some years it was not possible to achieve the precise target rates, but the annual average pig slurry rates over the study period were 29 and 52 m^3 ha^{-1} yr^{-1}, which were rounded to approximate the PS30 and PS50 target rates, respectively. The amounts of N applied are summarized in Table 3. Mineral N was applied as NH_4NO_3 (33.5%) divided into two equal side-dressings applied at the V3–V4 and V5–V6 stages of maize development. Phosphorus and/or potassium fertilizers were applied before planting every 1–3 years at rates of 150 kg P_2O_5 ha^{-1} and 250 kg K_2O ha^{-1} to ensure a sufficient supply. Soil levels were always kept above 25 mg P kg^{-1} (Olsen method) and 200 mg K kg^{-1} (ammonium acetate method) following local recommendations for maize (Villar-Mir and Villar-Mir, 2016).

Maize hybrids were derived from FAO cycle 600–700 and were planted between the first and second week of April at a density of 8–9.5 plants m^{-2} with a row spacing of 72 cm. Maize hybrids were changed every 2 years based on technical reports for the area (Betsebé et al., 2006; López et al., 2009). The field was kept free of weeds, pests and diseases by adopting local agricultural practices. Maize was harvested at physiological maturity, and stover was removed from the field during the first 4 years of the experiment (2002–2005) and incorporated during the remaining years (2006–2007 and 2010–2015).

2.2 Measurements

Grain yield was measured by harvesting two central rows (1.4 x 15 m) of each plot (15 x 11 m) with an experimental plot combine. Grain moisture was measured in a 300-g sample from each plot using a GAC II device (Dickey-John, Auburn, IL, USA) and the grain yield was adjusted to 14% moisture. Subsamples were taken and grain N levels were determined by near infrared (NIR) spectroscopy, using a calibrated Bran+Luebbe IA 500 device. Grain N uptake was calculated by multiplying the grain yield and N content. The aboveground biomass yield was determined at physiological maturity by harvesting plants from two central rows (1.4 x 4 m) at ground level. Subsamples were chopped and dried to determine the dry matter content of the biomass and whole-plant N concentrations (by NIR spectroscopy as above). The total plant N uptake was calculated by multiplying the N concentration and biomass production. The amount of chlorophyll (in SPAD units) was measured in 10 plants per plot at the R1 stage on the ear leaf using a Minolta SPAD-502 manual leaf chlorophyll meter.

Soil samples for the measurement of nitrate levels were taken before planting and fertilizer application (N_{ini}) and after harvesting (N_{res}) at three horizons (0−30, 31−60 and 61−90 cm). The nitrate levels are presented as the sum of the three horizons (0–90 cm). Soil nitrates were extracted with deionized water and measured using test strips in a Nitracheck® device calibrated according to the standard procedure (Bischoff et al., 1996). Soil samples from each plot contained a mixture of five cores per plot. Ammonium levels were not taken into account because the quantity of ammonium in the soil is negligible compared to nitrate based on our previous research and other studies in the same region (Villar-Mir et al., 2002; Berenguer et al., 2008). End of season BSN samples were collected at physiological maturity by cutting the stalk 15 and 35 cm above the ground and removing dried leaves from the resulting 20-cm stalk segment. The samples were milled and sieved before nitrate extraction and measurement as described above for soil nitrates. BSN samples from each plot contained a mixture of 10 stalks per plot.

The inclusion of a control treatment (N0) allowed us to estimate the amount of N supplied by the soil (N_{min}) in the absence of N fertilization. The N_{min} value includes mineralized N, N provided by biological fixation, N provided by atmospheric deposition, and ammonia released from the interlaminar spaces within the clay. N_{min} was calculated for the control plots (N0) using the following equation (Jarvis et al., 1996): $N_{min} = N_{res} + N_{plant} – N_{ini.}$

NUE (kg kg^{-1}) was defined as the ratio between N output (in the grain) and N input (organic and/or mineral fertilizer) as previously described (EUNEP, 2015). NUE_{gy} is the NUE for grain yield (kg kg^{-1}) and was defined as the ratio of the grain yield (14% moisture) to the N supply, where the N supply was the sum of the soil inorganic N in the 0–90 cm horizon at planting (N_{ini}), the mineralized soil N (N_{min}) and the total amount of N applied as mineral fertilizer (López-Bellido et al., 2005). NUE and NUE_{gy} were calculated for each plot from 2003 to 2015.

The NFV of pig slurry was based on the rate of side-dress mineral N that results in the same grain yield as that achieved by a pig slurry treatment, and was calculated by linear regression between two mineral N treatments (Schröder, 1997; Nevens and Reheul, 2002).

2.3 Statistical analysis

The results were analysed using the Proc Mixed procedure in the Statistical Analysis System (JMP 12, SAS Institute 2012). Means were separated by LSMeans Tukey's HSD test ($p < 0.05$) and levels not connected by the same letter are significantly different.

3. Results

3.1 Crop yield

The average grain yield and aboveground dry matter were affected by both the pig slurry and mineral N rates, and ranged from 6.8 to 14.9 Mg ha^{-1} and from 16.6 to 29.5 Mg ha^{-1}, respectively (Table 4). Figure 2 shows the grain yield time course of the most representative treatments in the experiment, the yields of which varied in different years. There was no response to fertilizer application in the first year of the experiment (2002) due to the initial high soil N content, but trends became evident in subsequent years. The control treatment (P0N0) resulted in a declining grain yield throughout the experimental period and stabilized at 4–6 Mg ha^{-1}. The P0N100 and P30N0 treatments, with average N inputs of 100 and 218 kg N ha^{-1}, respectively, and average grain yields of 13.1 and 13.4 Mg ha^{-1}, respectively, followed comparable trends in most years, and there was no significant difference between them in terms of the average grain yield (Table 4). Similarly, the results of the P0N200 and P50N0 treatments were also comparable, with average N inputs of 200 and 389 kg N ha^{-1}, respectively, and average grain yields of 13.6 Mg ha^{-1}. This similar behaviour among treatments became closer after the first 6 years of the experiment. The P50N200 treatment, with an average N input of 589 kg N ha^{-1}, achieved the highest grain yields, ranging from 13.1 Mg ha^{-1} in 2010 to 17.8 Mg ha^{-1} in 2015. The average grain yield of 14.9 Mg ha^{-1} was significantly higher than the other treatments (Table 4). The aboveground dry matter yields behaved in a similar manner to the grain yields. Accordingly, the NFVs of the P30N0 and P50N0 treatments based on grain yields as determined by linear regression between the P0N100 and P0N200 treatments (Eq. [1]) were 111 and 197 kg N ha^{-1}, respectively.

Yield (Mg ha^{-1} at 14%) = 8.6 + 0.0251 x mineral N applied (kg N ha^{-1})

[Eq. 1].

3.2 N uptake

The amount of N in the whole plants and grains (N uptake) varied from year to year, and was influenced by both the pig slurry and mineral N application rates (Table 4). In the first year (2002), there was no difference among treatments in terms of whole-plant and grain N uptake (data not shown) due to the high initial soil N content, and the values ranged from 131 to 323 kg N ha^{-1} and from 69 to 194 kg N ha^{-1}, respectively, depending on the treatment. The highest N uptake (~300 kg N ha^{-1}) was associated with highest grain yield (~14.5 Mg ha^{-1}) and highest N application rates, and the mean separation was very similar to that observed for grain yield. The ratio of grain N uptake to whole-plant N uptake in the control treatment was 0.53. This was significantly lower than the ratio in all treatments involving N application, where the values were very similar regardless of the treatment and the average was 0.62 (Table 4).

3.3 Soil N levels

The initial nitrate levels in the soil were high the first year of the experiment (2002) in all plots because of previous management practices in the experimental field and averaged 317 kg NO_3^- (Berenguer et al., 2008). During the 12-yr experimental period, N depletion occurred in some treatments, but high N soil levels were still observed in treatments receiving large N application rates (Table 4). The average initial nitrate levels ranged from 76 to 266 kg NO_3^- ha^{-1}, whereas residual levels ranged from 70 to 375 kg NO_3^- ha^{-1}. The lowest values were observed in the P0N0 control treatment and the highest values were observed in the P50N200 treatment.

3.4 NUE

During the first 2 years of the experiment, NUE and NUE_{gy} values were higher than average in most treatments. This reflected the high initial N levels in the soil at the beginning of the experiment, but they stabilized and became effectively constant as this initial nitrogen was depleted. The average NUE ranged from 1.20 kg kg^{-1} in the N100 treatment to 0.35 kg kg^{-1} in the P50N200 treatment. The treatments with the highest grain yields showed the lowest NUE values of 0.35–0.46 kg kg^{-1} (Table 4), i.e. only 35–46% of the applied N was present in the harvested product (EUNEUP, 2015). The average NUE_{gy} ranged from 39.9 kg kg^{-1} in the N0 control treatment to 16.1 kg kg^{-1} in the P50N200 treatment.

3.5 Chlorophyll levels

The average SPAD units recorded at stage R1 varied from year to year (Table 4) and ranged from 40 in the N0 control to ~57 in the N200, P30N100, P30N200, P50N100 and P50N200 treatments. Increasing the N application rate resulted in higher concentrations of chlorophyll in the leaves, although saturation occurred at ~57 SPAD units.

3.6 End of season BSN levels

The average BSN levels varied in different years and among the different fertilizer treatments, ranging from 544 mg NO_3^- kg^{-1} in the N0 control to 4283 mg NO_3^- kg^{-1} in treatments with the highest N inputs (Table 4). The highest grain yields were associated with higher average BSN levels and higher residual soil N levels. Figure 4 shows a correlation of R^2 = 0.57 between the residual soil N content and the BSN content. The clustering of data is greater for low values of residual soil N and BSN, whereas there is more dispersion when these values increase.

Figure 5 shows the relationship between BSN values and relative grain yields. This reveals two phases, one in which the BSN content is ~0 and the relative grain yield is 0.2–1, and a second in which the BSN content is 0–7000 mg NO_3^- kg^{-1} and the relative grain yields generally fall within the range 0.8–1. In other words, N fertilizer application rates required to attain near maximum grain yields (below ~0.8) result in low BSN levels that are similar among treatments, whereas application rates in excess of this amount result in proportionally higher BSN levels (Table 4 and Figure 5). Our results therefore establish three BSN categories: low (<750 ppm NO_3^-), optimal (750–2500 ppm NO_3^-) and excessive (>2500 ppm NO_3^-).

4. Discussion

4.1 Crop yield

The relative NFV (ratio of NFV to total N applied) was 51% for the P30N0 and P50N0 treatments. The value was higher at the beginning of the experiment but declined in the second year and became effectively stable thereafter due to soil N depletion. About half of the N applied as pig slurry is therefore lost to the environment (primarily by NH^3 volatilization) and the remainder stays in the soil as organic N and generates a residual effect. The NFV of manure in long-term experiments is more reliable because it considers this residual effect, although this residual effect is lower with slurries (Schröder, 2005). The relative NFV of manures tends to be 20–60% currently but should reach 80% in the near future (Schröder, 2005).

Decree 136/2009 (Generalitat de Catalunya, 2009) controlling the fulfilment of the EU Nitrates Directive 91/676 in the study area allows the application of 200 kg N ha^{-1} yr^{-1} of mineral N to maize crops, or up to 170 kg N ha^{-1} from organic sources which can be complemented with mineral N to a maximum of 300 kg N ha^{-1} in total. This maximum level is represented by our N200 treatment, and is also similar to the P30N100 treatment, which shows that compliance with the Nitrates Directive under our study conditions achieves satisfactory maize grain yields of ~13.5 Mg ha^{-1}, although not the maximum yields of ~15 Mg ha^{-1} (Table 4).

Another study in the same field, adjacent to the plots used in the current experiment and with the same management strategy and experimental period, has shown that a non-limiting N treatment of 300 kg ha^{-1} of mineral N (P0N300) resulted in a linear annual increase in the grain yield of 192 kg ha^{-1} yr^{-1} (unpublished data). Similar results were achieved with the P50N200 treatment in the current study, resulting in linear annual increase in the grain yield of 234 kg ha^{-1} (Figure 6). This is likely to reflect both genetic improvement and changing agronomic practices, such as increased planting densities (Lobell et al., 2014).

Pig slurry applications in the study area have been shown to increase yields compared to mineral fertilizers due to increases in the amount of soil organic matter and other nutrients (Daudén et al., 2004; Yagüe and Quílez, 2010a; Yagüe and Quílez, 2010c). Our P50N200 treatment achieved significantly higher yields than the previously-described P0N300 treatment (unpublished data) in 6 of the 12 years during our experiment (Figure 6). The differing performance in different years is likely to reflect the impact on N fertilization rates and climatic conditions during the growing season on the yield potentials and NUE of the hybrids (Ma and Dwyer, 1998; Khaliq et al., 2009).

4.2 N uptake

Our grain and whole-plant N uptake values match those reported by others working with similar pig slurry application rates in the same study area (Berenguer et al., 2008; Yagüe and Quílez, 2010a). As expected, applications of more than 400 kg N ha^{-1} (combined pig slurry and mineral N fertilizer) did not significantly increase the N uptake (Table 4) because the total applied N was greater than that required by the maize plants for optimal growth (Schröder et al. 2000). Our results also agree with those reported under other growing conditions for similar grain yields, such as an experiment in Argentina (Barbieri et al., 2006). However, we achieved considerably higher maximum grain yields (~14.5 Mg ha^{-1}) than reported in the Argentinian experiment (~10 Mg ha^{-1}) probably due to our higher maximum N uptake values, as reflected by the production of more biomass (Table 4).

4.3 Soil N levels

The fertilizer treatments which achieved the highest grain yields in our experiment (P30N200, P50N100 and P50N200) also resulted in the highest levels of residual N in the soil, and were the only treatments that resulted in significant differences between the initial and residual N levels (Figure 3). This suggests that substantial amounts of N were lost during the winter period (November to March) where the only soil cover was provided by crop residues. The estimated average N loss was 117, 106 and 109 kg N ha^{-1} for the P30N200, P50N100 and P50N200 treatments, respectively. Berenguer et al. (2008) reported an overall N balance during the first 3 years of their experiment but subsequent N losses varied widely in different years, ranging from 35 to 438 kg N ha^{-1}. It was therefore unclear whether the application of fertilizer to maize in order to achieve maximum yields and profits is environmentally sustainable (Yagüe and Quílez, 2010a).

Significant amounts of initial nitrate were found annually in the 0–30 cm horizon when applying 100 kg N ha^{-1} or more (Figure 3). Until the V3 stage, maize plants take up less than 0.5 kg N ha^{-1} per day (Schröder, 1999). For maize grown under sprinkler irrigation, which allows N applications according to demand, it is therefore sufficient to provide the first N application as a side-dressing at stage V4 or later to improve NUE. N uptake increases during stages V6–V8, and N should be made available during this period to avoid yield penalties (Hanway, 1966; Ritchie, 1997).

There was no statistically significant difference in either grain yield or the initial and residual soil N content between the P0N100 and P30N0 or the P0N200 and P50N0 treatments despite nearly doubling of the amount of applied N in pig slurry treatments (Table 4). The average initial and residual nitrate distributions across the 0–30, 31–60 and 61–90 cm soil horizons was similar in our petrocalcic calcixerept soil with no statistically significant differences among the horizons for any treatment (data not shown). This suggests that sampling the soil to a depth of 30 cm can provide reliable information about the N level in maize monoculture after 3 years. In the same area Cela et al. (2013) reported that it would not be profitable for farmers to sample to layers deeper than 0–30 cm to predict maize yields from the soil N content, as also reported for wheat (Tugues et al., 2017). Sampling to 30 cm was also deemed sufficient in Iowa, USA (Binford et al., 1992a) and in the Netherlands (Schröder et al., 1998) although other authors have reported that under their experimental conditions sampling to 60 cm may be necessary (Ehrhardt and Bundy, 1995; Sims et al., 1995).

4.4 NUE

The application of slurry resulted in a lower NUE than mineral fertilizer for similar total N inputs. This probably reflects the loss of NH_3 during the period between application and incorporation into the soil, which can amount to 20% of the total N in the first 4 h after application with a splash plate (Yagüe and Bosch-Serra, 2013b). Moreover, the incorporation of slurry into the soil does not always stop further NH_3 volatilization (Huijsmans et al., 2003). Furthermore, 33% of the N in the pig slurry was organic (Table 2). These combined factors probably contributed to the lower NUE of the pig slurry treatments. The NUEs calculated for the treatments achieving the highest growth yields (P30N200, P50N100 and P50N200) were near the threshold at which NUE is considered inefficient (<50%) by the EUNEP (2015). NUE_{gy} values for pig slurry were similar to those reported by others under low-efficiency Mediterranean conditions, e.g. Di Paolo and Rinaldi (2008) applied 300 kg mineral N ha^{-1} to maize plots and reported average grain yields of 10 Mg ha^{-1}.

The NUE for the P50N0 treatment (389 kg N ha^{-1}) resulted in a lower NUE than the N200 mineral N side-dressing, but there was no significant difference in grain yields or the residual N content in the soil (Table 4). This suggests that the maximum 170 kg N ha^{-1} for pig slurry applications (~25 m^3 ha^{-1}) allowed under the Nitrates Directive could be increased without additional nitrate leaching. However, we consider it likely that increasing the application rate could result in the accumulation of soil phosphorus (Cela et al., 2010), and that alternative application methods at current rates should be considered instead, such as direct incorporation into the soil or trail hose application (Huijsmans and De Mol, 1999; Schils and Kok, 2003; Schröder, 2005; Yagüe and Bosch-Serra, 2013b). This would help to increase the NUE of pig slurry applications without increasing application rates.

4.5 Chlorophyll levels

There is a close relationship between chlorophyll levels in leaves (measured in SPAD units) and the grain yield of maize crops (Piekielek et al., 1995; Rostami et al., 2008). As shown in Table 4, we confirmed this relationship in our long-term experiment: the treatments achieving the lowest grain yields (N0, N100 and P30N0) produced lower SPAD meter readings, but there were no statistically significant differences among the treatments that achieved high grain yields exceeding ~13.5 Mg ha^{-1}. The most likely explanation is that not all N is converted into chlorophyll during the R1 stage, when large amounts of N are available (Dwyer et al., 1995; Ritche, 1997; Varvel et al., 1997a). Our results are similar to the optimum of 52–56 SPAD units initially reported by Piekielek et al. (1995) and the 50–61 SPAD units reported by Berenguer et al. (2009). Differences among these reports may reflect variations in the experimental parameters, such as the use of different maize hybrids (Schepers et al., 1996; Sunderman et al., 1997), planting times (Jemison and Lyttle, 1996), growth yields and site year effects (Blackmer and Schepers, 1995; Varvel et al., 1997b).

4.6 End of season BSN test

The relationship we observed between N fertilizer application rates and BSN levels (Table 4) under Mediterranean conditions is similar to that reported for maize grown in Iowa, USA (Binford et al., 1992b). The optimal BSN level, based on economic profit or N requirements, has been proposed as 700–2000 mg NO_3^- kg^{-1} (Binford et al., 1992b) or 750–2000 mg NO_3^- kg^{-1} (Havlin et al., 2005). Most authors agree that maximum BSN levels should not exceed 2000 mg NO_3^- kg^{-1} (Blackmer and Mallarino, 1996; Lawrence et al., 2013). However, the treatments that achieved satisfactory grain yields in our experiment while complying with the Nitrates Directive (e.g. P0N200 and P30N100) generated average BSN concentrations greater than 2000 mg NO_3^- kg^{-1}. Indeed, under our conditions, the optimal BSN concentration was ~2500 mg NO_3^- kg^{-1}, which agrees with the range 1785–2624 mg NO_3^- kg^{-1} proposed by Villar et al. (2015) for a similar experimental area. The differences in optimal ranges discussed above may reflect the different yields under rain-fed conditions (9–13 Mg ha^{-1}) and in irrigated plots (13–15 Mg ha^{-1}).

The correlation between soil residual N and BSN levels suggests that the BSN test can highlight excess N applications, providing a valuable tool that can be used to complement soil tests and other strategies used to recommend fertilizer application rates for maize, as previously suggested (Fox et al., 2001). Stringent sampling is necessary for BSN tests to account for variability, e.g. 15 basal stalks in a uniform 6-ha field (Lawrence et al., 2013).

5. Conclusions

The application of fertilizers to maize in order to achieve maximum yields is not always environmentally sustainable using the application rates described herein (>300 kg N ha^{-1}) because high levels of residual N remain in the soil after harvest but decline significantly by the next season, suggesting large amounts leach into groundwater. The NFV of the two pig slurry application rates was 51% relative to the mineral N fertilizer side-dressing. Application techniques other than the surface splash plate method should therefore be considered in order to increase the NFV of pig slurry. Application rates that comply with the Nitrates Directive achieved satisfactory grain yields (~13.5 Mg ha^{-1}) but not the highest yields reported in the area (~14.5 Mg ha^{-1}). The maximum N application rate for combined slurry and mineral fertilizer in vulnerable zones defined by Catalan

Decree 136/2009 resulted in similar residual soil N levels to a single application of mineral fertilizer at the highest allowed rate. However, the NUE was significantly lower for treatments including pig slurry compared to mineral N fertilizer alone. End-of-season BSN levels can identify N deficiencies as well as N excess after harvest, and can facilitate N management in subsequent maize crops under Mediterranean conditions. The maximum optimal BSN under our conditions was 2.5 g NO_3^- kg^{-1}, higher than the 2.0 g NO_3^- kg^{-1} reported in the literature.

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Table 1. Major soil properties at the beginning of the experiment (2002).

	Horizon depth (cm)		
	Ap 0–23	Bw 23–69	Bk 69–117
Sand, g kg^{-1}	390	380	450
Silt, g kg^{-1}	400	420	380
Clay, g kg^{-1}	210	200	170
pH*	8.3	8.3	83
Ec, dS m^{-1}	0.2	0.34	0.59
CEC, cmolc kg^{-1}	24	-	-
Organic carbon, g kg^{-1}**	13	8	4
Bulk density, g cm^{-3}	1.40	1.56	1.63
Available water holding capacity, mm	29	67	54
P, mg kg^{-1} ***	31	-	-
K, mg kg^{-1} ****	217	-	-

* Water (1:2.5)

**Walkey-Black method

*** Olsen method

**** Ammonium acetate method

Table 2. Average composition (wet matter) of applied pig slurry (2002–2007, 2010−2015).

Characteristic	**Average**		
Dry matter, g kg^{-1}	100.3	±	2.8
Organic matter, g kg^{-1}	78	±	4
pH	8.4	±	0.5
C/N	13.9	±	2.7
NH_4^+, g N kg^{-1}	5.0	±	1.0
Total N, g kg^{-1}	7.5	±	1.4
P, g kg^{-1}	1.6	±	0.5
K, g kg^{-1}	4.3	±	1.2
CE, dS m^{-1}	6.9	±	2.5

Table 3. Amounts of inorganic N and total N applied to maize in each treatment.

			Average year	
Treatment	PS target* rate (m^3 ha^{-1})	Mineral N rate (kg N ha^{-1})	Inorganic N** (kg N ha^{-1})	Total N (kg N ha^{-1})
PS0N0	0	0	0	0
PS0N100	0	100	100	100
PS0N200	0	200	200	200
PS30N0	30	0	141	218
PS30N100	30	100	241	318
PS30N200	30	200	341	418
PS50N0	50	0	252	389
PS50N100	50	100	352	489
PS50N200	50	200	452	589

PS refers to pig slurry before sowing, and mineral N to ammonium nitrate applied as a side-dressing

* Average PS application rates were 29 ± 5 m^3 ha^{-1} for the target of 30 m^3 ha^{-1} and 52 ± 8 m^3 ha^{-1} for the target of 50 m^3 ha^{-1}.

** Inorganic N refers to ammonium in the case of pig slurry and to ammonium nitrate in the case of mineral N fertilizer.

Table 4. Effect of pig slurry and mineral N fertilization rates on grain yield, biomass yield, plant N uptake, grain N uptake, soil initial N, soil residual N, nitrogen use efficiency (NUE), grain yield nitrogen use efficiency (NUE_{gy}), chlorophyll level (SPAD units) and basal stalk nitrate (BSN) content.

Pig slurry (m^3 ha^{-1})	N rate (kg N ha^{-1})	Grain yield (Mg ha^{-1})	Biomass yield (Mg ha^{-1})	Plant N uptake (kg N ha^{-1})	Grain N uptake (kg N ha^{-1})	Soil initial N (kg N ha^{-1})	Soil residual N (kg N ha^{-1})	NUE (kg kg^{-1})	NUE_{gy} (kg kg^{-1})	SPAD-units	BSN (mg NO_3^- kg^{-1})
0	0	6.8 e	16.6 e	131 f	69 e	76 c	70 c	-	39.9 a	40 c	544 d
0	100	11.1 d	22.7 d	189 e	120 d	102 c	112 b	1.20 a	37.6 a	53 b	668 d
0	200	13.6 bc	26.9 bc	273 bc	166 c	145 bc	165 b	0.83 b	31.9 b	57 a	2143 c
30	0	11.4 d	24.8 cd	230 d	132 d	115 bc	108 bc	0.64 c	27.08 bc	52 b	739 d
30	100	13.6 bc	27.6 ab	276 bc	171 bc	151 bc	174 b	0.57 d	24.9 cd	57 a	2481 bc
30	200	14.3 abc	28.8 ab	307 ab	187 ab	216 ab	333 a	0.46 e	20.0 def	57 a	3399 ab
50	0	13.6 bc	27.1 abc	254 cd	167 bc	152 bc	185 b	0.51 e	21.9 de	56 a	2305 c
50	100	14.4 ab	29.0 ab	292 abc	190 a	237 ab	343 a	0.40 ef	17.8 ef	57 a	3817 a
50	200	14.9 a	29.5 a	323 a	194 a	266 a	375 a	0.35 f	16.1 f	57 a	4283 a
Block		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pig slurry (PS)		**	**	**	**	*	*	**	**	**	**
Error a											
Mineral N (N)		**	**	**	**	**	**	**	**	**	**
PS x N		**	**	**	**	NS	*	**	**	**	*
Error b											
Year (Y)		**	**	**	**	**	**	**	**	**	*
PS x Y		**	**	**	**	NS	**	**	**	**	*
N x Y		**	**	**	**	**	*	**	**	**	*
PS x N x Y		**	NS	NS	**	NS	**	**	**	**	NS

*, ** Significant at the 0.05 and 0.01 levels, respectively. NS - not significant. Letters show LSMeans separation by Tukey HSD Test.

Figure. 1. Annual precipitation and mean temperature for the experimental period (2002-2007, 2010-2015) and the historical period (1989-2015). Maize Growing Period Precipitation (GP P), Winter Period Precipitation (WP P), Maize Growing Period Temperature (GP T) and Winter Period Temperature (WP T). Growing Period and Winter Period, from April to September and October to March, respectively.

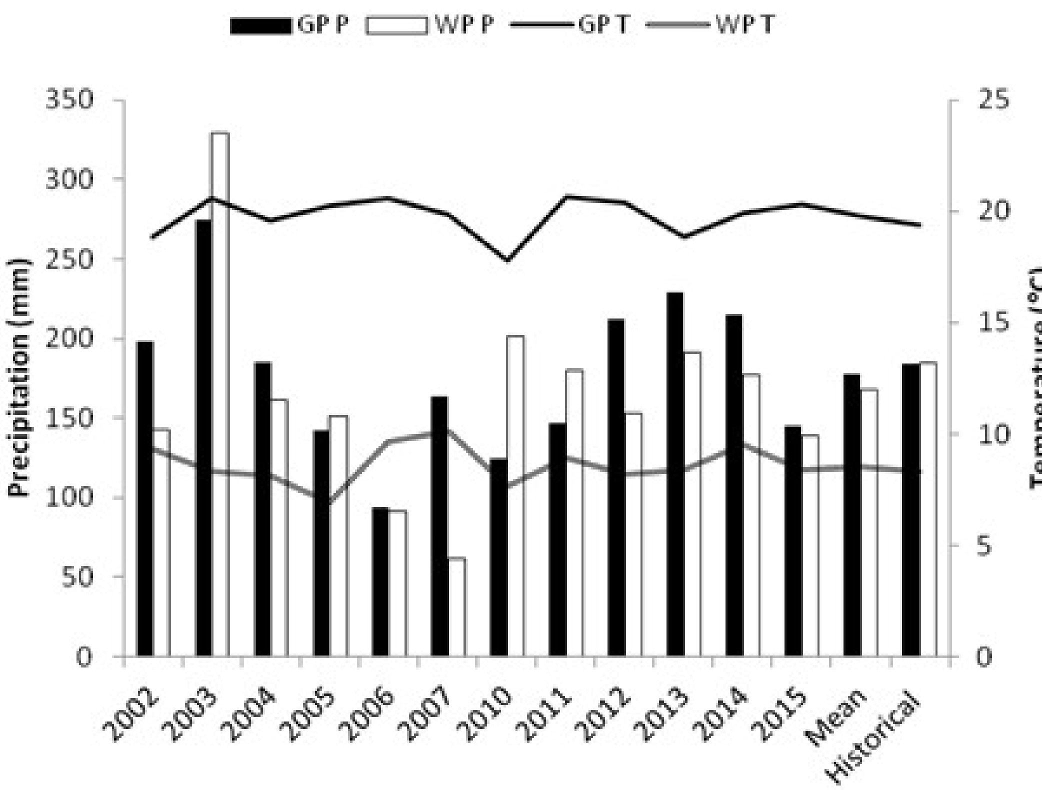


Figure 2. Grain yield time course during the experimental period (2002–2007, 2010–2015).

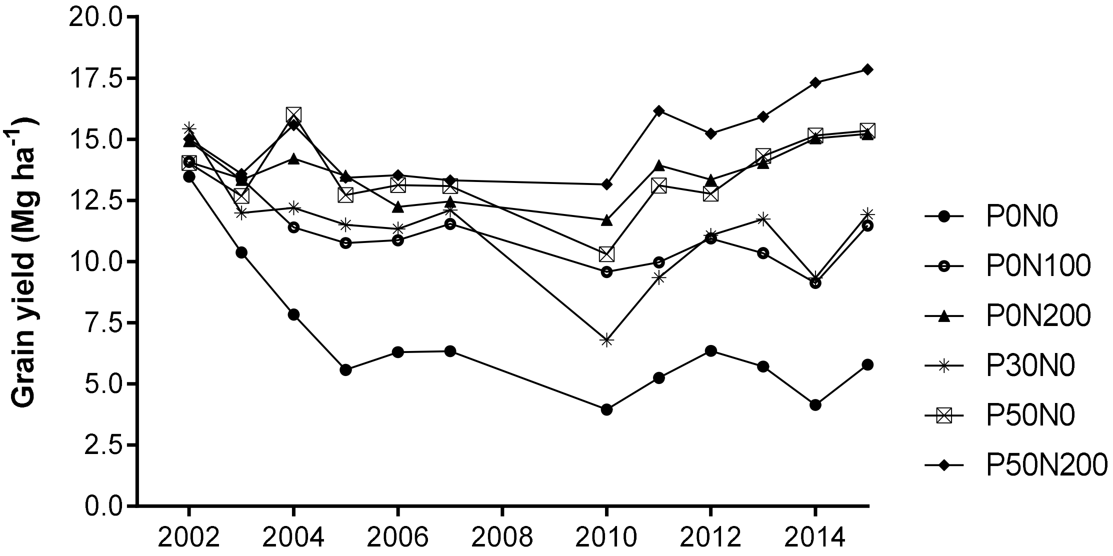


Figure 3. Average nitrate levels in the 0–30, 30–60 and 60–90 cm soil horizons before planting and fertilizer application (initial) and after harvesting (residual). Significance refers to comparison between initial and residual nitrate levels: **significant at p<0.01, ns = not significant.

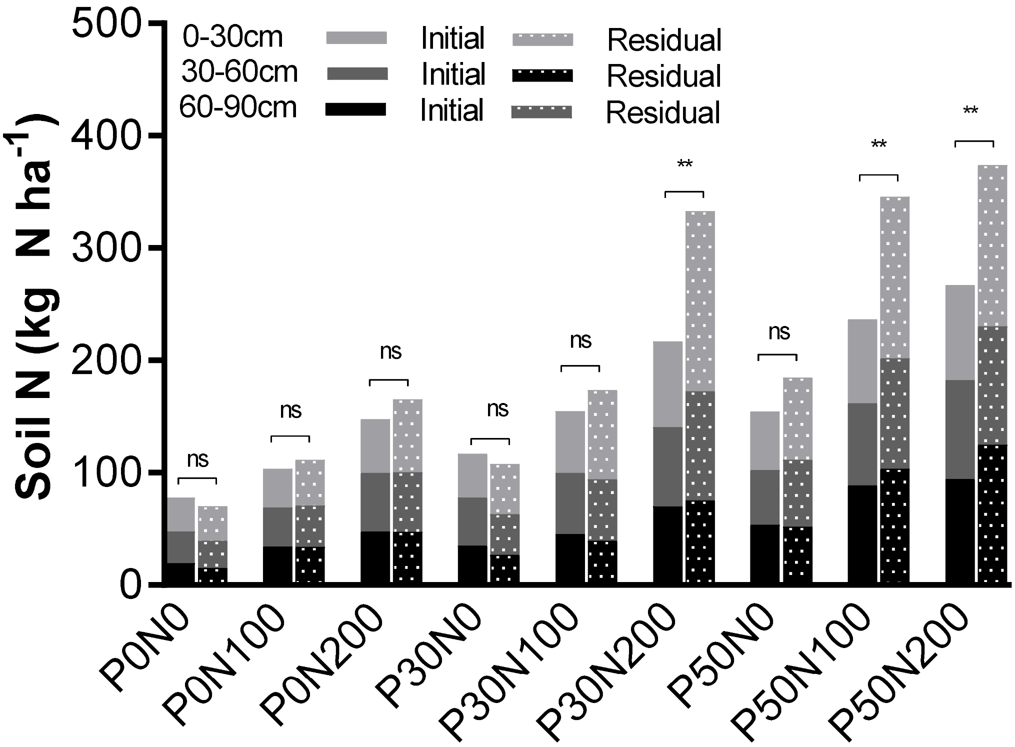


Figure 4. Relationship between soil residual N (0–90 cm) and basal stalk nitrate (BSN).

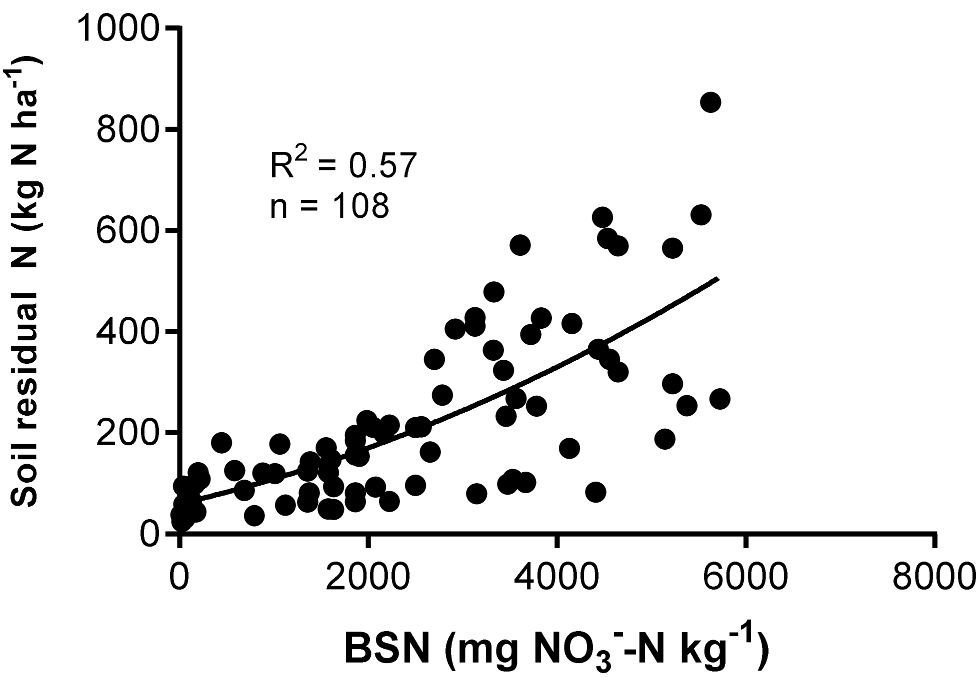


Figure 5. Relationship between relative yield and basal stalk nitrate (BSN) for all years (2002–2007, 2010–2015).

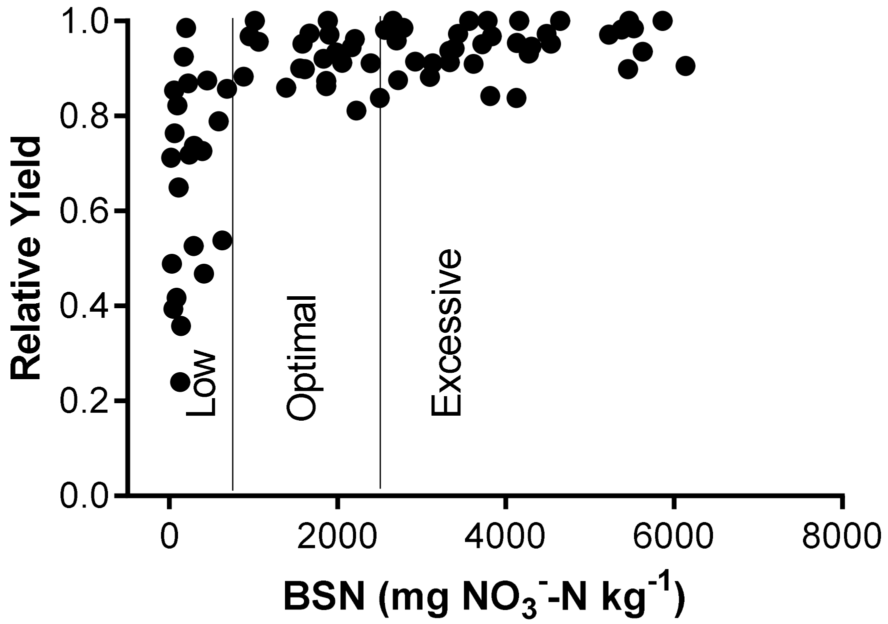
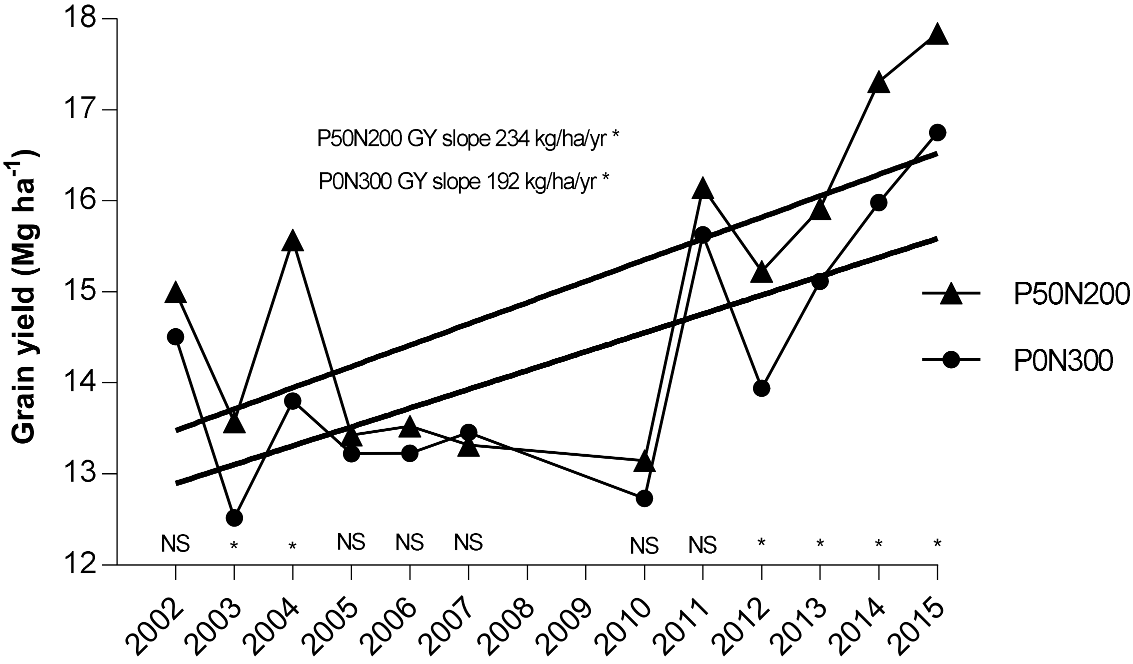


Figure 6. Grain yield at 14% moisture (GY) time courses for the treatment providing 300 kg mineral N ha^{-1} yr^{-1} (P0N300) and the P50N200 treatment throughout the experimental period (2002–2007, 2010–2015).



*: significant at p<0.05. NS: not significant.

CHAPTER III

THE EFFECTS OF LONG-TERM SWINE SLURRY MANURE APPLICATIONS ON SOIL AND MAIZE PLANT CU AND ZN AND SOIL ORGANIC CARBON

The effects of long-term swine slurry manure applications on soil and maize plant Cu and Zn and soil organic carbon

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Key words: organic fertilisation, pig slurry, heavy metals, 12-year experiment

Under review

Abstract

Cu and Zn are used in swine (*Sus crofa domesticus*) feed, so the application of liquid swine manure (LSM) to meet the nitrogen (N) requirements of maize crops and to address waste disposal needs can lead to the accumulation of these heavy metals in the soil. A field experiment was conducted between 2002 and 2015 in the Ebro Valley (north-east Spain) to determine the effects of long-term LSM application at two rates (30 and 50 m^3 ha^{-1}, equivalent to ~170 and ~270 kg N ha^{-1}) compared to 0 and 300 kg N ha^{-1} mineral fertiliser. The average annual applications of Cu and Zn at the high LSM rate were 1.8 and 7.2 kg ha^{-1}, respectively, resulting in soil accumulation of 1.3 kg ha^{-1} Cu and 5.8 kg ha^{-1} Zn per year. The low LSM rate (which complies with the EU Nitrates Directive) resulted in the accumulation of lower levels of both metals. Cu concentrations in whole plants (including grain) and grain alone increased with the LSM and N applications. The highest plant Cu concentration (5.03 mg kg^{-1}) was observed following the 300 kg N ha^{-1} treatment. The different treatments did not affect plant Zn levels (~21.1 mg kg^{-1}). However, the grain Zn concentration decreased with increasing N application rates. All plant and grain metal concentrations obtained were lower than the threshold values for animal and human ingestion (30 mg Cu kg^{-1} and 500–1300 mg Zn kg^{-1}). After 12 years, the soil organic carbon (SOC) stock of 63.42 Mg ha^{-1} in the first year (2002) increased by 6% and 28% at the low and high LSM rates, respectively. Mineral fertilisation (300 kg N ha^{-1}) achieved a 7% increase in SOC but the 0 kg N ha^{-1} control treatment caused the SOC to fall by 5%. Maize yields were not negatively affected by LSM: the grain yields were 13–15 Mg ha^{-1} over the 12-year study period, confirming the absence of phytotoxicity caused by Cu and Zn. Indeed, the soil Cu and Zn concentrations did not reach phytotoxic levels as stipulated in current legislation. The application of LSM at the rates discussed herein therefore poses no risk of Cu and Zn phytotoxicity or adverse effects in consumers.

1. Introduction

Fertilising crops with liquid swine manure (LSM) can cause effects such as NO_3^- leaching, phosphorus accumulation and run-off, soil salinity and the accumulation of nutrients (Diez et al., 2004; Moral et al., 2008; Yagüe and Quílez, 2010). However, LSM can also increase the soil organic carbon (SOC) content and introduce a wide range of micronutrients to improve soil quality and crop production. LSM is also an economical form of manure management which can reduce fertilising costs by replacing mineral fertilisers (Edmeades, 2003; Sánchez and Gonzalez, 2005; Biau et al., 2012).

One of the research topics related to manure management is the effect of LSM on heavy metal (copper and zinc) and soil phosphorus accumulation, especially in relation to continuous and long-term LSM applications (Brock et al., 2006; Berenguer et al., 2008; Panagos et al., (2012). These elements often accumulate in maize fields when large LSM applications are used (Smith et al., 1998; Eghball and Power, 1999).

Significant amounts of copper and zinc may be present in LSM because an feed salts containing these nutrients are often added to swine feed to improve animal health and weight gain (Hill et al., 2000). Swine metabolism leads to the excretion of more than 90% of ingested Cu and Zn (Nicholson et al., 1999; Dourmad and Jondreville, 2007). Considerable amounts of these non-assimilated elements are deposited on farmland where swine are reared when large and continuous LSM applications are used. Recent EU feed additive regulations have taken this into account, with revised maximum levels for Cu, Zn, Fe and Mn supplements to reduce the amounts that are excreted (European Union, 2003). European regulations set maximum amounts of 12 kg Cu ha^{-1} yr^{-1} and 30 kg Zn ha^{-1} yr^{-1} that can be applied to basic calcareous agricultural land (European Union, 1986; Gobierno de España, 1990). Although these regulations apply to sewage sludge and not LSM, they are usually considered as reference values due to the absence of legislation regulating the application of heavy metals in manures.

Heavy metals such as Cu and Zn tend to be chemically inert in soils, limiting their mobility and uptake by crops (Förstner, 1995). However, one they reach certain thresholds in the soil they can become phytotoxic and they can also be transferred into the food chain (Harris and Gitlin, 1996; Olivares and Uauy, 1996). Current regulations state that concentrations greater than 210 mg Cu kg^{-1} and 450 mg Zn kg^{-1} in soils with pH values up to 7 are likely to cause phytotoxicity (Gobierno de España, 1990). However, lower phytotoxicity thresholds of 60 mg Cu kg^{-1} and 300 mg Zn kg^{-1} have been reported in some experiments (Ruiz et al., 2009).

The application of LSM to maize fields has been shown to increase metal concentrations in the soil. In China, Xu et al. (2013) reported increases in total soil Cu and Zn in a maize field after LSM applications of 250 kg N ha^{-1} yr^{-1} or higher. In the USA, Brock et al. (2006) also observed soil Cu and Zn accumulation in a study of more than 100 fields where manure was applied at average rates of 8.7 kg Cu ha^{-1} yr^{-1} and 5.1 kg Zn ha^{-1} yr^{-1}. In France, Coppenet et al. (1993) reported increased levels of Cu and Zn over a 15-year period in agricultural soils where LSM had been applied. In Spain, Berenguer et al. (2008a) also reported Cu and Zn accumulation in maize fields treated with LSM containing 36.5 mg kg^{-1} Cu and 71.8 mg kg^{-1} Zn at rates of 30 and 60 m^3 ha^{-1} yr^{-1}.

This study reports the results of a long-term experiment (2002–2015) in which LSM was applied to maize fields in the Ebro Valley (north-east Spain) where ~9.9 million swine are reared per year (Magrama, 2015). Large amounts of LSM are produced and more than 90% is applied to agricultural land (Sisquella et al., 2004). The area has been designated as a zone vulnerable to nitrate leaching (European Union, 1991) and regional regulations are in place to ensure the fulfilment of the Nitrates Directive and to set maximum N application rates, depending on the area and crop (Generalitat de Catalunya, 2009). LSM comprises 90% water and long-distance transport tends to be expensive, leading to excess application in fields close to the livestock farms (Nolan et al., 2012). LSM is traditionally applied to irrigated maize because of its lodging tolerance to high N doses (Rajkumara, 2008). The application rate can be determined based on the N concentration and compliance with the Nitrates Directive (European Union, 1991) but limiting the N rates can also reduce the quantity of micronutrients applied to the soil.

The objective of this study was to evaluate the Cu, Zn and organic carbon accumulation profiles in the soil, as well as the concentrations of Cu and Zn in whole plants and grains, following the continuous application of LSM at two rates. The low rate is equivalent to the permitted dose in nitrate-vulnerable zones: 170 kg N ha^{-1} yr^{-1} (~30 m^3 ha^{-1} LSM). The high rate represents a more typical application rate: 270 kg N ha^{-1} yr^{-1} (~50 m^3 ha^{-1} LSM). These LSM rates were compared with treatments of 0 and 300 kg ha^{-1} yr^{-1} mineral N, in sprinkler-irrigated maize under Mediterranean conditions.

2. Materials and methods

2.1. Experimental design

The field experiment was conducted over a 14-year period (2002–2015) although no LSM was applied in 2008 and 2009 so the experimental duration was 12 years. The experiment was carried out at the IRTA Research Station in Gimenells (41°65′N, 0°39′E) on a Petrocalcic Calcixerept soil (Soil Survey Staff, 2014). The study was part of a long-term project relating to the effects of N fertilisation on maize (Berenguer et al., 2008a; Berenguer et al., 2008b). Table 1 summarises the main soil characteristics at the beginning of the experiment.

The statistical design was a complete randomised block with four replications. The treatments were randomised in the first year (2002), and were applied to the same plots each year thereafter. The plot size unit was 165 m^2 (15 m x 11 m). The fertilisation treatments comprised two LSM rates of 30 and 50 m^3 ha^{-1} yr^{-1}, and two inorganic N fertiliser rates of 0 and 300 kg N ha^{-1} yr^{-1}. The treatment groups are abbreviated to LSM30, LSM50, N0 and N300, respectively.

Maize was grown throughout the study period (2002–2015) although wheat and maize were grown in 2008 and 2009, respectively, without LSM or N fertilizer. To minimise year-to-year variations in nutrient concentration, LSM was always obtained from the same source, a neighbouring swine fattening farm. Each year, LSM was spread in March using the same commercial spreading machine, 3–4 weeks before maize planting, normally on a breezy or slightly cloudy day, and incorporated (20 cm depth) into the soil by cultivation within a few hours of application. Even though LSM was applied by a well-trained commercial applicator and practice runs were performed in neighbouring plots, the accurate application of specific doses of LSM to meet predefined targets was sometimes challenging. Daudén and Quílez (2004) also noted this issue when applying LSM using a commercial spreading machine. For this reason, in some years it was not possible to achieve the exact target rates of 30 and 50 m^3 ha^{-1} yr^{-1}. Indeed, the annual average LSM rates over the 12-year experimental study were 29 and 52 m^3 ha^{-1} yr^{-1}, and these are rounded to the target rates. Nutrient concentrations in the LSM (Table 2) were within normal ranges for slurries in the production systems of the area (Moral et al., 2008).

Nitrogen side-dressing (in the N300 treatment) was divided into two equal applications, first at the V3-V4 stage and second at the V6-V7 stage (Ritchie and Hanway, 1989).

Phosphorus and potassium fertilisers were applied to all plots with and without organic applications before planting, at rates of 65 kg P ha^{-1} and 207 kg K ha^{-1}, every 3 years.

2.2. Maize management

Maize was planted between the first and second week of April (depending on the year), at a rate of 8–9 plants m^{-2} with a row distance of 70 cm. Several maize cultivars were used during the 12-year experimental period, with a different cultivar introduced every 2 years (commercial maize catalogues change almost every year). All cultivars were FAO cycles 600–700. Maize stover was chopped and taken after harvest in the first 6 years of the experiment and incorporated into the soil in the final 6 years. A pre-emergence herbicide (1 L ha^{-1} 96% Metolachlor and 3 L ha^{-1} 47.5% Atrazine) was applied to control weeds for the first 6 years of the experiment, and in the last 6 years this was replaced with 3.3 L ha^{-1} of Trophy (40% Acetoclor plus 6% Diclormid). Banvel (20% Fluoxypyr) was also applied at 1 L ha^{-1} as a post-emergence herbicide to control *Abutilon theophrasti* Medik, if needed. The herbicides did not contain detectable levels of Cu or Zn.

Maize was sprinkler-irrigated, with approximately 7000 m^3 ha^{-1} yr^{-1} of good-quality irrigation water depending on the climatic conditions. The irrigation water did not contain detectable levels of nitrates.

2.3. Measurements

Maize was harvested for grain at maturity, between September 15th and October 15th, depending on the year. Grain yield was measured by harvesting two complete central rows (1.4 m x 11 m) from each plot with an experimental plot combine harvester. Grain moisture was determined in a 300-g sample from each plot using a GAC II (Dickey-John, Auburn, IL, USA) and the grain yield was then adjusted to 14% moisture. The aboveground biomass was measured at physiological maturity by harvesting plants from two central rows (1.4 m x 4 m) at ground level. The dry matter (DM) weight was calculated from two plants in each plot, after chopping them and drying 300 g of green tissue in a forced convection oven at 70ºC for 48 h. From these grain and biomass samples, subsamples were taken to determine Cu and Zn concentrations by inductively coupled argon plasma spectrophotometry (Polyscan 61E, Thermo Jarrell-Ash Corp., Franklin, MA, USA) after digesting the calcined plant ash with nitric acid (Mills and Jones, 1996).

Soil samples were taken after harvest in 2006, 2007, 2014 and 2015, at a 0–30 cm depth because soil retains most Cu and Zn in the 0–20 cm layer (plough layer) when LSM is applied (Martínez and Peu, 2000; Brock et al., 2006). The soil samples comprised four mixed cores per plot. Total Cu and Zn extracted with aqua regia (Ministry of Agriculture and Fisheries and Food, 1986) and Na_2-EDTA extractable Cu and Zn (Ministry of Agriculture, Fisheries and Food, 1986) concentrations were determined by a reference laboratory using atomic absorption spectrophotometry (PerkinElmer 2100, Norwalk, CT). The LSM nutrient composition was determined each year by atomic absorption spectrophotometry (as above) after ash extraction with 1 M HCl and dilution with distilled water. The SOC content at the beginning of the experiment in 2002 and at the end in 2015 was measured by dichromate oxidation (Walkley and Black, 1934) in a mix of four soil samples per plot from the 0–30 cm soil layer.

2.4. Calculations

Annual input rates (AIRs) of Cu and Zn (Table 3) were estimated by dividing the total amount of these metals applied during the experiment by 12 (the duration of the experiment in years). The Cu and Zn annual soil accumulation rates (ASARs) were estimated using the following equation (Berenguer et al., 2008a):

ASAR (mg kg^{-1}) = [(final metal soil concentration in LSM-fertilised plots – final metal concentration in N0 control plots) / number of years of the experiment].

Annual biomass uptake (ABU) and annual grain uptake (AGU) were estimated by multiplying the average dry matter biomass and grain yield of the last 2 years of the experiment by the average Cu and Zn concentrations in each treatment. The grain-biomass uptake ratio (GBUR) is the AGU/ABU ratio for each metal.

The stock of SOC in the first 30 cm was calculated using the soil bulk density (1.40 Mg m^{-3}) and SOC concentration.

2.5. Statistical analysis

Statistical analysis of variance was carried out using the mixed model procedure in the Statistical Analysis System (JMP PRO 12, SAS Institute 2012). The LSM rate and inorganic N input (regardless of origin) were considered as fixed factors and the replication as a random effect.

For clarity, the results for 2014 and 2015 were analysed separately. Means were separated by the LSMeans Tukey HSD test ($p < 0.05$), where levels not connected by the same letter are significantly different. A Dunnet contrast was used to compare plant and grain Cu and Zn concentrations with the N0 control treatment, where values beyond the upper or lower detection limit (UDL and LDL, $p < 0.05$) are significantly different (JMP 12, SAS Institute 2012).

3. Results

3.1. ASAR, ABU and AGU

A positive annual soil accumulation rate (ASAR) indicates soil accumulation whereas a negative value indicates depletion. The N300 treatment resulted in negative ASAR values for Cu and Zn, suggesting that Cu and Zn levels decline if LSM is not applied (Table 3). LSM applications achieved positive soil ASAR values for both Cu and Zn, but the values for LSM30 and LSM50 were very similar. The annual input rate (AIR) was higher for both metals under the LSM50 treatment (1.8 kg Cu ha^{-1} yr^{-1} and 7.2 kg Zn ha^{-1} yr^{-1}).

The annual biomass uptake (ABU) for both Cu and Zn varied among the treatments (Table 3). The highest ABU values for Cu were observed for the N300 and LSM50 treatments (0.13 and 0.12 kg Cu ha^{-1}, respectively) whereas similar ABU values for Zn were observed in all three treatments involving N applications and only the N0 control showed a significant difference. The ratio between grain and biomass uptake (GBUR) for Cu did not differ significantly between treatments (Table 3). However, the GBUR for Zn was highest in the N300 and LSM50 treatments (0.54 and 0.40, respectively).

3.2. Soil organic carbon, pH and EC

The N0 control treatment resulted in a small SOC loss of 0.8 g C kg^{-1} (–5%) over the study period, whereas the N300 treatment resulted in a small increase of 0.9 g C kg^{-1} (+6%) compared to 2002 (Table 4). Both LSM applications also increased the SOC (Table 4). The LSM30 treatment increased the SOC levels by 0.9 g C kg^{-1} (+6%) whereas the higher rate (LSM50) increased the SOC by 4.1 g kg^{-1} (+28%), representing an estimated annual increase of 0.34 g C kg^{-1} ha^{-1} yr^{-1} (Table 4). The pH values decreased slightly in the LSM plots, although only the higher LSM rate caused a significant change in pH

(Table 4). Most long-term studies with LSM have reported no changes in pH (Ndayegamiye and Cote, 1989). No significant differences between treatments were observed for soil EC values (Table 4).

3.3. Total Cu and Zn content of the soil

The total Cu and Zn concentrations in the soil in 2006, 2007, 2014 and 2015 are compared in Figure 1. Significant differences between fertiliser treatments were observed for both metals. The total Cu concentrations differed significantly between the LSM treatments and the N300 treatment in 2014 and 2015, but there were no significant differences between the lower LSM treatment and the N0 control (Figure 1a). The total Zn concentrations differed significantly between the LSM and mineral treatments in the last two years of the experiment. The highest soil concentrations we observed were 23.75 mg Cu $kg^{-1}$and 93.33 mg Zn kg^{-1} in the LSM50 treatment, although these values are considered normal for the region (López Arias and Grau-Corbí, 2004).

3.4. EDTA-Cu and EDTA-Zn content of the soil

Extractable Cu and Zn levels in the soil after 12 years of LSM applications were similar in 2014 and 2015 (Figure 2). LSM had a significant effect on the levels of extractable Cu and Zn suggesting that the application of slurry increases the amount of EDTA in the soil. The highest EDTA-Cu and EDTA-Zn concentrations were 8.5 and 19.2 mg kg^{-1}, respectively. Compared with the N0 control, the LSM30 treatment increased the amount of EDTA-Cu by 78% and the amount of EDTA-Zn by 334%, whereas the LSM50 treatment increased the amount of EDTA-Cu by 113% and the amount of EDTA-Zn by

543%. The N300 treatment resulted in the lowest levels of both EDTA-Cu and EDTA-Zn, as also observed for the total Cu and Zn levels (Figure 1).

3.5 Grain and biomass yields

The grain and biomass yields did not decrease following the application of LSM (Figure 3) as previously reported by Berenguer et al. (2008a). The average grain yields across treatments ranged from 10.88 to 12.53 Mg ha^{-1}. The LSM50 and N300 treatments resulted in statistically similar high yields whereas the N0 control resulted in the lowest yields (Figure 3). The biomass was also unaffected by the application of LSM. The average biomass yields across treatments ranged from 22.42 to 23.87 Mg ha^{-1}. There were no statistically significant differences among the LSM50, LSM30 and N300 groups in 3 of the 4 years, and the N0 control again resulted in the lowest yields (Figure 3).

3.6. Cu and Zn concentrations in the whole plant and grain

Whole-plant Cu and Zn concentrations (all aboveground biomass, including grain) were determined after 11 and 12 years of LSM applications (2014 and 2015). The N300 and LSM50 treatments resulted in the highest levels of Cu in 2014 and 2015: 5.03 and 4.68 mg kg^{-1} Cu, respectively (Figure 4). Although the N300 treatment resulted in the lowest soil total Cu and EDTA-Cu concentrations in 2014 and 2015 (Figures 1 and 2), this was not the case for Cu levels in the grain. Whole-plant Zn concentrations were not statistically different among the different treatments.

Grain Cu concentrations in 2014 and 2015 did not differ significantly among the treatments involving N applications, whereas the N300 treatment resulted in the lowest grain Zn concentration in both years.

4. Discussion

4.1. ASAR, ABU and AGU

Our ASAR values for Cu were similar to those reported by Berenguer et al. (2008a), who carried out a similar experiment although the Cu and Zn levels in the slurry may differ. However, our ASAR values for Zn were higher, probably reflecting the higher Zn content in the swine slurry in the last 6 years of the experiment (Table 2). This average concentration of Zn in our LSM (1397 mg kg^{-1} DM, Table 2) was similar to that reported by Comas et al. (2014) for the same region, i.e. north-east Spain (1335 mg kg^{-1} DM). The Spanish government has set a Zn threshold of 200–1000 mg kg^{-1} DM for elaborated organic fertilisers (Gobierno de España, 2013), which is more restrictive than the 2500–4000 mg kg^{-1} DM threshold set for sewage sludge in Directive 86/278/EEC (European Union, 1986). LSM is not an elaborated fertiliser, so this legislation does not affect the amount of LSM that can be applied to soil. In some treatments, the ASAR of total Cu and Zn was higher than the AIR (Table 3) which agrees with previous studies (Berenguer et al., 2008a; Brock et al., 2005; Martinez and Peu, 2000). This suggests that the levels of Cu and Zn can recover in soils and also that these elements remain in the soil when applied as LSM. The partitioning of Cu between the grain and biomass was not affected by N application, but a clear partitioning effect was observed for Zn Our Cu and Zn GBUR values are similar to those reported for maize by Lavado et al. (2001) in Argentina and Gigliotti et al. (1996) in Italy. In China, Xu et al. (2013) reported that Cu uptake was two to three times higher in maize stalks than grain.

4.2. Soil organic carbon

As well as causing the lowest grain and biomass yields (Figure 3), the N0 treatment also caused the SOC to decline at the end of the experiment (Table 4). The soil quality therefore becomes worse without mineral N or manure applications (Manna et al., 2007). The increase in SOC following the N300 treatment could reflect the greater crop productivity, i.e. the production of more roots and biomass (Izaurralde et al., 2001). Maize stover was incorporated into the soil during the last 6 years of the experiment (in all treatments). This suggests that mineral fertiliser may contribute to the maintenance of SOC levels if stover is incorporated, and should therefore be considered as a strategy to maintain soil quality.

Our results agree with previous reports showing that moderate LSM applications can maintain or even increase SOC levels, whereas large applications can increase the SOC substantially (Cote, 1989; Biau et al., 2012). Maize fields provided with maximum N rates from organic materials and also regularly incorporating crop residues do not experience a decline in SOC levels. Wilhelm et al. (2007) reported that 5.25–12.5 Mg ha^{-1} of maize aerial residues are required to maintain SOC levels in the United States, and such amounts are similar to or even lower than the amounts incorporated during the 12 years of our experiment.

4.3. Total Cu and Zn content of the soil

Berenguer et al. (2008a) reported increases in the levels of Cu and Zn similar to those observed in our experiment, which was carried out in the same field. They applied LSM at the same rates for 6 years, and found that the total soil Cu and Zn content increased to maximum levels of 19.4 and 91.6 mg kg^{-1}, respectively. Cu and Zn therefore do not appear to have accumulated further in the soil during the last 6 recent years of LSM applications combined with stover incorporation. However, during the last 6 years of our experiment, the average Cu concentration in the LSM was very similar to the value reported by Berenguer et al. (2008a), whereas the average Zn concentration in our LSM was 90% higher (Table 2). Yagüe and Quílez (2013) reported similar results in a 4-year experiment with maize and LSM in a nearby region. The application of mineral fertiliser achieved significantly lower values for Cu and Zn than the LSM treatments.

The average total Cu and Zn levels in the soil following the LSM treatments were 20.86 mg kg^{-1} and 81.28 mg kg^{-1}, respectively. These values were much lower than the local phytotoxic thresholds of 60 mg Cu kg^{-1} and 175 mg Zn kg^{-1} (López-Arias and Grau-Corbí, 2004) and the national limits of 210 mg Cu kg^{-1} and 450 mg Zn kg^{-1} (Gobierno de España, 1990). Mantovi et al. (2003) reported concentrations of 125 mg Cu kg^{-1} and 100 mg Zn kg^{-1} in silty clay loam soils used to grow maize in northern Italy following 10–15 years of swine and calf slurry applications containing 150–350 kg N ha^{-1} and Cu and Zn contents very similar to our LSM (Table 2). For slurry applications providing more than 350 kg N ha^{-1}, they reported soil concentrations of up to 200 mg Cu kg^{-1} and 125 mg Zn kg^{-1}. These Cu values would be considered phytotoxic in basic calcareous soils under Spanish regulations (Gobierno de España, 1990) although the Cu was derived from organic applications containing more than the 170 kg N ha^{-1} yr^{-1} allowed in our region. These data suggest that high application rates of animal slurries (350 kg N ha^{-1}) could result in soil contamination.

Our results differ from those reported by Coppenet el al. (1993) in a 15-year study in northern France, which suggested a risk of Cu and Zn phytotoxicity in their soils (pH 6) in fields treated with LSM at rates of 50–100 m^3 ha^{-1} yr^{-1}. In our experiment, the highest total soil Cu concentrations were associated with the highest SOC levels (Table 4 and

Figure 1). One potential explanation is the strong association between Cu and SOC because the latter behaves as an inner-sphere complex that binds Cu, reducing its availability and allowing vertical movement through the soil predominantly in association with soluble organic compounds (Hann et al., 2001; De Termmernan et al., 2003). In contrast, there is a weaker association between Zn and SOC because the latter behaves as an outer-sphere complex, thus increasing the availability of Zn (Kabata–Pendias, 2001). The high SOC levels resulting from LSM applications can increase the dissolved SOC content of the drainage solution causing more Zn to leach through the soil profile (Bril and Salomons, 1990; Japenga and Harmsen, 1990).

Maize can experience phytotoxic effects in the presence of ~21 mg Cu kg $^{-1}$ (Jarausch-Wehrheim et al., 1996) and 100 mg Zn kg^{-1} (Mantovi et al., 2003). After 12 years of LSM applications, the levels of Cu and Zn in our soil (23.75 mg Cu kg^{-1} and 93.33 mg Zn kg^{-1}) came near these levels, thus the composition of the slurry in our area is such that the treatment needed to meet N requirements delivers much more Cu and Zn to the soil than the maize plants can assimilate. The application of Cu and Zn is beneficial if soils lack sufficient quantities of these metals, but it is unclear whether the introduction of excess Cu and Zn is likely to inhibit crop productivity and/or cause environmental concerns due to runoff, as is the case for phosphorus levels resulting from high LSM application rates.

4.4. EDTA-Cu and EDTA-Zn content of the soil

The application of inorganic fertiliser (N300) resulted in the lowest concentrations of soil EDTA-Cu and EDTA-Zn in 2014 and 2015, probably reflecting the higher biomass production and the consequent enhanced mineral uptake (Table 3 and Figure 3). Plots treated with LSM became enriched with Cu-EDTA and Zn-EDTA, as reported by Mattias et al. (2009) in Brazil.

Berenguer et al. (2008a) reported increases in the concentration of EDTA-Cu that were similar to our observations, but the concentration of EDTA-Zn was lower, particularly in the LSM50 treatment. The Zn concentrations in our LSM increased significantly after 2007, averaging 71.8 ppm from the 2002–2007 period and 199.5 ppm (wet matter) for the 2010-2015 period (Table 2). These values were lower than those reported by Comas et al. (2014) for slurries in Catalonia, with average concentrations of 1335±783 mg kg^{-1} DM. These data suggest that the higher Zn content of our slurry explains the higher EDTA-Zn levels in our soil compared to the values reported by Berenguer et al. (2008a).

Caution must be exercised when comparing total metal concentrations and the concentration of EDTA complexes in different studies because the extraction method has a substantial effect on the recorded values, as reported by Petruzzelli et al. (1989). The acid extraction method used in this study was designed to extract micronutrients from deficient calcareous soils, but O'Connor (1988) claims that this method cannot accurately predict the uptake of metals into plants because the correlation between plant uptake and acid-extractable Cu and Zn levels becomes weaker when soils are no longer deficient in Cu and Zn.

4.5. Grain and biomass yields

The grain and biomass yields in 2014 and 2015 were higher in the plots receiving the N-rich N300 and LSM50 treatments (Figure 3). These treatments achieved biomass yields of 29.5 and 28.7 Mg ha^{-1}, respectively, and grain yields of 16.8 and 15.4 Mg ha^{-1}, respectively. The LSM50 treatment produced slightly lower yields probably because it represented ~270 kg N ha^{-1} yr^{-1} (applied before seeding) compared to the 300 kg N ha^{-1} yr^{-1} of the N300 treatment (applied as side-dressing). The grain yields were higher than those reported by Berenguer et al. (2008a) in 2006 and 2007, confirming the absence of crop phytotoxicity in our trials (Figure 3).

4.6. Cu and Zn concentrations in the whole plant and grain

The Cu and Zn concentrations in the whole plant and specifically in the grain were similar to those reported by Berenguer et al. (2008a) and Mantovi et al. (2003). Petruzzelli et al. (1989) reported higher maize grain Cu concentrations (4 ppm of Cu and 36 ppm of Zn), although their methodology involved nitric-perchloric acid digestion rather than our nitric acid method.

Andrfts et al. (1993) reported that higher rates mineral N fertilisation reduced the concentration of Zn in the grain, which agrees with our results. Mantovi et al. (2003) observed similar Zn concentrations in maize plants regardless of differences in the slurry application rates, and the resulting differences in N supply, which also agrees with our results.

The highest concentrations of Cu and Zn we observed in whole plants and grain (4.68 mg Cu kg^{-1} and 26.5 mg Zn kg^{-1}) following the application of LSM for 12 years, are not considered as a significant phytotoxic risk according to current European legislation (European Union, 2003). Our plant and grain concentrations were also lower than the maximum tolerable concentrations for animal species set by the National Research Council (2005): 25 mg Cu kg^{-1} (for sheep) and 200 mg Zn kg^{-1} (for fish). The maximum Zn concentration in this study was 26.5 mg kg^{-1}.

5. Conclusions

After 12 years of continuous LSM applications, the soil in our study did not accumulate sufficient Cu or Zn to exceed the threshold of phytotoxicity established in current legislation. The highest LSM application rate resulted in soil levels of 19.4 mg Cu kg^{-1} and 91.6 mg Zn kg^{-1} in the earlier years of application (2006 and 2007), and 23.75 mg Cu kg^{-1} and 93.33 mg Zn kg^{-1} in the later years (2014 and 2015). We therefore conclude that the application of Cu and Zn to soil at the concentrations we used, which are representative LSM concentrations in the Ebro Valley that comply with the Nitrates Directive, does not pose a direct risk to agricultural soil quality in the area. The maximum Cu and Zn levels in the whole plant or the grain in LSM plots were 4.68 mg kg^{-1} and 26.5 mg kg^{-1}, respectively, which are significantly lower than the maximum tolerable concentrations established by the National Research Council (2001) for animals. Given these results, the application of LSM to maize fields in our region even at twice the rate allowed by the EU in nitrate-vulnerable zones will not result in soil contamination or the accumulation of these metals to toxic levels in the whole plant or grain. The LSM application rate of 50 m^3 ha^{-1} yr^{-1} corresponded to annual input rates that were much lower than the maximum levels allowed by EU directives for sewage sludge, i.e. <15% of maximum levels for Cu and <24% of maximum levels for Zn. Our maize yields were not affected by the application of LSM. We therefore conclude that the application of LSM at the rates described herein does not pose any risk of phytotoxicity or adverse effects in consumers.

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Table 1. Soil properties at the beginning of the experiment (2002).

	Horizon depth, cm		
	Ap	**Bw**	**Bk**
	0-23	**23-69**	**69-117**
Sand, g kg^{-1}	390	380	450
Silt, g kg^{-1}	400	420	380
Clay, g kg^{-1}	210	200	170
pH†	8.3	8.3	8.3
EC, dS m^{-1}	0.2	0.34	0.59
CEC, $cmol_c$ kg^{-1}	24	-	-
Organic carbon, g kg^{-1}‡	13	8	4
Bulk density, g cm^{-3}	1.40	1.56	1.63
Available water holding capacity, mm	29	67	54
P, mg kg^{-1} §	31	-	-
K, mg kg^{-1} ¶	217	-	-

† Water (1:2.5).

‡ Walkey-Black method.

§ Olsen method.

¶ Ammonium acetate method.

Table 2. Average composition (wet matter) of applied liquid swine manure (LSM) (2002–2015).

Characteristic	**Mean**	**Std Dev**
Dry matter, g kg^{-1}	10.3	2.8
Organic matter, g kg^{-1}	78	4
pH	8.4	0.5
C/N	13.9	2.7
NH_4^+-N, g N kg^{-1}	5.0	1.0
Total N, g kg^{-1}	7.5	1.4
P, g kg^{-1}	1.6	0.5
K, g kg^{-1}	4.3	1.2
CE, dS m^{-1}	6.9	2.5
Cu, mg kg^{-1}	33.7	10.7
Zn, mg kg^{-1}	136	77

Table 3. Total input rate (TIR), average annual input rate (AIR), annual soil accumulation rate (ASAR), average annual biomass uptake (ABU) and grain uptake (AGU) and grain biomass uptake ratio (GBUR) for Cu and Zn in 2014 and 2015.

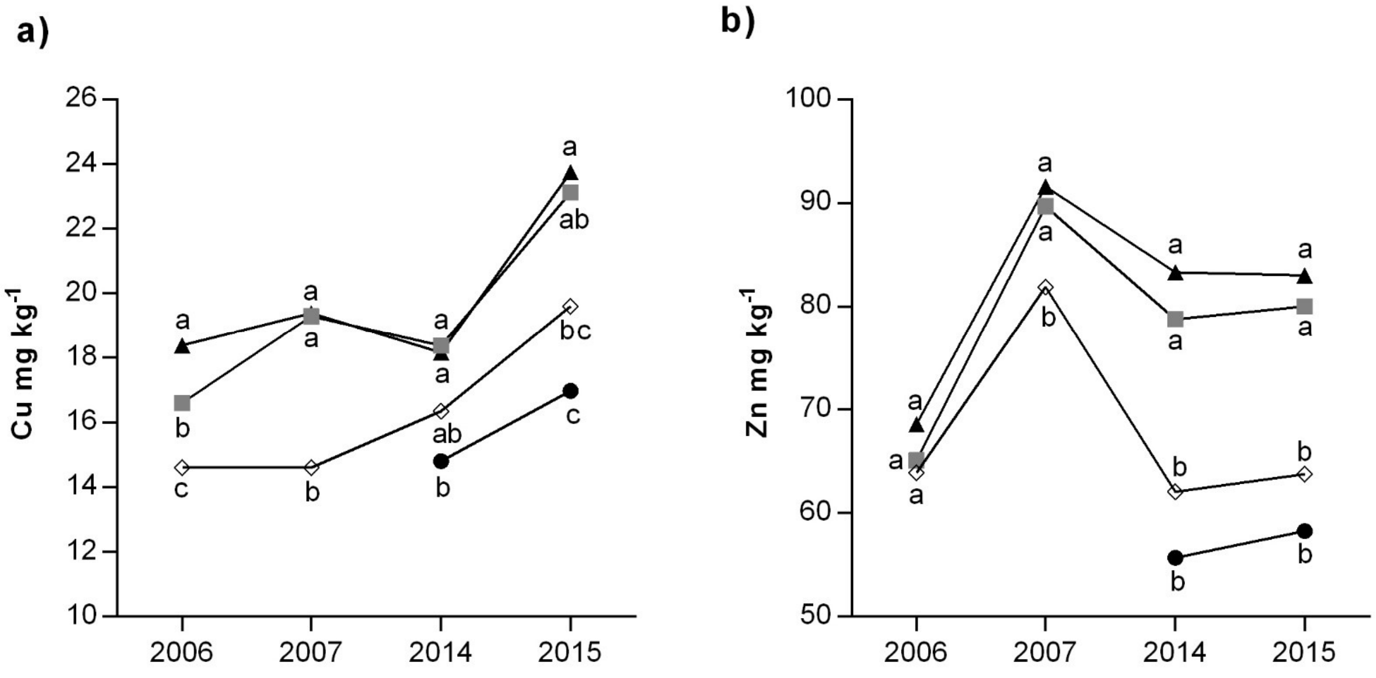
	Treatment	TIR	AIR	ASAR	ABU	AGU	GBUR
		---------------------------- kg ha^{-1}					-------------------------
Cu	N0	-	-	-	0.04 c	0.01 b	0.34 a
	N300	-	-	-0.93 b	0.13 a	0.04 a	0.33 a
	LSM30	12.2	1.0	1.47 a	0.08 b	0.03 a	0.36 a
	LSM50	21.7	1.8	1.25 a	0.12 a	0.04 a	0.34 a
Mean		-	-	-	0.09	0.03	0.34
Zn	N0	-	-	-	0.28 c	0.10 c	0.36 c
	N300	-	-	-0.95 b	0.50 b	0.27 a	0.54 a
	LSM30	48.9	4.1	5.06 a	0.58 ab	0.19 b	0.33 c
	LSM50	86.8	7.2	5.77 a	0.65 a	0.26 a	0.40 b
Mean		-	-	-	0.50	0.21	0.42

Table 4. Soil organic carbon (SOC), SOC stock, pH and electrical conductivity (EC) at the end of the experiment under the different treatments.

Treatment	**SOC**	**SOC stock***	**pH**	**EC**
	g kg^{-1}	Mg ha^{-1}		dS m^{-1}
N0	14.4 b	58.83 b	8.34 a	0.23 a
N300	15.8 a	66.28 a	8.33 a	0.20 a
LSM30	15.4 ab	64.68 ab	8.24 ab	0.20 a
LSM50	16.4 a	68.96 a	8.21 b	0.21 a
Mean	16.5	69.09	8.29	0.21

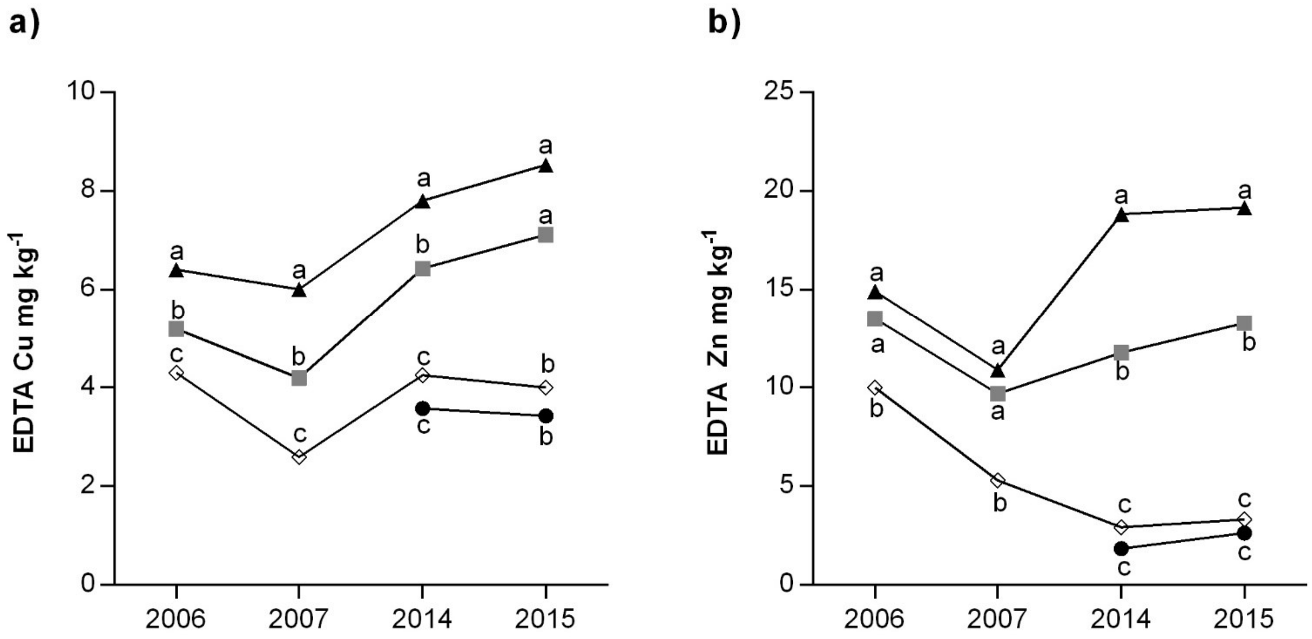
*Initial carbon stock of 53.59 Mg ha^{-1}. Letters show means separation by Tukey's HSD test.

Figure 1. Soil total Cu and Zn level in the first 0–30 cm soil depth, after 5, 6, 11 and 12 years (2006, 2007, 2014, 2015) of the experiment.



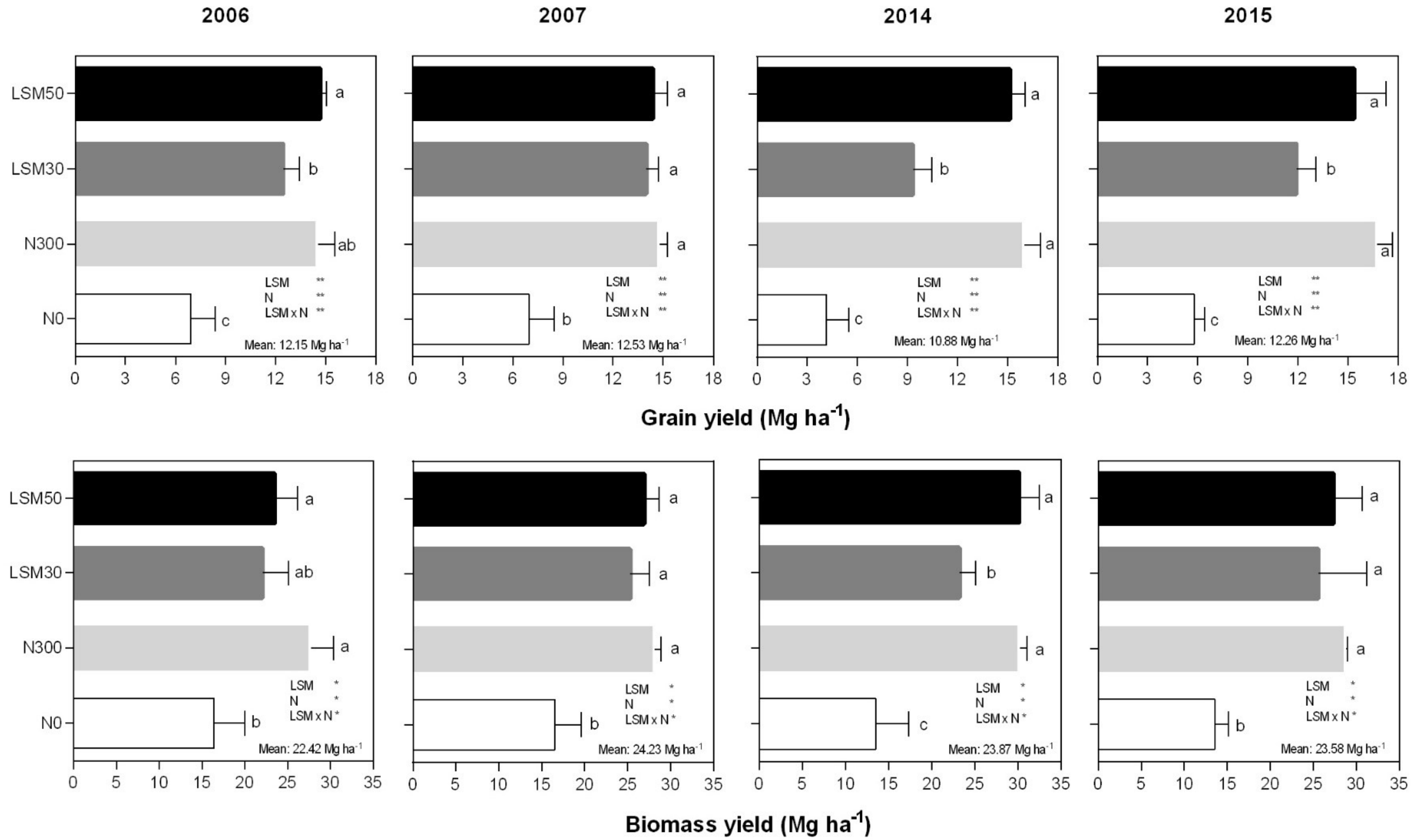
Markers represent the different treatments: black triangles = LSM50, grey squares = LSM30, white diamonds = N0 and black circles = N300. Letters show means separation by Tukey's HSD test.

Figure 2. Soil EDTA-Cu and EDTA-Zn levels in the first 0–30 cm soil depth, after 5, 6, 11 and 12 years (2006, 2007, 2014, 2015) of the experiment.



Markers represent the different treatments: black triangles = LSM50, grey squares = LSM30, white diamonds = N0 and black circles = N300. Letters show means separation by Tukey's HSD test.

Figure 3. Grain and biomass yields in 2006, 2007, 2014 and 2015 for the four treatments: LSM50, LSM30, N300 and N0.



Letters show means separation by Tukey's HSD test. LSM: Liquid swine manure. N: inorganic N (mineral fertiliser and NH_4^+-N). *,**: Significant at the 0.05 and 0.01 levels. Error bars indicate standard deviations of the mean.

GENERAL DISCUSSION

GENERAL DISCUSSION

The purpose of the present thesis is to contribute to the development of more sustainable agricultural systems, especially in terms of nitrogen (N) use in maize production. Through the knowledge generated as a result of the present research, the aim is to provide guidelines for a proper N fertilization management.

The use of livestock manure and mineral fertilizer or a combination of both in maize results, in general, in several benefits (Schröder, 2005). However, we have to be aware that the adoption of optimal N application rates and correct management are both economically and environmentally beneficial.

Many different factors affect the utilization and effects of manures and mineral fertilizers, and these factors, including N composition of the fertilizer source, soil N levels and crop yield level, should be considered when recommending N application rates and methods (Blackmer et al., 1989; Berenguer et al., 2008a). In the experiments described here, the maize yield response to different N rates, nitrogen use efficiency (NUE), soil residual N and the N rates allowed by the current legislation were studied.

The importance of determining soil initial N

In pig slurry (PS) and mineral N fertilization trials, grain yield response was greatly affected by initial soil N content and N fertilization (organic or mineral) rates, and varied between years. For this reason, N fertilization recommendations in maize production may vary greatly from one year to another in a given field and between fields in the same area, confirming reports by other authors (Binford, 1992; Nevens and Reheul, 2005; Halvorson et al., 2005; Cela et al., 2013).

In the field trials described here, no yield response to N fertilization was observed in the first year of the experiment because of the very high initial soil N content, 317 kg NO_3^--N ha^{-1} (0-90 cm), at the beginning of the experiment. A response to N fertilization was gradually observed over time as soil NO_3^--N became depleted, and depended on the initial soil NO_3^--N content.

In the 12 year experiment with mineral N in sprinkler irrigation conditions, it was determined that at planting time 203 kg N ha^{-1} of available N (soil NO_3^--N + mineral N fertilizer) in the 0-30 cm soil layer were needed to achieve maximum maize grain yields (~14 Mg ha^{-1}), with an R^2 of 77%. This value of available N is quite similar to those reported previously for sprinkler irrigation in the Ebro Valley (Isla and Quílez, 2011; Cela et al., 2013), but higher, compared to other growing situations, than those reported by Blackmer et al. (1989) and Binford et al. (1992) in Iowa (USA), or Cui et al. (2008) in the North China Plain. Other situations, with different yield levels, irrigation systems, soil types, and organic matter levels may need different available N for highest yields in maize.

Sampling to a 0-30 cm soil depth provided very similar correlations, from 76% to 79%, between grain yield and soil available N to sampling to 0-60 or 0-90 cm depths. This suggests that, in our conditions, sampling deeper than 30 cm for N recommendations for maize would not be necessary. Some authors have reported similar results to ours in their conditions, while others found it sensible to sample to a 60 cm depth (Sims et al., 1995a, Schröder et al., 1998).

Organic fertilization: cattle manure and pig slurry

In the experiment with PS applications under sprinkler irrigation conditions in a loam soil, the PS application rates of 30 (P30N0) and 50 m^3 ha^{-1} (P50N0) showed similar effects on maize grain yield during the experimental period (12-yr). The relative nitrogen fertilizer value (RNFV) was on average 51% for both the P30N0 and P50N0, compared to mineral N fertilizer applied at sidedress. The RNFV could improve and approach 80%, a value considered by some authors as desirable for manures in a near future (Schröder, 2005). For this, we think that other PS application methods should be studied. The P50N0 (total of 389 kg N ha^{-1}), almost double the amount of N allowed by the EU directives, allowed to obtain satisfactory maize grain yields, but not maximum ones. The highest average grain yields were obtained with combinations of organic and mineral fertilizer, but at N rates above 400 kg N ha^{-1} yr^{-1}, that resulted in residual N contents (0-90 cm) higher than 300 kg N ha^{-1}, which on average, significantly decreased from harvesting to the next maize planting time.

Mineral N fertilization

Mineral N fertilization gave satisfactory maize grain yields. Under sprinkler irrigation conditions in a loam soil, the application rate of 200 kg N ha^{-1} at sidedress was on average considered the most appropriate rate in our growing conditions, when considering grain yield (13.6 Mg ha^{-1}), residual N content (165 kg N ha^{-1}) and NUE (0.83 kg kg^{-1}), and at the same time coincides with the maximum N rate allowed by current legislation (Table 1). However, an increase in the maize yield potential of the hybrids used was observed during the experimental period (2002-2015), showing in the treatment of 300 kg N ha^{-1} an annual linear grain yield gain of 192 kg ha^{-1} yr^{-1} during the 12-year experimental period. If this situation of increased grain yields continues, we wonder if in a near future, because the last hybrid used in our experiments was registered in 2009, the most appropriate N rate could be around 250 kg N ha^{-1}. It will be interesting to assess the maize yield response to N fertilization, residual N contents and NUE at different N rates with the latest genetic material in the coming years.

Complementing organic fertilization with mineral fertilizer was needed at the 30 m^3 PS ha^{-1} rates in order to achieve high yields.

Table 1. Maximum amounts of N that can be applied to maize in nitrate vulnerable zones. OF: organic fertilizer. MF: Mineral fertilizer.

	Maximum allowed N (kg N ha-1)		
Irrigation	Total	OF	MF
Rainfed	210 (250*)	170	150 (170*)
Irrigated	300 (350**)	170	200

* Vulnerable zone 7 ** Vulnerable zone 1

Source: Generalitat de Catalunya, 2009. Decret 136/2009

N use efficiency

Nitrogen use efficiency (NUE) decreased when increasing N rates, and for mineral N applications ranged from 1.20 to 0.59 kg kg^{-1}. Organic applications always showed lower NUE values than mineral applications for similar N inputs, with values for the case of 200 kg mineral N ha^{-1} and 30 m^3 PS ha^{-1} (218 kg N ha^{-1}) of 0.83 and 0.64 kg kg^{-1}, respectively.

Soil organic carbon

SOC increased with PS and mineral fertilization; stover was chopped after harvesting and incorporated into the soil. The absence of fertilization resulted in a slight increase (10%) over the 12 years. Pig slurry rates influenced SOC stocks, where increasing the rate resulted in higher SOC sequestration rates. The application of 50 m^3 PS ha^{-1} yr^{-1} resulted in a SOC sequestration rate of 1.28 Mg C ha^{-1} yr^{-1}. Mineral fertilization also resulted in an increase in SOC contents, with the rate of 300 kg mineral N ha^{-1} resulting in sequestration rate of 1.06 Mg C ha^{-1} yr^{-1} (0-30cm). Soil type, climate conditions and tillage also influence the amount of stover or carbon needed to increase SOC levels (Vance et al., 2000; Wilhelm et al., 2007).

Effects of continuous pig slurry applications on Cu and Zn contents

After continuous PS applications, the application of 50 m^3 ha^{-1} resulted in soil levels of 19.4 mg Cu kg^{-1} and 91.6 mg Zn kg^{-1} in the 5^{th} and 6^{th} year of application (mean of 2006 and 2007), and 23.75 mg Cu kg^{-1} and 93.33 mg Zn kg^{-1} in the 11^{th} and 12^{th} years (mean of 2014 and 2015). These values are lower than the national limits of 210 mg Cu kg^{-1} and 450 mg Zn kg^{-1} (Gobierno de España, 1990). An accumulation of both metals was observed if comparing the PS treatments with the plots where PS was not applied. This concurs with reports from other authors (Coppenet et al., 1993; Mantovi et al., 2003; Berenguer et al., 2008b).

The maximum Cu and Zn levels in the whole plant or grain in PS plots were 4.68 mg kg^{-1} and 26.5 mg kg^{-1}, respectively, which are significantly lower than the maximum tolerable concentrations established by the National Research Council (1980) for animals, 30 mg Cu kg^{-1} and 500 mg Zn kg^{-1}.

Given these results, the application of PS to maize fields in our region, even at twice the rate allowed by the EU in nitrate-vulnerable zones, will not result in soil contamination of Cu or Zn in the near future (~100 years), or the accumulation of these metals to toxic levels in the whole plant or grain.

Current legislation about N applications in maize

The maximum amounts of N derived from organic sources that can be applied in nitrate vulnerable zones in Catalonia is 170 kg N ha^{-1}, a value which rises to 210 kg N ha^{-1} in non-vulnerable zones (Generalitat de Catalunya, 2009). Most farmers in irrigated areas apply more than 30 m^3 PS ha^{-1} yr^{-1} (~200 kg N ha^{-1} yr^{-1}) to maize (Sisquella et al., 2004). It has been shown in this thesis that application rates of 50 m^3 PS ha^{-1} yr^{-1} (~400 kg N ha^{-1} yr^{-1}) did not result in higher residual soil nitrate contents than the allowed application rate of 200 kg mineral N ha^{-1}, which could lead to think that the maximum allowed N rate for organic sources by the Directive of Nitrates could be raised for the case of maize. However, taking into account the possible accumulation of P, not evaluated in our study but reported by Cela et al. (2010) in the area, a reduction in the usual amounts of PS applied to maize and complying with the Directive of Nitrates would prevent soil P accumulation and diminish the risk of P losses and eutrophication. Introduction of legislation limiting P applications, crops with higher P uptake than maize, or double cropping systems, could help to decrease soil P levels in some fields.

Pig slurry is subject to variability in its nutrient composition, and the use of *in situ* devices, like the conductivity meter, which estimate the N concentration of PS could be useful for better N evaluation and to reduce the risk of N and P surpluses. Also, other PS application methods, facilitating the application of predefined PS rates, reducing ammonia emissions and improving application uniformity compared to the surface splash plate method, including the trail hose technique or direct incorporation, could result in a more efficient use of PS.

Future research

The results obtained in this thesis can provide guidelines for future research. Points in which we suggest that further research be considered include:

- Assessment of other PS application methods than the splash plate technique, including by trail hose (surface) application, or direct incorporation. Determination of the RNFV, ammonia losses and application uniformity. Evaluation of new commercial products containing nitrification inhibitors, such as DCD and DMPP, added to PS.

- Determination of yield response, NUE and residual soil N contents under different rates of N fertilization with the latest high productive maize genetic materials.

- Determination of the amount of N needed to obtain maximum yields in different situations, with different irrigation systems, N management systems, SOC levels and soil types.

- Revision of whether the maximum N rates allowed by current EU legislation remain optimal in a near future.

- Determination of SOC sequestration rates in different soils and management conditions, and their long-term evolution.

- Follow-up of Cu and Zn long-term evolution in soil after continuous applications of PS.

- Trapping of unused or escaping nitrate-N using cover crops or drainage control structures.

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GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

The main conclusions of the research carried out in this thesis are:

- Determination of initial (before planting) soil NO_3^--N content is a very useful tool for N recommendations in maize production. Recommendations should not be based on fixed rates because initial soil NO_3^--N, which influences the response of the maize to different fertilization rates, can vary greatly from year to year.

- The measurement of soil N availability (soil mineral N + fertilizer mineral N) before planting or side-dressing in the 0–30 cm horizon appears to be a suitable method for specification of optimal N fertilization applications for maize with sprinkler irrigation under Mediterranean conditions. It is just as accurate as sampling the soil to a 60 or 90 cm depth. It was determined that 203 kg N $ha^{-1}$of available N in the 0-30 cm soil layer were needed to achieve maximum average maize grain yields (~14 Mg ha^{-1}).

- The rate of 200 kg mineral N ha-1 in sprinkler irrigation was sufficient to produce optimal GY while also achieving relatively high NUE and moderate soil residual N levels. This N rate is similar to the maximum N application rates allowed under current local legislation.

- Maize hybrids became more productive over the course of the study. A linear annual grain gain of 192 kg ha^{-1} yr^{-1} was observed in a non-N-limiting mineral N treatment (300 kg ha^{-1}) during the 12-yr experiment.

- The end-of-season maize basal stalk nitrate test can highlight deficiencies and excess of N applications, and can complement soil tests and other strategies. Maximum maize basal stalk nitrate contents under our growing conditions should not exceed 2500 mg nitrate-N kg^{-1}.

- The measurement of chlorophyll levels can predict soon N deficiencies, but not excess N.

- The relative nitrogen fertilizer value of pig slurry applied by splash-plate and incorporated within 3-4 hours after application, compared to ammonium nitrate applied at two equal sidedressings (V3-V4 and V5-V6) was 51%. Other application methods, such as direct incorporation or trail hose (surface) should be assessed in order to increase the relative nitrogen fertilizer value of pig slurry.

- It is necessary to complement pig slurry applications at the maximum allowed rates of organic N (170 kg N ha^{-1}), about 25 m^3 ha^{-1}, with mineral N at sidedress in order to obtain satisfactory grain yields.

- After 12 years of continuous pig slurry applications at the allowed N rate or twice this rate, the soil did not accumulate sufficient Cu or Zn to exceed the phytotoxicity threshold established in current legislation.

- Copper and Zn concentrations in maize grain and whole plant in plots receiving pig slurry were significantly lower than the maximum tolerable concentrations established for humans or animals.

- At very high N application rates (combinations of organic and mineral fertilizer) significant differences were found between soil residual N and initial N contents.

- At similar N application rates, organic fertilization showed lower nitrogen use efficiency than mineral fertilization.

- It is possible to obtain appropriate grain yields by fertilizing with organic materials and at the same time having similar or lower risks of N leaching than when applying mineral fertilization. High manure or pig slurry application rates did not result in higher residual soil N contents than the application of similar or even lower N rates with mineral fertilizer.

- The application of fertilizers to maize in order to achieve maximum yields is not always environmentally sustainable, because high levels of residual N remain in the soil after harvest.

- Mineral and pig slurry fertilization resulted in increases in the soil organic carbon content in the upper 30 cm soil layer, indicating that mineral and organic fertilization should be considered as a strategy to improve or maintain soil quality.

- In general, the optimal N rate considered as the minimum N rate (organic or mineral) to achieve maximum grain and biomass yields coincided closely with minimum environmental impacts and the highest N efficiencies, suggesting that the optimal N rate was also the most environmentally friendly.

PICTURES


MINOLTA
SPAD
62.7


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MOTOR
159


Nitrachek 404
181 ppm


09/09/2014


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