Universitat de Lleida

Stover Management, Organic and Mineral Nitrogen Fertilization effects on Maize Production and Soil Quality under Irrigated Mediterranean Conditions

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Universitat de Lleida

UNIVERSITAT DE LLEIDA

DEPARTAMENT DE PRODUCCIÓ VEGETAL I CIÈNCIA FORESTAL

Stover Management, Organic and Mineral Nitrogen Fertilization effects on Maize Production and Soil Quality under Irrigated Mediterranean Conditions

Anna Biau Aresté

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A la vida, per ensenyar-me aprendre.

A la vida, per ensenyar-me a créixer.

AGRAÏMENTS

En aquesta vida no ens valem de nosatres mateixos per anar avançant en el camí, en ocasions assolim els nostres objectius gràcies a les crítiques, ajuda i col·laboració de moltes persones. Per aquest motiu vull expressar el meu agraïment a tots els que meu ajudat a arribar fins aquí:

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LIST OF CONTENTS

SUMMARY

Crop stover plays an important role in the maintenance of the soil fertility, and consequently has an important influence in present and future crop production. Returning crop stover to the soil favorably influences its organic matter (OM) levels, and consequently the structure, water storage and water and air movement, and other determinants of soil productivity. Corn stover returned to the soil also contribute to carbon (C) sequestration and hence help to reduce the release of greenhouse gases. Interactions between crop stover management and nitrogen (N) fertilization could therefore help to improve N use efficiency while increasing crop production and maintaining the sustainability of the cropping systems. Mineral fertilization constitutes a significant fraction of total corn production cost. Thus, the high concentration of swine farms in the Ebro Valley area allows an attractive fertilization strategy of using animal manure for reducing the costs of corn production. In order to investigate the effects of stover management on corn production and their possible interaction with the N fertilization, two field experiments were conducted from 2010 to 2012 in the irrigated areas of the Ebro valley. The study analyzed the interaction between corn stover management (incorporated vs removed) and N fertilization treatments (organic v.s. mineral) on corn production, and on selected soil quality indicators (dehydrogenase activity (DHA), microbial biomass carbon (MBC) and earthworm abundance). The N fertilization treatments consisted of 60 m^3 ha^{-1} $year^{-1}$ (about 386 kg N ha^{-1}) of cow slurry (CS) and mineral N fertilization rates of: 0 (control), 100, 200 and 300 kg N ha^{-1} $year^{-1}$ (0N, 100N, 200N, and 300N). Apart from these two field trails, the thesis also evaluated the impact of long-term (from 2002 to 2011) fertilization, organic (pig slurry, PS) and mineral (300N) on corn production and soil quality indicators (as acid-phosphatase activity, earthworm abundance, CO_2 Flux, Shannon H' diversity index (H'), number of utilized substrates (NUS), MBC, resistance to penetration (RP) and OM, among others). Furthermore, we studied the whole plant N concentration with different plant material preparation systems. Under our conditions, our data suggested that returned stover to the soil (as average 14 Mg of stover ha^{-1} $year^{-1}$) and organic fertilization had a positive impact on soil quality without grain yield penalties. Grain yield after three years of stover incorporation ranged from 16 to 20 Mg ha^{-1} depending on the N fertilization source.

RESUM

El rostoll dels cultius te un paper molt important en el manteniment de la fertilitat del sòl. L'incorporació del rostoll al sòl afecta favorablement els nivells de matèria orgànica (MO) i per tant l'estructura del sòl, l'emmagatzematge i el moviment d'aigua i aire, i altres determinants de la productivitat del sòl. Retornar el rostoll de blat de moro al sòl també contribueix al segrest de carboni (C) afavorint la reducció de l'emissió de gasos d'efecte hivernacle. La interacció entre la gestió del rostoll i la fertilització nitrogenada (N) pot ajudar a millorar l'eficiència d'ús de N, alhora que augmentar la producció i el manteniment de la sostenibilitat dels sistemes de cultiu. La fertilització mineral, sobre tot la nitrogenada, representa una part significativa del cost total de la producció de blat de moro. Per tant, l'alta concentració de les explotacions porcines a la zona de la Vall de l'Ebre permet que la utilització de purins sigui una estratègia de fertilització atractiva per reduir els costos de producció. Per tal d'investigar els efectes de la gestió del rostoll en la producció de blat de moro i la seva possible interacció amb la fertilització N, es van dur a terme dos assajos de camp des de 2010 fins a 2012 en zones de regadiu de la Vall de l'Ebre. Es va analitzar l'efecte de la interacció entre la gestió del rostoll (incorporat o eliminat) i els tractaments de fertilització N (orgànic o mineral) en la producció de blat de moro, i en alguns indicadors de qualitat del sòl (activitat deshidrogenasa (ADH), carboni de la biomassa microbiana (CBM) i l'abundància de cucs). El tractament de fertilització orgànica va consistir en 60 m^3 ha^{-1} any^{-1} (~ 386 kg N ha^{-1}) de purí vaquí (CS) i de fertilització mineral: 0 (control), 100, 200 i 300 kg N ha^{-1} any^{-1} (0N, 100N, 200N i 300N). A part d'aquests dos assaigs de camp, també es va avaluar l'impacte a llarg termini (de 2002 a 2011) de la fertilització orgànica (purí de porc, PS) i mineral (300N) sobre la producció de blat de moro i dels indicadors de qualitat del sòl (com l'activitat de la fosfatasa-àcida, l'abundància de cucs de terra, Flux CO_2, l'índex de diversitat H de Shannon (H'), el nombre de substrats utilitzats (NUS), CBM, la resistència a la penetració (RP) i MO, entre d'altres). A més, des de el punt de vista metodològic, es va mesurar la concentració total de N a la planta amb diferents mètodes de preparació de les mostres. Els resultats suggereixen que, sota les nostres condicions, incorporar el rostoll al sòl (~14 Mg de rostoll ha^{-1} any^{-1}) juntament amb la fertilització orgànica té un impacte positiu en la qualitat del sòl sense penalitzacions en el rendiment de gra. El rendiment de gra, després de tres anys d'incorporació del rostoll, es mou entre 16 i 20 Mg ha^{-1} depenent de la font de fertilització de N.

RESUMEN

El rastrojo de los cultivos tiene un papel muy importante en el mantenimiento de la fertilidad del suelo. Incorporar el rastrojo al suelo puede afectar favorablemente los niveles de materia orgánica (MO) y por tanto la estructura del suelo, el almacenamiento y el movimiento de agua y aire, y otros determinantes de la productividad del suelo. Devolver el rastrojo de maíz al suelo también contribuye al secuestro de carbono (C) favoreciendo la reducción de la emisión de gases de efecto invernadero. La interacción entre la gestión del rastrojo y la fertilización nitrogenada (N) puede ayudar a mejorar la eficiencia de uso del N, al mismo tiempo que aumenta la producción y el mantenimiento de la sostenibilidad de los sistemas de cultivo. La fertilización mineral, sobre todo la nitrogenada, representa una fracción significativa del coste total de la producción de maíz. Por ello, la alta concentración de las explotaciones porcinas en la zona del Valle del Ebro permite que la utilización de purines sea una estrategia de fertilización atractiva para reducir los costes de producción. Con el fin de investigar los efectos de la gestión del rastrojo en la producción de maíz y su posible interacción con la fertilización N, se llevaron a cabo dos ensayos de campo, desde 2010 hasta 2012, en las zonas de regadío del Valle del Ebro. Se analizó el efecto de la interacción entre la gestión del rastrojo (incorporado o eliminado) y los tratamientos de fertilización N (orgánico o mineral) en la producción de maíz y en algunos indicadores de calidad del suelo (actividad deshidrogenasa (ADH), carbono de la biomasa microbiana (CBM) y la abundancia de lombrices). La fertilización orgánica consistió en 60 m^3 ha^{-1} $año^{-1}$ (~ 386 kg N ha^{-1}) de purín vacuno (CS) y la fertilización mineral: 0 (control), 100, 200 y 300 kg N ha^{-1} $año^{-1}$ (0N, 100N, 200N y 300N). También, se evaluó el impacto a largo plazo (de 2002 a 2011) de la fertilización orgánica (purín de cerdo, PS) y mineral (300N) sobre la producción de maíz y los indicadores de calidad del suelo (como la actividad de la fosfatasa-ácida, la abundancia de lombrices, Flujo CO^2, el índice de diversidad H de Shannon (H '), el número de sustratos utilizados (NUS), CBM, la resistencia a la penetración (RP) y MO, entre otros). Además, se midió la concentración total de N en la planta con diferentes métodos de preparación de las muestras. Los resultados sugieren que, bajo nuestras condiciones, devolver el rastrojo al suelo (~ 14 Mg de rastrojo ha^{-1} $año^{-1}$) junto con la fertilización orgánica tiene un impacto positivo en la calidad del suelo sin penalizaciones en el rendimiento. El rendimiento de grano, después de tres años de incorporación del rastrojo, se mueve entre 16 y 20 Mg ha^{-1} dependiendo de la fuente de fertilización de N.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The Ebro Valley is an extensive area located in the northeast of Spain (Fig. 1), characterized by a semiarid climate, with average annual rainfall ranging from 200 to 400 mm. Despite its semiarid environment, this valley is one of the most important areas for livestock farming and agriculture in Spain due to the presence of considerable irrigation infrastructure. The two largest autonomous communities in this area: Aragon and Catalonia, respectively contained about 399,045 ha and 207,035 ha of irrigated land in 2011. The prevailing method of irrigation is flood-irrigation, based on systems built during the late 19th and early 20th centuries. Even today, this infrastructure still represents more than 50% of the irrigated lands of Aragon and Catalonia (MARM, 2011). The newly irrigated areas are mainly under sprinkler irrigation and represent about 20% of the total irrigated land in this area (MARM, 2011).

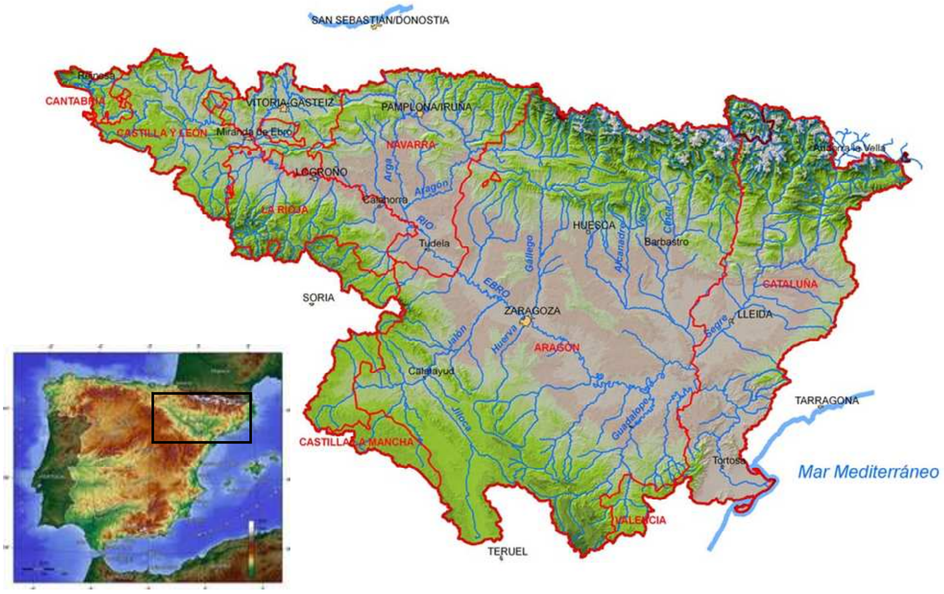

Fig. 1. Ebro river basin (MAPA).

Source: Confederación Hidrográfica del Ebro.

With about 105,694 ha (MARM, 2011), maize is the most important field crop cultivated in the Ebro Valley area. It is mainly used for animal consumption, constituting an important element in feed for pigs, cows and poultry. Generally speaking, average crop yields in the area range from 10 to 15 t ha^{-1} (14% moisture) under sprinkler irrigation (Cela et al., 2011; Boixadera et al., 2005; Daudén and Quílez, 2004), although under good agronomical conditions, the most efficient farms can produce up to 19 t ha^{-1} (Biau et al., 2011). High yielding maize crops grown in Spanish agro-systems require water but also a satisfactory input of available nitrogen (N) and a long growing season.

Surveys conducted in the Ebro Valley (Sisquella et al., 2004) show that about 50% of the maize-producing land is only fertilized with mineral N fertilizer. The rest of the area is fertilized with manure, mainly pig slurry (PS), which is applied before sowing the maize crop, normally complemented with mineral N fertilizer, applied at sidedress. In general, N is applied at rates of over 300 kg N ha^{-1} in fields that are only fertilized with mineral N and at more than 400 kg N ha^{-1} in those fertilized with manure. Spain is the second largest intensive swine producer in the EU (MARM, 2010) and the Ebro Valley contributes 40% of Spanish swine production. However, the excessive application of N fertilizer in agricultural systems has produced a possible reduction in the profitability of farms as well as the pollution of water and the atmosphere. For this reason, an EU nitrate directive (European Union, 1991) now limits the amount of N that can be applied to soil in many of the irrigated areas within the Ebro Valley. In this area, ground waters are frequently polluted with nitrate (Ferrer et al., 2003), with concentrations often exceeding 50 mg NO^{-}_{3} L^{-1}, the maximum level permitted by the European Union (1991). Consequently, as in many other regions of the EU, some areas of the Ebro Valley have been declared nitrate vulnerable. In these areas, it is not permitted to apply more than 350 kg N ha^{-1} year^{-1}, of which no more than 180-210 kg N ha^{-1} year^{-1} should derive from organic materials (Diari Oficial de la Generalitat de Catalunya, 2004).

The agricultural areas with the highest nitrate exports are those associated with irrigated agricultural systems growing crops with a high N use, such as maize. There is therefore a need for further studies into the development of agronomic practices that would enable these areas to increase the efficiency of their N fertilization while at the same time reducing nitrate leaching and N gas emissions from maize plots to water bodies.

Stover management is an important aspect of maize production because, apart from its agronomical effects relating to soil improvement, it may have also help to increase the profitability of maize farming. For instance, last year (2012) maize stover produced in the Ebro Valley commanded prices of around 18 € t^{-1}. The quantity of maize stover produced in the Ebro valley is large, ranging from about 14-17 t ha^{-1} year^{-1} (Lloveras et al., 2012) depending on total maize production. According to a survey by Sisquella et al. (2004) stover is removed on 50% of the land in the Ebro Valley and the amount of stover available as feedstock has been estimated at around 40%, with only a relatively small portion of this stover being available as pasture (Sisquella et al., 2004). These proportions of stover management can change from year to year and according to the price. Other aspects of maize stover management are its interaction with N fertilization (Wilhelm et al., 2004) and its impact on greenhouse gas emissions into the atmosphere and carbon (C) sequestration. Agricultural practices have been cited as both sources and sinks for greenhouse gases, especially CO_2 (Follett and Hatfield, 2001; Lal et al., 1998). Stover management and soil organic matter (SOM) content account for a significant portion of total terrestrial C (Wilhelm et al., 2004). By increasing the amount of maize stover returned to the soil, SOM content could be increased and, as a consequence, the quality of the soil, while at the same time reducing atmosphere C pools.

As researchers who work directly with agricultural producers, we are keenly aware of the need for farmers to have sustainable production systems and to maintain or increase their maize yields and the economic profitability of their farms. For this reason, we would like to emphasize the use of maize stover within the management practices applied in the Ebro valley.

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GENERAL OBJECTIVES

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The main objective of this thesi was to contribute to the improvement of the N management and profitability of high yielding irrigated maize, while reducing its soil and environmental impacts.

In order to achieve this main objective, we conducted several field trails: a trial initiated in the year 2002, (and conducted from 2002 to 2011) and two other trials undertaken from 2010 to 2012. The first trial consisted of an evaluation of the long-term impact on soil quality of applying different (organic and mineral) N fertilization sources. The other two field trials focused on the effects of stover management and its interactions with different mineral N fertilization rates on maize production and soil quality.

The main objectives were to:

1. Evaluate the management of maize stover and its interaction with mineral N fertilization in irrigated high-yielding crop systems.
2. Study the effects of short-term crop management and different (organic and mineral) N fertilization sources on selected soil quality parameters.
3. Study the effects of long-term use of organic and mineral N fertilizers on the productivity of irrigated maize and on selected physical, chemical and biological indicators of soil quality.
4. i) Compare the N content determined for the whole maize plant when the stover and grain were separately ground and analysed using the NIRS and Dumas combustion methods; ii) to compare the N content determined for the whole maize plant when the stover and grain were ground and analysed together using the NIRS and Dumas combustion methods; and iii) to investigate whether the

NIRS method is sensitive enough to detect differences in N concentrations in tissue associated with different rates of N application.

This document consists of four independent chapters presented in the format of a journal article. For this reason, some parts, such as the material and methods section, may contain a certain degree of repetition.

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

CHAPTER I

Stover management and nitrogen fertilization effects on corn production

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ABSTRACT

The incorporation of crop stover into the soil improves soil fertility and crop productivity by increasing carbon (C) sequestration and reducing the emission of greenhouse gases among other parameters. Interactions between crop stover management and nitrogen (N) fertilization could help to improve C sequestration while increasing productivity. The objective of this study was to evaluate the impact of incorporating or removing corn (*Zea mays* L.) stover, in combination with different N fertilization rates (0, 100, 200 and 300 kg N ha^{-1}), on corn production, soil organic carbon (SOC), and soil mineral N (SMN) in high production areas. We carried out two field experiments (Experiment 1 and Experiment 2) for three years under sprinkler irrigation. Over the duration of the experiment (short term period), stover management did not affect corn production or SMN levels, while high average grain yields were achieved (16–20 Mg ha^{-1}) when N was applied. After three years, removing the stover reduced SOC levels by approximately 0.82 and 1.06 g C m^{-2} (0-30 cm depth) in 2012 in Experiment 1 and 2, respectively. The amounts of corn stover incorporated were higher than 16 Mg ha^{-1} year^{-1} of dry matter. Our data suggest that returning stover to the soil has a positive short-term impact on soil quality without grain yield penalties. Although selling the stover provides a short-term economic advantage, continuous stover removal may cause significant soil degradation in the future.

Abbreviations: SOM, soil organic matter; SOC, soil organic carbon; SMN, soil mineral nitrogen.

The emerging bioenergy market based on corn (*Zea mays* L.) stover has encouraged many farmers to harvest and sell their stover rather than incorporate it back into the soil (Frossard et al., 2012). Selling the stover provides short-term economic gains, but according to Wilhelm et al. (2004) its incorporation increases soil productivity, thus it should not be considered entirely as a waste product (Blanco-Canqui, 2012). The incorporation of stover has many benefits including the prevention of soil erosion, the maintenance of soil organic matter (SOM) and soil structure by humification, and is a source of energy for soil biota (Lal, 2005). Stover is also an important source of macronutrients (NPK) and micronutrients such as S, Cu, B, Zn and Mo (Mubarak et al., 2002).

Apart from these benefits, corn stover has a long term positive impact on soil organic carbon (SOC) levels, and continuous removal can result in a progressive decline in yields (Wilhelm et al., 2004) by reducing SOC levels until the production capacity of the soil becomes limiting (Johnson et al., 2006; Mann et al., 2002). SOC is a key CO_2 sink, maintaining the productivity of agriculture while reducing greenhouse gas emissions and mitigating global climate change (Christopher et al., 2009). The benefits of higher SOC levels include the sequestration of atmospheric CO_2 as well as better soil quality (Benjamin et al., 2010; Blanco-Caqui and Lal, 2009). Furthermore, corn stover contains 17.7 g N kg^{-1}, 1.82 g P kg^{-1}, and 28.36 g K kg^{-1} of the fertilizer applied to crops (Johnson et al., 2010). However, not all the nutrients are available to the following crops. Most of the N remains in organic forms and mineralization is required before absorption, leading to a short-term N deficit affecting grain yield in the following crop (Van Den Bossche et al., 2009). Interactions between stover management and N fertilization have been reported by several authors (Karlen et al., 2011; Power et al., 1998; Maskina et al., 1993). The combination of corn stover and N fertilizer can influence SOC storage, but most of these studies involve US cropping systems (Mann et al., 2002), with grain yields of approximately 10 Mg ha^{-1} and N fertilization rates of 0–280 kg N ha^{-1} (Clapp et al., 2000). This productivity is much lower than in the Ebro Valley (North-East Spain), where average grain yields under irrigation are typically 14–16 Mg ha^{-1} (Cela et al., 2011; Berenguer et al., 2009). The higher yields in this area of Spain reflect the larger amounts of stover (HI of 50 %) and N fertilization applied to the soil (300–350 kg N ha^{-1}) and the use of irrigation (Quílez and Yagüe, 2010).

There is little information about the interaction between stover management and N fertilization rates in irrigated, high-yielding corn crops such as those in Spain. It is unclear whether the results from the US can be extrapolated to these conditions. Previous studies have compared the effects of stover incorporation with reduced tillage agriculture but little is known about the impact of stover management and conventional tillage (Karlen et al., 2011; Graham et al., 2007; Allmaras et al., 2000; Linden et al., 2000). A better understanding of the effects of corn stover management and its potential interaction with N fertilization could help to improve N use efficiency, soil quality and increase crop productivity in high-yielding environments.

The objective of this study was to evaluate the effects of two contrasting corn stover management practices (incorporation or removal) in combination with different N fertilization rates, and the impact on corn production (grain yield, biomass at maturity, and plant N content), SOC and soil mineral N (SMN) under conventional tillage.

MATERIALS AND METHODS

Two field experiments (Experiment 1 and Experiment 2) were conducted from 2010 to 2012 in Almacelles (NE Spain, 41º43'N, 0º26'E). Both experiments were in the same geographical region and were irrigated by sprinkler. The altitude of Experiment 2 was ~324 m amsl and that of Experiment 1 was ~271 amsl. The location is characterized by a semiarid climate with low precipitation (192 mm) and high temperature (19.1 ºC) during the corn growing period (Fig. 1). Experiment 1 had a slightly higher temperature than Experiment 2 because of the lower altitude. The soil in each experiment was well drained without salinity problems and major characteristics are listed in Table 1.

Experimental treatments consisted of corn stover management and different N fertilization rates. The stover management practices were stover removal from the field after harvest each year and stover incorporation with conventional tillage (by disk ploughing) to a depth of 25–30 cm. These practices were combined with N fertilization rates of 0, 100, 200, and 300 kg N ha^{-1}, referred to as 0N, 100N, 200N, and 300N, respectively. The N fertilizer was applied as ammonium nitrate (33.5% N) in two side-dressing doses applied using a small drop-type hand-driven fertilizer spreader, 50% at V3–V4 and 50% at V5–V6 (Ritchie et al., 1989). P and K fertilizers were applied annually before planting at rates of 150 kg P_2O_2 ha^{-1} and 250 kg K_2O ha^{-1}. The experimental design was a split-plot, with three replications, completely randomized treatments in the first year and the same treatments applied to the same plots thereafter. The stover management practices represented the main plots and the N fertilization rates the sub-plots. The experimental plot dimensions were 18 x 17 m.

Corn was planted in the first week of April at a rate of 80,000 plants ha^{-1} with 71 cm between rows in both experiments. The corn hybrids used in the experiment belong to the 600-700 FAO cycle. The varieties planted are among the most productive in regional variety tests (Lopez et al., 2011). Plots were sprinkler-irrigated 2-3 times per week, with approximately 700 and 1000 mm of water per season (lacking nitrate) in Experiments 1 and 2, respectively. The applied amounts depended on the climatologic conditions of the season. Both experiments were treated with 3.3 l ha^{-1} of the pre-emergence herbicide Trophy (Acetochlor 40% + Dichlormid 6%) and 1 l ha^{-1} of the post-emergence herbicide Fluoxypyr 20% (to control *Abutilon theophrasti* M.) plus 1.5 l ha^{-1} of Nicosulfuron to control *Sorghum halepense.*

Aboveground biomass and N content were evaluated at physiological maturity by harvesting 4 m of the central row from each plot. Two entire plants were chopped and dried to determine the moisture content, and aboveground plant N content was determined by near infrared spectroscopy (NIRS) (InfraAlyzer 2000 spectrometer, Bran+Luebbe, Norderstedt, Germany). Corn stover was either incorporated into the soil (that is, the whole plant aboveground biomass minus the grain biomass) or all of the corn stover was removed using commercial machinery and the rest of the residues were removed manually.

Corn was harvested in the second week of September and grain yield was measured by harvesting two complete central rows (1.42 x 17 m). Grain moisture was determined in a 300 g sample from each plot and the grain yield was adjusted to 14% moisture (GAC II, Dickey-John, Auburn, IL, USA). The grain N content was measured by NIRS as above.

Soil nitrate content (NO_3-N) was determined before planting (initial NO_3-N) and after harvesting (residual NO_3-N). Soil samples were taken from each plot (0–90 cm depth at 30 cm intervals). The nitrate was extracted in deionized water and measured using Nitrackek (KPG Products Ltd., Hove, East Sussex, UK) test strips (Bischoff et al., 1996) calibrated according to the standard procedure (Bremner, 1965).

The soil ammonium content (NH_4^+-N) was considered negligible (Berenguer et al., 2009) and was only measured at the beginning of the experiment in 2010 (0–30 cm depth). The mean of soil residual NH_4^+-N was 16 and 15 kg ha^{-1} in Experiment 1 and 2, respectively. As a consequence, the soil nitrate content was considered the soil mineral nitrogen (SMN) content. The SOC in the top layer of the soil (0–30 cm) was determined by measuring organic carbon using the dichromate oxidation procedure in which residual dichromate is titrated against ferrous sulfate (Walkley and Black, 1934).

A general linear model was used to determine if there were the statistically significant differences in the agronomic parameters yield, aboveground biomass, and plant N content. Soil mineral N and SOC were statistically analyzed as split-plot in time using the PROC MIXED procedure of SAS (Littell et al., 1998). In the mixed model, stover, N dose, and years were considered fixed variables, while replication was considered a random effect. Treatments were compared by Tukey's mean separation procedure ($p< 0.05$). All the analysis were performed using the SAS statistical package (SAS, 1999-2001).

RESULTS

Corn production

Stover management did not significantly affect corn yields in any of the growing seasons i.e. grain yields were whether the stover was removed or incorporated (Table 2). In both experiments and in all three growing seasons, there was no significant effect of the interaction between stover management and N fertilization rates on yield except in Experiment 2 in 2012 (Table 2).

In Experiment 2, the N fertilization rate affected corn production significantly, whereas in Experiment 1 grain yield did not respond to the different N fertilization rates (Table 2). Stover management had a significantly lower impact than N fertilization rates in both experiments (Table 2). Stover management did not affect biomass yields at maturity in either experiment. However, in both Experiment 1 and 2 in 2011 and in

Experiment 2 in 2010, a significant rise in biomass was observed with increasing N rates (Table 2).

Nitrogen fertilization rates significantly affected plant N content in Experiment 2 for all three growing seasons. By contrast, in Experiment 1 the N fertilization rate had no significant effect in any of the growing seasons.

Soil mineral nitrogen

At the beginning of the experiment in 2010, initial NO_3^-N was 282 g kg^{-1} in Experiment 1 and 118 g kg^{-1} in Experiment 2, at a depth of 0–90 cm. SMN was generally lower in Experiment 2 than 1, perhaps reflecting the use of cow slurry (CS) applications in Experiment 1 in previous years (Table 1). Incorporating or removing corn stover had no significant impact on SMN. However, in the plots that were not treated with mineral N fertilizer over the three growing seasons, stover incorporation reduced the residual SMN levels compared to plots from which stover was removed (Table 3).

Soil organic carbon

SOC levels in Experiments 1 and 2 remained stable when the stover was incorporated into the soil (Table 4). When the stover was removed, SOC levels declined (from an average of 21.1 to 18.8 g Kg^{-1} in Experiment 1, and from 19.3 to 16.8 g Kg^{-1} in Experiment 2) over the three years of the study (Table 4). Consequently, a significant interaction was observed (year*stover) in both Experiments (Table 4). SOC content was not affected by N fertilization rates (Table 4).

DISCUSSION

Grain yield was not significantly influenced by stover management treatment. The stover incorporated in our experiments was higher than 16 Mg ha^{-1} yr^{-1} of dry matter (Table 2). Rainfall in the region occurs mainly during the spring season which is normally unsuitable for decomposition (Figure 1) of the previous year's stover because of the relatively low temperatures. For this reasons, stover can immobilize a significant amount of SMN reducing its availability to the corn crop. However, stover management did not present any statistical differences in grain yield as a consequence of the possible immobilization of N (Table 2). Reports from studies in the US have demonstrated a positive impact when corn stover is returned to the soil at a rate of 7–10 Mg ha^{-1} (Clapp et al., 2000; Linden et al., 2000; Power et al., 1998) and some effect on yield might have been expected in the present study. One possible reason for the lack of yield response to stover management could be due to the high amount (for the area of our study) of SOC.

Only in 2012, in Experiment 2, after three years of trials, was a significant interaction (stover*N rates) observed. This shows that stover incorporation reduced grain yields at the lowest N rates, possibly due to N immobilization.

Biomass production was high with average yields of 32.4 Mg ha^{-1} in Experiment 1 and 34.24 Mg ha^{-1} in Experiment 2, with a harvest index of about 0.50. As a result of the high grain yields, biomass production was also high.

The average aboveground plant N content ranged from 8.2 to 13.4 g N kg^{-1} (Table 2). These levels can be considered in line with previous studies in the same area (Berenguer et al., 2009; Daudén and Quílez, 2004).

Soil organic C contents provide a measurement of soil organic matter status. The SOC content in both Experiments decreased over the period of the trials when the stover was removed in contrast with the stover incorporated treatments (Table 4). When the stover was incorporated (amounts higher than 16 Mg ha^{-1} yr^{-1} of dry matter) SOC content improved or remained stable. Similar results of no SOC increase under stover incorporation were also observed by Bundy et al. (2011). This study carried out in Wisconsin (USA) over a 10-yr period with grain yields ranging from 6 to 14 Mg ha^{-1} yr^{-1} did not find any significant increase in soil C levels at the end of the experiment. No increases in SOC in stover incorporated treatments have also been reported by other authors (Blanco-Canqui and Lal, 2007; Mann et al., 2002; Powlson, et al., 2011) who accepted the concept of soil C saturation level proposed by Six et al. (2002). At this point the soil cannot absorb more C because SOC is in equilibrium with the atmosphere and it returns as much C as it absorbs.

N fertilization can increase biomass production and the amount of crop stover available for reincorporation, but an imbalance of nutrients may limit the amount of C incorporated into the soil matrix or sequestrated into the soil (Kirkby et al., 2011). Although selling stover produces short-term economic gains, in the conditions of our study this practice will encourage soil degradation in the future. The mean SOC values for 2012 (Table 4) show that after three experimental years stover removal caused a reduction in SOC levels of approximately 0.82 and 1.06 g C m^{-2} in Experiment 1 and 2, respectively. According to Powlson et al. (2011) and Huggins et al. (2010), these small changes could have disproportionally large negative effects on the soil structure including its aggregate stability, water infiltration rate, etc.

Nitrogen use efficiency ranged from 56 kg N kg^{-1} in 300N in Experiment 1 to 182 kg N kg^{-1} in 100N in Experiment 2. The low N efficiency in Experiment 1 was mainly due to the high soil N content. This was because of the excessive application of N fertilizer which additionally results in contamination of water and the atmosphere (Yagüe and Quílez, 2010). The average aboveground plant N content ranged from 9.2 to

12.6 g N kg^{-1} (Table 2). These levels can be considered normal compared with previous studies in the same area (Berenguer et al., 2009; Daudén and Quílez, 2004). Nevertheless, Ciampitti and Vyn (2012) reported similar values of aboveground plant N content using modern-era corn hybrids. Whereas many studies of plant N content have been based on grain yields of up to 10–14 Mg ha^{-1} (Liu et al., 2006; Duivenbooden et al., 1995), this present study investigated corn production in areas with a higher yield potential. Due to the present high corn prices, producers normally apply high amounts of N to ensure they obtain high grain yields.

There was no significant effect of the interaction between stover management and N fertilization on grain yields, which was unexpected because more than 16 Mg ha^{-1} yr^{-1} of dry matter was incorporated into the soil (Table 2). This could be explained by the high SMN levels which have been reported in many areas of the Ebro Valley (Vazquez et al., 2006; Abad et al., 2004; Villar-Mir et al., 2002). No significant interaction between stover management and N fertilization in SMN was observed, because the N initial and N residual levels followed similar tendency under the two stover management systems. However, the SMN values with increasing N rates were much higher in Experiment 1 compared with Experiment 2. This discrepancy could be explained by significant N losses due to leaching (Berenguer et al., 2008), predominantly in Experiment 2, the soil type and water use. The optimal N fertilization rate varied depending on the experiment, showing that N fertilization recommendations should not be based on fixed rates of N application.

CONCLUSIONS

Our study considers corn crops with high grain yields (16–20 Mg ha^{-1}) and suggests that, in the short term, farmers can incorporate stover without yield or biomass penalties, while improving the SOC level of the soil. No significant effects were observed as a result of the interaction between stover management and N fertilization rates, indicating that stover incorporation has minimal impact on C and N storage in the short term (three study years), though stover removal did result in a small reduction in SOC. However, these small changes could have disproportionally large negative effects on soil quality. Farmers must therefore choose between selling the stover for short-term economic gain or incorporating the stover and improving the soil properties for future growing seasons. These conclusions are based on the results from two experiments lasting three years in which different corn stover management practices were tested at different N fertilization rates. The findings should be validated by field testing over longer duration.

Table 1. Chemical and physical soil properties in Experiment 1 and Experiment 2 at the beginning of the study (2010)

	Experiment 1			**Experiment 2**			
Depth (cm)	0-30	31-102	103-130	0-22	23-45	46-110	>111
Sand (g kg^{-1})	280	300	320	420	430	170	170
Silt (g kg^{-1})	420	460	470	330	360	630	650
Clay (g kg^{-1})	300	240	210	250	210	200	180
pH	8.4	8.2	8.3	8.2	8.4	8.4	8.4
E.C. (dS m^{-1})	0.21	1.57	1.73	0.19	0.17	0.22	0.22
Organic matter (g kg^{-1})	35	-	-	33	-	-	-
Bulk density (g cm^{-3})	1.4	-	-	1.64	-	-	-
P (Olsen) (mg kg^{-1})	122	-	-	90	-	-	-
K (NH_4Ac) (mg kg^{-1})	420	-	-	383	-	-	-
Soil type*	Gypsic haploxerept			Typic Calcixerept			
Precedent crop	Corn-corn			Corn-corn			
Previous manure application	Yes (cow slurry)			No			
Previous mineral N application	~200 kg N ha^{-1} year^{-1}			~300 kg N ha^{-1} year^{-1}			

*(Soil Survey Staff, 2003)

Table 2. Corn yield (140 g kg^{-1} seed moisture content), aboveground biomass (0 g kg^{-1} moisture content), aboveground biomass and plant N content at maturity for the different stover management practices and N fertilizer application rates in 2010, 2011, and 2012, in Experiment 1 (low altitude) and Experiment 2 (high altitude).

		Experiment 1									**Experiment 2**								
Stover management	**N rate (kg N ha^{-1})**	**Yield (Mg ha^{-1})**			**Biomass (Mg ha^{-1})**			**Plant N content (g kg^{-1})**			**Yield (Mg ha^{-1})**			**Biomass (Mg ha^{-1})**			**Plant N content (g kg^{-1})**		
		2010	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**
Incorporated	**0**	13.9	13.9	16.6	29.4	27.6	30.5	11.8	9.0	10.7	14.0	11.0	16.1	26.1	21.5	26.7	9.3	8.2	9.7
	100	13.9	15.1	17.1	34.1	33.1	30.0	12.9	11.2	10.1	18.5	17.1	18.3	35.1	32.2	38.3	11.0	8.8	10.8
	200	13.2	18.8	16.4	26.2	41.7	34.1	12.8	9.9	11.3	19.0	20.0	20.5	31.0	37.9	37.2	11.5	9.6	11.5
	300	13.3	18.6	17.1	30.7	41.3	28.4	12.4	9.7	11.4	18.7	20.0	20.8	33.0	38.5	35.8	11.7	10.2	10.6
Mean		13.6	16.6	16.8	30.1	36.0	30.8	12.5	9.9	10.9	17.6	17.0	19.0	31.3	32.5	34.5	10.9	9.2	10.6
Removed	**0**	14.8	18.3	16.7	31.3	37.0	28.3	12.3	9.9	10.9	16.0	13.1	17.6	28.4	26.3	35.8	11.2	9.4	9.3
	100	13.0	18.6	16.2	29.2	36.5	26.7	11.5	9.7	11.3	18.1	18.5	20.2	37.2	31.5	42.7	11.8	9.7	10.4
	200	14.2	18.8	14.1	25.6	38.3	33.6	13.4	10.4	11.0	19.0	18.8	20.6	30.4	35.6	41.2	11.5	9.6	10.4
	300	15.4	18.8	17.8	29.8	38.8	34.9	12.7	10.4	10.6	17.1	19.9	21.3	35.7	42.1	41.8	11.4	10.4	10.7
Mean		14.4	18.6	16.2	29.0	37.7	30.9	12.5	10.1	10.9	17.6	17.6	19.9	32.9	33.9	40.4	11.4	9.8	10.2
Block		NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	*	NS
Stover (S)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error a		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N rate (N)		NS	NS	NS	NS	*	NS	NS	NS	NS	**	**	**	**	**	NS	**	**	*
SxN		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS

*,**Significant at the 0.05 and 0.01 levels, respectively. NS not significant

Table 3. Soil mineral nitrogen (N) (g kg^{-1}) before planting and applied N fertilizer (Nini), and residual N after harvest (Nresi) (depth 0-30 cm) for 2010, 2011, and 2012 in Experiment 1 and Experiment 2.

		Experiment 1						**Experiment 2**					
		2010		**2011**		**2012**		**2010**		**2011**		**2012**	
Stover management	**N rate (kg N ha^{-1})**	**Nini**	**Nresi**	**Nini**	**Nresi**	**Nini**	**Nresi**	**Nini**	**Nresi**	**Nini**	**Nresi**	**Nini**	**Nresi**
Incorporated	**0**	96±18	63	127	58	86	47	54±11	48	52	58	69	37
	100		108	198	100	145	94		28	52	33	71	27
	200		226	228	273	159	237		59	73	53	92	37
	300		225	334	375	254	269		91	75	132	97	103
Mean			155	222	202	161	161		57	63	69	82	51
Removed	**0**		117	138	116	151	93		27	65	36	59	17
	100		174	208	225	214	101		49	69	33	52	24
	200		199	314	230	185	250		66	92	58	57	30
	300		269	354	328	220	175		156	96	134	100	37
Mean			190	253	225	192	155		74	81	65	67	27

	Experiment 1		**Experiment 2**	
	Nini	**Nresi**	**Nini**	**Nresi**
Block	NS	NS	NS	NS
Stover (S)	NS	NS	NS	NS
Error a	-	-	-	-
Nrate (N)	**	**	NS	**
SxN	NS	NS	NS	NS
Error b	-	-	-	-
Year (Y)	**	NS	**	NS
YxS	NS	NS	*	NS
YxN	**	NS	*	NS
Y*S*N	NS	NS	NS	NS

*,**Significant at the 0.05 and 0.01 levels, respectively. NS not significant

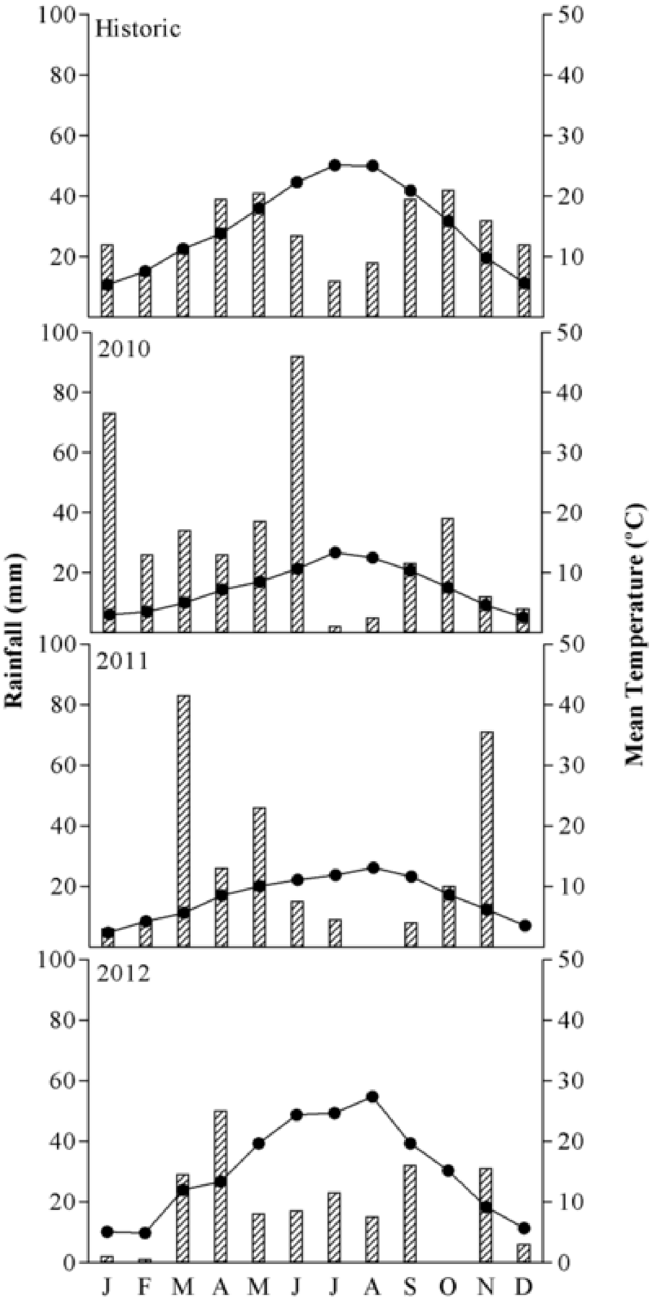
Table 4. Soil organic carbon (g kg^{-1}) after harvest following different stover management practices and N fertilizer application rates in 2010, 2011, and 2012, in Experiment 1 and Experiment 2.

		Experiment 1			**Experiment 2**		
Stover management	**N rate (kg N ha^{-1})**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**
Incorporated	**0**	16.9	18.8	19.6	16.9	19.8	19.1
	100	20.9	20.1	20.0	16.4	19.7	17.9
	200	19.3	22.3	20.7	18.6	19.0	20.0
	300	19.9	21.9	21.6	18.4	17.9	19.5
Means		19.3	20.8	20.5	19.1	19.1	19.1
Removed	**0**	22.9	23.9	21.2	18.9	17.3	17.0
	100	20.2	22.1	18.9	20.2	18.8	19.5
	200	19.9	21.3	20.4	16.9	21.1	18.4
	300	21.5	21.4	20.2	17.7	20.1	17.4
Means		21.1	20.2	18.8	19.3	18.1	16.8

	Experiment 1	**Experiment 2**
Block	NS	NS
Stover (S)	NS	NS
Error a	-	-
Nrate (N)	NS	NS
SxN	*	*
Error b	-	-
Year (Y)	NS	NS
YxS	*	*
YxN	NS	NS
Y*S*N	NS	NS

*Significant at the 0.05. NS not significant

Figure 1. Monthly rainfall and mean temperature for the historic period (1989–2012) and for the experimental period (2010–2012).



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CHAPTER II

Mineral Fertilizer and Organic Manure effects on Soil Chemical and Biological Properties in Irrigated Maize

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ABSTRACT

Soil amendments with mineral and organic fertilizers together with maize stover incorporation is nowadays a common practice that may favorably affect several soil properties, including soil biological activity. In this research, a field experiment was conducted under a continuous maize monoculture in semiarid irrigated conditions. The objective of the study was to investigate the effect of mineral and organic fertilizer and its interaction with stover management on grain yield and selected soil quality parameters. Treatments tested were stover management (incorporated or removed) and nitrogen (N) fertilization: mineral (300N) and organic (cow slurry, CS), and a control (0N). Selected soil quality parameters were measured at the end of the experiment after three years of maize production: dehydrogenase activity (DHA), microbial biomass carbon (MBC), earthworm abundance, and soil organic carbon (SOC) content, apart from grain yield and whole plant biomass at physiological maturity. All soil parameters measured showed higher values when the stover was incorporated. The stover production that ranged between 9.9 and 20.7 Mg ha^{-1} yr^{-1} was sufficient to maintain SOC levels in agricultural systems. Although MBC was higher under mineral N fertilization, an increase of 44 mg C kg^{-1} was observed after three years of stover incorporated. Grain yield presented a significant interaction between N fertilization and stover management. In contrast, dehydrogenase activity (22 and 30 mg INTF kg^{-1} dry soil h^{-1} under stover incorporation in 300N and CS, respectively), earthworm abundance (7 and 12 earthworm m^{-2} in the plots where the stover was incorporated in 300N and CS, respectively), and SOC followed a similar trend (0N<300N<CS). The results indicated that organic manure, under incorporated stover, was the treatment with the highest beneficial effects on soil quality parameters.

Keywords: Maize; Cow Slurry; N fertilization; Soil Quality

INTRODUCTION

Agricultural soils in semiarid Mediterranean areas of the Ebro Valley (Spain) are characterized by low organic matter (OM) contents mainly due to the warm and dry climate, the cultivation systems and because of the extended exposure to erosion and degradation (Plaza et al., 2004; Garcia et al., 1994). On the other hand, the Ebro Valley is an area with high concentration of livestock that produces large amounts of manure. More than 100,000 Mg year^{-1}of nitrogen (N) disposed in the Ebro Valley fields come from livestock production (Teira, 2008). Animal manure is a source of N and other nutrients when applied to crops being a cost-effective way to utilize this animal waste (Salmerón et al., 2010). Also, manure and other materials of organic origin are frequently applied to the soil to increase the levels of plant nutrients and to improve the physical, chemical and biological soil properties that directly affect soil fertility (Bohme et al., 2005). Particularly, applications of organic amendments, such as cow slurry (CS), produce positive effects on microbial activity that are very important in regulating soil properties (Dick, 1992). Moreover, understanding soil microbial activity is increasingly recognized as important factor for the restoration and sustainability of ecosystems (Potthoff et al., 2006; Steenwerth et al., 2002). Since the microbial community plays a critical role regulating some soil processes such as decomposition of OM and nutrient cycling, there is a keen interest in better understanding the factors that regulate its size, activity and structure (Zeller et al., 2001). The importance of the size of microbial biomass is emphasised by the fact that this is the eye of the needle through which all organic material that enters the soil must pass (Jenkinson and Powlson, 1976). A variety of microbial parameters have the potential for use as diagnostic indicators of soil quality such as microbial biomass and microbial diversity (Bending et al., 2004; Anderson, 2003; Sparling et al., 1997). In contrast, nutrients contained in organic fertilisers that have to be released by microbial metabolism to make most of them available to plants, the nutrients in inorganic fertilizers can be directly taken up by plants. This is why inorganic fertilisers directly affect crop yields and it is the main reason for its use (Böhme et al., 2005). Moreover, the presence of these organic and inorganic nutrients substances in the soil is associated with a general increase in nutrient contents and with their subsequent effects on some properties such as microbial activity, the humus fraction, soil structure and saturation of the ion-exchange system (Kirchner et al., 1993). On the other hand, while changes in the soil OM content occur very slowly, the soil microbial biomass carbon (MBC) responds much more rapidly to changes in managements that alter the annual input of organic material into the soil (Powlson and Jenkinson, 1981). Thus, changes in MBC measured over relatively short periods can indicate trends in total OM content. Although the long-term effects of organic and inorganic fertilization on the soil's physico-chemical properties have been characterised (Biau et al., 2012; Bundy et al., 2011; Mijangos et al., 2010), less is known about the effects of CS farming system in irrigated Mediterranean semi-arid zones. Therefore, MBC content, microbial activity (as dehydrogenase activity, DHA) and, the earthworm abundance are feasibly believed to be appropriate, sensitive and reliable indicators that can be used to monitor the microbial response to the organic amendment and, in the end, to evaluate the impact in the productive capacity of the soil.

The objective of this research was to study the effect of maize stover and N fertilization source (organic and mineral) on selected soil quality parameters (MBC, DHA, SOC content and, earthworm abundance) in high-yielding maize irrigation systems.

MATERIALS AND METHODS

Field experiments

A maize field trial was conducted from 2010 to 2012 in Almacelles (NE Spain, 41º43'N, 0º26'E) under sprinkler irrigation. The location of the experiment is representative of the region, with semiarid climate with a high mean temperature of 19.1ºC and precipitation of 192 mm during the maize growing season. The study was conducted under sprinkler irrigation providing approximately from 900 to 1000 mm of water per year during the growing period. The soil is well drained with no problems of salinity and is classified as Typic Calcixerept (Soil Survey Staff, 2003). Soil quality indicators were taken at the end of the field trials in 2012, after three years of experiments. Selected physico-chemical parameters were also measured at the beginning of the experiment in 2010. The measurements were soil texture, pH, electrical conductivity (EC), cation exchange capacity (CEC), bulk density, water holding capacity, available P (Olsen P) and extractable K (NH_4Ac) (Table 1).

Maize was planted during the first week of April every year at a rate of 80,000 plants ha^{-1} with 75 cm space between rows. The maize cultivars used were PR33P67 in 2010 and PR32G49 in 2011 and 2012. Experimental treatments consisted on stover management and N fertilization. The stover management practices were i) stover removal from the field after harvesting every year and, ii) stover incorporation with conventional tillage (with disk ploughing) to a depth of 25-30 cm. Fertilizer treatments comprised: i) a single application of CS before planting and ii) a mineral dose of 300 kg N ha^{-1} year^{-1} (300N) (33.5% ammonium nitrate) split twice in V3–V4 and V5–V6 developing stages (Ritchie et al., 1989). A zero N rate was included as a control (0N). The applied rate of CS was about 60 m^3 ha^{-1} year^{-1}, meaning and average of about 386 kg N ha^{-1} year^{-1}. The amount of N applied derived from CS is show in Table 2. The slurry was applied with a commercial spreader and was ploughed into the soil after 3–5 hours to reduce ammonia (NH_3) volatilization losses. The mineral fertilizer was applied using a small, drop-type hand-driven spreader. All plots were also fertilized before planting with phosphorus (P) (150 kg P_2O_5 ha^{-1} year^{-1}) and potassium (K) (250 kg K_2O ha^{-1} year^{-1}) to avoid mineral deficits of these elements.

The plot dimensions were 10 x 18 m (with 15–16 rows per plot) and were arranged in a randomized block design with three replicates. The plots were randomized the first year and in the following years the treatments were always applied at the same plot.

Analysis of plant and soils samples

Maize was harvested the second week of September each year. Grain yield was measured by harvesting two complete central rows (1.50 m x 10 m). Grain moisture was determined for each plot from a 300 g sample and grain yield was adjusted to 14% moisture (GAC II, Dickey-John, Auburn, IL, USA). The soil physical and biological parameters measured were soil organic carbon (SOC), MBC, DHA and the abundance of earthworms (at a soil sampling depth of 0-30 cm).

The SOC in the top layer of the soil (0–30 cm) was determined by measuring organic C using the dichromate oxidation procedure in which residual dichromate is titrated against ferrous sulfate (Walkley and Black, 1934). Microbial biomass carbon and DHA were determined according to Vance et al., (1987) and Tahlmann (1968), respectively. The abundance of earthworms (depth 0-30 cm in 25 x 25 cm areas) was measured according to Baker and Lee (1993). The results were subjected to analysis of variance with the General Linear Model procedure of the Statistical Analysis System (SAS, 1999-2001).

RESULTS AND DISCUSSION

Average grain yield from 2010 to 2012 was 17.5 Mg ha^{-1} and presented a significant interaction between stover management and N fertilization source (Table 3). The highest grain yields were obtained with the incorporation of the stover under mineral N fertilization. The mineral N fertilizer had greater effects than CS on the average grain yield (Table 3). According to previous studies of Biau et al. (2012) this could be attributed to the differing application strategies, because CS was applied before planting whereas the mineral N was applied at sidedress. However, initially the highest yields were expected with slurry application because of the high amount of total N applied with CS (Table 2). Thus, this result suggests that some N was loss during and after the CS application. Consequently, is crucial the immediately incorporation of slurry into the soil to avoid N-NH_4 volatilization. Previous study (Piñol et al., 2007) under similar environmental condition suggests that about 15-50% of the N applied with the manure could be lost by volatilization. Furthermore, according to Schröder et al. (2005) organic fertilizer has a residual N effect after the year of its application to land, because the decomposition of organic material usually takes longer than a year. For this reason, when organic fertilizers are used repeatedly, residual effects accumulate and significantly increase the availability of N (Whitmore and Schröder, 1996; Wolf and Van Keulen, 1989).

Biomass production at maturity varied according to the N source. The control treatment (0N) presented lower values than fertilizer treatments (either organic or mineral) in both stover management practices (Table 3).

Soil organic carbon

The effect of stover management and N fertilization on SOC was evaluated using data from the last year (2012) after the maize harvest. Soil organic C content vary significantly with the stover management, although it did not vary with N source (Table 4). Comparing both stover management practices, all treatments when the stover was incorporated had an increase in SOC of about 2, 3, and 1 g of C kg^{-1} of soil in 0N, 300N and CS, respectively (Table 4), as it would be expected after three years of incorporated important amounts of stover (between 9.9 and 20.7 Mg ha^{-1} year^{-1}) (Table 3). These results are consistent with the observations of Johnson et al. (2006), in a study of major grain crops (barley, maize, oat, sorghum, soybean, sunflower, and wheat) in the USA, who reported that the amount of crop stover needed to maintain SOC can range from 5.25 to 12.50 Mg ha^{-1} depending on the cropping system and tillage practices. Nevertheless, Wilhem et al. (2007) showed that these values exceed the crop stover required to control erosion in Corn Belt soils. Although the lack of directly applicable data on crop stover return on SOC under Mediterranean conditions, Shukla et al. (2006) recently stated that SOC is the best single measure of soil quality. In fact, long term studies using slurry as fertilizer showed enhancement of organic C in amended soils. For instance, Hountin et al. (1997) observed, in a maize production study, an increase of SOC with organic-rich manure after 14 years of application to a poorly-drained area of Quebec (Canada). However, Rochette et al. (2000) measured no significant increase of SOC after 19 consecutive years of slurry application in a maize study in the same area. Thus, different effects may be observed on SOC as a function of slurry applied and soil characteristics and climatic conditions in which experiments are conducted. Furthermore, the results of SOC content follow the same trend than previous long-term studies in the Ebro Valley (Biau et al., 2012) although in that case it was used pig slurry as organic fertilizer.

Microbial biomass carbon

According to Garcia et al. (2000), the MBC can be a better indicator of variations in soil fertility than the SOC because it responds rapidly and with greater sensitivity to soil changes. Thus, short-term measurements of MBC can reflect the long-term tendency of the OM (Kirkby et al., 2011). Furthermore, the soil MBC, which represents about 1-5% of total SOC, can provide an effective early warning of the improvement or deterioration of soil quality as a result of different management practices (Powlson et al., 1987). In our study, MBC content was positively influenced by the stover incorporation, although we did not observe significant differences due to the stover management (Table 4). This higher amount of MBC was expected because higher amounts of stover were incorporated (9.9, 14.4, and 17.1 Mg ha^{-1} year^{-1} in 0N, 300N, and CS, respectively). Microbial biomass carbon was significantly greater in 300N (Table 4). This result was not totally expected because, according to Saha et al. (2008) and Belay et al. (2002), the most N-containing fertilizers tend to acidify soil. The acidifying probably resulted in the appearance of unfavorable conditions to MBC, and thus avoids the growth of the autochthonous microorganisms of the soil (Kaur et al., 2005). In these situations the rate of decomposition of the existing OM is decreased rather than the more normal effect of enhancing the rate of OM addition. However, our result is consistent with the observations of Mandal et al. (2007) who reported that MBC was greater in soils with additions of 34 years of stover plus inorganic NPK. In contrast, Hao et al. (2008) observed that the MBC was considerably greater in soils receiving manure than in plots receiving merely NPK fertilizer in three subtropical paddy soils. Similarly to the results of Kaur et al. (2005) the MBC, in our study, was low in the control 0N than in the N fertilization treatments (300N and CS) in both stover management practices (Table 4). As shown in Table 4, the averages of 251 and 207 mg C kg^{-1} of MBC found in the plots where stover was incorporated or removed, respectively, is very low compared to the values obtained by other long term studies (> 2051 mg C kg^{-1} of MBC) in quite similar soils in the same area (Chapter III). These differences could be explained due to the accumulation effect and the soil characteristics, that tend to illustrate the importance of long-term additions of organic materials to soil for maintaining land productivity.

Dehydrogenase activity

Dehydrogenase activity is often used as the indicator of soil fertility and it also can denote the amount and activity soil microbes (Gil-Sotres et al., 2005). The activity of this enzyme depends on the metabolic state of soil microorganisms. Noteworthy, higher DHA found in our study under CS treatments indicating that this source of fertilization, together with stover incorporation, was more beneficial to microbial activity than mineral N fertilization (300N) applications (Table 4). Generally the enzyme activities in the soil are closely related to the SOC content (Beyer et al., 1993). It has been reported that the application of balanced amounts of nutrients and manures improved the SOC and MBC status of soil, which corresponded with higher enzyme activities (Mandal et al., 2007). In our study, similar trend was also observed in both stover management practices (Table 4). Hence, according to García et al. (1994), DHA can be used as an indicator of microbial activity in semiarid soils. In agreement with Pancholy and Rice (1973), dehydrogenase activity is influenced more by the quality than by the quantity of OM incorporated into soil. Thus, the stronger effects of CS on dehydrogenase activity might be due to the more easily decomposable components of CS on the metabolism of soil microorganisms. The observation that DHA is poorly affected by mineral N fertilization is in the same line that the study of Marinari et al. (2000).

Earthworm abundance

The stover management affected positively, although without statistical significance, the earthworm abundance (Table 4) in our study. It is well-known that earthworm activity in the soil enhances microbial population and biomass (Aira et al., 2002). Similarly, that it happened in the DHA and SOC levels, the earthworm abundance followed the tendency: N0<N300<CS. Many authors have reported that organic fertilization caused an increase in soil biological activity (Marinari et al., 2000; Fraser et al., 1994; Kirchner et al., 1993). Moreover, the organic fertilizers supplied phosphate to the soil, giving a more balanced nutritional status than mineral fertilizers. Earthworms were in general highly present in organically fertilized plots (CS) (Table 4), emphasizing the beneficial effect of slurry on soil biology. However, Curry (1976) reported that slurry may be toxic to earthworm in the short term we did not find a negative effect of slurry on earthworm abundance after maize harvesting. In fact, our study is in agreement with Mijangos et al. (2010) where fertilization with CS seemed to increase earthworm population. Regarding that, Ebro Valley is an area characterized by an intensification of the livestock sector and detachment of agriculture, for this the supply of slurry in these areas is a profitable option to improve soil biological properties.

CONCLUSIONS

Higher values of soil quality parameters (SOC, MBC, DHA, and earthworm abundance), although not always significant, were observed when the maize stover was incorporated than when it was removed. Manure (CS) and mineral fertilizers (300N) had similar effects on maize production, relative to applying no N (0N). Thus, the addition of nutrients in either form is essential for the maintenance of soil quality.

There was no significant interaction between stover management and N fertilization in any of the soil quality parameters analyzed. However, the application of organic fertilizer (CS) under stover incorporation showed the highest values of soil quality parameters as SOC, DHA, and earthworm abundance. Furthermore, the soil quality parameters measured (SOC, DHA, and earthworm abundance) followed the same tendency: 0N<300N<CS either of the stover management practices. These results showed that important functional soil microbial properties were affected by organic N fertilization treatments as CS, in the high-yielding maize irrigated areas of the Ebro Valley. The set of soil parameters analyzed in this study contributed to a better understanding of fertilization effects on the size and activity of microbial communities in soils. Overall, three years of continuous CS applications under semiarid conditions produce beneficial effects on soil properties in the short term.

Finally, organic treatments (CS) together with stover incorporated stimulated soil biological activity probably due to the synergism of soil and organic material micro-organisms or a stimulation of microbial growth by organic compounds added with the CS. Thus, this change, in the long-term, are believed to have significant influence on the quality and productive capacity of the soil.

Table 1. Physico-chemical parameters at the beginning of the experiment in 2010.

	Horizon			
Depth (cm)	0-22	23-45	46-110	>111
Sand (%)	42	43	17	17
Silt (%)	33	36	63	65
Clay (%)	25	21	20	18
pH	8.2	8.4	8.4	8.4
E.C. (dS m^{-1})	0.19	0.17	0.22	0.22
Organic matter (%)	3.30	-	-	-
Bulk density (g cm^{-3})	1.64	-	-	-
P (Olsen) (mg kg^{-1})	90	-	-	-
K (NH_4Ac) (mg kg^{-1})	383	-	-	-

Table 2. Applied nitrogen derived from cow slurry (CS).

Target PS rate (m^3 ha^{-1})	N applied (kg N ha^{-1})							
	2010		2011		2012		Mean	
	NH_4^+-N	Total N	NH_4^+-N	Total N	NH_4^+-N	Total N	NH_4^+-N	Total N
60	160	345	191	429	154	383	168	386

Table 3. Effect of nitrogen fertilization source and stover management on grain yield, biomass at maturity, and estimated stover incorporated or removed. Average from 2010 to 2012.

Stover management	N source	Grain yield (GY) (Mg ha^{-1})	Biomass at maturity (BM) (Mg ha^{-1})	Estimated stover incorporated or removed (Mg ha^{-1} year^{-1}) ((BM – GY) - 10%[†])
Incorporated	0	14	25	9.9 [‡]
	300	20	36	14.4
	CS	17	36	17.1
	Average	17	32	13.8
Removed	0	16	30	12.6
	300	19	40	18.9
	CS	18	41	20.7
	Average	18	37	17.4
ANOVA				
Block		NS	NS	
Stover (S)		NS	NS	
Error a		-	-	
N rate (N)		0.0001	0.0041	
S*N		0.0226	NS	

[†]Estimated losses of stover [‡](*i.e.* 25- 14 = 11 - 1.1). NS: not significant.

Table 4. Effect of nitrogen source and stover management on biological selected soil quality indicators after maize harvest in 2012.

Stover management	N source	Dehydrogenase Activity (mg INTF kg^{-1} dry soil h^{-1})	MBC (mg C kg^{-1} soil)	Earthworms (m^{-2})	SOC (g C kg^{-1} soil)
Incorporated	0	19	215	8	19
	300	22	275	7	20
	CS	30	263	12	20
	Average	24	251	9	19.7
Removed	0	15	127	4	17
	300	17	267	3	17
	CS	19	227	10	19
	Average	17	207	6	17.6
			ANOVA		
Block		NS	NS	NS	NS
Stover (S)		0,0025	NS	0,0374	0,0025
Error a		-	-	-	-
N rate (N)		0,0046	0,0404	NS	0,0046
S*N		NS	NS	NS	NS

NS: not significant.

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CHAPTER III

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Original article

The impact of organic and mineral fertilizers on soil quality parameters and the productivity of irrigated maize crops in semiarid regions

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ABSTRACT

Pig slurry (PS) is widely used as a fertilizer for the production of maize in Spain. Field testing was carried out over a ten-year period to compare the performance of maize fertilized with PS (45 m^3 ha^{-1}, equivalent to 315 kg nitrogen (N) ha^{-1} year^{-1}) (PS45) and mineral fertilizer (300 kg N ha^{-1}) (N300) along with a N-free control (N0). Grain yield, biomass at physiological maturity, plant N uptake and soil nitrates (NO_3^-–N) were measured as agronomic properties. Soil physical, chemical and biological parameters (as acid-phosphatase activity, earthworm abundance, CO_2 Flux, Shannon H′ diversity index (H′), number of utilized substrates (NUS), microbial biomass carbon (MBC), resistance to penetration (RP) and organic matter (OM) among others) were measured at harvest in the last two years of the experiment. The mineral fertilizer promoted the highest grain yield and N uptake by the plants, but also resulted in the highest residual NO_3^-–N levels in the soil. Interestingly, most of the indicators revealed no statistically significant differences between the treatments in either test years, although a general trend was observed (N0 < N300 < PS45). The repeated application of PS had a beneficial impact on the soil quality over time but did not improve grain yields.

1. Introduction

Intensive pig farming is an important agricultural and economical activity in Europe, and Spain is the second European country in pig production after Germany [1]. Intensive pig farming has therefore a strong economic impact, not least because the resulting pig slurry (PS) is a valuable source of nitrogen (N) for crops [2]. Pig slurry can fully or partially replace mineral fertilizers depending on the crop, the soil type and the climate [3–5].

Maize is widely cultivated in the irrigated areas of the Ebro Valley, and N inputs are crucial to maintain productivity [6]. Organic fertilizers such as PS are beneficial because they are less expensive than mineral sources of N and the use of PS solves the problem of disposal in the livestock sector [7]. However, it has been necessary to apply much higher doses of PS than recommended for the region in European Union (EU) Directives in order to maintain high grain yields [2,8,9]. The mobility and fate of N applied as PS is complex due to its “residual effect” [10] and this has caused nitrate (NO_3^-) levels in drinking water to exceed permitted limits in the EU (50 mg NO_3^-/L) [11]. Moreover, uncontrolled applications can lead to environmental problems such as greenhouse gas emissions and eutrophication of water bodies among others [12]. It is therefore necessary to minimize the adverse environmental effects of N from PS, particularly in the waters and soils of NO_3^--sensitive areas.

Soil properties also play an important role in agronomic management. Pig slurry is particularly valuable as a fertilizer in soils with a low content of organic matter (OM), such as the soils in the region where the study was conducted which have often an OM content of <2% [13]. The application of excess PS does not generally increase grain yield [14], but the higher OM content improves the physical, chemical and biological properties of the soil thus making it more resilient under adverse conditions [15–17]. This improvement in soil quality is essential to maintain productivity and is also beneficial to the environment, e.g. by increasing microbial biomass and activity in the soil which is an important quality indicator [18,19]. Pig slurry and other animal manures can therefore provide a rich source of both macronutrients and micronutrients for agro-ecosystems with low fertility levels.

Long-term field experiments can help assess the effects of different types of nutrient management on the ability of cropping

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Abbreviations: AWCD, average well color development; H′, Shanon diversity index; MBC, microbial biomass carbon; NUS, number of used substrates; RP, resistance to penetration.

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systems to maintain productivity, and can also help to determine the relationship between productivity and soil OM content [20,21]. The potential benefits of long-term organic fertilizer application have been widely reported [20,22,23], but few studies have considered the impact of organic fertilizers on continuous maize crops cultivated under irrigated conditions in highly productive areas, and the effect on soil biota [24,25]. The objective of this study was therefore to evaluate and compare the effects of long-term organic and mineral N fertilizers on the productivity of irrigated maize in a semiarid area, focusing on selected physical, chemical and biological soil quality indicators.

2. Materials and methods

2.1. Field experiments

Field experiments were conducted at Gimenells Research Station in north-east Spain (41°65′N, 0°39′E) between 2002 and 2011, although in 2008 and 2009 there were no experimental treatments of the maize plots. The field site is characterized by well-drained Petrocalcic Calcixerept soil with no significant salinity [26]. Soil quality indicators were carried out in soil samples taken in 2010 and 2011, the last two years of the experiment. Selected physicochemical parameters were also measured in 2002 (at the start of the experiment) using standard methods [27]. These comprised soil texture, pH, electrical conductivity (EC), cation exchange capacity (CEC), bulk density, water holding capacity, available P (Olsen P) and extractable K (NH_4Ac) (Table 1). The test region has a semiarid climate with a high mean temperature of 19.1 °C and low precipitation (192 mm) during the maize growing season. Irrigation is therefore required to achieve high grain yields. The study was carried out under sprinkler irrigation system providing approximately 700 mm of water over the maize growing season, combined with conventional tillage with moldboard ploughing to a depth of 25–30 cm.

Fertilizer treatments comprised a single application of PS before planting (composition summarized in Table 2) and a mineral N fertilization (33.5% ammonium nitrate) applied twice in V3–V4 and V5–V6 developing stages [28]. A zero N rate was included as a control (N0). Pig slurry was applied at a dose of 45 m^3 ha^{-1} (PS45), which is equivalent to approximately 315 kg ha^{-1} $year^{-1}$ of N. The slurry was applied with a commercial spreader and was ploughed into the soil after 3–5 h to reduce ammonia (NH_3) volatilization losses [29]. The mineral fertilizer was applied using a small, drop-type hand-driven spreader at a dose equivalent to 300 kg N ha^{-1} $year^{-1}$ (N300), which is within the normal range for the Ebro Valley and it is sufficient to achieve average maize grain yields in the region [30]. The soil N and ammonium (NH_4^+) content was determined using the Kjeldahl method (Pro-Nitro A, model 4002430). All plots were also fertilized before planting with phosphorus (P) (65 kg P ha^{-1} $year^{-1}$) and potassium (K) (207 kg K ha^{-1} $year^{-1}$) to avoid mineral deficits. The elemental plot dimensions were 15 × 11 m (with 15–16 rows per plot) and were arranged in a randomized block design with three replicates.

Table 1
Chemical and physical soil properties at the beginning of the experiment (2002).

	Horizon		
	Ap, 0–23 cm	Bw, 23–69 cm	Bk, 69–117 cm
Sand (%)	39	38	45
Silt (%)	40	42	38
Clay (%)	21	20	17
pH	8.3	8.3	8.3
EC (dS m^{-1})	0.20	0.34	0.59
C.E.C. (mequiv. 100 g^{-1})	24	–	–
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	–	–

Table 2
Average composition of pig slurry (PS) applied from 2002 to 2011.

Characteristic	Average from 2002 to 2011
pH	8.3 ± 0.5
Dry matter (g kg^{-1})	75 ± 19
NH_4^+–N (g kg^{-1})	4.8 ± 1.0
Total N (g kg^{-1})	7.2 ± 1.2
P (g kg^{-1})	1.5 ± 0.4
K (g kg^{-1})	4.5 ± 1.3
OM (t ha^{-1})	3.9

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 7 years of the experiment, and in the final 3 years it was applied 3.3 L ha^{-1} of Trophy (40% Acetocloro plus 6% Diclormid). It was also applied 1 L ha^{-1} 20% Fluoxypyr as a post-emergence herbicide to control *Abutilon theophrasti Medik* as needed. Maize was sown during the first week of April at a density of ~80,000 ha^{-1} with 75 cm between rows, and was harvested during the first week of October. The crop residue was removed from the field after harvest every year.

2.2. Analysis of plant and soil samples

Crop biomass was estimated at physiological maturity by hand-cutting 4 m from a central row of each plot (to avoid border effects) then chopping three plants into pieces in order to determine the dry matter content. Total N content was determined by near infrared spectroscopy (NIRS), using a previously-calibrated 500 Infrared Analyzer (Bran + Luebbe, Norderstedt, Germany). Total N uptake was calculated by multiplying the N content by the biomass at physiological maturity. Grain yield was determined by harvesting two complete central rows (1.5 m × 15 m) with a small plot combine, and 300 g of grain was taken from each plot to determine the grain moisture content and to adjust grain yield to 14% moisture (GAC II, Dickey-John, Auburn, IL, USA). Soil NO_3^-–N levels were determined before planting (N_{ini}) and after harvesting (N_{resi}) in samples taken at three depths (0–30 cm, 31–60 cm and 61–90 cm); the NO_3^-–N results are presented as the addition from 0 to 90 cm. Soil NO_3^-–N were extracted using deionized water and were measured using test strips in a Nitracheck® device calibrated according to the standard procedure. Soil NH_4^+–N was considered to be negligible in the test area [31]. The N budget was calculated for each plot in each year of the experiment. Mineralization (N_{min}) was estimated for the N0 treatment using the equation $N_{min} = N_{resi} + N_{plant} - N_{ini}$ [32] assuming zero N losses from unfertilized plots. N_{plant} was the aboveground plant N uptake at maturity. Nitrogen losses were estimated from the N budget in the fertilized plots [33] using the formula: $N\ loss = N_{final} + N_{plant} - N_{ini} - N_{min} - N_{fert}$, where N_{fert} was the N applied as organic (PS) or mineral fertilizers. Nitrogen losses were considered to be the sum of leached NO_3^-–N, N lost by volatilization and denitrification and N that remained unaccounted. A negative N loss value was interpreted as a loss from the soil–plant system, whereas a positive value was interpreted as uncounted N inputs.

Soil resistance to penetration (RP) was determined by carrying out five *in situ* cone index measurements (0–50 cm) for each plot, using a Rimik CP40 cone penetrometer to measure soil compaction. The instrument recorded cone index values and used an ultrasonic transducer to measure the depth of insertion. The OM in the top layer of the soil (0–30 cm) was determined by measuring organic carbon using the dichromate oxidation procedure in which residual dichromate is titrated against ferrous sulfate [34]. Some studies indicate organic carbon recuperation of 60–86%, so an adjustment factor was required [35]. For the analysis biological quality indicators, 15 soil samples (core diameter 3 cm) from a depth of 0–10 cm were randomly collected from each plot and pooled. Fresh soils (field moisture) were sieved using a 2 mm mesh and stored at 4 °C before testing (within 2 months) for acid phosphatase activity [36], mineralizable N (N_{min}) [37], microbial biomass carbon (MBC) [38], average well color development (AWCD) and microbial functional diversity on Eco-BIOLOG® plates, including number of utilized substrates (NUS) and Shannon H′ diversity index (H′), at mid-exponential phase [39]. It was also determined the abundance of earthworms (depth 0–30 cm in 25 × 25 cm areas, soil replaced after sampling to preserve integrity) [40].

Soil CO_2 emissions (CO_2 Flux) were determined *in situ* at three sampling areas in the middle of each plot using a PP-Systems EGM-4 IRGA® linked to a cylindrical soil respiration chamber SRC-1 (diameter 10 cm, height 15 cm). The soil microbial metabolic quotient (qCO_2) was inferred directly by dividing the CO_2 Flux by the MBC. It was also carried out gravimetric analysis of the soil moisture content (GSM) by oven drying soil taken from each sampling point at 105 °C.

The results were analyzed statistically as repeated measure data using the Proc Mixed procedure in SAS [41] and the SAS statistical package [42].

3. Results and discussion

3.1. Agronomic properties

The mineral N fertilizer had greater impact than PS on the average grain yield and plant N uptake from 2002 to 2011 (Table 3). This may reflect the differing application strategies, with PS45 applied once before sowing and mineral N split into two doses. PS45 applied before sowing was not used efficiently by the crop, suggesting a significant proportion of the N in PS45 is probably lost by volatilization in the 3–5 h before ploughing. The average temperature during application was 12–18 °C depending on the year, and previous studies under these conditions suggest that 15–50% of the N applied in PS45 could be lost by volatilization [43]. The emission of NH_3 from animal manures during and after spreading depends on the temperature, humidity, precipitation and wind-speed, suggesting that the response of maize crops to different forms of N fertilizer will depend on both agronomic management practices and the climate [44].

Table 3
Effect of nitrogen (N) Fertilization rate on grain yield (Yield), biomass at maturity and plant N uptake, average from 2002 to 2011.

	Treatments	Yield ($mg\ ha^{-1}$)	Biomass maturity ($mg\ ha^{-1}$)	Plant N uptake ($kg\ ha^{-1}$)
Average from 2002 to 2011	N0	4.28c[a]	11.2b	133c
	N300	15.8a	27.3a	386a
	PS45	12.2b	25.2a	301b
Block		NS	NS	NS
Treatment		**	*	*
Year		**	**	*
Year * Treatment		**	*	*

Mean values ($n = 3$), NS: not significant, * significant at the 0.05 level, ** significant at the 0.01 level.

[a] Values in each column followed by the same letter are not significantly different at the 0.05 profitability level.

The N uptake rate in our maize plants (Table 3) was similar to the rates reported in other studies, i.e. 155–300 kg N ha^{-1} [6,45]. The higher uptake rate at higher doses of N suggested some degree of 'luxury consumption', but it was observed no differences in the biomass of mature plants treated with PS45 and N300 (Table 3). This could reflect experimental error because the area used for biomass sampling in each plot was smaller (3 m^2) compared to that assessed for grain production (23 m^2).

The NO_3^-–N levels in the soil were high for all treatments in 2002 but in 2003 the NO_3^-–N levels were affected by the nature of the fertilizer treatment in the previous year (Table 4). After a further 8 years of fertilizer applications, it was observed a decline in residual soil NO_3^-–N levels in the N0 and PS45 plots but an increase in the N300 plots from 279 kg ha^{-1} of N in 2002 to 439 kg ha^{-1} in 2011 (Table 4), which is in-line with previous reports of extensive N mineralization achieved using mineral fertilizers in the same climate [31]. This could increase the risk of N leaching during the autumn and winter, with a negative impact on the environment. The relatively low level of residual NO_3^-–N in the PS45 plots in 2010–2011 (Table 4) suggests that the N not used by the crop and not lost through volatilization is immobilized in the soil, which could account for up to 25% of the total NH_4^+–N applied as PS45 [46]. The $NH4^+$–N content of the soil in 2002 was 24 kg ha^{-1} of N [14] and the residual values in 2011 were 0.97 and 1.77 kg ha^{-1} for N300 and PS45, respectively, which can be considered negligible.

The general trend shown by the N budget was a higher N loss in the PS45 plots than the N300 plots (Table 5). However, these data could be misinterpreted because, as previously reported [47], NH_4^+–N can be fixed in the interlayers of the clay minerals. Daudén and Quílez [2] found that the immobilization and/or fixation of N when using PS fertilizers could play and important role in the N budget under similar climatic conditions.

3.2. Soil quality indicators

Table 6 summarizes the effect of the different fertilizer treatments on the physical, chemical and biological soil quality indicators tested. For most of the parameters, there were no significant differences ($p > 0.05$) among the treatments, the exceptions being the mineralizable N content and earthworm abundance. However, there was an interesting trend (N0 < N300 < PS45) over both years monitored that might indicate a long-term benefit from fertilizer treatments, especially PS45.

The mineralizable N content reflects the amount of biologically active N in the soil [48], i.e., the N that can be progressively mineralized by microorganisms and thus made available to growing crops. The mineralizable N content increased significantly when fertilizer was applied, particularly PS45. This contrasts with the higher residual soil NO_3^-–N content in the N300 plots, which increases the risk of leaching. This combination of abundant mineralizable N but scarce mineral N suggests the N cycle is more tightly coupled to plant requirements in the PS45 plots, with concomitant positive effects on soil fertility and the environment. Similar results were reported in previous studies comparing organic and mineral fertilizers for maize production [49].

CO_2 emissions and phosphatase activity were measured as indicators of general and specific soil microbial activity, respectively. Microbial phosphatases play a central role in the mineralization of P by catalyzing the hydrolysis of esterified phosphoric acid and releasing phosphate that can be taken up by microbes and plants [50]. Microbial activity was higher in the N300 and PS45 plots than the N0 plots in both years, but especially in 2011 when

Table 4
Evolution of soil nitrate (NO_3^-) content (kg NO_3^-–N ha^{-1}) before planting and, fertilizing (Ini) and after harvest (Resi) (depth 0–90 cm) from 2002 to 2011.

Treatment	2002		2003		2004		2005		2006		2007		2010		2011	
	Ini	Resi	Ini	Resi	Ini	Resi	Ini	Resi	Ini	Resi	Ini	Resi	Ini	Resi	Ini	Resi
N0	247	150	159	29	53	21	47	59	16	83	50	82	55	59	96	51
N300	279	544	497	246	157	200	266	295	105	370	195	308	75	361	145	439
PS45	377	441	416	252	179	133	237	208	57	202	143	149	97	71	119	65
Means	301	378	357	176	130	118	183	187	59	218	129	180	76	164	120	185

ANOVA	Ini	Resi
Block	NS	NS
Treatment	*	*
Year	**	*
Year*Treatment	*	NS

Mean values ($n = 3$), NS: not significant, * significant at the 0.05 level, ** significant at the 0.01 level.

Table 5
Effect of nitrogen (N) Fertilizer sources on estimated N losses from 2002 to 2011.

Treatment	N losses (kg ha^{-1})							
	2002	2003	2004	2005	2006	2007	2010	2011
N0	0	0	0	0	0	0	0	0
N300	−70	−379	−54	−99	128	21	164	361
PS45	−142	−328	−262	−255	−119	−232	−253	−191
Means	−106	−354	−158	−177	5	−105	−45	85

ANOVA	
Block	NS
Treatment	*
Year	*
Year * Treatment	*

Mean values ($n = 3$), NS: not significant, * significant at the 0.05 level.

CO_2 emissions increased by >60% and phosphatase activity by >40%. This is consistent with previous studies showing that the continuous addition of manure had a significant impact on basal respiration [22] and soil biological activity in general [51]. In contrast, Aira et al. [52] found that the application of PS reduced microbial biomass and microbial activity in the short term. It was found no significant difference between the two fertilizer treatments for these parameters, perhaps due to the heterogeneity of replicate soil samples (Table 6). Phosphatase activity can be repressed by a feedback mechanism induced by the reaction product phosphate, as shown when microbes are transferred from phosphate-free to phosphate-supplemented medium [53]. Chunderova and Zubeta [54] showed that after 4 years of cropping, high phosphate concentrations at field testing sites inhibited microbial phosphatase activity. However, no such effects were observed in N300 or PS45 plots even after 8 years of continuous applications, and others have published similar results [55]. Indeed the treated plots showed a higher phosphatase activity than control plots, probably due to the presence of phosphatase in maize roots. High levels of soluble inorganic phosphate are probably required to inhibit phosphatase activity in the soil [54,56].

Microbial biomass carbon is an indicator of microbial abundance in the soil and responds rapidly to disturbances caused by tillage or fertilizers [57]. Short-term measurements of MBC can therefore reflect the long-term measurements of the organic material in the soil [58]. It was observed a slight (but not statistically significant) increase of the MBC in N300 and PS45 plots compared to control plots, but surprisingly a statistically significant increase in MBC was observed from 2010 to 2011 corresponding to a significant reduction in gravimetric soil moisture (<14%) and a concomitant increase in soil compaction throughout the 0–50 cm profile (Table 6). The application of PS reduced soil compaction slightly in both years (Table 6). Soil compaction can affect seeding emergence, infiltration or water movement within the soil profile [59], yet it appears from our MBC data in 2011 that these limiting factors for plant growth do not affect soil microbial abundance. This suggests that soil microorganisms adapt more readily to limiting abiotic conditions by optimizing their metabolism, resulting in the lower qCO_2 observed in 2011 soils. Following the same trend, the community level physiological profiles revealed by the Eco-BIOLOG® plates suggest that neither the functional/metabolic diversity of the microbial community (indicated by the NUS and Shannon H′ diversity index) nor its average capacity to metabolize different carbon substrates (AWCD) are negatively affected by drought or soil compaction,

Table 6
Effect of nitrogen (N) Fertilizer rate on physical, chemical and biological soil quality indicators in 2010 and 2011.

Year	Treat.	N_{min} (mg N–NH_4 kg^{-1})	Acid phosphatase (mg 4-NP kg^{-1}dry soil h^{-1})	Earthworms (m^{-2})	CO_2 flux (gCO_2/m^2 h)	AWCD ($t = 2$)	H′ ($t = 2$)	NUS ($t = 2$)	MBC (mg C/ kg soil)	0–10 RP (Kpa)	11–20 RP (Kpa)	21–50 RP (Kpa)	GSM (%, p/p)	qCO_2	OM (%)
2010	N0	48	109	1	0.30	0.22	3.03	9	2051	2439	2697	3752	14	0.16	1.49
	N300	69	143	4	0.34	0.26	3.41	12	2297	1973	2801	4200	15	0.15	1.76
	PS45	81	152	11	0.54	0.31	3.64	14	2160	1808	2516	3751	16	0.25	1.56
2011	N0	51	86	3	0.39	0.74	4.27	22	6560	3093	3624	4316	12	0.06	1.50
	N300	77	121	4	0.64	0.79	4.13	21	8059	2664	4033	4716	11	0.08	1.82
	PS45	89	124	9	0.62	0.90	4.35	23	9763	2464	3801	4610	13	0.06	1.54
ANOVA															
Block		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Treat		*	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Year		NS	*	NS	*	**	*	**	*	*	*	NS	**	*	NS
Year * Treat		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS

NS: not significant, * significant at the 0.05 level, ** significant at the 0.01 level.

since both values increase significantly from 2010 to 2011. In both years, the abundance of earthworms varied significantly among the treatments (N0 < N300 < PS45) (Table 6). This agrees with previous reports showing that slurry treatments have long-term beneficial effects on earthworms [60], although the NH_4^+–N content may be toxic to earthworms in the shorter term [61].

Finally, it is noted with interest that the repeated application of PS at a dose equivalent to 32 tons ha^{-1} of OM over 8 years was not sufficient to increase the OM content of the soil above that of the N300 plots (Table 6). This contrasts with the results of previous studies, albeit conducted under different environmental conditions [24,49,62]. One potential explanation is that the N richness of PS45 reduces the amount of OM that must be applied to achieve N fertilization requirements (Table 2). Alternatively, the lability of organic substrates in PS (and its low C/N ratio) may facilitate the mineralization of OM by microorganisms and subsequent volatilization [12,63]. This may explain why the highest CO_2 flux was detected in the PS45 plots (Table 6). Our hot climate favors the loss of organic matter by accelerating microbial activity, thus increasing its oxidation and volatilization. Finally, the higher yield in the N300 plots increases soil carbon inputs from crop residues and root exudates, but the high C/N ratio of crop residues reduces the decomposition rate leading to an increase in the OM content. These combined factors may account for the similarity between the PS45 and N300 plots in terms of OM content and other biological indicators of soil quality, given that the abundance, activity and diversity of soil microbes are closely linked to the OM content [64].

4. Conclusions

The application of PS as a fertilizer (45 m^3 ha^{-1}) in a sprinkler-irrigated maize crop did not improve yields compared to plots treated with mineral fertilizer containing a similar dose of N (300 kg ha^{-1}). A better strategy is therefore required to maximize the benefits of PS in the Ebro Valley, e.g. novel application methods such as immediate ploughing to prevent volatilization of the N content. The positive effects of organic fertilizers may take several years to realize, possibly because the nutrients are initially immobilized (as organic compounds and microbial biomass) but are later released causing the soil quality to improve. The application of PS resulted in marginally higher soil quality parameters in both test years, but the most significant impact was on earthworm abundance, a key soil quality indicator that is linked to other parameters such as soil moisture and resistance to penetration. The most important findings were that the repeated application of PS to soil under irrigated semiarid conditions has a beneficial impact on the soil quality without increasing maize yields, and that earthworm abundance is an inexpensive and early indicator of soil quality.

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CHAPTER IV

Determining total Nitrogen Concentration in whole Maize plant using Near-Infrared Spectroscopy

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ABSTRACT

In this study, near infrared spectroscopy (NIRS) was compared to the Dumas combustion method for estimation of whole maize plant nitrogen (N) concentration. Two methods for sample preparation prior to analysis were compared; samples were either whole plant (whole-plant) ground and analyzed together or grain and stover were ground and analyzed separately (stover-grain). Treatments were the 0 (0N) and the 300 kg N ha^{-1} (300N). The results showed that there were not significant differences in plant tissue N concentration determined with NIRS when the whole-plant (9.68 g N kg^{-1}) group was compared with the stover-grain (10.13 g N kg^{-1}) group. In addition, plant N concentration reported by NIRS (9.9 g N kg^{-1}) and by the Dumas combustion (9.68 g N kg^{-1}) were not statistically different from each other. However, both methods were able to detect a significant difference between plant tissue N concentration between the treatments.

Key words: maize; whole plant; nitrogen content; NIR spectroscopy

INTRODUCTION

Intensified crop production to maximize crop yields is becoming more reliant on methods that allow farmers to quickly determine causes for potential yield loss with rapid problem solving capability. For example, nitrogen (N) content which is one of the most important aspects of maize production and many studies (Berenguer et al., 2009; Yagüe and Quílez, 2010) have been conducted about its plant content and soil extractions. Therefore, methods that are reliable, effective, fast, and not cost prohibitive should be developed to facilitate and speed up the process of plant tissue analysis. Near infrared spectroscopy (NIRS) is a simple, fast, and non-destructive method that can be used to measure, at a low cost, the nutrient composition in the different parts of the plant (Sileoni et al., 2010). In NIRS analysis, samples are irradiated with light at wavelengths ranging from 800-2500 nm. This light reflected off powdered solids, contains compositional information which can be unraveled by a computer to report multiple analyses under one minute (Murray, 1986). By calibrating the NIR spectrum for plant tissue of different composition it is possible to use NIRS to predict the nutrient status of plants, which could therefore substitute current chemical methods (Pfitzner et al., 2001). NIRS has become a useful approach to determine N concentration in maize tissue and especially in maize grain (Melchinger et al., 1986; Volkers et al., 2003; Baye et al., 2006). An advantage of using NIRS compared to other destructive methods for elemental composition analysis is that the NIR spectrum also provides information about organic structure and composition of the material being analysis (i.e. protein, starch, and lipid contents) (Orman and Schumann, 1991; Orman and Schumann, 1992; Cogdill et al., 2004; Sileoni et al., 2010). This ability to make multiple predictions from one spectrum further enhances the value of the NIRS technique. Plant breeders can also benefit from NIRS analysis. Analysis of maize grains can provide the uniformity of seed samples with respect to specific quality traits; this advantage allows breeders to select lines on the basis of comprehensive quality data in the short time between harvesting of one generation and sowing of the next (Osborne, 2006). Because of these benefits, NIRS has been used extensively in maize breeding programs aiding in the registration of cultivars throughout Canada and Europe (Valdes et al., 1987; Barriere and Argillier, 1993). For maize production, total N concentration in plant tissue is an important factor used to forecast crop productivity, to evaluate N uptake in the stover residue and, consequently it is also useful for environmental protection purpose (Wilhelm et al., 2004). The estimation of whole plant N uptake is labor intense, as in most cases researchers need to determine it by first determining the N contents of grains and stover separately (Quílez and Yagüe, 2010); then adding the two fractions together to give the total N uptake. To maximize the efficiency in determining total N uptake by maize plants, some researchers have estimated N uptake in the whole maize plant, without separating the grain from the stover (Cela et al., 2011). The process of grinding whole maize plants for total N analysis can result in a non-homogenous sample, due to the differences in particle sizes resulting from the two different types of plant tissue. The high variability in particle size between the maize grain and stover could then lead to higher variability in the results than what should be expected. However, it is unknown whether the problem of different particle sizes in sample would also present a significant problem for NIRS analysis. Therefore, this research was developed to compare two types of plant sampling preparation and the use of NIRS compared with the Dumas combustion method to determine total N concentration and uptake by maize plants.

The objectives of this study were to: i) compare the N content determined in whole maize plant when stover and grain were ground and analyzed separately by NIRS and by the Dumas combustion method; ii) compare the N content determined in whole maize plant when stover and grain were ground and analyzed together by NIRS and by the Dumas combustion method; and iii) investigate if NIRS is sensitive to detect differences in N concentrations tissue due to different N application rates. The hypothesis is that NIRS is sensitive to detect differences in N concentration due to different N application rates in two types of whole maize plant sampling preparation.

MATERIALS AND METHODS

The maize plant samples used for this study were collected from two maize N fertility trials conducted in the northeast of Spain (41º43’N, 0º26’E) in 2011. In those trials, mineral N was applied at the rates of 0, 100, 200, and 300 kg ha^{-1}, rates that supplied N amounts that ranged from limiting to non-limiting for optimum maize growth and yield (Berenguer et al., 2008). For each trial, the experimental design was a completely randomized design with six replications. Plants collected were used for plant and grain N uptake determined by NIRS and by the combustion method of Dumas (Dumas, 1831). Samples were only collected from the plots receiving 0 and 300 kg N ha^{-1}, namely 0N and 300N, respectively. In total, 24 plots were sampled (12 plots from each N treatment); four plants were harvested form each plot and separated into two groups of two plants each, which gave a total of 96 samples being analyzed. In one group, the whole plant, stover and grain, were ground together and the ground material was used for analysis of tissue N concentration, this group will be referred to as whole-plant. In the other group, the grain and stover were separated prior to tissue N concentration analysis, this group will be referred to as stover-grain. For the stover-grain group, the results were expressed as a weighed average of grain plus stover so that they could be compared with the plant N concentration determined in the whole-plant group. The weighed average was calculated as:

$$y = \frac{[(Np * DWp) + (Ng * DWg)]}{(DWp + DWg)}$$

where y is the plant N content, Np is the N content (%) of the whole plant without grain, DWp is the dry weight (g) of the whole plant without grain, Ng is the N content (%) of the grain, and DWg is the dry weight (g) of the grain.

The plant samples used for NIRS analysis were ground three times using a commercial VIKING-STIHL garden shredder GE-205 (Langkampfen, Austria). After grinding, the particles size ranged from 1 mm in diameter for the grain to 2 cm in diameter for the stover part. Because of the large difference in particle size distribution, for the whole-plant group the ground material was manually mixed so that a homogeneous mixture was obtained prior to NIRS analysis. For the Dumas analysis, ground samples of whole plant, stover, and grain (approx. 300 g chopped material per sample) were weighted and dried in an oven at 65ºC for 48 h to determine the moisture content. After drying, about 100 g of samples were ground one more time in a laboratory mill fitted with a 1 mm screen to assure homogeneity of samples prior to analysis.

For the NIRS analysis the N content of the tissues in the whole-plant and stover-grain groups were analyzed using an InfraAlyzer 2000 spectrometer (Bran+Luebbe, Norderstedt, Germany). This spectrometer has a tungsten lamp as energy source and is equipped with a filter wheel to allow absorbance reading of a given sample at 19 different wavelengths (1445, 1680, 1722, 1734, 1759, 1778, 1818, 1940, 1982, 2100, 2139, 2180, 2190, 2208, 2230, 2270, 2310, 2336 and 2348 nm) in each recorded spectrum. This spectrometer was previously calibrated for N contents in standard whole maize plant samples (including grain) using the N values obtained with the Dumas method. Samples were analyzed in triplicates. All the instrument management and spectral data recording was monitored with SESAME software from Bran+Luebbe. ANOVA was used to determine if there were significant differences in total N concentration between the NIRS and Dumas methods and also if there were differences in N concentration due to different N application rates. All statistical analysis was performed using SAS (2001).

RESULTS AND DISCUSSION

The whole maize plant N concentration ranged from 9.18 to 10.18 g kg^{-1} as determined with NIRS and from 9.6 to 10.2 g kg^{-1} as determined with Dumas combustion method (Table 1). The weighted average N values ranged from 10.03 to 10.23 g kg^{-1} and from 9.28 to 10.08 g kg^{-1} with NIRS and Dumas combustion methods, respectively (Table 1). These results are consistent with previous studies from Boixadera et al.(2005; 2010) and Yagüe and Quílez (2010), who reported maize tissue N concentration ranging from 7.7 to 13.5 g kg^{-1} in similar environmental conditions using the Dumas combustion method. These results show that using the whole plant or weighted average as well as the two methods of N analysis (NIRS and Dumas combustion) provide results that were comparable.

There was a significant N rate effect on plant tissue N concentration as determined by both methods for N analysis (Table 2). The plant that received the treatment 300N had higher N content than plants that received 0N, as would be expected. However, there were no significant differences when comparing the different methods for N content analysis (Table 2).

Measurement of nutritional status, as indicated by whole plant tissue N concentration in maize production, is crucial to provide N recommendations. Thus, analyzing plant N content is essential when trying to improve N use efficiency of crops through the adjustment of N fertilizer applications (Gastal and Lemaire, 2002), and therefore, to improve N management in agricultural systems (Huang et al., 2011). At present, N application represents about 20 % of production fix costs (Lloveras et al., 2012) (without counting the water and irrigation expenses), and improved N use efficiency can reduce risks of environmental contamination with nitrate and also reduce production costs by reducing the amount of N applied.

According to Cozzolino *et al.*(2001) quality parameters in whole maize plants can be accurately predicted using NIRS when samples are well ground and they are taken carefully. Thus NIRS can potentially become a valuable tool to evaluate the N content of whole plants, and also, this procedure can be time effective compared with the weighted average method. This is consistent with the results shown in Figure 1. This figure plots the average of NIR spectrum for a set of 24 samples scanned for whole plant, stover and grain, separately, at the nineteen different wavelengths. The main spectrum of stover and whole plant samples were much more similar than the mean spectrum of grain samples (Figure 1). This could be related to the different properties of the grain samples, such as higher N concentration. The stover and whole plant absorbencies presented similar spectra (Figure 1 a, b), which means that grain N concentration is diluted upon mixing with the stover sample.

CONCLUSIONS

The results reported on maize tissue N concentration using NIRS showed sufficient accuracy in the analysis of whole plant N concentration for maize, suggesting this technique could be used in further experiments to quickly access the N status of the crop. In fact, this technique is widely accepted as one of the most promising process control, nondestructive and, accurate for monitoring chemical parameters in maize production. Furthermore, this research showed that using NIRS on stover and grain samples ground together was as reliable and useful as using the weighted average method. In conclusion, the NIRS approach was as reliable in determining maize tissue N concentration as was the Dumas combustion method.

Table 1. Mean (g Kg^{-1}), standard deviation and analyses of variance of the method of N analysis (NIR vs Dumas), the sample processed (whole plants and weighted average) and its interaction.

Sample processed	Method NIR	 Dumas
	N (g Kg^{-1})	
Whole plant	9.68±0.5	9.9±0.3
Weighted average	10.13±0.1	9.68±0.4
Effect		
Replication	NS[†]	
Sample processed[¶]	NS	
Method of N analysis	NS	
Method of N analysis x Sample processed	NS	

[†] NS, not significant.

[¶] Samples processed refers to tissue and grain sample being ground together or separated.

Table 2. Mean (g Kg^{-1}), standard deviation and analyses of variance of the method of N analysis (NIR vs Dumas), N treatment (0N and 300N) and its interaction.

	Method	
N Treatment	NIR	Dumas
N (Kg ha^{-1})	N (g Kg^{-1})	
0N	9.23±0.3 b†	9.08±0.3 b
300N	10.43±0.3 a	10.58±0.3 a
Effect		
Replication	NS	
Method of N analysis	NS	
N Treatment	**†	
Method of N analysis x N Treatment	NS	

† **Significant at the 0.01 level. NS, not significant. † Means followed by the different letter are significantly different according to Tukey multiple range test (0.05).

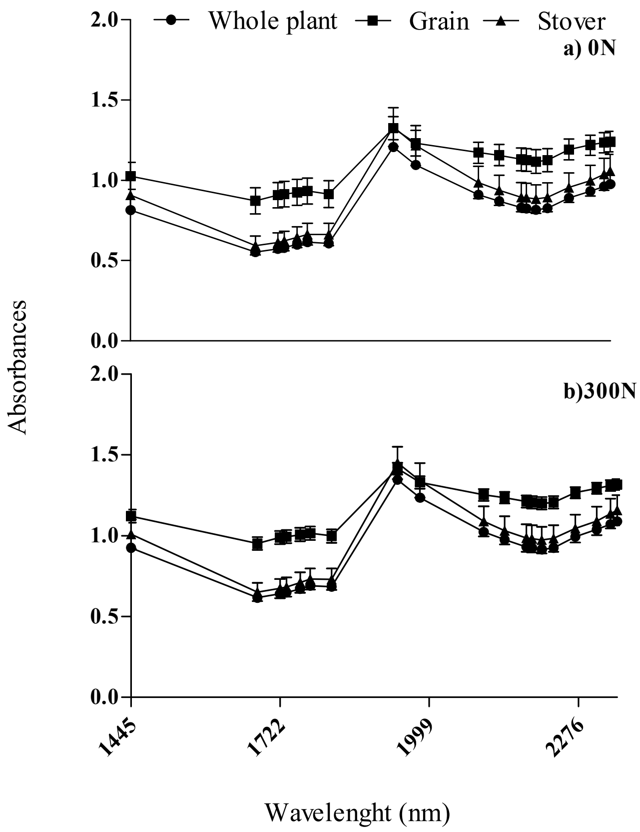

Figure 1. Mean spectra of the calibration samples of whole plant (WP), grain and stover (without grain) for a) 0N and b) 300N. Bars represent one Standard Error from twelve replications.

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GENERAL DISCUSSION

The Challenge

The present thesis seeks to contribute to the development of more sustainable agricultural systems in terms of crop production and soil management. Through the knowledge generated as a result of the present research, we have sought to interest farmers in maintaining the sustainability of their soils. At the same time, we have challenged them to take interest in a new potential source of income: crop stover. We have also taken on the more difficult challenge of trying to make realistic recommendations relating to how stover could be managed to offer a reasonable land use.

The impact of removing crop stover on soil quality and crop productivity must be assessed before appropriate decisions can be made and policy decisions taken with respect to the emerging biofuel industry. Finally, we must remember and maintain our social mandate to develop a safe agricultural system that can help to prevent increases in the level of greenhouse gases released into the atmosphere.

GENERAL DISCUSSION

In the short-term, our results demonstrated that the incorporation of maize stover did not penalise maize production and indeed enhanced nutrient cycling. However, in their review of USA trials, Wilhelm et al., (2004) showed that the removal of maize stover had a negative influence on crop production. The proportion of maize stover needed to return into the soil may depend on such factors as soil type, climate and yield expectations, etc. The actual amount of maize stover that must be incorporated in order to control soil erosion has been estimated at about 70% of total production (McAloon et al., 2000). Returning maize stover to the soil increased the soil organic carbon (SOC) content with respect to the removal of stover. Changes in SOC were therefore proportional to the amount of crop stover returned to the soil. Layese et al. (2002) also reported a positive interaction between nitrogen (N) application and the return of maize stover, indicating that N fertilization was insufficient to sustain SOC without the return of maize stover.

Maintaining SOC levels should be a primary consideration of agricultural producers when they design cropping systems for specific locations and soil types. Evidently, crops that produce more stover will offer a greater potential for maintaining, or indeed increasing, SOC than those that produce less stover. The removal of maize stover for off-field uses such as energy production or livestock feed should only be considered when it comes from fields that provide an excess with regard to what is needed to maintain the existing SOC level (Benjamin et al., 2010). In a review of the long-term effects of different agricultural systems on soil quality parameters, Dick (1992) reported that there is generally a positive relationship between soil carbon (C) content and microbial biomass and concluded that the incorporation of stover into the soil increases biological activity.

In the case of fertilizers, our results showed that in comparison with mineral N fertilizers, the use of manure increased the soil organic matter (OM) content when stover was incorporated. This is consistent with other evidence (Dick, 1992) and is not surprising given that manure supplies the soil with OM. For example, in our study, the application of fresh farmyard manure (in the form of pig slurry, PS) at a rate of 45 m^{-3} ha^{-1} $year^{-1}$ supplied about 3.8 tonne ha^{-1} $year^{-1}$ of OM. However, taking into account the results presented in Chapters I and II, we note that manure does not generally confer any advantages in terms of maize yields. This may reflect the different strategies applied;

manure was applied before sowing, while mineral N was applied at sidedress and then split into two doses. A significant proportion of the N applied in the form of manure was therefore potentially lost due to volatilization (between 15 and 50% of the N applied) before ploughing (Piñol et al., 2007). Despite the fact that the amount of manure applied is known, it is an arduous task to calculate the exact amount of N applied by organic fertilizers that is available to the crop. This suggests that when manure is applied at similar rates to mineral fertilizers, it does not have similar effects to maize yields and is demonstrated by the results reported in Chapters I and II. However, the beneficial impact of applying manure was seen in the soil quality (Haynes et al., 1995; Fraser et al., 1994; Perrott et al., 1992). The conclusion that manure applications do not have a generally beneficial effect on maize production is not a surprising given that we do not know the exactly amount of N applied.

Relative to mineral N fertilizers, we found that the N requirements for a high-yielding maize crop grown in the Ebro Valley fell within a common range of 300-350 kg N ha^{-1} (Yagüe and Quílez, 2010). Although stover management and mineral N fertilization did not present a significant interaction (Chapter I, Table 2), incorporating the stover at an application rate of 300 kg N ha^{-1} produced the most advantageous results in terms of maize production.

Overall, our results confirmed the positive effect that the application of manure can have on soil quality parameters. For instance, as shown in Chapter I, the incorporation of maize stover in combination with organic N fertilization (in the form of cow slurry, CS) improved the chemical and biological properties of soil (such as SOC, microbial biomass carbon (MBC), dehydrogenase activity (DHA), and earthworm abundance) that directly affect soil fertility. These observations are consistent with those of Böhme et al. (2005). Moreover, the results obtained in Chapter II were confirmed by those in Chapter III, where long-term applications of organic fertilizer (in this study, PS was used) resulted in higher values for soil quality parameters than for mineral N fertilizers. Although this Chapter (III) extensively studied the impact of organic and mineral fertilizers on soil quality parameters, the most important finding was perhaps the relevance of earthworm abundance when evaluating soil quality. These results clearly demonstrated the superiority of the integrated use of organic manure over that of inorganic fertilizers, confirming those reported in other studies (Liu et al., 2010; Gong et al., 2009). Both trials also showed an interesting trend in soil quality parameters relating to the source of the fertilizer applied (0N< mineral< organic). This trend indicates the benefits that can be obtained from fertilizer treatments and especially from organic sources (such as CS or PS).

Finally, grain yields in the AP field were significantly increased by N fertilization (Chapter I, Table 2). Very high maize grain production (> 19 Mg ha^{-1}) was obtained in this field and plant N content ranged from 9.0 to 13.4 g of N kg^{-1} (Chapter I, Table 2). Studies involving modern-era maize hybrids have also reported similar values (Ciampitti and Vyn, 2012). Whereas many studies have determined the N contents of grain and stover separately, with the posterior use of a weighted average to obtain the whole-plant N content, we used whole maize plants to determine the N concentration. We therefore considered it relevant to validate this method for use with important field grain productions. Our results reported that determining whole maize tissue N concentrations using the NIRS approach offered as reliable and useful a method as that of Dumas. The NIRS method is also sensitive and accurate enough to detect differences in N concentrations. According to Cozzolino *et al.* (2001), the quality parameters of whole maize plants can be accurately predicted using the NIRS method when samples are well-ground and carefully processed. NIRS could therefore potentially become a valuable tool for evaluating the N content of whole maize plants, in order to calculate the equivalent to the weighted average of the grain and stover parts processed separately.

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GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

The main conclusions of this thesis are:

1. In the short-term, in high yielding (16-20 Mg ha^{-1}) sprinkler-irrigated maize and high SOC content, applying typical fertilization rates (200-300 kg N ha^{-1}), farmers can incorporate stover without yield or biomass penalties and thereby improve the SOC levels of their soils.
2. There was no significant interaction between stover management and N fertilization rates. This showed that stover incorporation had only a minimal impact on C and N storage in the short-term (during the three years of study) but that stover removal caused a reduction in the SOC level. However, at low N fertilization rates (0 and 100 kg N ha^{-1}) grain yields were generally lower under stover incorporation.
3. Selling stover implies a short-term economic gain, but the incorporation of stover improves soil properties for future growing seasons.
4. Indicators of soil quality parameters (SOC, MBC, DHA, and earthworm abundance) tended to be higher when the stover was incorporated than when it was removed.
5. Manures (such as CS) and mineral fertilizers (300N) had similar effects on maize production relative to not applying N fertilizer (0N). However, SOC, DHA, and earthworm abundance exhibited the same tendency (0N<300N<CS) for each of the stover management practices applied. In short-term CS applications under semiarid conditions produced beneficial effects in terms of soil quality.

6. Continuous maize fertilization with PS (45 m^3 ha^{-1} $year^{-1}$) (~ 315 kg N ha^{-1} $year^{-1}$), under sprinkler irrigation did not improve grain yields compared to plots treated with mineral fertilizer containing a similar dose of N (300 kg ha^{-1} $year^{-1}$).
7. When 8 years of repeated PS applications to a monoculture maize crop were compared with mineral fertilization, we noted higher levels of soil quality parameters. The most significant impact was on earthworm abundance, which is an inexpensive and early indicator of soil quality.
8. NIRS is an accurate, non-destructive technique for determining the N concentration in maize tissue. This technique could therefore be used in further experiments to quickly assess the N status of a given crop.
9. Using NIRS to evaluate whole-plant maize N in stover and grain samples ground together offered an equally reliable and useful methodology as determining the N content of grain and stover separately (and applying a weighted averaging). At the same time, applying the NIRS approach to whole-plant maize offers a reliable way to determine the maize tissue N concentration as the Dumas combustion method.