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ORIGINAL ARTICLE

Nitrogen fertilization with pig slurry in a barley-sorghum double-annual forage cropping system

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Abstract The high concentration of pig farms in NE Spain makes pig slurries an attractive fertilizer to reduce costs of agricultural production. However, inadequate management of fertilization with pig slurry can cause negative environmental consequences. In this context, a 4 year field trial was carried out to evaluate several fertilization strategies, using pig slurry, for a double-annual forage cropping rotation with barley (*Hordeum vulgare* L.) and sorghum (*Sorghum bicolor* L.) under sub-humid Mediterranean conditions. Four nitrogen (N) fertilization treatments applied as pig slurry (0, 170, 250 and 330 kg N ha^{-1} year^{-1}) were applied and their effects on yield, N uptake, unrecovered N, and residual NO_3-N in soil were evaluated for each crop and each rotation. The 4 year average dry matter (DM) forage yield of sorghum was 9.3 Mg ha^{-1} in all N fertilization treatments, except for the control (0 kg N ha^{-1}) which was 6 Mg ha^{-1}. However, barley DM yields varied among N treatments. The highest barley yield (8.7 Mg DM ha^{-1}) was achieved with the application of 330 kg N ha^{-1} year^{-1}. The barley yields were reduced by a 26% (6.9 Mg DM ha^{-1}) and a 64% (5.3 Mg DM ha^{-1}) with N rates of 250 and 170 kg N ha^{-1} year^{-1}, respectively. The average total annual yield was 17.8 Mg DM ha^{-1} for the maximum N rate tested (330 kg N ha^{-1} year^{-1}). Indeed, the application of N rates above the maximum amount allowed by the Nitrates Directive in areas vulnerable to nitrate contamination (NVZ) (170 kg N ha^{-1} year^{-1} in form of pig slurry), improved the total annual DM yield by 10–18%. However, N rates of 250 kg N ha^{-1} year^{-1} increased by 69% the unrecovered N compared to applying 170 kg N ha^{-1} year^{-1}. This could lead to N losses to the environment, probably by nitrate leaching and/or volatilization.

Keywords Biomass · N efficiency · N fertilization · Rainfed · Soil residual N

Abbreviations

DM	Dry matter

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PS	Pig slurry
N	Nitrogen
NVZs	Nitrate vulnerable zones
ANRF	Apparent nitrogen recovery fraction
ANE	Agronomic nitrogen efficiency

Introduction

The pig population in Spain is around 30.1 million, being the EU-28 country with the largest pig census, followed by Germany (26.4 million; EUROSTAT 2018). The Northeast region of Spain contains 51% of the total Spanish pig census; 28% in Catalonia and 23% in Aragon (MAGRAMA 2015).

These areas produce large amounts of pig slurry that can be used as organic fertilizer for crops (Yagüe and Quílez 2010; Martínez et al. 2017a). However, improper management of pig slurry can have environmental consequences (Mosier et al. 1998; Amon et al. 2006; Petersen and Miller 2006). One of the most documented environmental problems is the loss of nitrogen (N) to the atmosphere during the storage and field application of the slurry (Bosch et al. 2014). The atmospheric deposition of N causes acidification or eutrophication of surface waters, and an increase in greenhouse gases, especially nitrous oxide (N_2O; Sakamoto et al. 2006; Ndegwa et al. 2008). Moreover, excessive slurry applications have a high risk of contamination of groundwater by nitrate leaching and surface runoff (Isidoro et al. 2006; Salmerón et al. 2010). Aware of this problem, the European Community (EEC) implemented the Nitrate Directive (EEC 1991). According to this regulation, each Member State must identify in its territory areas which are vulnerable to nitrate contamination (NVZ) where the amount of animal manures applied to soil must not exceed 170 kg N ha^{-1} $year^{-1}$.

Some farms in NE Spain practice double-annual cropping strategies to maximize the productivity of the land (Maresma et al. 2019a). Farms combine a winter cereal followed by a summer cereal in the same year, and in many cases both crops are used as forage feed for livestock. However, in areas where double cropping is not practised, the crop cycle can be completed with either winter cereal or summer cereal in irrigated areas to obtain grain. In these cases, this production can be used to feed the intensive farm pigs within the same region. In this way, the high availability of livestock manure and its economic advantage over mineral fertilizers makes pig slurry the most used fertilizer in local agricultural systems. The most common double-annual cropping strategy combines maize (*Zea mays.* L.) with a winter cereal such as barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum*) or triticale (×*Triticosecale Wittmack*). However, scarce and irregular summer precipitation, mainly in rainfed Mediterranean environments, has provoked a search for alternative summer crops to maize (Ejeta and Knoll 2007; Ramazanzadeh and Asgharipour 2011; Reddy et al. 2008). Sorghum (*Sorghum bicolor* L.) has increased in popularity among farmers due to its higher resistance to drought than maize, although, at certain phenological stages, sorghum can also be sensitive to drought stress (Tuinstra et al. 1997; Reddy et al. 2009).

Several fertilization studies in double-annual cropping systems have been carried out using mineral fertilizers (Guo et al. 2008; Shang et al. 2014; Maresma et al. 2019a), and organic fertilizers (Grignani et al. 2007; Ovejero et al. 2016; Perramon et al. 2016; Yagüe and Quílez, 2010). Indeed, studies on double-annual cropping systems in Mediterranean irrigated environments (winter crop + maize) have been recently published (Tomasoni et al. 2011; Giola et al. 2012; Cavalli et al. 2016; Demurtas et al. 2016; Maresma et al. 2019a). Moreover, only a few recent studies have used sorghum as a double-annual cropping alternative to maize, combined with oats (Perramon et al. 2016), barley (González-García et al. 2016) or triticale (Lyons et al. 2019a). Sorghum could provide a competitive alternative to corn silage under irregular rainfed conditions due to its higher water use efficiency and drought tolerance when water is scarce (Merrill et al. 2007). Therefore, there is a need to evaluate the double-annual cropping strategy (sorghum + winter crop) in rainfed Mediterranean environments.

The hypothesis of this study is that the N rate allowed by the Nitrates Directive in NVZ (EEC, 1991) (170 kg N ha^{-1} $year^{-1}$ of organic fertilizer) may be appropriate if only one crop is grown per year, whether it is a winter or summer cereal. However, in NVZ with high livestock density, when an annual double crop forage rotation is established, the demand for N increases and this limit can cause a deficit in the adequate supply of nutrients to the crops. Indeed, the European Union (EU) has allowed derogations of the Directive concerning this specific aspect (Van der Straeten et al. 2012), and in some regions or countries it allows the application of higher N amounts of organic origin, up to a maximum of 250 kg N ha^{-1} year^{-1} (European Union 2005, 2006, 2007a, b, 2008, 2011). Situations for derogation include crop rotations with long growing seasons, crops with high N uptake, or soils with high denitrification capacity. Therefore, in these agricultural systems, it should be possible to exceed the threshold of the amount set by the European Directive, maintaining high productivity and minimizing the environmental impact on groundwater.

The main objective of this study was to assess and optimize N fertilization in terms of dry matter yield, N balance, and N efficiency comparing fertilization strategies using pig slurry (PS) in a 4 year double-annual forage cropping system under rainfed sub-humid conditions.

Materials and methods

Experimental site

A 4 year study (2012–2016) was carried out in an experimental field located at Torelló 42°2′0'' N, 2°15′12'' E) (NE, Spain). Different fertilization strategies were evaluated in a common double-annual forage crop rotation in the area, barley and sorghum. The summer cereal (sorghum) is usually seeded in June after the forage winter cereal harvest (barley), and similarly, winter cereals are seeded in October–November after the forage sorghum harvest. Previously, other experiment consisting in double-annual forage cropping rotation of maize and triticale was practiced between 2006 and 2012 (Ovejero et al. 2016). The soil in the experimental field is calcareous, with a moderately-alkaline pH and a loam texture. The main soil properties of the field site are presented in Table 1.

Table 1 Selected soil properties for different soil depths at the beginning of the experiment (2012)

	Depth		
	0–0.3 m	0.3–0.6 m	0.6–0.9 m
pH	8.3	8.3	8.3
EC $_{1:5}$ (dS m^{-1})	0.22	0.15	0.17
Organic matter (%)	1.75	1.23	0.88
P (mg kg^{-1})			
T0$^{\alpha}$	28	12	5
T1$^{\alpha}$	37	14	5
T2$^{\alpha}$	39	20	10
T3$^{\alpha}$	42	12	7
K (mg kg^{-1})			
T0	114	60	54
T1	118	63	51
T2	100	56	46
T3	112	57	55
Sand (%)	31.7	32.1	34.6
Silt (%)	47.2	44.9	44.9
Clay (%)	21.1	23	20.5

$^{\alpha}$The acronyms of the treatments are described in Table 2

Climate and weather conditions during the experimental period

The climate in the study area is humid sub-Mediterranean with continental tendency of transition between the Mediterranean climate and that of Central Europe. The average annual temperature of the area ranges around 12–13 °C, with an annual rainfall of 650 mm.

Total precipitation and the average temperature recorded by an automatic weather station (ECRN-50 Rain Gauge, Decagon Devices) during the experimental period are presented in the Supplementary Material (Figure SM1). Total annual precipitation was highly variable among the 4 years of the experiment. Rainfall was higher in the 2012/13 growing season (775 mm) and 2013/14 growing season (997 mm) than the average rainfall from 1985–2006 (651 mm). However, precipitations during the 2014/15 and 2015/16 growing seasons were below average (610 and 643 mm, respectively). In the 2013/14 growing season most of rainfall occurred during the growth of sorghum (752 mm from June to October), which is 400 mm above the average rainfall of the same months in the period 1985–2006 (352 mm).

The difference in the average temperature between the growing seasons during the experimental period was relatively small, varying from 13.1 °C in 2012/13–13.7 °C in 2013/14. The average annual temperature throughout the experimental period was 13.5 °C.

Experimental design

The experiment was designed as a randomized complete block with four pig slurry (PS) treatments and three replications. Each experimental plot was 12 m wide by 40 m long. The PS treatments were:

- T0—Control: Only was applied K in order to avoid deficits of this mineral in these plots.
- T1—170 kg N ha^{-1} year^{-1}: Corresponds with the maximum N organic rate allowed according by Nitrates Directive 91/676 (EEC, 1991).
- T2—250 kg N ha^{-1} year^{-1}: Corresponds with the N rate which we hypothesize to match the double-annual cropping strategy (barley + sorghum) requirements.
- T3—330 kg N ha^{-1} year^{-1}: Corresponds to N application rate above theoretical crop requirements.

The application of each pig slurry fertilizer rate was carried out in the same experimental plots every year. In addition, prior to the establishment of the experiment, the plots were fertilized with PS for 6 years with the N fertilization dose described in Ovejero et al. (2016). Table 2 details the N application rates that were applied in each treatment and crop throughout the trial (2012–2016).

The PS was obtained every year from the same pig-fattening farm located near the experimental field, and it was applied using commercial machinery for slurry bandspreading with 15 cm of separation between bands. Pig slurry was sidedressed in February to the barley at tillering phase (20–29 stage (Zadoks et al. 1974)). A second application of PS was done before the sowing of sorghum in June. The PS application before sorghum was done on bare soil and incorporated to 15 cm by a chisel type cultivator blade immediately after application to minimize ammonia volatilization losses. The corresponding N rates of each fertilization treatment were applied adjusting the speed of the tractor, whereas the N concentration of the PS was determined 'in situ' by the electrical conductivity and density of the PS in the tank (Provolo and Martínez-Suller 2007).

Table 2 Distribution of the N fertilization (kg N ha^{-1}) in the double-annual cropping system for each treatment and crop in the experimental period 2012–2016. Fertilizer was applied in form of pig slurry. Barley received the N application at sidedress time and sorghum just before sowing

Treatment	Barley (kg N ha^{-1})	Sorghum (kg N ha^{-1})	Total annual (kg N ha^{-1})
T0	0	0	0
T1	0	170	170
T2	80	170	250
T3	80	250	330

The plots with the control treatment (T0 were fertilized only with 60% potassium chloride (hand applied, no incorporated into the soil) before sowing the sorghum to compensate the potassium extractions of the double-annual forage cropping system.

Double cropping system management

Both barley and sorghum were managed according to the common practices in the area. After the harvests of both crops a chisel cultivator (15 cm depth) was passed to prepare the sowing of the next crop. The barley cv. *Cometa* was sown in November with a 12 cm row-space planter at a rate of 180 kg seeds ha^{-1} and was harvested at the beginning of June in the early dough stage (stage 83; Zadoks et al. 1974). Sorghum hybrid cv. Grass II was sown in June using a 75 cm row-space planter and with 18 cm between the seeds in a row (45 kg seeds ha^{-1}). The sorghum was harvested in October at the hard dough stage (stage 8 of the Vanderlip scale; Vanderlip 1993). The exact dates of sowing and harvesting are showed in Table 3. An herbicide was applied each year to combat broadleaf weeds during summer cereal cropping. The forage biomass obtained normally is used in the area for dairy cattle feed (silage).

Physicochemical analysis of pig slurry

During application, the tank of the spreader was calibrated to apply the target PS rates by measuring the electrical conductivity and density of PS in order to adjust the speed of the tractor. Also, each PS tank applied in the experimental field was sampled to determine nutrient concentrations. These samples were tagged and frozen at −20 °C until chemical analysis. The real amount of N applied was calculated according to the physicochemical characteristics of

Table 3 Date of sowing and harvest of barley and sorghum from 2012 to 2016, and days of growing period for each crop

Growing season	Crop	Date of sowing	Date of harvest	Days from sowing to harvest
2012/13	Barley	23/11/2012	04/06/2013	193
2013	Sorghum	28/06/2013	08/10/2013	102
2013/14	Barley	6/11/2013	02/06/2014	208
2014	Sorghum	20/06/2014	23/10/2014	125
2014/15	Barley	19/11/2014	05/06/2015	198
2015	Sorghum	03/07/2015	26/10/2015	115
2015/16	Barley	17/11/2015	06/06/2016	202
2016	Sorghum	11/07/2016	27/10/2016	108

Table 4 Average physicochemical characteristics of pig slurry applied from 2012 to 2016 in the experimental field

Parameter	Average from 2012 to 2016
pH	8.4 ± 0.1
Electrical conductivity (dS m^{-1})	21.9 ± 2.3
Dry matter (kg DM Mg^{-1})	37.4 ± 12.7
Organic matter (kg OM Mg^{-1})	43.8 ± 15.5
Ammonium-N (kg N m^{-3})	2.6 ± 0.2
Organic N (kg N m^{-3})	1.0 ± 0.4
Total N (kg N m^{-3})	3.3 ± 0.7
P (kg m^{-3})	0.5 ± 0.2
K (kg m^{-3})	2.2 ± 0.2

the PS (Table 4), and this information was considered for calculations in this paper.

Yield determination and N plant concentration

To determine the barley forage yield, the central part of the experimental plot was harvested with a 6-disc grooming mower (2.25 m width). The mowed area was 68.4 m^2 (30.4 m × 2.25 m) per plot. Forage sorghum was harvested with a conventional reaping-chopping machine (5 m width) together with a self-weighing trailer (built-in scale). The mowed area was about 152 m^2 (30.4 m × 5 m). After both harvests (sorghum and barley) subsamples were taken to determine the dry matter (DM) amount in the laboratory. The subsamples were dried in a forced air oven at 60 °C for at least 48 h. Biomass N concentration was determined by NIR spectroscopy, using a previously calibrated 500 Analyser (Bran + Luebbe, Norderstedt, Germany). The N uptake was calculated in each plot by multiplying plant N concentration by DM at harvest.

Soil mineral nitrogen analysis

Soil NO_3^--N concentration was determined three times during each year; after the barley harvest (June), after the sorghum harvest (October) and at barley tillering stage before PS application (February). Two soil samples were taken at three depths: 0.0–0.3, 0.3–0.6 and 0.6–0.9 m in each plot with a sampling auger with a diameter of 2 cm (Eijkelkamp®). A single composite sample was obtained for each depth and plot by mixing the two soil samples. NO_3^--N was analysed after extracting the fresh sample in deionized water and followed by a filtration, using a colorimetry method (Kempers 1974) with a continuous flow autoanalyzer. Total soil NO_3^--N concentration from 0.0 to 0.9 m, was calculated by adding the NO_3^--N in the three sampled depths. Bulk density was measured using the metal cylinder method at the beginning of the experiment in order to calculate NO_3^--N (kg ha^{-1}). Soil NH_4^+-N was considered insignificant compared with nitrates in the experimental area (Villar-Mir et al. 2002; Berenguer et al. 2008) and it was not measured in this study.

N balance

The N balance was calculated at every growing season for each plot, combining the data of both crops. The net N mineralization (N_{min}) from the soil organic matter was estimated in the control plots (T0) according to Eq. 1 (Sexton et al. 1996). Nitrogen losses (nitrate leaching, ammonia losses and N from rainfall water) were not measured in the study so the chosen equation does not include those terms.

$$N_{min}\left(\text{kg N ha}^{-1}\right) = N_f + N_u - N_i \quad (1)$$

where N_f is post-harvest soil NO_3^--N; N_u is the plant *N* uptake; and N_i is pre-plant soil NO_3^- -N. A negative value of N_{min} was interpreted as N that could have been lost by the uncontrolled factors mentioned above (leaching, volatilization, etc.; Sexton et al. 1996).

Unrecovered N was estimated from the *N* balance for the fertilized plots (Berenguer et al. 2009; Cela et al. 2011) according to Eq. 2:

$$\text{Unrecovered N}\left(\text{kg N ha}^{-1}\right) = N_f + N_u - N_i - N_{min} - N_{fert} \quad (2)$$

where N_{fert} is the *N* applied by fertilization. A negative value for the unrecovered *N* represents the sum of leached NO_3^--N, N lost by ammonia volatilization and denitrification, applied organic *N*, immobilised NH_4^+-N and clay-fixed NH_4^+-N. Whereas a positive value is interpreted as surplus *N*.

Nitrogen efficiency

The following N-efficiency parameters (López-Bellido and López-Bellido 2001a) were calculated for each fertilized treatment in both crops:

(1) Apparent N recovery fraction (ANRF; %): (N uptake in fertilized plots—N uptake in unfertilized plots) / N applied to fertilized plots.
(2) Agronomic N efficiency (ANE; kg kg^{-1}): (DM yield in fertilized plots—DM yield in unfertilized plots) / N applied to fertilized plots.

Statistical analysis

A mixed-design analysis of variance model (ANOVA), considering the growing seasons as repeated measures, was carried out to evaluate the response of the variables measured to PS fertilization. In the mixed-design ANOVA model, the PS treatment was a between-subjects variable (a fixed effects factor), and the growing season was a within-subjects variable (a random effects factor). If the interaction between the growing seasons and the treatment was significant, separate analyses of each growing season were performed (one-way ANOVA). When significant differences between treatments were detected, a multiple comparison of means was performed according to the Duncan test with a 95% confidence interval. The variables DM yield, N uptake, ANRF and ANE were analysed by crop and the total cropping system (both crops). N concentration and residual soil NO_3^--N only was analysed by crops. In the case of unrecovered N was analysed considering the total period of both crops. Statistical analyses were performed using the IBM SPSS software for Windows, version 28.0 (IBM Corp., Armonk, NY, USA).

Results

Crop yield

Overall, fertilization treatment, growing season and its interaction affected significantly to DM yields of both barley and sorghum (Table 5). The 4 year average annual DM yield of the control treatment (0 N) (8.5 Mg ha^{-1}) was significantly lower than the yield in the rest of the fertilization treatments. The highest N treatment (T3) yielded a total (sorghum + barley) of 17.8 Mg DM ha^{-1} yr^{-1} on average, being significantly higher than the yields of the T1 and T2 treatments (14.6 and 16.2 Mg DM ha^{-1}, respectively) (Table 5). The growing season strongly affected the total annual yields. In the 2012/13 growing season the total annual yield for the T3 treatment was 25.2 Mg DM ha^{-1}, whereas in the 2015/16 growing season the same treatment yielded only 8.6 Mg DM ha^{-1} (Fig. 1). In the 2012/13 and 2015/16 growing seasons, barley yielded more DM than the sorghum (Fig. 1).

Barley DM yields were affected by the fertilization treatment, the growing season and the interaction between these two factors. The increase of the fertilization N rate significantly increased average barley yields, ranging from 2.5 Mg ha^{-1} in T0 to 8.7 Mg ha^{-1} in T3 (Table 5). In the 2012/13 growing season, barley yields were considerably higher than in the other 3 years of the study for all the treatments (Fig. 1).

Sorghum DM yields were affected by fertilization treatments. However, only significant differences were observed between the control (T0; 6.0 Mg DM

Table 5 Average effect of the nitrogen fertilization rates with pig slurry on, dry matter yield (DM), whole plant N content, N uptake, residual soil NO_3^- -N content, apparent N recovery fraction (ANRF), agronomic N efficiency (ANE) and unrecovered N for each crop and the sum of both in the whole of the 4 years of the experimental period (2012–2016)

Crop	Treat[α]	DM yield (Mg ha^{-1})	N concentration (%)	N uptake (kg ha^{-1})	Residual soil NO_3^--N (kg ha^{-1})	Unrecovered N (kg ha^{-1})	ANRF (%)	ANE (kg kg^{-1})
Barley	T0	2.5d	0.99	23d	30c	–	–	–
	T1	5.3c	0.96	51c	36b	–	–	–
	T2	6.9b	1.01	71b	38ab	–	57.7b	52.3b
	T3	8.7a	1.08	99a	51a	–	90.0a	71.9a
Mean		*5.8*	*1.01*	*61*	*39*	–	*73. 8*	*62.1*
Treatment (T)		***	NS	***	*	–	*	*
Growing season (GS)		***	*	***	***	–	*	*
T x GS		**	NS	*	*	–	NS	NS
Sorghum	T0	6.0b	0.97c	55c	34c	–	–	–
	T1	9.3a	1.34b	111b	85b	–	36.2	22.5a
	T2	9.3a	1.39b	115ab	89b	–	39.9	22.2a
	T3	9.2a	1.53a	128a	155a	–	31.5	13.7b
Mean		*8.5*	*1.31*	*102*	*91*	–	*35.9*	*18,5*
Treatment (T)		***	***	***	***	–	NS	*
Growing season (GS)		***	***	***	***	–	***	***
T x GS		**	NS	*	*	–	NS	NS
Barley + Sorghum	T0	8.5d	–	77d	–	–	–	–
	T1	14.6c	–	163c	–	−92b	55.8	40.1a
	T2	16.2b	–	186b	–	−156a	46.7	33.4b
	T3	17.8a	–	227a	–	−238a	46.1	28.9b
Mean		*14.3*	–	*163*	–	*−162*	*49.5*	*34.4*
Treatment (T)		***	–	***	–	*	NS	*
Growing season (GS)		***	–	***	–	NS	***	***
T x GS		**	–	**	–	NS	NS	*

[α] The acronyms of the treatments are described in Table 2

Absence of value (-) indicates that there was no fertilization in that period

NS: not significant. *, ** and ***significant for $P<0.05$, $P<0.01$ and $P<0.001$, respectively

Within each variable and for each crop, the different letters indicate significant differences between fertilization treatments according to the Duncan test for $P<0.05$

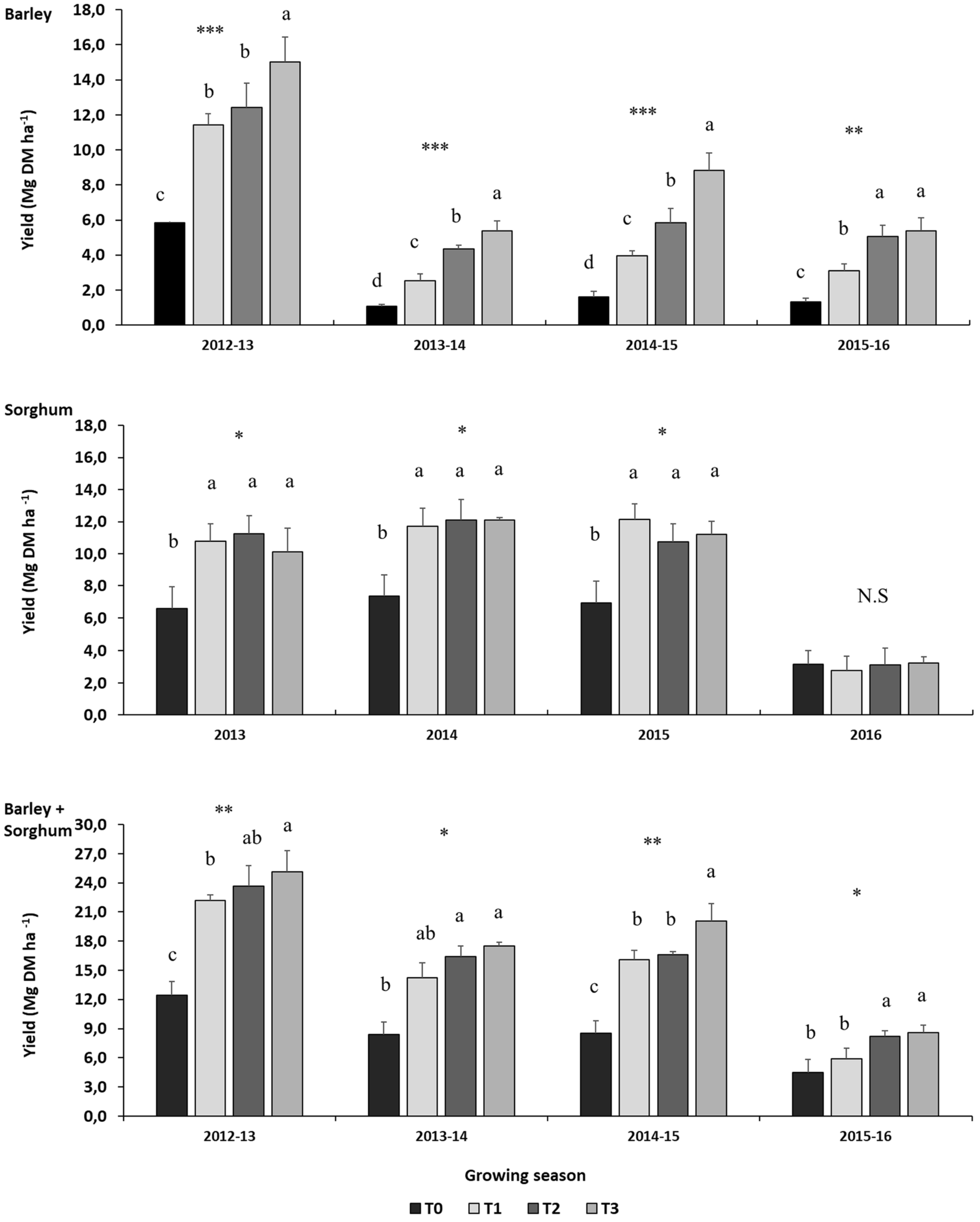
Italics indicate the mean of the four treatments

ha^{-1}) and the rest of fertilization treatments T1, T2 and T3 (9.3, 9.3 and 9.2 Mg DM ha^{-1}, respectively) (Table 5). The effect of the growing season and its interaction with the N fertilization were significant on DM yield. Sorghum DM yields in 2016 were lower than all the other growing seasons, and no differences were found among N treatments (Fig. 1).

Plant N concentration and N uptake

In barley no significant differences were found between the different N fertilization treatments, however in sorghum, there were significant differences between treatments, except in T1 and T2 (Table 5).

Overall, the plant N concentration in barley was more stable than the N concentration in sorghum (Table 6). In the 4 year experiment, average barley N concentration varied among years from 0.89 to


Barley
Yield (Mg DM ha-1)
18,0
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
***
c b b a
2012-13
***
d c b a
2013-14
***
d c b a
2014-15
**
c b a a
2015-16
Sorghum
Yield (Mg DM ha -1)
18,0
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
*
b a a a
2013
*
b a a a
2014
*
b a a a
2015
N.S
2016
Barley + Sorghum
Yield (Mg DM ha -1)
30,0
27,0
24,0
21,0
18,0
15,0
12,0
9,0
6,0
3,0
0,0
**
c b ab a
2012-13
*
b ab a a
2013-14
**
c b b a
2014-15
*
b b a a
2015-16
Growing season
■ T0 □ T1 ■ T2 ■ T3

◂**Fig. 1** Yield during the experiment from 2012 to 2016 for different PS fertilization rates. T0, T1, T2 and T3 correspond to control treatment, 170, 250, and 330 kg N ha^{-1} yr^{-1} rate of N in form of pig slurry treatments, respectively. Error bars indicate standard deviation of the mean. Within growing seasons, different letters indicate significant differences between treatments according to Duncan´s multiple range test ($p < 0.05$). NS: not significant. *, ** and ***significant for $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

1.07%, whereas sorghum N concentration varied from 0.89 to 1.88% (Table 6).

The total annual N uptake increased with higher N fertilization rates (Table 5). The effect of the growing season was significant as well as the interaction between growing season and fertilization treatment. The pattern of differences between N treatments was similar to that commented above for the total DM yield of both crops. The average N uptake in T3 (227 kg N ha^{-1}) was significantly higher than the rest of treatments (Table 5).

The average N uptake of the barley was affected by the fertilization treatment and growing season, as well as their interaction (Table 5). The N uptake varied significantly among treatments, with a gradual increase from the control (T0) to the high N rate (T3) (Table 5). These significant differences between treatments were observed in all growing seasons of the experiment (Table 6). The maximum N uptake of the barley was 202 kg ha^{-1} (T3, 2012/13 growing season) and the minimum 9 kg ha^{-1} (T0, 2013/14).

In sorghum, the N fertilization treatment significantly affected the N uptake (Table 5), except in 2016, where no differences between treatments were found (Table 6). The N uptake in the control (T0; 55 kg N ha^{-1}) was significantly lower than the rest of treatments. The average N uptake of T1, T2 and T3 were 111, 115 and 128 kg N ha^{-1}, respectively, being only significantly different T1 respect to T3 (Table 5).

Soil NO_3^--N concentration.

The fertilizer rate significantly affected the soil NO_3^--N (Table 5). The residual soil NO_3^--N concentration was variable throughout the 4 year experimental period (Fig. 2). The highest residual soil NO_3^--N concentrations were found every year in October, after the sorghum harvest. Up to 487 kg NO_3^--N ha^{-1} were calculated for the T3 treatment in the entire depth profile (0.0–0.9 m) (Fig. 2). However, except for 2012, the residual soil NO_3^--N levels were always below 240 kg NO_3^--N ha^{-1} considering the whole profile (0.0–0.9 m depth; Fig. 2). Overall, soil NO_3^--N concentration was higher in T3 compared to the other treatments, but these differences were smaller in February (Fig. 2).

Unrecovered N and N efficiency

The unrecovered N was affected by the fertilization treatment (Table 5). The unrecovered N of the double-annual cropping system in T3 (238 kg N ha^{-1}) was significantly higher than T1 (92 kg N ha^{-1}, respectively).

The average ANRF for the double-annual cropping system (49.5%) and for the sorghum (35.9%) did not vary significantly among the different fertilization treatments (Table 5). However, there were significant differences between the ANRF in the barley N treatments (57.7% in T2 and 90.0% in T3). The growing season significantly affected the ANRF of barley, sorghum and barley + sorghum.

The ANE of the barley, sorghum and barley + sorghum, was affected by the fertilization treatment and the growing season (Table 5). Overall, barley showed higher ANE than sorghum, 62.1 kg kg^{-1} compared to 18.5 kg kg^{-1}.

Discussion

Crop yield, N concentration and N uptake

Barley yield responded to N fertilization (Pardo et al. 2009; López-Bellido et al. 2001b), and to the residual N of the sorghum. Even though the N rate applied to barley was the same in the treatments T0-T1 and T2-T3 (Table 2), barley yields were significantly different among all the treatments, except for the last year of the experiment (Fig. 1). The DM yield differences between the barley treatments that received the same rate of N showed the importance of the effect of the residual N from the fertilizer applications to sorghum in the following crop (Maresma et al. 2019a; Salmerón et al. 2011). For instance, despite barley received 80 kg N ha^{-1} in T2 and T3, the barley yields in T3 were 23% higher than in T2. This fact

Table 6 N concentration and N uptake for each crop and the sum of both, in the different N rates tested during the 4 years of the experiment

Crop	Treatment[α]	N concentration(%)				N uptake (kg ha $^{-1}$)			
		2012/13	2013/14	2014/15	2015/16	2012/13	2013/14	2014/15	2015/16
Barley	T0	0.83	0.83	1.12	1.17	49c	9b	18c	16c
	T1	0.97	0.79	0.99	1.10	111b	20b	39b	34b
	T2	1.09	0.96	0.97	1.01	136b	41a	57b	51a
	T3	1.34	0.97	1.00	0.99	202a	53a	88a	53a
	Mean	*1.06*	*0.89*	*1.02*	*1.07*	*124*	*31*	*51*	*39*
	Significance	–	–	–	–	***	**	**	***
		2013	2014	2015	2016	2013	2014	2015	2016
Sorghum	T0	0.63	0.67	1.25	1.32	41c	49c	86b	42
	T1	0.84	0.88	1.63	2.01	90b	103b	197a	55
	T2	0.91	1.08	1.55	2.02	101ab	130a	166ab	62
	T3	1.20	1.05	1.72	2.14	124a	127a	192a	69
	Mean	*0.89*	*0.92*	*1.53*	*1.88*	*89*	*102*	*160*	*57*
	Significance	–	–	–	–	**	**	*	NS
		2012/13	2013/14	2014/15	2015/16	2012/13	2013/14	2014/15	2015/16
Barley + Sorghum	T0	–	–	–	–	90c	58c	104c	57c
	T1	–	–	–	–	201b	123b	236b	89b
	T2	–	–	–	–	237b	171a	223b	114a
	T3	–	–	–	–	326a	180a	281a	122a
	Mean	–	–	–	–	*213*	*133*	*211*	*96*
	Significance	–	–	–	–	***	***	***	***

[α] The acronyms of the treatments are described in Table 2

NS: not significant.*, ** and ***significant for $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

Within each variable and for each crop, the different letters indicate significant differences between fertilization treatments according to the Duncan test for $P < 0.05$

Italics indicate the mean of the four treatments

was similarly observed in the previous 6 year study by Ovejero et al. (2016).

Barley yields were probably affected by the rainfall (water availability) during the growing season. Low precipitations during the growing period (November–May), and particularly in May, during the grain filling, had an effect in DM yields (Fig. 1). However, despite the effect of the growing season, the tendency of increasing yields with N application at sidedress was maintained (Fig. 1). The yield differences among the fertilizer treatments could be attributed partially to the tillering ability of barley. The higher N treatments afforded better conditions for tiller development in early stages (Alzueta et al. 2012), which were translated into higher yields at the harvest time (Fig. 1).

In barley plant N concentrations, no significant differences were found between the different N fertilization treatments. This fact was probably due to the barley harvest (Table 3) occurred at practically the same growing stage each year, with only a few days of difference among the 4 years considered. Maresma et al. (2019a) reported N concentrations in barley biomass similar to our study (1.10–1.39 mg kg^{-1}), with no effect of the fertilization treatment. Therefore, the barley N uptake of each treatment was mostly determined by the DM yields.

The average sorghum yields throughout the experiment were similar in each of the growing seasons except for 2016. In the last growing season, the precipitation during June, July and August, was considerably lower (133 mm) than the historical precipitation in the area (212 mm) and the DM

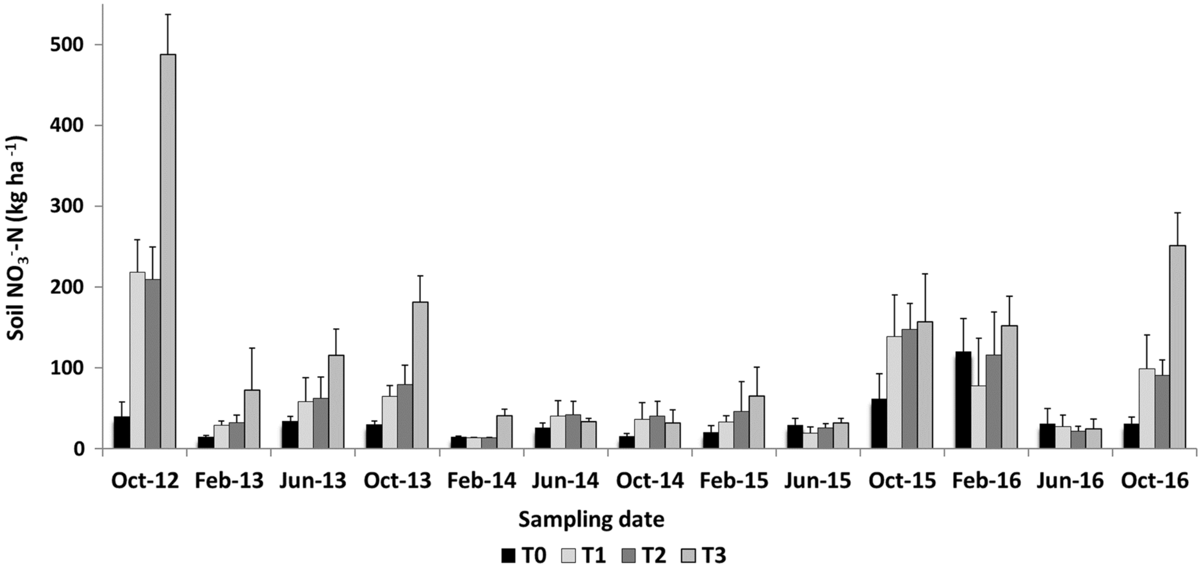


Fig. 2 Soil NO_3^--N concentration (0–0.9 m) in different treatments over the period of 4 years. T0, T1, T2 and T3 correspond to control treatment, 170, 250, and 330 kg N ha^{-1} yr^{-1} rate of N in form of pig slurry treatments, respectively. Error bars indicate standard deviation of the mean

yields were negatively affected. The yields of the other 3 years of the experiment showed that precipitations of 385 mm were enough to achieve maximum sorghum yields, and that higher precipitations (> 500 mm; such as 2014) did not increase the sorghum yield. Therefore, it could be considered that 385 mm of water could be the response threshold, beyond which sorghum was non-responsive to extra precipitation in the conditions of the study. In contrast, previous research in same conditions has shown that maize yield is more dependent on summer precipitation and sowing date (Maresma et al. 2019b). Ovejero et al. (2016) showed considerably DM yields in maize (14.8 Mg DM ha^{-1}) with high precipitations in summer (500 mm), suggesting that, in contrast to sorghum, maize is in fact responsive to extra precipitation under the same conditions.

The maximum sorghum DM yield in the 4 year study was 11.4 Mg DM ha^{-1}, which is higher than the 8.2 Mg DM ha^{-1} obtained by Perramón et al. (2016) in a double crop with oats in an area close and similar the region of our study. However, González-García et al. (2016), in different double-annual cropping system study carried out in the same region of our study, reported sorghum yields of 12.7 Mg DM ha^{-1}.

The N concentration in sorghum was lower than in barley (Table 6). The stage of maturity of the crop at harvest is one of the most important factors for the N levels in crops. While barley was always harvested in same maturity stage, sorghum maturity at harvest was more irregular (Table 3). Indeed, sorghum has been reported as a crop with high variation of N concentration depending on the stage of maturity at harvest (Atis et al. 2012; Lyons et al. 2019b).

Sorghum absorbed more N than the barley except for the first year of the study, when the residual N prior to barley sowing was high (Fig. 2). Even in 2016, when sorghum DM yields were lower than the barley yields, the total N uptake of sorghum was higher due to its higher concentration of N in the plant.

Previous research has reported the high N efficiency of double-annual cropping systems using mineral N fertilizers (Maresma et al. 2019a), or livestock slurry (Trindade et al. 2009). In this sense, Perramon et al. (2016) concluded that in double-annual cropping systems the application of rates higher than those allowed by the Nitrates Directive could be feasible for improving yields while maintaining a low risk of environmental pollution.

Overall, the application of the maximum allowed N rate by the NVZ reduced the total annual DM yields by 18% compared to the T3 (330 kg N ha^{-1} yr^{-1}) in our study. The highest average yield in the double-annual cropping system was achieved with the T3 treatment (17.8 Mg DM ha^{-1}), and the total N uptake was 227 kg N ha^{-1}, with an annual N application of 330 kg ha^{-1}. The N application exceeded the crop demands in the T3 and some N losses to the environment are expected (Miguez 2005).

Considering the results of each crop, in sorghum, there were no significant differences among T1, T2 and T3 fertilizer treatments. In contrast, barley yields showed a tendency of increased yields with higher N rates (with statistical differences among treatments). PS fertilization applied before sorghum sowing could have led an important residual effect on the subsequent barley. Consequently, these results suggest that the distribution of the N between crops could be improved by applying a higher N rate to barley and reducing the N rate to the sorghum. This fact could avoid over-fertilization in sorghum and incomplete fertilization in barley, and consequently, increase the efficiency of crop N uptake and reducing the risk of NO_3^-- N lixiviation. In this way, the high variability of precipitation among years makes the determination of an optimum N rate for the studied double-annual cropping system difficult.

Soil NO_3^--N concentration

At the beginning of the experiment (October 2012), soil NO_3^--N concentrations were the highest observed in the study (~500 kg ha^{-1} in T3), representing a high risk of N leaching if heavy rain events occur (Liu et al. 2003; Nevens and Reheul, 2005; Brye et al. 2003; Martínez et al. 2017b). The soil NO_3^--N levels were higher because there were no N extractions from the previous crop (maize) due to a lack of precipitation during its growing season that resulted in low germination and a failed harvest (Ovejero et al. 2016).

The higher application of N in the summer crop (Table 2) together with the higher mineralization of the organic matter during the summer period (Magdoff et al. 1984; Qiu et al. 2012; Yagüe and Quílez 2015) produced higher accumulation of soil NO_3^--N after the sorghum harvest than after the barley harvest (Fig. 2). The soil NO_3^--N levels after sorghum harvest were reduced below 100 kg N ha^{-1} in February (Fig. 2). This fact can either be explained by N losses throughout the winter or by crop N absorption during the early stages of development.

The total amount of N applied to the cropping system affected the residual soil NO_3^--N. Higher soil NO_3^--N concentrations were found after sorghum and when higher N rates were applied. This strengthens the discussion that the distribution of the N fertilizer could be improved by applying higher percentage of the total fertilizer to the barley.

Previous research in N fertilization strategies for double-annual cropping systems under irrigated conditions have proved the importance of applying higher N rates to the summer crops (Maresma et al. 2019a; Iguácel et al. 2010; Yagüe and Quílez, 2013). However, the uncertain precipitation during the growing period of the summer crop in our conditions reduces the reliability of the summer harvest. Therefore, and from a management standpoint, more importance and attention should be given to the yield of the winter crop.

Unrecovered N and N efficiency

The unrecovered N of treatments T2 and T3, which exceed the legal maximum N rate allowed by the Nitrates Directive in ZVN (EEC 1991), increased the risk of N losses to the environment by leaching of NO_3^--N to groundwater or by volatilization of ammonia. However, part of the unrecovered N could be immobilized and fixed NH_4^+-N in the clays, being in part available for the next crop of the rotation in double cropping systems in sub-humid Mediterranean conditions (Schröder 2005; Hartmann et al. 2014). The unrecovered N was affected by the precipitation during the growing seasons, especially in summer. The lack of precipitation affects crop growth, and consequently, crop yield. For instance, the low yields achieved in 2016 were translated into high unrecovered N.

The N efficiencies decreased as the N rate increased, agreeing with the trend reported by Fageria and Baligar (2005) for cereal crops. Indeed, double-annual cropping has been reported to improve the N use efficiency compared to an annual cropping system (Zavattaro et al. 2012) and increase the yield (Borrelli et al. 2014). In our study, higher N efficiencies were determined for barley as compared to sorghum (Table 5), probably owing to the presence of higher residual NO_3^--N in the soil after the sorghum harvest and the lower N fertilizer applications in barley.

Moreover, the high N use efficiency of the barley could have been favoured by the sidedress application of the fertilizer in a period of low temperatures (February). Sidedressing has been shown to reduce N losses compared to pre-sowing fertilizer application on bare soil (Sieling et al. 2014). Our study showed the potential of the double-annual cropping system to increase yields up to a 11% and a 18% with N rates above the Nitrates Directive (250 and 330 kg N ha^{-1} $year^{-1}$, respectively) (Table 5). However, N rates above the legal limit (170 kg N ha^{-1} $year^{-1}$) almost doubled and tripled the unrecovered N after each growing season (156 and 238 kg N ha^{-1} $year^{-1}$, for T2 and T3, respectively) (Table 5).

Conclusions

In this 4 year experiment of a barley-sorghum double-annual cropping system, DM yields were very variable depending on the precipitation during the growing season of each crop.

The N application in form of pig slurry at rates higher than those legally permitted by the Nitrates Directive slightly increased DM yields of the double-annual cropping system. When analysing each crop of the rotation, in barley DM yields varied significantly between treatments depending on the dose of fertilizer applied. In contrast, in sorghum, the productions were similar throughout the experiment, and the yield did not vary significantly between the different N treatments applied.

PS fertilization applied before sorghum sowing had an important effect on DM yield on the subsequent barley due to a residual effect. In this way, residual soil N was higher after the sorghum harvest than after the barley harvest, especially in dry summer periods where the sorghum yielded poorly.

Unrecovered N after each growing season was considerably higher with N rates above the legal limit (170 kg N ha^{-1} $year^{-1}$) and may lead to increase the risk of N losses to the environment by leaching of NO_3^--N to groundwater.

In view of these results, to avoid part of these N losses, it is suggested a reduction in the dose of N fertilization in the summer crop, applying higher percentage of the total fertilizer to the winter crop.

Further studies are necessary to establish optimum N fertilization rates from livestock manures, taking into account the potential N residual effects, N use efficiency and N mineralization/immobilization.

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Data availability The datasets analysed during the current study are available from the corresponding author on reasonable request.

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