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ORIGINAL PAPER

Liquid Cattle Manure Effect on Corn Yield and Nutrients' Uptake and Soil Fertility, in Comparison to the Common and Recommended Inorganic Fertilization

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Abstract
Liquid dairy cattle (*Bos taurus*) manure effect on corn (*Zea mays* L.) yield and nutrients' uptake and soil fertility were studied, in comparison to the crop's common and recommended inorganic fertilization, by means of a 5-year field experiment. The treatments applied each year in the same plots were: (i) manure, (ii) common inorganic fertilization, (iii) recommended inorganic fertilization, and (iv) no fertilization. Each year from each plot, surface soil samples were collected before sowing, corn above-ground biomass was collected at silage, they were analyzed, and grain yield was determined at harvest. Upon all kinds of fertilization, corn silage yield increased in comparison to control and ranged between 51 and 194, 50 and 190, and 39 and 189% for the manure, recommended, and common inorganic fertilization treatment, respectively. Similarly, grain yield and the macro- and micronutrients' plant uptake were increased. Soil fertility improved regarding the NO_3-N, which upon organic or inorganic fertilization increased 10–46% in comparison to the control. Manure application significantly increased K by 32–81%. However, in the case of P, an excessive increase was observed, which was two to three times higher than the inorganic fertilization (30–44 mg kg^{-1}). Consequently, repeated annual applications of liquid cattle manure to soil can enhance crop yield, nutrients' uptake, and soil fertility, at levels higher or similar to the common or recommended inorganic fertilization for the crop. However, the possibility of P build up should also be considered.

Keywords Corn · Inorganic fertilization · Liquid cattle manure · Plant essential nutrients

1 Introduction

The application of organic fertilizers instead of inorganic fertilizers is one of the environmental long-term approaches to sustainable agriculture and at the same time, it is economically feasible (Wang et al. 2018). Animals produce significant amounts of wastes, as animal breeding is highly developed in Greece (Vlyssides et al. 2015). The Greek livestock system consists of cows, calves, sheep, goats, swine, and pullets breeding (Skoulou and Zabaniotou 2007; Vlyssides et al. 2015). Based on the FAO statistical data, intensive livestock produces a substantial amount of manure in Greece, which from 1996 to 2013 produced approximately 80,000 Mg of N, and almost 35% of it came from cattle breeding (FAO 2019). At the same time, non-renewable resources like phosphate rocks, which are used in the industry of P fertilizers, are expected to become scarce in the future (Gasparatos et al. 2019; Li and Marschner 2019). Furthermore, the mining processes disturb the environment (Golroudbary et al. 2019; Grigatti et al. 2015).

In general, returning organic matter to soil plays a key role in waste management and at the same time, as wastes have significant amounts of essential macro- and micronutrients for plants, they represent a nutrient source for crops, with the ultimate goal of nutrient cycle closure (Grigatti et al. 2015; Schlegel et al. 2017; Yang et al. 2016). Application of manure to agricultural fields has several benefits like the improvement of soil chemical, physical, and biological properties (Martinez et al. 2017). However, attention should be drawn to the

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amounts of nutrients added, because application may exceed crops' needs (Schlegel et al. 2017). Environmental impacts like water pollution and eutrophication by NO_3 leaching and P run off, NH_3 emissions, etc. should be taken under consideration (Schlegel et al. 2017; Yang et al. 2016). Furthermore, another issue that should be taken into account is heavy metals' accumulation to soil and their possible entrance to the food chain through agricultural products (Guan et al. 2018; Zhao et al. 2014).

One of the main reasons that prevent farmers from using organic fertilizers is their slow and variable effects on crops' yield in short time (Wang et al. 2018). Nevertheless, animal manures are an appropriate N source due to their ability to supply crops with readily available inorganic N, but also because they contain organic N, which affects crops and soils in the long term (Cavalli et al. 2017; Zhang et al. 2018). Nitrogen efficiency is affected by the liquid or the solid fraction of the manure, with liquid slurries having high N efficiency due to their low dry matter content, low value of C/N, and high inorganic N content (Cavalli et al. 2017; Marchezan et al. 2020; Marzi et al. 2020). Therefore, research on the soil application of animal manure should provide information about the change of soil chemical properties and fertility and the studies should be performed in medium to long-term periods (Marchezan et al. 2020; Martinez et al. 2017; Schlegel et al. 2017).

Corn is extensively cultivated in Europe and especially in Greece more than 200,000 ha year^{-1} were cultivated from 2009 to 2013 and the average grain yield was above 10 Mg ha^{-1} year^{-1} (FAO 2019). An appropriate management of fertilization has the vital purpose of reducing undesirable environmental impacts. As reported by Halvorson et al. (2005), N from soils as well as from organic or inorganic fertilizers is not used efficiently in most crop production systems. Moreover, N budget must be calculated, resulting in a better N management in agriculture and in crop production system particularly, and soil tests need to be performed, so that fertilizer recommendations are adjusted to obtain the best economic and environmental results (Halvorson et al. 2005). The production of corn and dairy cattle are two economically important activities, often related in many agricultural lands and variable findings about beneficial or no effect on corn yield, after medium- to long- or short-term application of cattle manure to soil, respectively, are reported in the literature (Martinez et al. 2017).

We hypothesized that in terms of corn productivity and soil fertility, liquid cattle manure could have a similar effect on the common inorganic fertilization used by the farmers in the area and on the recommended inorganic fertilization according to the results of pre-plant soil analysis, conducted in the beginning of corn growing season each year. In addition, a continuous 5-year field application of liquid cattle manure on irrigated maize could provide information about environmental risks that may arise. In view of the above, the objectives of the study were to: (i) investigate the effects of liquid dairy cattle manure on corn yield and nutrients' content and uptake, in comparison to the common and recommended inorganic fertilization for corn, by means of a 5-year field experiment and (ii) evaluate the effect of manure application on soil available macro- and micronutrients mainly, but also on soil chemical properties. The field experiment of the present study was a continuation of a similar experiment since 1996.

2 Materials and Methods

2.1 Details of the Present Experiment and the Background of the Experimental Field

The experiment of the current study was conducted in a field of the Farm of Aristotle University of Thessaloniki, Greece, during the years of 2009–2013. The site of the experimental field was located at 22° 59′ 6.17″ E, 40° 32′ 9.32″ N and the soil was a calcareous loam ($pH = 8.3 \pm 0.1$), classified as Typic Xerorthent (Matsi et al. 2003). Monthly total rainfall and mean temperature during the years of 2009–2013 were recorded and are presented in Table 1. In addition, the 30-year average rainfall and temperature for the region, where the experiment was established, were 437 mm and 15.4 °C, respectively. The size of the experimental plots was 44.8 m^2 with a 2-m buffering zone and the experimental design was the randomized complete block design (RCBD) with four fertilization treatments replicated six times.

The treatments, which were established before sowing each year in the same experimental plots, were the following: (i) liquid cattle manure (manure), (ii) inorganic N-P fertilization applied at rate common for the farmers in the area (common IF), (iii) inorganic N-P-K fertilization applied according to pre-planting soil analysis (recommended IF), and (iv) unfertilized soil (control).

Liquid dairy cattle manure consisted of excrement plus urine, bedding material, and cleaning water. Manure was applied at the rate of 80 Mg ha^{-1} year^{-1} (wet weight basis) and provided 250 kg N ha^{-1} year^{-1}, 55 kg P ha^{-1} year^{-1}, and 200 kg K ha^{-1} year^{-1}. Manure's application rate was based on its Kjeldahl-N content and its N application rate was similar to that of the common IF, according to the results of analyses conducted previous years of experimentation. Those analyses showed that manure was alkaline in reaction ($pH = 7.8 \pm 0.1$) and its dry and organic matter content and total amounts of the macronutrients N, P, K and of Cu, Zn, and B from the micronutrients were almost constant (Table 2) (Matsi et al. 2003, 2015). Manure was applied at the surface of the plots and then, it was incorporated into the soil after it has been dried, commonly 2 to 3 days after its application.

Table 1 Monthly total rainfall and mean temperature, during the years of 2009–2013

Month	Total monthly rainfall (mm)				
	2009	2010	2011	2012	2013
January	79.4	9.2	13.8	35.8	23.1
February	2.5	94.0	20.2	8.4	97.2
March	43.4	32.4	30.6	28.4	27.4
April	18.2	19.4	22.2	52.6	11.0
May	18.6	49.4	128.6	59.6	7.4
June	48.4	55.2	28.0	7.4	16.2
July	6.0	31.4	2.4	0.0	70.2
August	74.0	4.4	13.0	4.6	11.8
September	37.6	12.0	46.8	31.0	25.4
October	22.0	154.4	26.6	26.7	7.4
November	26.8	15.2	12.4	42.2	23.2
December	42.2	13.6	22.2	48.0	32.8
Total	419.1	490.6	366.8	344.7	355.1
	Mean monthly temperature (°C)				
	2009	2010	2011	2012	2013
January	14.1	6.2	6.3	3.8	6.9
February	6.2	8.8	7.2	6.2	8.9
March	10.1	10.3	9.6	11.2	11.0
April	14.0	15.0	13.3	15.1	15.3
May	20.4	19.9	18.2	19.7	22.0
June	23.9	23.9	23.8	26.6	24.8
July	27.5	26.8	27.6	29.7	27.0
August	26.0	28.8	26.6	28.0	27.7
September	21.6	22.6	23.9	23.5	22.8
October	17.4	15.0	14.7	19.4	17.1
November	12.4	14.9	8.6	13.9	13.8
December	10.4	8.1	7.6	5.9	6.4

According to the common farming practice in the area, inorganic N-P fertilizers in the common IF treatment supplied 260 kg N ha^{-1} $year^{-1}$ and 57 kg P ha^{-1} $year^{-1}$. Due to high content of illite in the soils of the area, K was not applied. Depending on the results of pre-plant soil analysis every year, N-P-K fertilizers in the recommended IF treatment applied at the ranges of 210–240 kg N ha^{-1} $year^{-1}$, 24–25 kg P ha^{-1} $year^{-1}$ the first two years and 0 kg P ha^{-1} $year^{-1}$ the last three years of experimentation and 152–208 kg K ha^{-1} $year^{-1}$. The calculation of the amounts of the three macronutrients to be added in the particular treatment was conducted by means of a software suitable for fertilization recommendation (in Greek) and it was based on the results of soil analysis, in respect to the available concentrations of the three macronutrients and certain general soil properties (i.e., pH, particle size distribution, organic matter content) and on crop's demand. More specific, the N-P-K sufficiency critical levels used for the fertilization recommendations were 10–19 mg kg^{-1} for NO_3-N extractable with KCl, 10–15 mg kg^{-1} for Olsen-P, and 150–200 mg kg^{-1} for K extractable with CH_3COONH_4. The inorganic N-P-K fertilizers used in the experiment were ammonium nitrate (33.5-0-0), super phosphate (0-20-0), and potassium phosphate (0-0-50). All fertilizers, organic and inorganic, were applied before corn sowing.

The field of the current study had been used in a similar fertilization experiment with liquid cattle manure since 1996. Initially (1996–2000), the field was cultivated with winter wheat (*Triticum aestivum* L.) (Matsi et al. 2003), then was left to fallow for 1 year (2001) and subsequently was cultivated with corn (2002–2008) (Lithourgidis et al. 2007; Matsi et al. 2015). During the corn period, all treatments except the third one (which was application of the common IF with split application of N) were identical to the aforementioned for the current experiment.

2.2 Soil and Plant Sampling and Analysis of the Current Study

Composite surface (0–30 cm) soil samples, consisted of three sub-samples each, were collected from each experimental plot before corn sowing, in May, every year, during the period of 2009–2013. The soil samples were air-dried, ground to pass a 2-mm sieve and analyzed for certain chemical properties and available macro- and micronutrients in two replications.

Table 2 Composition of the liquid dairy cattle manure, expressed on wet weight basis

Dry matter	Organic matter	Kjeldahl-N	NH_4-N	P	K
(g kg^{-1})					
80[†]±3[‡]	56 ± 1	3.1 ± 0.1	1.3 ± 0.1	0.68 ± 0.02	2.5 ± 0.1
Cu	Zn	Fe	Mn	B	
(mg kg^{-1})					
8.8 ± 0.4	19 ± 1	99 ± 12	2.3 ± 0.3	5.3 ± 0.4	

Average values of the years 1996 and 1997 reported by Matsi et al. (2003)

[†] Mean

[‡] Standard deviation ($n = 6$)

Specifically, pH was determined in soil suspension with water (1:2 w/v soil to water ratio), electrical conductivity was determined in the saturation extract (EC_{se}), total N was determined by the Kjeldahl method (Bremner 1996), and organic C was determined by the wet oxidation method (Walkley and Black 1934). Moreover, NO_3-N extractable with KCl (Mulvaney 1996), Olsen-P (Kuo 1996), K extractable with CH_3COONH_4 (Thomas 1982), Cu, Zn, Fe, and Mn extractable with DTPA (Lindsay and Norvell 1978), and hot water extractable B were determined (Keren 1996).

Corn aboveground biomass was collected at silage (R3 growth stage) each year and was dried at 65 °C and the silage yield was calculated. The plant samples were ground and analyzed in two replications for total N by the Kjeldahl method and after dry ashing (Mills and Benton-Jones 1996) for P by the ascorbic acid method (Kuo 1996), K by flame photometry, Cu, Zn, Fe, and Mn, by atomic absorption spectrometry, and B by the azomethine-H method (Keren 1996). In addition, nutrients' plant uptake was calculated at silage and grain yield was determined at harvest (R6 growth stage), adjusting the grain moisture to 15%.

For the aboveground biomass collected at the R3 growth stage, the apparent N recovery (ANR) for all fertilization treatments and the mineral fertilizer equivalent (MFE) of manure were estimated, using the equations:

$$\text{ANR }(\%) = 100 \times \left[\text{NU}_{+\text{N}}\ \left(\text{kg ha}^{-1}\right) - \text{NU}_{-\text{N}}\ \left(\text{kg ha}^{-1}\right)\right] / \left[\text{N applied}\ \left(\text{kg ha}^{-1}\right)\right]$$

$$\text{MFE }(\%) = 100 \times \text{ANR}_{\text{M}} / \text{ANR}_{\text{IF}}$$

where NU_{+N} is the N uptake by corn for organic or inorganic fertilization treatment, NU_{-N} is the N uptake by corn for the control treatment, "N applied" is the total amount of N applied to soil by manure or inorganic fertilizer, and ANR_M and ANR_{IF} are the apparent N recoveries of manure and each of the inorganic fertilizer (common and recommended IF) treatments.

2.3 Statistical Analysis

For each parameter, repeated measures analysis of variance (ANOVA) was conducted using the 5 years as repeated measures. The Bartlett's and Mauchly's tests were conducted to check for homogeneity of variances and covariances for each parameter and the protected least significant difference (LSD) test at $p = 0.05$ was used for the mean comparisons.

3 Results

3.1 Effect of Fertilizers' Application on Corn Yield and Nutrient Uptake

Corn silage (Fig. 1) and grain yield (Fig. 2) were affected by treatment at all years from 2009 to 2013. Both yields, which

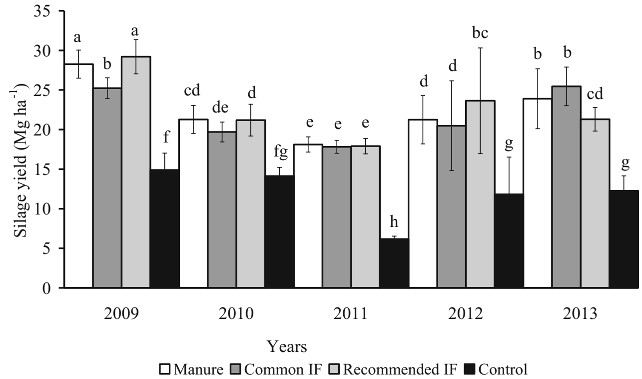

Fig. 1 Dry aboveground biomass yield of corn at the R3 growth stage (silage), the years of 2009–2013. Manure, liquid cattle manure; common IF, inorganic N-P fertilizer applied at a rate common for the farmers in the area; recommended IF, inorganic N-P-K fertilizer applied according to pre-planting soil analysis; control, unfertilized soil. Lines at the surface of bars represent the standard deviation ($n = 6$). Means indicated with different letters are statistically different using the LSD test, at $p \leq 0.05$

were obtained from the manure treatment, significantly increased in comparison to the control and ranged at levels similar to both inorganic fertilization treatments, common and recommended IF. Also, it is noteworthy that both inorganic fertilizations affected in a similar manner both silage and grain yield.

The concentrations of the macro- and micronutrients, in corn biomass at the R3 growth stage collected in the aforementioned years, were not significantly affected by the fertilization treatments and ranged for the macronutrients N-P-K at 7.1–10.4, 0.94–2.00, and 4.5–9.5 g kg^{-1}, respectively, and for the micronutrients Cu, Zn, Fe, Mn, and B at 2.0–5.6, 28.6–55.0, 26.6–73.0, 15.7–23.4, and 6.4–13.0 mg kg^{-1}, respectively (data not shown). However, macro- and micronutrients' uptake by corn significantly differentiated among fertilization treatments and increases were obtained upon manure or both

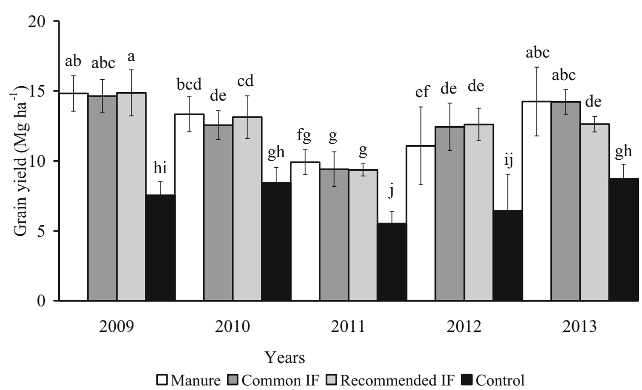

Fig. 2 Grain yield of corn at the R6 growth stage (harvest), the years of 2009–2013. Manure, liquid cattle manure; common IF, inorganic N-P fertilizer applied at a rate common for the farmers in the area; recommended IF, inorganic N-P-K fertilizer applied according to pre-planting soil analysis; control, unfertilized soil. Lines at the surface of bars represent the standard deviation ($n = 6$). Means indicated with different letters are statistically different using the LSD test, at $p \leq 0.05$

common and recommended IF treatments. This was attributed to the increase of corn silage yield at the particular treatments (Tables 3 and 4).

As shown in Table 5, in general, the ANR values of the manure treatment were at levels comparable to the inorganic fertilization. However, an increasing trend of the ANR values was evidenced for the manure and recommended IF treatments relative to the common IF treatment most of the years, whereas the opposite trend was evidenced for the last year of experimentation and this trend was significant in certain cases. The calculated values of MFE of the manure treatment in respect to both the inorganic fertilization treatments were close to 100% or even higher and the average value over the whole period was 112 ± 24%.

3.2 Effect of Fertilizers' Application on the Soil Properties

Although soil pH was significantly affected, it remained strongly alkaline and ranged from 7.9 to 8.5 through all years and treatments. The same stands for EC_{se}, which ranged from 0.35 to 0.97 dS cm^{-1}. As far as soil organic C and total N are concerned, a significant increase was observed upon manure's addition compared to the rest of the treatments, within each year, in only certain years and their respective concentration ranges in manure treated plots were 7.6–11.2 and 0.84–1.24 g kg^{-1} (data not shown).

Soil available NO_3-N, P, and K were significantly affected by the manure or inorganic fertilizers application each year of experimentation (Table 6). The soil available NO_3-N of the manure treatment was higher than the control and it was similar or higher than both inorganic fertilization treatments. Manure-treated plots had significantly higher concentrations of soil available P in comparison to the control and both inorganic fertilization treatments (Table 6). As far as the soil available K is concerned, a consistent increase of its concentration in the treatments of manure or recommended IF was observed through the years (Table 6). The soil available Cu, Zn, and B were significantly affected by treatment during the years and the application of liquid cattle manure resulted in increased concentrations in comparison to the control and both the inorganic fertilization treatments as well (Table 7). The soil available Fe and Mn showed no significant differences among all treatments through the years (Table 7).

4 Discussion

Taking into consideration the aforementioned results about the corn (Figs. 1 and 2) and macronutrients' uptake for the

Table 3 Macronutrients' uptake by corn plants at the R3 growth stage (silage), the years of 2009–2013

	Year				
Treatment	2009	2010	2011	2012	2013
	N (kg ha^{-1})				
Manure	291[†]±52[‡] ab*	200 ± 28 de	179 ± 22 ef	188 ± 30 ef	206 ± 15 de
Common IF	254 ± 35 bc	196 ± 17 e	132 ± 44 gh	186 ± 55 ef	235 ± 38 cd
Recommended IF	276 ± 25 ab	201 ± 32 de	156 ± 17 fg	191 ± 84 ef	172 ± 33 ef
Control	126 ± 30 ghi	100 ± 17 hi	45 ± 2 j	103 ± 66 hi	93 ± 28 i
	P (kg ha^{-1})				
Manure	27 ± 5 efgh	37 ± 6 bcd	30 ± 4 defg	43 ± 8 ab	44 ± 9 ab
Common IF	27 ± 4 efgh	31 ± 6 def	29 ± 1 efg	39 ± 14 abc	43 ± 5 ab
Recommended IF	30 ± 5 defg	32 ± 9 cdef	31 ± 3 def	46 ± 13 a	34 ± 8 cde
Control	18 ± 2 ij	25 ± 8 fghi	11 ± 2 j	23 ± 15 ghi	21 ± 7 hi
	K (kg ha^{-1})				
Manure	170 ± 43 bc	206 ± 79 ab	157 ± 11 cd	127 ± 26 defg	108 ± 20 efgh
Common IF	133 ± 11 de	94 ± 24 fghi	163 ± 20 cd	118 ± 49 efg	113 ± 6 efg
Recommended IF	236 ± 69 a	178 ± 39 bc	170 ± 28 bc	129 ± 29 def	100 ± 6 efgh
Control	91 ± 9 ghij	104 ± 38 efgh	55 ± 6 j	74 ± 60 hij	58 ± 12 ij

Manure, liquid cattle manure; common IF, inorganic N-P fertilizer applied at a rate common for the farmers in the area; recommended IF, inorganic N-P-K fertilizer applied according to pre-planting soil analysis; control, unfertilized soil

[†] Mean

[‡] Standard deviation ($n = 6$)

*Means followed by different letters, within the same parameter, are statistically different using the LSD test, at $p \leq 0.05$

Table 4 Micronutrients' uptake by corn plants at the R3 growth stage (silage), the years of 2009–2013

Treatment	Year 2009	2010	2011	2012	2013
	Cu (g ha^{-1})				
Manure	133[†]±44[‡] a*	92 ± 32 bcd	67 ± 7cdefgh	71 ± 51 cdef	100 ± 28 bc
Common IF	116 ± 51 ab	67 ± 31defgh	80 ± 25 cde	68 ± 48 defgh	70 ± 29 cdefg
Recommended IF	118 ± 33 ab	73 ± 20 cdef	65 ± 19 defgh	56 ± 30 efghi	69 ± 11 defg
Control	40 ± 11 ghi	28 ± 6 i	26 ± 4 i	44 ± 22 fghi	38 ± 6 hi
	Zn (g ha^{-1})				
Manure	898 ± 135 bcde	788 ± 164 cdefg	885 ± 215 bcde	872 ± 257 bcde	1338 ± 746 a
Common IF	927 ± 353 bcd	560 ± 83 fghi	838 ± 138 cdef	795 ± 242 cdefg	1270 ± 442 a
Recommended IF	929 ± 247 bcd	620 ± 114 defgh	959 ± 317 bc	824 ± 163 cdefg	1171 ± 372 ab
Control	520 ± 96ghi	611 ± 86 efgh	300 ± 70 i	471 ± 312 hi	560 ± 181 fghi
	Fe (g ha^{-1})				
Manure	754 ± 157 cde	835 ± 294 bcd	1052 ± 85 ab	804 ± 285 bcd	974 ± 512 abc
Common IF	873 ± 144 abc	770 ± 309 cde	1130 ± 339 a	964 ± 227 abc	806 ± 224 bcd
Recommended IF	1007 ± 88 abc	817 ± 240 bcd	902 ± 138 abc	1049 ± 302 ab	790 ± 193 bcd
Control	432 ± 78 f	571 ± 163 def	452 ± 118 f	514 ± 380 ef	347 ± 93 f
	Mn (g ha^{-1})				
Manure	510 ± 218 abcd	476 ± 64 abcde	285 ± 65 gh	430 ± 196 bcdef	524 ± 141 abc
Common IF	578 ± 321 a	379 ± 88 defgh	322f ± 57 gh	412 ± 193 cdef	594 ± 109 a
Recommended IF	554 ± 237 ab	433 ± 99 bcdef	331 ± 136 fgh	366 ± 64 efgh	492 ± 102 abcde
Control	243 ± 36 hi	270 ± 53 h	128 ± 24 i	259 ± 195 hi	244 ± 27 hi
	B (g ha^{-1})				
Manure	229 ± 82 ab	270 ± 66 a	136 ± 35 efg	196 ± 60 bcd	205 ± 45 bcd
Common IF	175 ± 59 cdef	203 ± 36 bcd	141 ± 20 efg	203 ± 64 bcd	236 ± 75 ab
Recommended IF	209 ± 66 bcd	217 ± 88 bc	160 ± 45 def	204 ± 62 bcd	165 ± 24 cdef
Control	95 ± 18 gh	185 ± 42 bcde	65 ± 14 h	128 ± 100 fg	96 ± 19 gh

Manure, liquid cattle manure; common IF, inorganic N-P fertilizer applied at a rate common for the farmers in the area; recommended IF, inorganic N-P-K fertilizer applied according to pre-planting soil analysis; control, unfertilized soil

[†] Mean

[‡] Standard deviation ($n = 6$)

*Means followed by different letters, within the same parameter, are statistically different using the LSD test, at $p \leq 0.05$

period 2009–2013 (Table 3), as well as those reported by Lithourgidis et al. (2007) and Matsi et al. (2015) for the years of 2002–2008, manure application affected positively corn silage and grain yield and N, P, and K uptake by corn plants for several consecutive years. The same stands for the inorganic fertilization. In agreement with these findings, Martinez et al. (2017) and Cavalli et al. (2016), who conducted field experiments in Mediterranean countries, found that manure treatments increased corn yield in comparison to the untreated control. Similar results for corn are also reported by Marchezan et al. (2020), after cattle slurry application to soil for 12 years. Moreover, Cavalli et al. (2016) reported that, after 3 consecutive years of manure (liquid, solid, untreated) application, N uptake by corn and Italian ryegrass (*Lolium multiflorum* Lam.) plants grown in plots, which had received almost similar amounts of NH_4-N in the form of manure or inorganic fertilization, were similar and significantly higher than the control. The researchers attributed this to the increase of the aboveground biomass yield. However, decline of crops' yield and adverse impact on the environment upon heavy application rates of cattle manure to soil are also reported in the literature (Schlegel et al. 2017).

The aforementioned results concerning the plant micronutrients (Table 4) are in agreement with the findings of Nikoli and Matsi (2011), who showed that although the application of manure or inorganic fertilization had no effect on micronutrients' absorption by corn plants, it increased micronutrients' plant uptake significantly compared to the control. However, Yolcu et al. (2010) reported increased Zn and Cu concentrations in common vetch (*Vicia sativa* L.) and

Table 5 The apparent N recovery (ANR) for inorganic fertilization and manure treatments and the mineral fertilizer equivalent (MFE) of manure, the years of 2009–2013

	Year				
Treatment	2009	2010	2011	2012	2013
	ANR (%)				
Manure	66[†]± 24[‡] a*	40 ± 18 cdef	54 ± 9 abc	34 ± 19 ef	45 ± 13 bcdef
Common IF	49 ± 22 bcd	37 ± 11 def	33 ± 16 f	32 ± 14 f	55 ± 14 ab
Recommended IF	65 ± 12 a	44 ± 9 bcdef	48 ± 7 bcde	38 ± 23 def	34 ± 9 ef
	MFE (%)				
Common IF	134 ± 23 ab	109 ± 40 bcd	161 ± 45 a	106 ± 57 bcd	83 ± 27 d
Recommended IF	101 ± 23 bcd	92 ± 55 cd	111 ± 12 bcd	89 ± 42 d	131 ± 15 abc

Manure, liquid cattle manure; common IF, inorganic N-P fertilizer applied at a rate common for the farmers in the area; recommended IF, inorganic N-P-K fertilizer applied according to pre-planting soil analysis

[†] Mean

[‡] Standard deviation ($n = 6$)

*Means followed by different letters, within the same parameter, are statistically different using the LSD test, at $p \leq 0.05$

barley (*Hordeum vulgare* L.) with the addition of liquid cattle manure in comparison to solid and mixed cattle manure, applied for 2 consecutive years.

As in the present study, Marchezan et al. (2020), who evaluated cattle slurry, pig slurry, and pig deep-litter as organic fertilizers for corn in comparison to inorganic fertilization, after field applications for 12 years, reported ANR values of the same magnitude for cattle slurry and N-P-K fertilization treatments. The respective values for the other two organic amendments were much lower. The higher ANR values

Table 6 Soil available macronutrients, the years of 2009–2013

	Year				
Treatment	2009[§]	2010	2011	2012	2013
	KCl extractable NO_3-N (mg kg^{-1})				
Manure	14.1[†]±1.5[‡] de*	9.4 ± 0.7 ghi	9.3 ± 2.0 ghi	24.0 ± 5.1 ab	22.7 ± 1.3 b
Common IF	11.2 ± 2.5 fg	8.2 ± 0.9 hi	7.8 ± 1.2 chi	26.3 ± 5.4 a	22.7 ± 2.8 b
Recommended IF	13.0 ± 2.8 ef	8.0 ± 2.0 hi	10.9 ± 1.9 fg	23.8 ± 4.4 b	19.0 ± 1.7 c
Control	9.8 ± 2.1 gh	7.1 ± 1.2 i	7.2 ± 1.7 i	19.0 ± 5.4 c	15.6 ± 2.3 d
	Olsen-P (mg kg^{-1})				
Manure	18.5 ± 5.3 d	39.2 ± 1.7 a	27.3 ± 6.5 b	37.1 ± 8.3 a	35.4 ± 3.2 a
Common IF	7.8 ± 3.2 fg	14.6 ± 2.3 de	9.3 ± 2.7 f	17.8 ± 3.2 d	22.8 ± 5.7 c
Recommended IF	8.5 ± 2.2 fg	10.7 ± 3.0 ef	10.5 ± 2.7 f	10.6 ± 2.1 f	10.0 ± 2.1 f
Control	4.9 ± 1.9 gh	5.1 ± 1.7 gh	3.8 ± 1.0 h	9.7 ± 2.5 f	8.5 ± 1.8 fg
	CH_3COONH_4 extractable K (mg kg^{-1})				
Manure	95 ± 30 efg	130 ± 11 cd	146 ± 19 bc	165 ± 20 ab	172 ± 17 a
Common IF	62 ± 8 i	89 ± 43 fgh	85 ± 12 fghi	89 ± 9 fgh	133 ± 26 cd
Recommended IF	64 ± 10 i	99 ± 25 ef	117 ± 17 de	128 ± 30 cd	147 ± 9 bc
Control	66 ± 19 hi	74 ± 4 ghi	80 ± 18 fghi	92 ± 8 fg	131 ± 31 cd

[§] From Matsi et al. (2015) (Copyright: Cambridge University Press 2014; with permission)

Manure, liquid cattle manure; common IF, inorganic N-P fertilizer applied at a rate common for the farmers in the area; recommended IF, inorganic N-P-K fertilizer applied according to pre-planting soil analysis; control, unfertilized soil

[†] Mean

[‡] Standard deviation ($n = 6$)

*Means followed by different letters, within the same parameter, are statistically different using the LSD test, at $p \leq 0.05$

Table 7 Soil available micronutrients, the years of 2009–2013

Treatment	Year				
	2009[§]	2010	2011	2012	2013
	DTPA extractable Cu (mg kg^{-1})				
Manure	2.9[†]± 0.6[‡] a	2.5 ± 0.2 bcd	2.5 ± 0.1 cdef	2.5 ± 0.2 bc	2.8 ± 0.1 ab
Common IF	2.4 ± 0.7 cdefg	2.2 ± 0.1 efgh	2.1 ± 0.3 gh	2.1 ± 0.3 h	2.4 ± 0.3 cdefg
Recommended IF	2.5 ± 0.2 bcde	2.2 ± 0.3 defgh	2.3 ± 0.2 cdefgh	2.1 ± 0.2 h	2.6 ± 0.2 bc
Control	2.6 ± 0.2 bc	2.4 ± 0.1 cdefgh	2.4 ± 0.2 cdefgh	2.2 ± 0.2 fgh	2.5 ± 0.2 bcd
	DTPA extractable Zn (mg kg^{-1})				
Manure	1.09 ± 0.30 d	1.66 ± 0.17 a	1.49 ± 0.13 b	1.35 ± 0.11 c	1.66 ± 0.15 a
Common IF	0.67 ± 0.11 fgh	0.76 ± 0.11 fg	0.70 ± 0.09 fgh	0.65 ± 0.10 ghi	0.95 ± 0.22 e
Recommended IF	0.58 ± 0.05 hi	0.65 ± 0.04 ghi	0.64 ± 0.11 ghi	0.58 ± 0.14 hi	0.80 ± 0.14 f
Control	0.53 ± 0.10 i	0.64 ± 0.11 ghi	0.64 ± 0.07 ghi	0.59 ± 0.05 hi	0.80 ± 0.10 f
	DTPA extractable Fe (mg kg^{-1})				
Manure	14.9 ± 3.9 fgh	17.9 ± 1.6 bcdef	20.2 ± 3.0 b	18.5 ± 1.5 bcde	19.8 ± 3.2 bc
Common IF	13.8 ± 5.5 h	17.7 ± 0.8 bcdef	19.9 ± 2.9 bc	17.2 ± 2.4 bcdefg	19.6 ± 0.8 bc
Recommended IF	14.4 ± 2.1 gh	17.5 ± 1.9 bcdef	25.2 ± 2.9 a	16.4 ± 1.7 defgh	19.1 ± 1.6 bcd
Control	15.7 ± 1.8 efgh	18.5 ± 1.5 bcde	19.4 ± 2.2 bcd	16.9 ± 2.3 cdefg	18.6 ± 1.4 bcde
	DTPA extractable Mn (mg kg^{-1})				
Manure	16.3 ± 1.4 de	9.5 ± 1.0 h	15.7 ± 2.3 de	21.5 ± 2.4 c	27.2 ± 2.1 b
Common IF	11.3 ± 3.7 gh	8.9 ± 0.7 h	14.2 ± 0.9 ef	17.9 ± 4.3 d	31.0 ± 6.1 a
Recommended IF	12.6 ± 1.4 fg	8.7 ± 0.5 h	15.2 ± 1.5 def	16.7 ± 3.7 de	26.7 ± 2.9 b
Control	10.9 ± 2.5 gh	9.7 ± 1.1 h	14.8 ± 1.0 ef	17.8 ± 2.5 d	27.3 ± 2.9 b
	Hot water extractable B (mg kg^{-1})				
Manure	0.84 ± 0.08 b	1.02 ± 0.12 a	0.72 ± 0.13 bcdef	0.80 ± 0.16 bc	0.73 ± 0.13 bcdef
Common IF	0.65 ± 0.09 efgh	0.78 ± 0.12 bcd	0.52 ± 0.11 ij	0.72 ± 0.05 bcdef	0.68 ± 0.11 cdefg
Recommended IF	0.69 ± 0.09 cdefg	0.74 ± 0.07 bcde	0.50 ± 0.07 j	0.61 ± 0.09 fghij	0.57 ± 0.08 ghij
Control	0.66 ± 0.11 defgh	0.70 ± 0.19 cdef	0.52 ± 0.06 ij	0.55 ± 0.08 hij	0.64 ± 0.05 efghi

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Manure, liquid cattle manure; common IF, inorganic N-P fertilizer applied at a rate common for the farmers in the area; recommended IF, inorganic N-P-K fertilizer applied according to pre-planting soil analysis; control, unfertilized soil

[†] Mean

[‡] Standard deviation ($n = 6$)

*Means followed by different letters, within the same parameter, are statistically different using the LSD test, at $p \leq 0.05$

observed in the manure and recommended IF treatments in comparison to the common IF the years of 2009–2012 (Table 5) could be attributed to the higher amount of N applied in the latter treatment since both yields and N uptake (Figs. 1 and 2, Table 3) were similar among all fertilization treatments. As far as the year of 2013 is concerned, the increased ANR value for the common IF treatment could be attributed to its significantly higher N uptake the specific year (Table 3), as well as the high MFE values obtained for manure could be attributed to its readily available N, but only partially. According to Matsi et al. (2003) and is shown in Table 2, almost 40% of the manure's Kjeldahl-N was NH_4-N. Apart from the considerable amount of plant available N in manure, the high MFE was likely due to in-season plant available manure N (i.e., NH_4-N that was not volatilized, due to surface application and a delayed incorporation, and mineralized manure organic N) and N from the mineralization of residual manure organic N.

As far as the soil properties are concerned, in all treatments, pH remained at levels similar to those of the original soil (8.3 ± 0.1) before the establishment of the initial experiment with wheat (Matsi et al. 2003). In addition, EC_{se} was far below the critical limit for salinity risk of 4 dS m^{-1} (Brady and Weil 2008). Similar findings are reported for the same soil depth and the previous years of experimentation (Lithourgidis et al. 2007; Matsi et al. 2003, 2015). Differences in soil pH of 0.5 unit, among cattle manure (liquid or solid) and control treatments, are reported for both field and incubation experiments

(Cavalli et al. 2017; Schlegel et al. 2017). The past years similar results, concerning EC_{se} under the critical limit for salinity risk, have been published, even upon heavier application rates of liquid beef manure than those used in this experiment, i.e., 336 Mg ha^{-1} year^{-1} (Evans et al. 1977). Moreover, similar findings in respect of the EC_{se} are reported in a recent study, after a 10-year field experiment with the addition of cattle manure and swine effluents (Schlegel et al. 2017). Since no constant significant increases were observed, within each year, either for the soil organic C or the total N upon addition of organic matter in the form of manure, no significant differences of the C/N values among treatments were obtained. In all cases, the C/N ranged at levels (7.1–12.7) (data not shown) common for inorganic soils (Brady and Weil 2008).

The significant increases of soil NO_3-N observed upon organic and inorganic fertilization (Table 6) are in agreement with the findings of Lithourgidis et al. (2007) and Matsi et al. (2003, 2015) regarding the same soil depth and the previous years of experimentation. Variable results are reported in the literature, concerning the effect of cattle manure on soil available N, depending on manure's origin, in comparison to inorganic fertilizers. For example, the use of solid cattle manure (Martinez et al. 2017) released less inorganic N than the inorganic fertilization even though the application rate of N was higher in the former case and this could be attributed to the slow mineralization of the manure's organic N. On the other hand, the use of the liquid fraction in un-separated digested manure and of only liquid manure provided high amounts of readily available N for crops, due to the considerable amounts of inorganic N contained in the liquid fraction of both manures (Cavalli et al. 2017).

The residual concentrations of NO_3-N of all treatments in the first 3 years of the current experimentation (Table 6) could be characterized relatively low, while they were considerable the last 2 years (2012–2013) (Dahnke and Johnson 1990). The latter period an increasing trend of NO_3-N was observed in all treatments, which could not be attributed to fertilization practices, since the N addition rates remained the same as the previous 3 years. Taking into account that the monthly total rainfall of the years 2011–2013 was considerably lower than that of the rest of the years (Table 1) and in addition, it was lower than the 30-year average rainfall (437 mm) of the region, this could result in reduced losses of NO_3-N through leaching and thus explain the increased concentrations of NO_3-N in all treatments, the last 2 years of experimentation.

Similarly to the previous years (1997–2008) of experimentation (Lithourgidis et al. 2007; Matsi et al. 2003, 2015), soil available P was found to be increased in the fertilized plots (Table 6). However, a trend of P build up in the manure treated plots was evidenced almost all years of the current study, while such a trend was not observed the previous years. More specific, although P was applied at equal amounts each year, for the manure (55 kg ha^{-1}) and common IF (57 kg ha^{-1}) treatments, the manure-treated plots had two to three times higher P concentration, which was also much higher than the lower limit of sufficiency critical levels (10 mg kg^{-1}) (Thomas and Peaslee 1973). This was probably due to the slow mineralization rate of manure's organic P, applied the years of the present study but also in the previous years, as is also reported as a possibility by Li and Marschner (2019). Recovery of P in manure treated soils has been reported to be even twice as much of soils received inorganic fertilization (Halajnia et al. 2009). Manures are usually applied to arable soils at rates equivalent to the N requirements of the crop and that could end up to soil P build up because the N/P ratio of manures range is 1.5–9, depending on the animal source, while at the same time, plant N/P uptake range is 4.5–9 (McCallister et al. 2010).

Since only in the treatments of manure and recommended IF K was applied, they contained significantly higher K amounts in comparison to the common IF and control treatments, the years of 2010–2013 (Table 6). This increase was desirable as K reached or even exceeded the minimum value of 110 mg kg^{-1} for adequate soil K (Haby et al. 1990). Only after the year of 2009, K was applied in the recommended IF treatment. Previously, only N-P were added (Lithourgidis et al. 2007; Matsi et al. 2003, 2015) in the same plots and that was the reason of no significant differences, in respect to soil available K, among both inorganic fertilization treatments and the control in the year of 2009 and also between the control and common IF treatments afterwards.

The increased concentrations of soil available Cu, Zn, and B upon manure's application (Table 7) were attributed to the micronutrients' content of the liquid cattle manure. In agreement with these findings, increases of Cu and Zn are reported by Schlegel et al. (2017) upon a 10-year application of manures, i.e., solid cattle manure and swine effluents, and by Guan et al. (2018) upon 23–34 years of sheep and cattle manure application. Moreover, the concentrations of Cu, Zn, and B were similar, whereas those of Fe and Mn were higher than the sufficiency levels reported by Sims and Johnson (1991), even in the control treatment. Although the Cu, Zn, Fe, and Mn are essential micronutrients for plants, these can also have adverse impact on the environment as heavy metals. In general, total heavy metals' concentrations are commonly used to evaluate soil quality in respect to pollution. However, their available fractions are referred as a more suitable index to estimate their transfer from soils to plants and eventually to human (Guan et al. 2018). Cattle manure can affect heavy metals' distribution to soil, as its organic matter can bind up heavy metals to soil in several cases, although organic Zn can be easily mineralized (Zhao et al. 2014). Based on the results of this study and the evaluation of micronutrients' concentrations reported previously, there was no indication of environmental risk concerning heavy metals, due to liquid cattle manure's agronomic use.

5 Conclusions

Based on this study, we can make certain recommendations concerning the use of liquid cattle manure as fertilizer for crops, consecutively for medium- to long-term period. Basically, manure's application to soil at rates comparable (regarding N) to the common or recommended inorganic fertilization (after pre-plant soil analysis) for crops can enhance crop's yield and macronutrients' and micronutrients' uptake, at levels higher or similar to the inorganic fertilization. At the same time, manure can maintain soil fertility in respect to macronutrients and micronutrients. However, attention should be given in the case of P. This is because manure's application to soil, at a rate based on its N content for several years, could cause P build up. In addition, heavy metals' accumulation to soil at unacceptable levels, due to repeated annual manure's application, is unlikely.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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