Scientia Horticulturae 322 (2023) 112426

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Partial replacement of chemical fertilizers with animal manures in an apple orchard: Effects on crop performance and soil fertility

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ARTICLE INFO

Keywords:
Chemical fertilisers replacement
Manure
Slurry
Acidified slurry
'Gala'
Fruit quality
Crop health
Soil fertility

ABSTRACT

The combined use of chemical fertilisers with organic materials in crop fertilization is an essential approach to transition towards a more sustainable and resilient agriculture in Europe. In an apple orchard, chemical fertilisers (CF) were partially (25 to 57%) replaced with animal manure (cattle slurry - CS, acidified cattle slurry - ACS, cattle solid manure - CsM, and poultry manure - PM), based on the crop's nitrogen (N) requirements. Apple production and soil properties were monitored during a 3-year experiment. At the end of the third year, leaf N was higher in the control treatment (CTRL, 100% CF). Apple production was, on average, higher in the CS treatment, although not significantly different from the CTRL. Fruit analysis showed that replacing CF with animal manures did not significantly impacted fruit quality (weight, ºBrix and firmness). Soil organic carbon (SOC), N and exchangeable potassium (K^+) were significantly higher in the manure treatments. The increase in soil K^+ in the manure treatments consequently increased soil K/magnesium (Mg) ratio, slightly mitigating K and Mg antagonism, as seen by the increase in fruit K. However, K content was still deficient in fruits and leaves in all treatments. It can be concluded that the partial replacement of chemical fertilisers by animal manures (CS, ACS and PM) had a positive effect on soil health with no decrease of apple production.

1. Introduction

The excessive use of chemical fertilizers (CF) has led to well-known environmental impacts and raised production costs (Wang et al., 2018; Fang et al., 2021). In recent years, the trend in Europe has shifted slightly, due to increased efforts from the European Union to promote more sustainable and resilient agriculture, which is reflected in the implementation of many new policies (Hendriks et al., 2022). One approach in this transition, according to the authors, is the exchange of nutrients within the agricultural and livestock sector, a common practice before the introduction of CF. Livestock manure production is at an all-time high, and the amount produced from cattle, pigs, and poultry is equivalent to 1–2 times of the nitrogen (N) and phosphorus (P) that are applied through chemical fertilizers annually (Fangueiro et al., 2021). Therefore, recycling nutrients from livestock production to crop production has great potential and benefits for both sub-sectors (Li et al., 2020).

However, excessive application of animal manure can also have negative impacts on the environment. European legislation, such as the nitrate directive, limits the use of manures to prevent soil contamination and groundwater eutrophication (Pedizzi et al., 2018). Hence, it is necessary to decrease the application rate of manures in areas with high inputs such as cereal crops and find alternative agricultural land for their application (Fangueiro et al., 2021). Utilizing animal manure in permanent crops, such as orchards, has significant potential, particularly because organic matter decreases as the orchard ages, due to minimal incorporation of organic matter and the removal of carbon from the trees' branches, leaves, and fruits (Li et al., 2018). This is especially important in Mediterranean regions, where organic matter depletion is a pressing concern (Fangueiro et al., 2021). While the effects of animal manure application to annual crops on soil fertility are well-studied, there is a lack of research on their effects in permanent crop systems (Villa et al., 2021). The joint application of CF and organic materials has become increasingly popular in recent years. Nevertheless, more studies are still needed, to fully assess the impact of CF replacement by animal manure on soil and apple productivity.

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https://doi.org/10.1016/j.scienta.2023.112426
Received 10 July 2023; Received in revised form 3 August 2023; Accepted 16 August 2023
Available online 22 August 2023

In the present study, animal manures (cattle liquid manure (i.e., cattle slurry), cattle solid manure and poultry solid manure) were used to partially replace CF to meet the nutrient requirements of an apple orchard located in Portugal. The impacts of replacing CF with animal manures on crop productivity, fruit quality and soil chemical properties were assessed during the three years of experiment, with more emphasis on the results from the final year. Additionally, the impact of using acidified cattle slurry with sulphuric acid was also assessed, as there is a lack of literature on the effects of acidified cattle slurry on permanent crops. Acidification is a common practice in manure processing, and the application of acid slurry to soil results in reduced ammonia emissions (Eriksen et al. 2012; Pedersen et al. 2017) and delayed nitrification, leading to lower N losses and increased N fertilizer value of the slurry (Fangueiro et al. 2013).

2. Materials and methods

2.1. Experimental site

The experiment was conducted in a 'Royal Gala' apple orchard (Malus x domestica Borkh.), grafted onto M7 rootstock, which was planted in 2016. The orchard is situated in Tapada da Ajuda, Lisbon, Portugal (38.706864, -9.183493) and has central leader trained trees with a spacing of 1 m within the row and 4 m between the rows. The alleyways are left with natural grass, which is maintained using a mower. The orchard is equipped with drip irrigation and fertigation, which are provided from bud burst (around March) to postharvest (around October). Herbicides are applied once at the beginning of the flowering stage, in a 1-meter-wide strip along the trees, and four phytosanitary treatments are applied to prevent pests and diseases. All these practices are consistent with the Portuguese Standards for Integrated Fruit Production (DGADR, 2012).

The climate in the area is characterized as Csa according to the Köppen-Geiger climate classification, owing to the temperate climate with hot and dry summers. The soil is classified as a Leptosol, according to the World Reference Base for soil classification (WRB-IUSS, 2014).

The average annual precipitation in the years 2020, 2021, and 2022 was 618.4 mm, 395.4 mm, and 537.8 mm, respectively.

2.2. Experimental design

The experiment comprised three years of data and was designed using a randomized block design with five fertilization treatments and four blocks. The five fertilization treatments included one control treatment (CTRL), that received only CF, and four manure treatments that received a combination of manures and CF (Table1). Within each block, each treatment consists of a plot with five trees (20 m^2), resulting in a total of 20 trees per treatment and 100 trees in the entire trial.

In this trial, cattle slurry (CS), acidified cattle slurry (ACS), cattle solid manure (CsM), and poultry solid manure (PM) were used as replacement for the CF. The replacement rate was based on the crop's requirements for available nitrogen (N_{avail}), which were obtained via the Portuguese Standards for integrated production of pome fruits (DGADR, 2012), considering the orchard's vigor, estimated productivity, and leaf nutritional status.

The application rate of the manures was based on the amount of N potentially released during the first year following the application to the soil (N_{avail}), which rely on the total nitrogen (TN) content and the N mineralization rate of the manures. The N_{avail} values for each material were estimated using the values specified in Portugal's Legislation (CBPA, 2018), considering the climate and the type of soil on which the manures are planned to be applied. Specifically, the value of N_{avail} for CS, CsM, and PM were determined as 65%, 40%, and 55% of TN, respectively.

In addition, the plants' needs for P and potassium (K) were considered. All treatments received 13 kg P ha^{-1} and 91 kg K ha^{-1}, except for CsM that was richer in P and K than the other manures and slurries, providing significantly more. The CS, ACS and PM treatments required K supplementation, which was applied in the form of potassium sulphate. No P supplementation was required in these latter treatments.

Table 1
Application rate of N_{avail} in the three years of experiment, in the form of CF and animal manures, and the N_{avail} content of the manures. [1]Mean values of three replicates.

Trial years	Treatments	N_{avail} (kg ha^{-1})		N_{avail} in manures (g kg^{-1})[1]
		Via CF	Via manures	
2020	CTRL	60	-	-
	CS	45	15	2.12
	ACS			2.12
	CsM			2.81
	PM			9.76
2021	CTRL	80	-	-
	CS	40	40	2.00
	ACS			2.00
	CsM			3.14
	PM			9.04
2022	CTRL	70	-	-
	CS	30	40	2.26
	ACS			2.26
	CsM			2.30
	PM			10.27

CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure.

The process of acidifying cattle slurry involved adding concentrated sulphuric acid (H_2SO_4, 95–97%) to raw cattle slurry at a ratio of 6 mL of acid to 1 L of slurry, to reach a pH of 5.5, following the method described by Fangueiro et al. (2013). The application rate for both ACS and CS was the same because ACS was only prepared on the day of application. The literature suggests that there are no significant differences between the two slurries in terms of TN content (Sorensen and Eriksen, 2009; Sigurnjak et al. 2017), hence the same application rate was used.

The manures and slurries were applied during the full bloom stage of the orchard, which varied depending on the year: in the first two years, manure application was in March, and in the third year, it was postponed until May, due to weather restrictions and late dormancy break. Manure was applied in a narrow strip 10 cm away from the trees using a ditch that was 30 cm deep and 20 to 30 cm wide. The ditch was created using a tractor and small plow, and then closed with a hoe to bury the materials. This process was only done once at the beginning of each growing season, unlike fertigation, which is applied throughout the season.

2.3. Analytical procedures

2.3.1. Manure analysis

A sample was taken from each material and analysed in triplicate for dry matter (DM), organic matter (OM), pH, electrical conductivity (EC), total nitrogen (TN), ammonium (NH_4^+-N), and total macro and micronutrient content (P, K, Ca, Mg, Na, S, Fe, Cu, Zn, and B). The procedures for these determinations were performed in accordance with Prado et al. (2022). The C/N ratio was estimated using the organic carbon (C) calculated from the OM content, using the 1.724 "Van Bemmelem Factor" (Fangueiro et al. 2016), and the TN content. The main physical and chemical characteristics are presented at Table 2 and the nutrients effectively applied, based on the materials' characteristics and application rate, are presented in the Supplementary Data (Supplemental Table S1).

2.3.2. Soil analysis

Soil samples were collected annually in January over the three years experiment. The samples were collected from the top 30 cm of soil using a probe, afterwards were air-dried until they reached a constant weight,

Table 2
Physical and chemical properties of the animal manures and slurries used in each year of trial. Mean values of three replicates.

Materials	pH	EC	DM	TN	NH_4^+-N	C/N	P
		mS cm^{-1}	%	g kg^{-1} (FM)			g kg^{-1} (FM)
2020 – 1st year of trial							
CS	7.45^C	15.06^A	8.52^D	3.32^C	1.43^B	9.76^B	0.56^D
ACS	7.37^D	15.54^A	8.99^C	3.32^C	1.51^B	10.06^B	0.59^C
CsM	8.68^A	5.01^C	27.13^B	7.03^B	0.81^C	16.09^A	1.39^B
PM	7.77^B	8.89^B	54.27^A	17.74^A	4.93^A	15.97^A	3.22^A
Signif.	*	*	*	**	*	*	*
2021 – 2nd year of trial							
CS	7.65^B	16.26^B	6.47^D	2.92^C	1.60^B	8.35^c	0.45^C
ACS	5.02^B	17.80^A	7.17^C	2.92^C	1.52^B	8.81^c	0.42^D
CsM	9.14^A	4.20^D	49.48^B	7.85^B	0.86^C	15.70^b	2.04^B
PM	9.20^A	9.59^C	72.16^A	16.44^A	4.38^A	20.84^a	3.13^A
Signif.	*	*	*	*	*	***	*
2022 – 3rd year of trial							
CS	7.99^C	13.21^A	13.82^B	3.86^C	1.62^C	9.40^B	0.59^B
ACS	6.74^D	13.36^A	13.93^B	3.86^C	1.79^B	9.79^B	0.55^B
CsM	8.98^A	6.86^B	29.03^A	5.76^B	0.74^D	17.57^A	2.38^A
PM	8.86^B	6.13^B	60.76^A	18.68^A	5.09^A	15.78^A	2.47^A
Signif.	*	*	*	*	*	*	*

Signif. – significance level in the ANOVA test or Kruskal-Wallis test; ns – not significant ($P>0.05$); * - significant at $P<0.05$; *** - significant at $P<0.001$; In each column, values followed by the same letter do not significantly differ by the LSD test at $\alpha=0.05$. Small letters represent differences obtained with the ANOVA test, and capital letters represent differences obtained with the Kruskal-Wallis test. CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure. EC – electrical conductivity, TN – total nitrogen, DM – dry matter, FM – fresh matter, OM – organic matter.

and then sieved through a 2 mm mesh.

Soil pH, electrical conductivity (EC), soil organic carbon (SOC), extractable P, exchangeable cations (K^+, Mg^{2+}, Ca^{2+}, Na^+) and soil particle size were determined using the same methodology as described in Esteves et al. (2022). Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) were extracted with 2 M KCl and measured using spectrophotometry through a segmented flow auto-analyzer (Skalar San Plus, Skalar Analytical B.V., Breda, the Netherlands), in accordance with Houba et al. (1998). The total mineral nitrogen (N_{min}) was calculated as the sum of ammonium and nitrate.

Micronutrient content (iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn)) were determined using the Lakanen and Ervio extraction method (Lakanen and Ervio, 1971). The nutrients in the extract were quantified by ICP-OES.

2.3.3. Leaf analysis

Leaf samples were collected during the period of 90 to 120 days after full bloom, following national recommendations for leaf analysis (DGADR, 2012). Healthy leaves were selected for each treatment and block, and oven dried at 65 °C until constant weight and grounded to pass through a 0.50 mm stainless steel mesh. The dried material was used for the determination of nutrient content: leaf nitrogen (N) was determined using the Dumas combustion method (Buckee, 1994), and the other elements (P, K, Ca, Mg, S, Fe, Cu, Zn, and B) were determined using the same procedure explained in Section 2.3.1. All elements are reported to the dry weight (DW) at 105ºC.

2.3.4. Fruit production and fruit physical and chemical properties

All ripe apples were harvested in August during each of the three years of the trial, in every treatment and block. Yield efficiency (YE) was calculated by dividing the average yield (kg) by the trunk cross-sectional area (TCSA, cm^2) (Milosevic et al. 2019). TCSA was obtained for each tree by measuring the trunk diameter, using an automatic pachymeter (± 0.01 mm), at 20 cm above the grafting point, at the end of the vegetative cycle. The trunk diameter was measured in two orientations of the tree, and the mean value was used for TCSA calculation.

Ten apples were randomly collected from each plot and each block for fruit quality assessment and chemical analysis. The fresh apples were refrigerated for at least one day and then used to measure fruit weight; fruit diameter with an automatic pachymeter; total soluble solids content (TSS) with a refractometer (HI 96801, Hanna Instruments, Rhode Island, USA) at room temperature; and flesh firmness using a fruit pressure tester with an 11 mm tip (FT 327, T.R. Turoni, Forlì, Italy), on two opposite sides of the apple after skin removal.

For chemical analysis, thin slices of the apples were cut, oven-dried at 65 °C until a constant weight was reached, and then ground for the determination of total N content and macro and micronutrients. Total N content was obtained through the micro-Kjeldahl method, which is a scaled-down version of the standard method (Horneck and Miller, 1998). After digestion, distilled water was added to reach a volume of 50 mL, and 10 mL of the solution were collected to measure the nutrient content through ICP-OES. The content of P, K, Na, Mg, Ca, S, Fe, Cu, Mn, Zn, and B in the fruits was determined using the same procedures as explained in Section 2.3.1. All nutrient contents were expressed in dry weight (DW) at 105ºC.

In the first and second year, fruits were harvest in a single harvest, whereas in the third year of the trial, the harvest was sectioned into two due to different apple ripening stages.

2.4. Statistical analysis

The results from the manure's chemical analysis followed a completely randomized design, while the remaining data followed a complete randomized block design. The normal distribution of data and the homogeneity of variances were ensured through the Shapiro-Wilk and Levene tests, respectively. We rejected the null hypothesis that the data are normally distributed, and that the variances are homogeneous when the P-value of each test was smaller than 0.05. Data meeting the assumptions were analysed through ANOVA, whereas those that did not were analysed through the Kruskal-Wallis test for the completely randomized design and the Friedman test for the complete randomized block design. Statistical differentiation between treatments was determined using Fisher's least significant difference (LSD) at a 0.05 probability level. The Pearson correlation coefficient (r) was used to obtain correlations between selected variables (* - significant at $P<0.05$, ** - significant at $P<0.01$; *** - significant at $P<0.001$). The RStudio software package (Massachusetts, USA) was used to test the entire dataset.

3. Results and discussion

3.1. Soil

3.1.1. Initial soil analysis and manure characteristics

Prior to the start of the trial, the orchard was sampled at random locations, and the aforementioned characteristics were analysed. The analysis showed that the soil belongs to the clay texture class, with 28.08%, 25.30%, and 46.62% of sand, silt, and clay, respectively. The soil in the orchard had a neutral pH of 7.35, an EC of 127.35 μS cm^{-1}, indicating non-saline soil, and a SOC content of 1.4%, classified as medium level for soils with a fine texture (INIAV, 2022). The nitrogen content was as follows: 8.73, and 5.30 mg kg^{-1} of NH_4^+-N, and NO_3^--N, respectively. The remaining extractable nutrient content was: 55.27 mg P kg^{-1}, 193.62 mg Fe kg^{-1}, 20.70 mg Cu kg^{-1}, 4.62 mg Zn kg^{-1}, and 540.56 mg Mn kg^{-1}. The exchangeable cation content for Na^+, K^+, Ca^{2+}, and Mg^{2+} was found to be 0.31, 0.76, 21.95, and 7.93 $cmol^+$ kg^{-1}, respectively. Nutrient content is classified as high or very high depending on the element according to the Portuguese legislation (INIAV, 2022). Only organic matter and Na^+ were classified as medium. The other soil exchangeable cations were classified as high (K^+) or very high (Ca^{2+} and Mg^{2+}). The Ca/Mg ratio was 2.77, which is considered adequate for soils and will not result in structural or nutritional

Table 3
Soil analysis after each year of trial. Below is shown soil pH, electrical conductivity (EC), soil organic matter carbon (SOC), nitrogen content (NH_4^+-N, NO_3-N and N_{min}), extractable P, micronutrient content (Fe, Mn, Cu and Zn), and exchangeable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}). Mean values of four replicates.

Treatments	pH	EC	SOC	N_{min}	NH_4^+-N	NO_{3}-N	P	Fe	Cu	Zn	Mn	Na^+	K^+	Ca^{2+}	Mg^{2+}	K/Mg
		µS cm^{-1}	%	mg kg^{-1} of dry soil								cmol$^+$ kg^{-1} of dry soil				
After the 1st year of trial																
CTRL	7.81	128.80	1.54	7.34	1.98	5.36	83.85	147.71	18.59	4.74	343.21	0.24	0.99	23.35	7.88	0.13
CS	7.61	111.65	1.77	8.37	2.29	6.08	62.07	141.35	17.57	4.21	346.83	0.29	1.10	21.46	8.78	0.13
ACS	7.69	111.08	1.55	5.87	1.41	4.46	60.07	144.30	17.29	4.06	350.92	0.35	1.09	21.55	8.63	0.13
CsM	7.62	116.88	1.42	8.09	1.69	6.39	79.90	135.97	16.65	3.92	331.49	0.31	0.96	22.24	8.48	0.12
PM	7.68	125.15	1.70	8.89	1.50	7.39	94.39	139.10	17.42	4.60	340.68	0.31	1.18	23.13	8.21	0.15
Signif.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
After the 2nd year of trial																
CTRL	7.46^a	129.65^c	1.48^b	6.37	0.39^D	5.98	86.18^b	106.95	16.17	2.09^C	186.97	0.24^D	0.98^D	21.90	8.72	0.11^D
CS	7.39^a	263.58^c	1.80^b	20.41	1.88^C	18.53	81.38^b	103.78	14.88	3.22^{BC}	197.53	0.41^B	1.38^{BC}	22.07	8.90	0.16^C
ACS	6.42^c	841.95^a	1.90^{ab}	10.36	$4.47B^C$	5.89	67.56^b	96.10	12.83	2.43^C	176.87	0.50^{AB}	1.26c	22.50	8.69	0.15^{CD}
CsM	7.47^a	391.30^{bc}	2.52^a	42.20	$5.49A^B$	36.71	265.69^a	105.63	13.00	11.48^A	161.09	0.61^A	2.58^A	21.04	8.77	0.30^A
PM	6.98^b	520.65^b	2.00^{ab}	21.23	12.35^A	8.88	139.18^b	105.61	16.04	7.73^{AB}	220.56	0.32^C	1.82^{AB}	22.23	8.22	0.22^B
Signif.	***	***	*	ns	**	ns	**	ns	ns	*	ns	**	**	ns	ns	**
After the 3rd year of trial																
CTRL	7.45^{ab}	143.78^C	1.56^c	14.88^c	3.71^b	11.16	77.23^b	84.78	18.15^a	2.56^D	159.62	0.41	0.84^C	22.36	9.32	0.09^C
CS	7.50^a	197.93^{AB}	1.93^{bc}	25.26^{ab}	9.54^a	15.73	116.42^b	80.27	15.12^b	6.25^B	150.79	0.62	1.33^B	21.57	9.27	0.15^B
ACS	7.12^b	185.28^{BC}	1.96^{bc}	20.94^{bc}	11.04^a	9.90	80.75^b	85.64	15.53^b	3.60^C	163.23	0.51	1.28^B	20.58	8.81	0.15^B
CsM	7.70^a	263.98^A	2.51^a	30.11^a	9.60^a	20.51	184.36^a	89.53	16.57^{ab}	10.33^A	145.31	0.84	1.90^A	22.31	9.60	0.21^A
PM	7.68^a	262.98^A	2.33^{ab}	27.88^{ab}	12.39^a	15.49	119.85^b	74.79	14.24^b	9.43^A	159.95	0.52	1.35^B	22.85	8.87	0.16^B
Signif.	*	*	**	*	*	ns	*	ns	*	**	ns	ns	*	ns	ns	**

Signif. – significance level by the ANOVA test or Friedman test; ns – not significant ($P>0.05$); * - significant at $P<0.05$; ** - significant at $P<0.01$; *** - significant at $P<0.001$; In each column, values followed by the same letter do not significantly differ by the LSD test at $\alpha=0.05$. Small letters represent differences obtained with the ANOVA test, and capital letters represent differences obtained with the Friedman test. CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure.

imbalances in the soil (INIAV, 2022); however, K/Mg ratio in the soil was 0.10, a low value that can induce K deficiency due to antagonism with Mg (Hannah, 2011; Xie et al. 2021).

The manures generally exhibited high pH values and nutrient content, whereas the slurries had high electrical conductivity (EC) values (Table 2). Except for N_{avail}, which was uniformly applied across all treatments (Table 1), the slurries provided more NH_4^+-N, acidified cattle slurry (ACS) provided more S, and cattle solid manure (CsM), due to its high nutrient concentration, was the treatment that provided higher quantities of macro and micronutrients, and added more OM. Poultry manure (PM) also provided the most micronutrients (Supplemental Table S1).

Additionally, the C/N ratio aligned with the mineralization rate specified by the regulations, as slurries had lower C/N and thus mineralized more quickly (Azeez and van Averbeke, 2010). The higher C/N ratios of CsM and PM could result in N immobilization in these treatments, due to their C/N ratios above 15 (Chadwick et al. 2000) which can limit the N provided to the crop and inevitably affect crop productivity, especially in CsM that had higher C/N ratio. This was taken into consideration when calculating the application rates of the manures and slurries, so that available N application is homogeneous in all treatments. However, the mineralisation rate used was only theoretical.

3.1.2. Soil pH and electrical conductivity (EC)

At the end of the second and third years of the trial, there were significant differences between treatments in terms of soil pH and EC (Table 3). These differences can be attributed to a cumulative effect resulting from the application of manure over two or three years, as manures have a residual effect (Cai et al. 2019), or to an increased replacement rate of the chemical fertilisers (CF) from 25% during the first year to 50% and 57% over the second and third year, respectively. This resulted in a greater quantity of manure introduced into the soil, potentially amplifying their impact (Amiri and Fallahi et al. 2009).

In the second year of the trial, the application of ACS and PM significantly increased the soil's EC. However, in the third year, soil EC in the ACS treatment was not significantly different from the control (CTRL, 100% mineral N), but was higher in the other manure treatments. The increase in soil EC with the application of manures can be due to the dissolved salts applied through the manures and slurries (Azeez and van Averbeke, 2012). Other studies have also found higher soil EC values in the acidified treatments, when compared to the non-acidified (Sigurjnak et al. 2017; Edesi et al. 2020), however, this only happened in the second year of this experiment. These differences between the years might be due to differences in meteorologic conditions that could have promoted leaching or run-off of salts.

Soil pH, in the second year, was lower in the ACS and PM treatments, and higher in the CTRL, cattle slurry (CS) and CsM treatments. In the third year there were less significant differences between the treatments, but ACS had again the lowest soil pH. Although the effect is small, CS, CsM and PM increased soil pH, probably due to the breakdown of organic compounds (Cai et al. 2019). Milosevic and Milosevic (2017) also found higher soil pH when manure was mixed with NPK fertilisers, in an apple orchard.

The literature shows inconsistent results regarding the effects of acidified slurry on soil pH and EC and seems to be dependent on trial duration. For instance, no significant differences in soil pH were obtained between acidified and non-acidified slurry in a 54-day pot experiment with lettuces (Sigurnjak et al. 2017), and in a two-year experiment with winter wheat (Edesi et al. 2020). But in a three-year field experiment, Fangueiro et al. (2018) observed a significantly lower soil pH in the acidified treatments, when compared to the untreated counterpart in a double cropping system with oats and maize. This is more in line with the present study results. The decrease in soil pH was less pronounced in the present study, due to a good soil buffer capacity in the orchard, consequent from high clay and organic matter content (Fangueiro et al. 2018).

Nevertheless, the decrease in soil pH in the ACS treatment was never below the threshold of 5.5, a point at which soil liming is recommended in fruit tree production (DGADR, 2012).

3.1.3. Soil organic carbon (SOC)

At the end of the second and third year of the trial, there was an overall increase in SOC in the manure treatments, when compared to the CTRL, although only significant in CsM and PM treatments (Table 3), due to the higher amount of organic matter applied in these treatments (Supplemental Table S1). This was corroborated by the significant correlation between the application rate of organic matter from manures and slurries and the increase in SOC (r=0.77** in the third year), which has been documented before (Amiri and Fallahi, 2009). The joint application of CF with organic materials has also resulted in increased SOC in another experiment with an apple orchard, resulting in higher soil water retention and optimized porosity, which lessened the effects of water scarcity in the authors' study (Li et al. 2017).

ACS, in the second year, presented slightly higher SOC than CS, which might be due to slower or retarded C mineralization rate (Fangueiro et al. 2013; Sorensen and Eriksen, 2009) and is in agreement with other trials (Fangueiro et al. 2018). However, after the third year, this difference was not observed.

Compared to pre-trial SOC values, the CS, ACS, CsM and PM treatments showed an increase of 38%, 40%, 80% and 66%, respectively, after three years of trial. This is in line with other studies where organic fertilization led to an increase in soil OM (Zhao et al. 2014; Martínez-Alcántara et al. 2016; Villa et al. 2021).

3.1.4. Soil nitrogen

After the second and third year of trial, soil NH_4^+-N was higher in the manure treatments than in the control, especially in PM and ACS (Table 3). Although CS and ACS provided more NH_4^+-N than other treatments, that was not reflected in the soil analysis, which can be due to crop absorption. This was expected as the slurries had higher NH_4^+-N/TN ratios and lower C/N ratios, indicating more readily available N at the moment of application (Chadwick et al. 2000; Gutser et al. 2005).

Nitrate content, on the contrary, was not significantly different between the treatments. This might be due to high variability within the treatments and can be explained by manure hotspots in the soil, which happen when manure is unevenly applied or not fully incorporated into the soil, resulting in areas with concentrated organic matter and increased microbial activity (Baral et al. 2017).

Even without significant differences, it was observed higher and lower concentrations of NH_4^+-N and NO_3-N, respectively, in the ACS treatment when compared to CS. This might be an indication of delayed nitrification in the ACS treatment (Fangueiro et al. 2013; Fangueiro et al. 2016), or initial N immobilization and decreased N mineralisation (Sorensen and Eriksen, 2009; Sigurnjak et al. 2017).

The high N content during the dormant stage may result in nutrient losses due to low crop needs, however, the weeds can utilize the remained N since they are not trimmed during this stage. Also, the trimmed weeds (trimmed during flower blooming) remained in the field, returning nutrients and organic matter to the soil, increasing water retention and help mitigate soil erosion in intensive orchards (Zipori et al. 2020).

3.1.5. Other soil nutrients

Soil extractable P was higher in the CsM treatment in the second and third year of experiment, reflecting the amount of nutrient applied via the manure (Supplemental Table S1), as confirmed by the significant correlation (r=0.65*) between manure-P applied and soil P content.

The concentrations of Soil Zn, exchangeable Na^+ and K^+ in the third year showed significant differences, with higher concentrations observed in treatments that supplied more of these nutrients (Supplemental Table S1). This was, again, confirmed by significant positive correlations between manure-applied nutrients and nutrient soil content. In the third year, only soil Zn and K^+ showed significant differences. Nielsen and Edwards (1982) found a relative abundance of exchangeable cations in the order Ca>Mg>K, which is also observed in the present trial, but proportion of Mg and K was lower and higher, respectively, when compared to the present study.

Soil K/Mg ratio in this orchard ranges between 0.09 (CTRL) and 0.21 (CsM) on the third year. Hannah (2011) found that soil K/Mg ratios below 0.3 resulted in Mg-induced K deficiency in vineyards and suggested an adequate ratio between 0.4 and 0.5 to avoid this type of K deficiency. The increase of K^+ in the manure treatments allowed for a significantly higher K/Mg ratio in the soil (Table 3), however, these values are still very low and below the threshold indicated by Hannah (2011). This threshold depends on the extraction used for nutrient determination, site location and crop used and well as soil texture (Laekemariam et al. 2018), however, compared to thresholds adopted by other authors (Laekemariam et al. 2018; Xie et al. 2021), the soil K/Mg ratio in this orchard still indicated Mg-induced K deficiency.

Soil Cu also showed significant differences after the three years of trial, with higher values in the CTRL and CsM treatments, although Cu content in the CsM was not significantly different from the other manure treatments. This was not reflected by the application rate of Cu by the manures.

The lower nutrient content in the CTRL treatment might be a result of faster nutrient absorption, as chemical fertilizers have a faster nutrient release than animal manures (Yang et al. 2022). And animal manures also increase soil's capacity to retain nutrients due to the increase in soil organic matter (Amiri and Fallahi, 2009), contributing to higher nutrient content in these treatments.

3.2. 3.2. Apple production

Apple production and yield efficiency (YE) in the three years of trial are presented in Table 4. Significant differences between the treatments were only noted in the third year of trial, however, a trend in crop production can be seen since the first year, with lower and higher values in the CsM and CS treatments, respectively.

In the third year, CS produced the highest quantity, although it was not significantly different from the control. On the other hand, CsM led to the lowest yield. In terms of yield efficiency, the results are similar, although both ACS and CsM had lower efficiencies. Contrary to the present trial, using cattle manure as partial replacement for chemical fertilisers in a 'Gala' apple orchard resulted in no significant differences between the control, which received only CF (Yang et al. 2022). On the other hand, Zhao et al. (2014) used swine manure as CF replacement in a 'Fuji' apple orchard and reported higher yields when compared to the use alone of CF.

ACS should generate lower N losses compared to CS, due to the acidification that decreases NH_3 volatilization and delays nitrification (Fangueiro et al. 2016, 2017). And so, a better or similar performance

Table 4
Yield (kg tree^{-1}) and yield efficiency (YE, kg cm^{-2}) per treatment in the three years of the trial. Mean values of four replicates.

Treatments	2020 – 1st year		2021 – 2nd year		2022 – 3rd year	
	Yield (kg tree^{-1})	YE (kg cm^{-2})	Yield (kg tree^{-1})	YE (kg cm^{-2})	Yield (kg tree^{-1})	YE (kg cm^{-2})
CTRL	5.33	0.38	8.36	0.60	8.18^{ab}	0.58^{ab}
CS	4.61	0.33	8.72	0.61	9.25^{a}	0.61^{a}
ACS	4.07	0.32	6.60	0.50	6.48^{bc}	0.44b^{c}
CsM	2.91	0.23	6.67	0.53	5.87^{c}	0.41^{c}
PM	3.20	0.24	7.47	0.55	6.99^{bc}	0.45^{bc}
Signif.	ns	ns	ns	ns	*	*

Signif. – significance level by the ANOVA test; ns – not significant (P>0.05); * - significant at P<0.05. In each column, values followed by the same letter do not significantly differ by the LSD test at α=0.05. CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure.

was expected from ACS when compared to CS. However, in this study, the manures and slurries were incorporated into the soil, minimizing NH_3 emission, and the soil used was a fine textured soil with high organic matter content, which are conditions that potentially minimize N losses from CS (Cameira et al. 2019). The underperformance of ACS, when compared to CS, might be due to a delay of organic matter mineralization and consequently lower nutrient availability, as mentioned before. Sigurnjak et al. (2017) also obtained lower yields in acidified treatments compared to untreated pig slurry, but in a short-cycle crop (lettuces), which is much more sensible to salinity and changes in soil. Further studies are needed to study ACS as crop fertiliser.

Although the significantly lower apple production in CsM treatment could be attributed to N immobilization or slower OM mineralisation, it is more likely that the lower apple production in the manure treatments might be due to an already highly fertile soil, with high clay content, making the extra nutrients provided by the manures of little relevance considering the soil's nutrient reserve. In less fertile sandy soils, the application of animal manures to increase soil fertility is more impactful (Amiri and Fallahi, 2009). Nevertheless, the results are still positive when considering CS, ACS and PM as CF replacements.

3.3. Leaf analysis

Only leaf N and S showed significant differences between treatments (Table 5). CTRL had the highest N content, although it was not significantly different from the CsM. Milosevic et al. (2022) also found lower foliar N content in the manure treatments than in the chemical treatments, which received calcium ammonium nitrate (CAN) and urea.

Over 50% of mineral N fertilization was replaced by manure N, but the differences in leaf N levels between manure treatments and the control were small. This suggests that manures were still able to provide nutrients to the crop during the growing season.

Nevertheless, insufficient leaf nitrogen was observed in all treatments, according to the reference values (Table 5), and adjustments in the fertilizer management should be made to tackle N deficiency.

Regarding leaf S, the CTRL and CS treatments had significantly higher content than the other treatments, except for ACS which did not differ significantly from the two. Interestingly, the higher application rate of S in the ACS treatment did not result in the highest leaf S values among the manure treatments. For instance, Sigurnjak et al. (2017), in lettuces, found higher leaf S contents in the acidified treatments when compared to the non-acidified counterparts.

The excessive presence of leaf Mg, along with leaf K deficiency, might be a result of the antagonistic relationship between the two cations, as high levels of soil Mg^{2+} inhibit plant K^{+} absorption (Hannan, 2011; Xie et al. 2021), and result in compensatory absorption of Mg (Nguyen et al. 2017). However, no correlation was found between leaf K and leaf Mg, or between leaf K and soil Mg^{+} (also not when considering the % of Mg in the total exchangeable cation content), contradicting previous experiments (Nielsen and Edwards, 1982). Aichner and Stimpfl (2002) advised that an appropriate ratio of leaf K/Mg would be 5.6, and in this experiment, K/Mg ratio ranged between 3.01 (CTRL) and 3.63 (CS), corroborating higher Mg absorption in relation to K absorption.

The improved soil K/Mg ratio in the manure and slurry treatments did not significantly improve K absorption, however, leaf K/Mg ratio slightly increased in the manure treatments. Application of S may help plant K absorption, by potentially decrease soil pH and consequently decrease Mg availability (Hannan, 2011). Leaf K and leaf Mg were slightly higher and lower, respectively, in the ACS treatment, although there were no significant differences. Further studies are necessary to evaluate the potential of ACS, acidified with sulphuric acid, on improving K plant absorption in high-Mg soils.

Very few significant correlations were found between soil and leaf nutrients, which can be attributed to large reserves of nutrients within the tree structure, the empirical data from soil test and the influence of cultural and environmental factors on how trees absorb and distribute nutrients (Haynes, 1990).

3.4. Fruit physical and chemical analysis

Both flesh firmness and total soluble solids (TSS) are important factors for consumers and are related to sense of texture and "sweet taste" in apples, respectively (Musacchi and Serra, 2018), hence affect the marketability of the fruits. Presented in the Supplementary Data are the results of fresh fruit analysis from the first and second years of the trial (Supplemental Table S2) and it is seen significant differences between the treatments in terms of flesh firmness and TSS. However, these results vary each year, as these characteristics depend on internal and external factors, such as environmental factors and maturity stage (Musacchi and Serra, 2018). Therefore, more focus was placed on the results of the third year of the trial, to discuss the cumulative effects of three years of manure application.

In the first harvest of the third year, significant differences were observed between the treatments, but only in fruit TSS, where the ACS and CTRL had the highest values (Table 6). In the second harvest, there were differences between the treatments in fruit diameter, firmness, and TSS: the diameter was slightly higher in the manure treatments comparing to the control, but both ACS and PM were not statistically different from CTRL. Fruit firmness was slightly lower in the ACS treatment and higher in the CsM treatment. In terms of TSS, the control had higher values, followed by PM.

Although there were differences between treatments, the differences observed were relatively small and variable with the fruit's ripening stage. Specifically, mean fruit weight and TSS were significantly higher in the second harvest ($P<0.05$ and $P<0.001$, respectively), while firmness was higher in the first harvest ($P<0.05$). On the other hand, fruit

Table 5
Mean foliar nutrient content of the leaves on the third year of trial (2022), reported to the dry weight (DW) at 105°C. Sample material was collected between 90 to 120 days after full bloom. It is also shown the national reference values for healthy apple cv 'Royal Gala' trees (DGADR, 2012). Mean values of four replicates.

Treatments	N	K	Ca	Mg	P	S	Fe	Cu	Zn	Mn	B
	$g\ kg^{-1}$ (DW at 105 °C)						$mg\ kg^{-1}$ (DW at 105 °C)				
CTRL	21.20^{A}	11.48	12.93	3.88	2.00	2.16^{a}	261.87	21.29	19.26	21.97	30.50
CS	19.37^{B}	11.59	10.19	3.20	2.25	2.07^{a}	401.93	20.34	19.69	21.56	30.61
ACS	19.23^{B}	11.84	10.12	3.38	2.26	2.02^{ab}	235.91	20.86	16.80	24.31	30.85
CsM	19.56^{AB}	11.11	11.03	3.48	2.31	1.88^{b}	375.59	20.72	22.23	23.43	30.37
PM	19.29^{B}	10.95	10.54	3.54	2.45	1.60^{c}	303.88	20.05	17.47	22.80	30.74
Signif.	*	ns	ns	ns	ns	***	ns	ns	ns	ns	ns
Reference values	25–30	13–20	9–16	2–3	1.4–2.0	2.2–3.0	>45	10–50	10–100	25–200	25–50

Signif. – significance level by the ANOVA test or Friedman test; ns – not significant ($P>0.05$); * - significant at $P<0.05$; *** - significant at $P<0.001$; In each column, values followed by the same letter do not significantly differ by the LSD test at $\alpha=0.05$. Small letters represent differences obtained with the ANOVA test, and capital letters represent differences obtained with the Friedman test. CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure.

Table 6
Mean fruit weight, diameter, firmness, and total soluble solids (TSS) in the two harvests of the third year of trial (2022). Mean values of ten replicates.

Treatment	Weight g	Diameter mm	Firmness kg cm^{-2}	TSS °Brix
1st harvest				
CTRL	127.58 ± 31.18	66.39 ± 5.41	8.97 ± 1.52	14.74^{ab} ± 1.13
CS	128.85 ± 22.00	66.54 ± 4.55	8.91 ± 1.28	14.60^{b} ± 1.11
ACS	124.41 ± 18.47	65.45 ± 4.47	9.27 ± 1.25	15.38^{a} ± 1.36
CsM	132.07 ± 21.42	67.06 ± 4.11	8.89 ± 1.06	14.39^{b} ± 1.00
PM	125.86 ± 21.16	66.36 ± 4.49	9.16 ± 1.09	14.56^{b} ± 1.07
Signif.	ns	ns	ns	*
2nd harvest				
CTRL	126.18 ± 26.32	65.12^{b} ± 5.03	8.75^{ab} ± 1.50	15.62^{a} ± 0.95
CS	132.36 ± 26.25	66.77^{ab} ± 4.39	8.81^{ab} ± 1.01	14.96^{b} ± 0.90
ACS	142.87 ± 32.66	68.53^{a} ± 5.23	8.43^{b} ± 0.99	14.98^{b} ± 0.87
CsM	133.77 ± 37.65	66.48^{ab} ± 7.19	9.31^{a} ± 1.55	14.99^{b} ± 1.20
PM	138.58 ± 20.48	68.40^{a} ± 4.47	8.61^{ab} ± 1.11	15.36^{ab} ± 1.15
Signif.	ns	*	*	**

Signif. – significance level by the ANOVA test; ns – not significant ($P>0.05$); * - significant at $P<0.05$; ** - significant at $P<0.01$; In each column, values followed by the same letter do not significantly differ by the LSD test at α=0.05. CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure.

diameter did not differ significantly between the two harvests ($P<0.05$). These variations between harvests were also reported in a previous study, where fruit firmness decreased and TSS increased with fruit maturity (Iglesias et al. 2008).

The values of fruit TSS and firmness were higher in the present study, when compared to the values of Mota et al. (2022) but are in line with the results obtained by Kumar (2018) for 'Royal Gala' apples. This variation in the literature is expected, as TSS and firmness are highly dependent on cultivar, location, and maturity (Musacchi and Serra, 2018). For instance, Hoehn et al. (2003) concluded that, for 'Gala', consumers prefer apples with TSS values of 12.3 ºBrix, which is slightly lower than the present results (Table 6). Despite of the significant differences between the treatments, it is unlikely that consumers will detect a significant change in terms of sensory experience (Amarante et al. 2008).

Average fruit size and weight play a significant role in a farmer's profitability since these characteristics set the price paid to the farmer for the 'Gala' apples (Mota et al. 2022). Since there were no significant differences between the treatments, it is reasonable to assume that farmers will not suffer a loss in profitability, in terms of the price received for the 'Gala' apples, if they choose to partially replace chemical fertilizers with animal manures and slurries. In 'Gala' apple trees the replacement of CF with cow manure decreased fruit weight (Yang et al. 2022), which contrasts with the present study's findings as no significant differences were found between the treatments. Comparing to Mota et al.'s (2022) results, the fruit weight is lower in the present experiment.

Similar to the fresh fruit analysis, the nutrient content of the apples also differed between the two harvests in the third year of trial. The third-year results are discussed herein; however, the first- and second-year results are presented in the Supplementary Data (Supplemental Table S3). No significant differences between the treatments were found in these two years, except for fruit Na in the second year, which was higher in the CTRL and CS treatments.

In the first harvest of the third year, only fruit K and Mn significantly differed between the treatments, whereas in the second harvest, only Ca and Na differed between treatments (Table 7). Fruit total N did not significantly differ between the treatments, and it was correlated to leaf N (r=0.59**), but only in the first harvest.

In the first harvest, the Mn content was lower in the CS treatment, and higher in the CTRL, ACS and PM treatments, although the differences were small. And in terms of fruit K, the nutrient content was higher in the manure treatments, particularly in the PM treatment. In the second harvest, Ca content was higher in the CTRL treatment, while Na was higher in the manure treatments, particularly in the solid manure treatments (CsM and PM).

It has been reported that apple fruits extract N and K from soil, with K being the most extracted element, followed by N (Nava et al. 2008; Nachtigall and Dechen, 2006). This was also observed in this experiment (Table 7). Kuzin and Solovchenk (2021) recommended that fruit K content should be within 0.6 and 1.1%, but in the present study, fruit K was below this threshold, again indicating poor K absorption. Fruit K and Mg showed a positive correlation (r=0.51* and r=0.49* in the first and second harvest, respectively), contradicting the hypothesis that Mg was negatively impacting K absorption. However, Xie et al. (2021) mentioned that when K/Mg ratio in the growing medium is unbalanced, the relationship between K and Mg is often antagonistic at the source organs (e.g. old leaves), but synergetic in the sinks (fruits). In this case, no correlation was found at the foliar level, but a synergetic relationship was found in the apples. Nevertheless, it is difficult to find correlations between fruit nutrients as nutrient content in the fruit is dynamic and its accumulation changes throughout fruit development (Casero et al. 2004).

Comparing to Kumar's (2018) results in a 'Royal Gala' apple, the values of fruit K and Ca are higher than in the present study, but Mg and micronutrients (Fe, Cu, Mn, and Zn) content are lower. Another study reported that the values for fruit Ca in 'Gala' trees ranged from 0.53 to 0.66 g kg^{-1} (fresh weight) (Neilsen et al. 1999), which are much higher than those obtained in the present study. Comparing to Nielsen and Edwards (1982), fruit Mg and K were lower and higher, respectively, when compared to the present experiment. However, the abundance of these nutrients was in the same order as presented here: K>Mg>Ca.

Our data show that both K and Ca were in deficit in the fruit, even though there was translocation of the nutrients from leaves to fruits, supported by the positive correlations between leaf and fruit nutrients (r=0.58** and r=0.46* for K and Ca, respectively). The fact that these positive correlations occurred mostly in the second harvest might be an indication that the first harvest was premature. This was also observed in fruit firmness and TSS on the second harvest, that indicated that fruits were more ripe compared to the first harvest.

Ca is the most relevant nutrient to impact fruit marketability and storage capacity (Casero et al. 2004) and the ratios of this nutrient with K and N provide information relating to fruit storage. N/Ca should be below 10 to avoid metabolic disorders, whereas K/Ca should be below 25 to maximize apple's shelf life (Mota et al. 2022). In the first harvest, values of these two ratios are above the author's recommendations (Supplemental Table S4). In the second harvest, significant differences in K/Ca ratio were observed between treatments, with CTRL having a significantly lower values than the manure treatments. The ratio N/Ca did not significantly differ between treatments in this harvest, and the values were only above 10 in the CsM treatment (Supplemental Table S4). Contrary to the present trial, Amarante et al. (2008) found lower N/Ca ratios in the organically managed orchard with 'Royal Gala' than in the conventional orchard.

The high values of these ratios in the present experiment might be problematic, especially in the manure treatments. The low Ca content in the fruits impacted these ratios, especially K/Ca ratio, and might possibly promote bitter pit incidence (Casero et al. 2004).

Nevertheless, the relationship between fruit nutrients, content and balance, and fruit quality is still not entirely clear. Nava et al. (2008)

Table 7
Mean nutrient content of the apples, in the first and second harvest of the third year of trial (2022). Content reported to the dry weight (DW) at 105ºC. Mean values of four replicates.

Treatment	Total N $g\ kg^{-1}$ (DW)	K	Ca	Mg	P	S	Na $mg\ kg^{-1}$ (DW)	Fe	Cu	Zn	Mn	B
1st harvest												
CTRL	2.19	4.46^{c}	0.16	0.33	0.69	0.25	88.86	32.27	13.29	2.03	2.37^{a}	25.87
CS	1.83	4.75^{bc}	0.12	0.33	0.71	0.23	88.51	36.42	12.27	1.43	2.05^{b}	26.50
ACS	1.48	5.16^{ab}	0.17	0.35	0.68	0.23	92.18	27.75	14.88	1.46	2.41^{a}	26.21
CsM	2.12	5.22^{ab}	0.14	0.35	0.73	0.24	101.40	29.44	14.89	1.90	2.28^{ab}	27.36
PM	1.84	5.28^{a}	0.14	0.33	0.73	0.23	105.01	29.14	17.01	1.73	2.42^{a}	27.29
Signif.	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
2nd harvest												
CTRL	1.72	4.69	0.25^{a}	0.33	0.69	0.26	106.02^{b}	35.45	14.68	1.25	2.68	27.18
CS	1.49	5.04	0.16^{b}	0.31	0.70	0.24	131.33^{ab}	39.01	16.91	1.22	2.60	26.08
ACS	1.71	5.33	0.19^{b}	0.33	0.72	0.25	124.63^{ab}	40.48	14.83	1.16	2.59	26.84
CsM	1.57	5.15	0.16^{b}	0.31	0.72	0.23	150.95^{a}	37.47	15.71	0.81	3.05	26.42
PM	1.60	5.11	0.18^{b}	0.32	0.69	0.23	151.51^{a}	42.32	15.06	1.20	2.73	24.63
Signif.	ns	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns

Signif. – significance level by the ANOVA test; ns – not significant ($P>0.05$); * - significant at $P<0.05$. In each column, values followed by the same letter do not significantly differ by the LSD test at $\alpha=0.05$. CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure.

reported K/Ca ratios above 38 in a long-term field trial with a 'Fuji' cultivar and did not observe any physiological disorders during harvest or storage of those fruits.

4. Conclusions

The replacement of conventional chemical fertilisers by almost 60% with solid and liquid animal manures resulted in significantly higher levels of soil organic matter and nutrients and slightly higher crop productivity when cattle slurry was used as a substitute. The replacement did not affect fruit's marketability and farmer's profitability, as evidenced by the small differences in fruit TSS and firmness, and similar fruit weight between the CTRL and the manure treatments.

We also found that the acidified cattle slurry performed poorly compared to the untreated cattle slurry, in terms of fruit production, likely due to inhibited N decomposition and nutrient release. However, further research is needed.

Soil analysis also revealed favourable conditions for Mg-induced K deficiency, as shown by the very low K/Mg ratio in the soil. The increase in soil K^{+} observed upon replacement with manures or slurries significantly increased the soil K/Mg ratio but did not seem to significantly increase leaf K absorption. However, this increase was reflected in the fruits, as fruit K was higher in the manure treatments. Nonetheless, K and Ca deficiencies were observed, which can potentially impact fruit's shelf life.

Further research is necessary to determine other factors that may be influencing plant nutrient absorption in this orchard. Ultimately, replacing chemical fertilizers with animal manures enhances C sequestration in orchards, maintains soil fertility in the long term, and potentially lowers production costs if manures are outsourced from nearby suppliers.

CRediT authorship contribution statement

Catarina Esteves: Investigation, Methodology, Formal analysis, Writing – review & editing, Writing – original draft. **David Fangueiro:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Project administration. **Mariana Mota:** Methodology, Formal analysis, Writing – review & editing. **Miguel Martins:** Formal analysis. **Ricardo P. Braga:** Writing – review & editing. **Henrique Ribeiro:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

Acknowledgement

This research was funded by 1) the Project Nutri2Cycle: H2020-SFS-30-2017- "Transition towards a more carbon and nutrient efficient agriculture in Europe", funded by the European Union, Program Horizon 2020 (grant agreement no 773682); 2) LEAF (Linking Landscape, Environment, Agriculture and Food Research Unit), funded by FCT (UIDB/04129/2020). This document reflects only the authors' view, and the Union is not liable for any use that may be made of the information contained therein. The authors would like to appreciate the graduate students Eva Costa and Miguel Mata. The authors also thank the advice and recommendations of the editor and the anonymous reviewers.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112426.

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