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Research article

Partial replacement of mineral fertilisers with animal manures in an apple orchard: Effects on GHG emission

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A B S T R A C T

Partial replacement of mineral fertilisers (MF) with animal manures is a good alternative to reduce MF use and increase both nutrient cycling in agriculture and soil organic matter. However, the adoption of this practice must not lead to increased environmental impacts. In this two-year study conducted in an apple orchard, MF were partially replaced with various animal manures, including cattle slurry (CS), acidified cattle slurry (ACS), solid cattle manure (CsM), or poultry manure (PM), and their impacts on greenhouse gas emission (GHG: CO_2, N_2O and CH_4) were examined. A control (CTRL) receiving only MF served as the baseline, representing the conventional scenario in orchard fertilisation. Overall, replacing MF with manures increased GHG emissions, with the magnitude of the impacts depending on the specific characteristics of the manures and the amount of nutrients and organic matter applied. Comparing to the CTRL, application of ACS and CS led to higher CH_4 and N_2O emissions, while PM application increased both N_2O and CO_2 emissions. In contrast, replacement with PM and CsM decreased CH_4 emissions. Nevertheless, results varied between the two years, influenced by several factors, including soil conditions. While acidification showed potential to mitigate CH_4 emissions, it also led to increased N_2O emissions compared to CS, particularly in 2022, suggesting the need for further investigation to avoid emission trade-offs. Replacement with CS (20.49 t $CO_{2\text{-eq}}$ ha^{-1}) and CsM (20.30 t $CO_{2\text{-eq}}$ ha^{-1}) showed comparable global warming potential (GWP) to the conventional scenario (CTRL, 19.49 t $CO_{2\text{-eq}}$ ha^{-1}), highlighting their potential as viable MF substitutes.

1. Introduction

Among growing concerns about global warming and the resulting climate change, scientific studies are increasingly focused on more sustainable alternatives for all human activities. A significant contributor to environmental challenges is the escalating emission of greenhouse gases (GHG) in recent decades, a trend in which food production systems play a notable role (Yoro and Daramola, 2020; Canadell et al., 2021). Substantial changes in food production systems are necessary to prevent global warming from surpassing the 1.5 °C target (IPCC, 2018).

A strongly suggested set of practices in Europe includes closing the nutrient cycles in agriculture by reintegrating animal manure as crop fertiliser (Hendriks et al., 2022). This approach not only promotes a circular economy in agriculture but also reduces reliance on mineral fertilisers and mitigates their associated impacts. The application of manure introduces organic matter and nutrients to the soil (Liu et al., 2015; Gautam et al., 2020), enhancing soil fertility (Meng et al., 2005; Steiner et al., 2007; Shakoor et al., 2021) and promoting carbon (C) sequestration (Maillard and Angers, 2014; He et al., 2016; Gautam et al., 2020).

The advantages of applying manure to enhance soil organic matter are particularly important in Mediterranean regions, which are characterised by low soil organic matter content and significant soil erosion challenges (Calleja-Cervantes et al., 2017; Francaviglia et al., 2019). Apple orchards, extensively cultivated in Mediterranean regions (Aguilera et al., 2013), offer significant potential for utilising animal manure. They excel in C storage within their perennial structures, capable of retaining 1.5% more C than arable crops (Francaviglia et al., 2019), making them ideal candidates for enhancing soil organic matter and mitigating global warming. Globally, apple production covers 4.8

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million hectares, producing 93.1 million tonnes of fruit in 2021 (Food and Agriculture Organization of the United Nations, 1997). Given the importance of apple production, not only at the regional but also at the global level, the adoption of such environmentally friendly measures in orchard crops shows significant potential to help control global warming while simultaneously addressing soil fertility and manure management challenges in agriculture.

However, the application of nitrogen (NH_4^+-N and NO_3^--N) and C through manure can stimulate the production of GHG (Chadwick et al., 2011; Xia et al., 2020; Ruangcharus et al., 2021). GHG emissions are also influenced by the production system implemented in the orchard, whether intensive or extensive (Alaphilippe et al., 2016), and whether conventional or organic (Keyes et al., 2015). Additionally, different management practices such as irrigation, mulching and cover crops (Fentabil et al., 2016; Gu et al., 2019; Tang et al., 2022), as well as soil characteristics (Smart et al., 2011), can impact GHG emissions. Moreover, nitrogen (N) application rates play a significant role, as N_2O emissions respond positively to increasing application rates of N fertilisers (Xie et al., 2017; Gu et al., 2019), and these rates can vary greatly across the different regions. For example, N application rates in apple orchards vary from 27 kg N ha^{-1} in Portugal (Figueiredo et al., 2013) to 1000 kg N ha^{-1} in China (Wang et al., 2016). Therefore, studying different management practices and fertilisation strategies, including manure application, is important to understand potential mitigation measures in orchards (Smart et al., 2011).

This work aimed to investigate, in a two-year study, the impacts of partially replacing mineral fertilisers (MF) with different animal manures in an apple orchard on GHG emissions. The first year (2021) focused on evaluating the short-term effects of this fertilisation strategy on GHG emissions, comparing various animal manures as potential replacements. In the second year (2022), our objectives broadened: 1) validation of the trends observed in 2021, 2) determination of the orchard's global warming potential (GWP) under different scenarios: either exclusively applying mineral fertilisers, or partially replacing MF with different animal manures, 3) assessment of soil N dynamics to explore potential correlations with N_2O emissions, and 4) calculation of emission factors (EF) for N_2O emissions associated with these fertilisation scenarios, which is an important addition for N_2O budget calculations (Gu et al., 2019).

2. Methods and materials

2.1. Experimental site and design, and manure application

The study was conducted in an apple orchard with the 'Gala' apple cultivar grafted onto M.7 rootstock, located at Tapada da Ajuda, Lisbon, Portugal (38.706864° N, −9.183493° W). The orchard was established in 2016 and had trees spaced 1 m apart within rows and rows spaced 4 m apart, covering a total area of 4000 m^2. The orchard is managed in accordance with the Portuguese standards for Integrated Pome Fruit Production (Direção-Geral de Agricultura e Desenvolvimento Rural, 2012) and has a fertigation system. The soil is classified as a Leptosol, according to the World Reference Base for soil classification (IUSS Working Group WRB, 2015). The local climate is classified as Csa according to the Köppen-Geiger climate classification, characterised by temperate climate with hot and dry summers (IPMA, 2011).

Soil moisture and temperature were monitored using a soil sensor (Enviropro MT 40 cm, EnviroPro Dielectrics Pty Ltd, Moonta, AU), which recorded data at 10-cm intervals up to a depth of 40 cm. Precipitation data were collected by a sensor (Adcon RG1, OTT HydroMet GmbH, Kempten, GER) positioned 1.8 m above the soil surface in an unobstructed area. These sensors transmitted data to a centralised platform, where irrigation events were also recorded. Soil temperature and moisture, precipitation and irrigation logs, measured from March 2021 to March 2023, are presented in the Supplementary Material (Figure S1 and S2). Irrigation (though a fertigation system with drip-irrigators) is active from bud burst until post-harvest, which is typically around November. This coincides with the warmest months of the year. Field capacity and water stress levels were calculated based on the soil's characteristics and crop's water demands. Irrigation events were then determined based on whether soil moisture levels exceeded or fell below these two established parameters.

The experiment followed a complete randomised block design with five fertilisation treatments with four blocks each. The treatments included a control (CTRL, receiving only MF), cattle slurry (CS), acidified cattle slurry (ACS), cattle manure (CsM) and poultry manure (PM). Each block of each treatment consisted of a plot with five trees, occupying a 20 m^2 area (Fig. 1). A detailed map depicting all treatments and blocks employed in the orchard experiment is available in the Supplementary materials (Supplemental Figure S3). This experiment spanned from 2020 to 2023 (Esteves et al., 2023), however, GHG emissions were only measured in the 2021 (March 2021 to April 2022) and 2022 (May 2022 to March 2023) campaigns.

The application rate of the fertilisers was based on plant N demands, which were estimated considering the projected crop production and the orchard's fertility (Direção-Geral de Agricultura e Desenvolvimento Rural, 2012). Regarding MF, the plant-available N (PAN) content was equivalent to the total amount of nitrogen. However, for manures and slurries, PAN was estimated from the total nitrogen (TN) concentration and standard mineralisation rates: 65%, 65%, 40%, and 55% of TN for CS, ACS, CsM, and PM, respectively (CBPA, 2018).

In 2021 and 2022, the N application rates were 80 kg PAN ha^{-1} and 70 kg PAN ha^{-1}, respectively. The replacement rate of MF with animal manures was 50% in 2021 and approximately 57% in 2022. It is noteworthy that during the first year (2020), a 25% MF replacement was carried out.

The manures and slurries were applied in a band on March 2nd, 2021, and on May 3rd, 2022, coinciding with the full bloom stage of the orchard. In 2022, the application of manure was later in comparison with 2021 due to a warm winter, which prolonged the dormancy period and delayed flower bloom. For manure application, a trench (Fig. 1) was opened approximately 10 cm away from the tree line. The trench measured 20–30 cm in width and 30 cm in depth and was created using a tractor and a small plough. The manures were then evenly spread within the trench and covered manually with soil.

ACS was prepared on the day of manure application each year. It was obtained by acidifying the raw cattle slurry with sulphuric acid (H_2SO_4, 95%) at a ratio of 6 mL of acid to 1 L of slurry, achieving a pH of 5.5 (Fangueiro et al., 2013). Throughout the acidification process, the slurry was periodically stirred to ensure the homogenisation of acid distribution.

2.2. Soil analyses

Soil samples were collected from the fertilised areas and in the interrow (IR), as illustrated in Fig. 1, during the summer of 2022 at specific intervals: 3, 7, 10, 15, 17, 21, 29, 35, 43, 49, 67, 78 days after manure application. Sampling was conducted using a probe, with samples taken from each block and treatment, from the top 30 cm of the soil layer. Samples were then oven-dried at 40 °C until reaching a constant weight. Prior to laboratory analysis, the samples were manually ground in a stone mortar and sieved to a size of 2 mm.

Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) levels were determined following the method described by Houba et al. (1989), involving extraction with KCl (2 M) and subsequent measurement using a segmented flow auto-analyser (Skalar San Plus, Skalar Analytical B.V., Breda, the Netherlands). Total mineral N (N_{min}) was then calculated by summing the NH_4^+-N and NO_3^--N values obtained from the analysis.

2.3. Manure analysis

Manure and slurry samples were taken from the containers, after

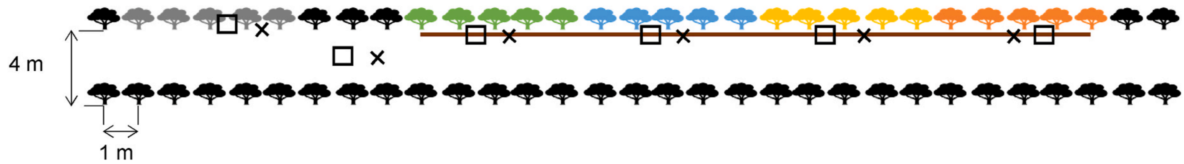

Fig. 1. Example of one row of trees from the trial: colourful trees represent different treatments, arranged in plots of five trees (black trees are not included in the trial's assessments). The brown line denotes the trench opened for manure application in a band. Squares and crosses mark the locations of the chambers used to sample GHG emissions and soil samples, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

homogenisation, and were then characterised in triplicate using the methods described in Prado et al. (2022). The manure's characteristics are presented in Table 1. The amount of nutrients effectively applied, calculated based on the manure's characteristics and the application rate, is provided in the Supplementary material (Supplemental Table S4).

2.4. GHG fluxes measurements

GHG measurements were carried out continuously from March 2021 to March 2023, to assess GHG emissions during the orchard's active stage (from manure application until fruit harvest in August of both years) and dormant stage (the remainder of the year). During the 2021 campaign (March 3rd, 2021 to April 18th, 2022), measurements were exclusively carried out in the treatment plots. However, during the 2022 campaign (May 4th, 2022 to March 3rd, 2023), measurements were carried out in both the treatment plots and in the IR, which had indigenous vegetation. The varying measurement durations were due to differences in the dates of manure application each year (Section 2.1).

Measurements were conducted using the static chamber method (Rochette and Hutchinson, 2005). Chambers were installed in the soil immediately after manure application in both years. In the CTRL treatment, chambers were positioned in the row of trees, between two drippers. In the manure-amended treatments, chambers were placed directly adjacent to the row of trees, covering the area supplied with fertigation and manure. In the IR treatment, chambers were positioned in the middle of the interrow (Fig. 1). Vegetation was removed from the CTRL and manure chambers before measurement but was left intact in IR chambers.

Measurements initiated precisely one day after manure application and were conducted between 8:00 and 12:00 p.m., with more frequent measurements following manure application. In the first year, PVC chambers (Supplemental Figure S5-A) were used, while in the second year, aluminium chambers (Supplemental Figure S5-B), covered with a reflective and insolating plastic to prevent overheating within the chambers, were used.

The concentration of CO_2, CH_4, and N_2O within each chamber was measured immediately after closing the chamber (T0), and again after 20 (T20) and 40 (T40) minutes of air accumulation. Air samples were collected from each chamber through a sampling tube (Teflon tube with an internal diameter of 3 mm and a length of 27 m). Gas concentration (mg m^{-3}) were determined using a photoacoustic gas equipment (Supplemental Figure S5-C, INNOVA 1512, Lumasense Technologies, Ballerup, Denmark). The photoacoustic gas monitor was equipped with an optical filter for water vapor (filter type SB0527), and the detection limits for N_2O (filter type UA0985), CO_2 (filter type UA0982), and CH_4 (filter type UA0982) were 0.03 ppm, 5.1 ppm and 0.4 ppm, respectively. Prior to the trial, the manufacturer calibrated the photoacoustic gas monitor, which was configurated to compensate for water interference and cross-interference.

Table 1
Mean values (n = 3) of the physical and chemical properties of the manures and slurries used in each year of trial.

Materials	DM	TN	NH_4^+-N	PAN[z]	C/N	P
	%	g kg^{-1} (FM)				g kg^{-1} (FM)
2021						
CS	6.47^D	2.92^C	1.60^B	2.00	8.35^c	0.45^C
ACS	7.17^C	2.92^C	1.52^B	2.00	8.81^c	0.42^D
CsM	49.48^B	7.85^B	0.86^C	3.14	15.70^b	2.04^B
PM	72.16^A	16.44^A	4.38^A	9.04	20.84^a	3.13^A
Signif.	*	*	*	–	***	*
2022						
CS	13.82^B	3.48^C	1.62^C	2.26	9.40^B	0.59^B
ACS	13.93^B	3.48^C	1.79^B	2.26	9.79^B	0.55^B
CsM	29.03^A	5.76^B	0.74^D	2.30	17.57^A	2.38^A
PM	60.76^A	18.68^A	5.09^A	10.27	15.78^A	2.47^A
Signif.	*	*	*	–	*	*

Signif. – significance level in the ANOVA test or Kruskal-Wallis test; * - significant ($p < 0.05$); *** - significant at $p < 0.001$; Within each column and for each year, values followed by the same letter do not significantly differ according to the LSD test at $\alpha = 0.05$. Lowercase letters represent differences identified using the ANOVA test, while uppercase letters represent differences identified using the Kruskal-Wallis test. CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure, IR – interrow. DM – dry matter, EC – electrical conductivity, FM – fresh matter, PAN - plant available nitrogen, C/N - organic carbon to total nitrogen ratio, P - total phosphorus.

[z] Considering the estimated mineralisation rates: 65% of TN in the CS and ACS, and 40% and 55% of total N in the CsM and PM, respectively.

2.5. Calculations and statistical analysis

Gas fluxes (G) were determined using the following equation (Fangueiro et al., 2017):

$$G\left(mg\ C\ or\ N\ m^{-2}day^{-1}\right) = m \times \frac{M_a}{M_m} \times \frac{V}{A} \times 1440 \tag{1}$$

Where m (mg m^{-3} min^{-1}) represents the linear regression of gas concentration at each sampling time (T0, T20, T40), V and A are the volume (m^3) and area (m^2) of the chambers, respectively. M_a is the atomic weight of C (12 g mol^{-1}) or N (14 g mol^{-1}), and M_m is the molecular weight of CO_2 (44 g mol^{-1}), CH_4 (16 g mol^{-1}) or N_2O (44 g mol^{-1}). Fluxes were then transformed into daily emissions (1440 min).

Cumulative emissions were estimated by averaging the flux between two sampling days and multiplying by the time interval between those days. GWP of each treatment was estimated by converting the emitted CO_2, N_2O and CH_4 into CO_2-equivalents ($CO_{2.eq}$) using the conversion factor of 1, 273 and 27.2 for CO_2, N_2O and CH_4, respectively (IPCC, 2021), which are the 100-year GWP values. For the yield-scaled emissions, crop productivity in each plot (20 m^2), reported in Esteves et al. (2023), and the gaseous emissions during the orchard's active stage, also reported to the 20 m^2 plot, were taken into consideration.

The emission factor (EF) for N_2O emissions in the whole orchard (during the entire measurement period in 2022–304 days) for each treatment was calculated as follow:

$$EF\ (\%) = \frac{total\ N(N_2O)_{treatment} - total\ N(N_2O)_{IR}}{N\ applied} \tag{2}$$

In the manure-amended treatments, the applied N consisted of the TN applied (61.54 kg TN ha^{-1} in the CS and ACS treatments, 100.00 kg TN

ha^{-1} in the CsM treatment, and 72.73 kg TN ha^{-1} in the PM treatment) plus the PAN applied from mineral N fertilisers (30 kg PAN ha^{-1}). In the CTRL treatment, the added N consisted of 70 kg of PAN ha^{-1}. The IR treatment was considered as the unfertilised treatment.

The GWP of each scenario, whether using only MF or partially replacing MF with manure, was calculated as follows:

$$GWP_{scenario}\left(g\ CO_{2-eq}\ ha^{-1}\right) = 0.25 \times GWP_{treatment} + 0.75 \times GWP_{IR} \quad (3)$$

Since the fertilised areas account for 25% of the total, while the unfertilised areas (IR) constitute the remaining 75% of the orchard's total area (4000 m^2). The logic behind this reasoning is based on the 1 × 4 m spacing between trees and rows, respectively. We determined that the fertilised area corresponded to a 1 m^2 area (50 cm to each side of the tree, in all orientations of the tree), with the unfertilised area comprising the remaining space (3 m^2). This approach was consistently applied to calculate the emissions of CO_2, N_2O, and CH_4 for each scenario. The EF and GWP calculations for each scenario were specifically conducted for the final year (2022) due to the inclusion of measurements in the IR.

To verify ANOVA assumptions, we conducted tests for normal distribution using the Shapiro-Wilk test and for homogeneity of variances using the Levene test. The null hypothesis (data normally distributed and homogenous variances) was rejected at $p < 0.05$. Data that met these assumptions were analysed using ANOVA. For data that did not meet these assumptions, we employed the Kruskal-Wallis test for manure analysis and the Friedman test for GHG emissions and soil analysis. Kruskal-Wallis and Friedman tests are rank-based, meaning that different letters in the tables indicate differences between the mean ranks. However, the tables present standard means. Statistical differentiation between treatments was determined through Fisher's least significant difference (LSD) at a significance level of 0.05. The relationship between emissions and soil and climate conditions and manure characteristics was assessed using the Pearson correlation coefficient, with a significance set at $p < 0.05$. Analysis was conducted using R (R Core Team, 2023) and RStudio (Posit team, 2023).

3. Results

3.1. Short term effect on GHG emissions

In 2021, the partial replacement with manures and slurries led to increased cumulative CO_2 emissions compared with the CTRL, but no significant differences were observed between the manure-amended treatments (Table 2). Considering only the orchard's active stage, CO_2 emissions were significantly higher in the PM and CsM treatments compared with ACS and CS. This difference might be due to higher CO_2 peaks in the CsM and PM treatments, significantly higher in the PM treatment on day 6 (16.15 g C m^{-2} d^{-1}, $p < 0.001$) and 45 (15.13 g C m^{-2} d^{-1}, $p < 0.001$) (Supplemental Figs. S6–A).

Similarly to CO_2 emissions, most of the N_2O emissions occurred within the first 100 days of the experiment, after manure application (Supplemental Figs. S6–B). Cumulative N_2O emissions were significantly higher in the PM treatment, followed by the slurries (ACS and CS), CsM and finally CTRL, which exhibited the lowest emissions (Table 2). The higher cumulative emissions in the former treatments can be attributed to the significant emission peaks observed: ACS peaked immediately after manure application, on day 1 (14.10 mg N m^{-2} d^{-1}, $p < 0.05$) and again on days 10, 17 and 45 (smaller peaks); while CS peaked on day 17 (25.59 mg N m^{-2} d^{-1}, $p < 0.05$); and PM reaching a significant emission peak of 86.36 mg N m^{-2} day^{-1} on day 70 ($p < 0.05$).

In contrast, CH_4 emissions remained consistently negative throughout the 2021 campaign, during both the active and the dormant stage (Supplementary Figure S6-C). However, an exception was observed in the CS treatment, which exhibited an initial CH_4 peak of 552.07 mg C m^{-2} day^{-1} on day 2. This led to positive cumulative CH_4 emissions in the CS treatment but negative cumulative emissions in the other treatments (Table 2). Replacement with ACS and CsM significantly increased the soil's ability to retain or oxidise CH_4, in comparison with the CTRL.

When considering yield-scaled emissions (Table 3), the CTRL treatment exhibited the lowest CO_2 and N_2O emissions in the first year, although the yield-scaled N_2O emissions in the CTRL were not statistically different from those in the CsM treatment. The manure-amended treatments did not significantly differ from each other in terms of CO_2, but PM led to the highest yield-scaled N_2O emissions. Regarding CH_4, only CS led to positive yield-scaled CH_4 emissions. ACS and CsM led to significantly lower yield-scaled CH_4 emissions compared with CTRL. The CTRL treatment resulted in the lowest yield-scaled GWP, while the other treatments did not differ significantly.

3.2. Medium-term effects on GHG emissions

Consistent with the 2021 findings, higher cumulative CO_2 emissions were also observed in the PM treatment in 2022 (Table 2). However, in this second campaign, ACS did not significantly differ from PM. Over the

Table 2
Mean values (n = 4) of the cumulative GHG emissions in the two years of measurement, both during the active stage of the orchard (175 and 106 days in 2021 and 2022, respectively) and during the entire measurement year (412 and 304 days in 2021 and 2022, respectively).

Treatments	CO_2 (g C m^{-2})		N_2O (mg N m^{-2})		CH_4 (mg C m^{-2})	
	Active stage	All year	Active stage	All year	Active stage	All year
2021						
CTRL	299.95^C	753.39^B	85.68^D	154.79^D	-865.00^B	-1220.96^B
CS	610.69^B	1300.95^A	413.15^{BC}	492.03^{BC}	1736.64^A	1229.56^A
ACS	525.01^B	1272.09^A	408.11^B	507.72^B	-1226.27^C	-1661.76^{CD}
CsM	743.66^A	1540.49^A	176.51^C	312.00^C	-1329.89^D	-2035.14^D
PM	904.66^A	1618.78^A	2831.63^A	3296.93^A	-1041.81^C	-1329.03^{BC}
Signif.	**	*	**	**	**	**
2022						
CTRL	158.53^A	466.77^c	105.12^B	151.43^{CD}	0.00^B	-205.03^b
CS	146.53^{AB}	558.97^{bc}	162.21^A	258.45^B	505.79^A	251.38^a
ACS	195.47^A	623.12^{ab}	248.42^A	459.48^A	283.16^A	34.64^a
CsM	171.92^A	555.54^{bc}	75.48^B	185.28^{BC}	-78.63^C	-656.94^c
PM	187.51^A	698.35^a	97.18^B	548.02^A	-90.20^C	-379.36^b
IR	71.91^B	540.11^{bc}	50.01^C	96.32^D	-80.33^C	-362.10^b
Signif.	*	**	**	**	**	***

Signif. – significance level in the ANOVA or Friedman test; * - significant at $p < 0.05$, ** - significant at $p < 0.01$, *** - significant at $p < 0.001$; Within each column and for each year, values followed by the same letter do not significantly differ according to the LSD test at $\alpha = 0.05$. Lowercase letters represent differences identified using the ANOVA test, while uppercase letters represent differences identified using the Friedman test. CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure, IR – interrow.

Table 3
Mean values (n = 4) of yield-scaled cumulative GHG emissions and GWP for each year, expressed as kilogram (kg) of fruit produced.

Treatments	CO_2 (g C kg^{-1})	N_2O (mg N kg^{-1})	CH_4 (mg C kg^{-1})	GWP (kg $CO_{2.eq}$ g kg^{-1})
2021				
CTRL	147.30^B	43.66^C	-410.74^B	0.54^B
CS	296.16^A	189.60^B	885.04^A	1.20^A
ACS	348.93^A	293.40^B	-825.08^C	1.38^A
CsM	642.19^A	168.13^{BC}	-927.72^C	2.42^A
PM	545.34^A	1645.54^A	-559.66^{BC}	2.69^A
Signif.	*	**	*	*
2022				
CTRL	86.66	63.44	0.00^B	0.34
CS	65.81	73.17	225.31^A	0.28
ACS	131.79	154.83	183.98^A	0.56
CsM	126.88	59.49	-67.68^C	0.49
PM	118.78	71.97	-61.58^C	0.46
Signif.	ns	ns	**	ns

Signif. – significance level in the Friedman test; ns – not significant ($p > 0.05$); * - significant at $p < 0.05$, ** - significant at $p < 0.01$; Within each column and for each year, values followed by the same letter do not significantly differ according to the LSD test at $\alpha = 0.05$. CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure.

entire measurement period in 2022, cumulative CO_2 emissions in the CTRL treatment were statistically comparable to those of the CS, CsM, and IR treatments. Considering only the orchard's active stage, cumulative CO_2 emissions in CTRL did not significantly differ from those observed in the manure-amended treatments and were significantly higher than in the IR treatment.

Nevertheless, in the beginning of the 2022 campaign there were notable CO_2 emission peaks in the manure-amended treatments (up to 4.25 g C m^{-2} d^{-1} on day 2 in the PM treatment) in comparison to the CTRL and IR treatments (between 0.90 and 1.60 g C m^{-2} d^{-1}). These peaks in the manure-amended treatments remained significantly higher until day 14 ($p < 0.05$). The lack of significant differences between the IR treatment and the fertilised treatments (excluding PM) over the entire measurement period (304 days), may be attributed to a pronounced emission peak observed during the dormant stage. This peak (3.88 g C m^{-2} d^{-1}) was statistically higher in the IR treatment on day 213 ($p < 0.001$) (Supplemental Figs. S7–D).

Significantly higher cumulative N_2O emissions were found in the manure-amended treatments compared to CTRL and IR, except for CsM which did not differ from the CTRL treatment (Table 2). Considering only the orchard's active stage, ACS and CS treatments led to significantly higher cumulative N_2O emissions, followed by CTRL, PM and CsM, and finally by IR, which exhibited the lowest cumulative emissions. The higher cumulative emissions in the CS and ACS treatments can be attributed to distinct N_2O peaks ($p < 0.01$) observed on day 9 (8.36 mg N m^{-2} d^{-1}) and day 17 (10.20 mg N m^{-2} d^{-1}) in the CS and ACS treatments, respectively (Supplemental Figs. S7–E). It is also noteworthy that the PM treatment also exhibited a significant N_2O emission peak during the dormant stage, on day 153 (3.76 mg N m^{-2} d^{-1}, $p < 0.05$), which contributed to the higher cumulative emissions during the entire measurement period (304 days) in this treatment.

In contrast to the findings of the 2021 campaign, ACS resulted in significantly higher N_2O emissions and similar CH_4 emissions when compared with CS. Only CS and ACS treatments resulted in positive CH_4 emissions during the entire measurement period, while the other treatments showed negative cumulative emissions (Table 2). In comparison with CTRL and IR, CsM resulted in significantly lower CH_4 emissions. However, considering only the orchard's active stage, cumulative CH_4 emissions were lower in the CsM, PM and IR treatments compared to CTRL. The higher cumulative CH_4 emissions observed in the slurry treatments can be attributed to the pronounced emission peaks on day 2 in the CS (304.19 mg C m^{-2} d^{-1}) and ACS (196.25 mg C m^{-2} d^{-1}) treatments (Supplemental Figure S7-F). Nonetheless, this initial CH_4 peak was significantly higher in the CS treatment ($p < 0.01$).

In this campaign, the yield-scaled emissions only exhibited significant differences between treatments concerning CH_4 emissions, with higher values observed in the CS and ACS treatments, and lower values in the CsM and PM treatments (Table 3). Replacing MF with animal manures did not increase the orchard's yield-scaled GWP, in contrast with the findings of the 2021 campaign.

3.2.1. Soil nitrogen

Soil N dynamics (Fig. 2) were examined during the initial 78 days after manure application to potentially correlate with N_2O emissions. Soil N was statistically lower in the IR treatment when compared to the other treatments, presenting values below 5.62 mg NH_4^+-N kg^{-1} and below 10.72 mg NO_3^--N kg^{-1}. An exception was CTRL, which showed little differentiation between IR.

Regarding the manure-amended treatments, PM consistently presented higher NH_4^+-N content, with higher differentiation compared with the other treatments after day 7. Soil NH_4^+-N in this treatment peaked on several occasions (reaching a maximum of 252.43 mg kg^{-1} on day 64), while NO_3^--N remained relatively low during the sampling period (<61.26 mg kg^{-1}). Soil N dynamics in the PM treatment did not align with N_2O peaks, whereas NH_4^+-N peaks observed in the CS and ACS treatments on day 7 coincided with N_2O peaks. However, no correlation was found between soil NH_4^+-N levels in the CS and ACS treatments and the corresponding N_2O emissions ($p > 0.05$).

Conversely, soil NO_3^--N in the CS treatment was negatively correlated with N_2O emissions ($r = -0.69$, $p < 0.05$), as higher emissions of N_2O occurred during periods with lower soil NO_3^--N. CsM consistently presented lower values of both NH_4^+-N and NO_3^--N.

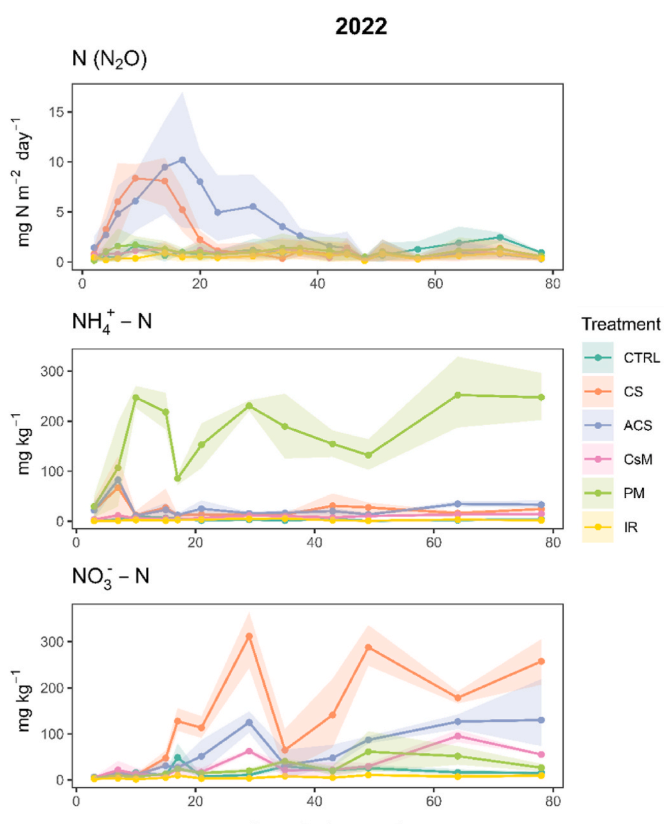

Fig. 2. N_2O emissions and soil dynamics of ammonium (NH_4^+-N) and nitrate (NO_3^--N) in the first 78 days after manure incorporation. Mean values of four replicates and confidence interval at 95% confidence (shaded area around treatment lines). CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure, IR – interrow.

3.3. GWP and emission factors of the different fertilisation scenarios

During the orchard's active stage, no significant differences were observed in CO_2 emissions and GWP between the treatments. However, replacing MF with CS and ACS led to higher N_2O and CH_4 emissions compared to the CTRL scenario, which exclusively received MF (Table 4). Conversely, both CsM and PM scenarios led to comparable N_2O emissions in relation to the CTRL scenario, while leading to higher CH_4 oxidation potential.

Considering the entire measurement period, both the ACS and PM scenarios exhibited increased CO_2 and N_2O emissions compared with the CTRL, whereas CS and ACS scenarios showed increased CH_4 emissions (Table 4). Conversely, CsM decreased CH_4 emissions compared with the CTRL. Therefore, GWP values were higher in the PM and ACS scenarios, and lower in the CTRL, CS, and CsM scenarios.

Emission factors for N_2O emissions, considering the applied N, were significantly higher in the PM and ACS scenarios compared with CsM, CTRL and CS treatments (Table 4).

4. Discussion

4.1. Nitrous oxide

4.1.1. Comparison between growing seasons

The N_2O emissions observed in the 2021 campaign were relatively higher than those observed in the 2022 campaign across all treatments. Such inter-year differences have been previously documented (Fentabil et al., 2016; Leytem et al., 2019) and can be attributed to various factors, such as differences in the composition of the applied manure (Chadwick et al., 2000b; Velthof et al., 2003), which varied between the two years (Table 1), variations in weather and soil conditions (Chadwick et al., 2000a; Akiyama et al., 2004; Alsina et al., 2013), and differences in soil status in terms of N and C content (Xie et al., 2017; Zhou et al., 2022).

Differences in N_2O peaks were also noted between the two years. In 2021, the PM treatment exhibited very high N_2O fluxes between day 45 and 93, while in 2022, a significant peak was only observed during the dormant stage. This led to significantly higher N_2O emissions in the PM treatment during the active stage of 2021, but not during the 2022 active stage. This discrepancy might be due to differences in soil moisture content and dry matter (DM) content of the manure.

During the 2021 active stage, soil moisture levels frequently exceeded field capacity, potentially promoting anaerobic conditions and increasing N_2O emissions (Alsina et al., 2013) from the available N in the PM treatment. However, no significant correlation was found between emissions in the PM treatment and soil moisture in 2021 (Supplemental Fig. S8).

In contrast, during the 2022 active stage soil moisture levels were low and it was the use of slurries, CS and ACS, that resulted in significantly higher N_2O emissions. This could be attributed to the higher water content in the slurries, facilitating a more effective distribution of their C and N components in the soil and leading to higher N_2O losses from anaerobic microsites (Velthof et al., 2003). The dry soil conditions prevalent during this period in 2022 could explain the lack of an emission peak in the PM treatment in this year and the higher emissions in the moisture-rich materials (CS and ACS). However, no correlation was found between N_2O emissions and manures' DM during this time. DM content was only significantly correlated with N_2O emissions during the orchard's dormant stage ($r = 0.75$, $p < 0.001$), which probably corresponds to the N_2O peak observed in the PM treatment during this period.

A consistent trend observed in both years was the lower emissions in both the CTRL and CsM treatments. However, in the 2021 campaign, emissions in the CTRL treatment were significantly lower compared with CsM.

4.1.2. Comparison between untreated slurry and acidified slurry

In 2021, the ACS treatment exhibited an earlier peaked compared with CS, unlike in 2022. The early peak of ACS in 2021 may have been influenced by acidification, as the reduction in slurry pH significantly decreases NH_3 emissions (Sørensen and Eriksen, 2009), leading to higher N availability for denitrification and subsequent loss as N_2O. Despite efforts to minimise NH_3 emissions by incorporating the slurries into the soil, there remains the possibility of small N losses via NH_3 volatilisation (Fangueiro et al., 2015b) in the CS treatment, from soil macropores or due to uneven slurry distribution in the trench, which potentially explains the earlier peak in ACS compared with CS. However, this effect was not consistent throughout the years.

In the 2021 campaign, ACS and CS emitted comparable cumulative N_2O emissions, while in 2022, ACS resulted in higher N_2O emissions than CS (considering the total measurement period). Additionally, ACS in 2022 also showed a trend of slightly higher CO_2 emissions (although not significantly) compared with CS, which could have contributed to O_2 depletion, leading to anaerobic conditions and subsequent stimulated N_2O emissions (Fangueiro et al., 2015a; Leytem et al., 2019). This discrepancy between ACS and CS in 2022 might have been exacerbated by the incorporation of the slurry, which could have increased anoxic conditions in the soil and consequently increase N_2O emissions. Seidel et al. (2017), who investigated the effects of acidification on NH_3 abatement and N_2O emissions in grassland, reported higher N_2O emissions in the acidified cattle slurry compared to non-acidified slurry. Similar findings were reported in a study using pig slurry, where acidified slurry led to higher N_2O emissions (Gómez-Muñoz et al., 2016). In 2022, N_2O emissions in the ACS treatment peaked later (day 17) compared with the CS treatment (day 9), suggesting delayed nitrification. The lower soil pH resulting from the acid conditions in the slurry may have inhibited the activity of nitrifying organisms (Fangueiro et al., 2015a), thus affecting microbial activity and nitrification processes. Indeed, soil nitrate levels were significantly lower ($p < 0.01$) in the ACS treatment compared with CS on several occasions, indicating reduced

Table 4
Mean values ($n = 4$) of GHG emissions and GWP (indicated by $CO_{2\text{-eq}}$ emission) for each scenario established in this experiment, both during the active stage of the orchard and during the entire measurement period. Additionally, the EF for N_2O emissions in each treatment, as a percentage of the total N added, is presented.

Scenarios		CO_2 (kg C ha^{-1})	N_2O (g N ha^{-1})	CH_4 (g C ha^{-1})	GWP (t $CO_{2\text{-eq}}$ ha^{-1})
Active growing stage (106 days)					
100% mineral N	CTRL	935.63	637.85^{B}	−602.50^{C}	3.68
57% replacement rate with manure	CS	905.64	780.58^{A}	661.98^{A}	3.68
	ACS	1028.00	996.11^{A}	105.40^{B}	4.20
	CsM	969.11	563.76^{B}	−799.08^{D}	3.77
	PM	1008.09	618.01^{B}	−827.99^{D}	3.93
Signif.		ns	*	**	ns

Scenarios		CO_2 (t C ha^{-1})	N_2O (kg N ha^{-1})	CH_4 (kg C ha^{-1})	GWP (t $CO_{2\text{-eq}}$ ha^{-1})	EF (% applied N)
Overall measurement period (304 days)						
100% mineral N	CTRL	5.22^{c}	1.10^{b}	−3.23^{b}	19.49^{c}	0.79^{b}
57% replacement rate with manure	CS	5.45^{bc}	1.37^{b}	−2.09^{a}	20.49^{bc}	1.77^{b}
	ACS	5.61^{ab}	1.87^{a}	−2.63^{a}	21.27^{ab}	3.97^{a}
	CsM	5.44^{bc}	1.19^{b}	−4.36^{c}	20.30^{bc}	0.68^{b}
	PM	5.80^{a}	2.09^{a}	−3.66^{b}	22.02^{a}	4.40^{a}
Signif.		**	**	***	**	**

Signif. – significance level in the ANOVA or Friedman test; ns – not significant ($p > 0.05$), * - significant at $p < 0.05$, ** - significant at $p < 0.01$, *** - significant at $p < 0.001$; Within each column and for each measurement period, values followed by the same letter do not significantly differ according to the LSD test at $\alpha = 0.05$. CTRL – control, CS – cattle slurry, ACS – acidified cattle slurry, CsM – cattle solid manure, PM – poultry manure. GWP - global warming potential, EF - emission factor.

nitrification rates. However, despite this observation, no correlation was found between soil N (NH_4^+ or NO_3^-) and N_2O emissions in the ACS treatment.

4.1.3. Impact of fertilisation treatments on N_2O emissions

CS and ACS resulted in higher N_2O emissions, particularly during the 2022 active stage. The higher water content in the slurries likely promoted anaerobic conditions compared to the drier manures (PM and CsM), further stimulating N_2O emissions (Velthof et al., 2003), as previously mentioned. Additionally, the authors found that lower C/N ratios and higher mineral N in the manure composition contributed to higher total N_2O emissions. These characteristics could explain the higher emissions in the slurry treatments, as slurries had the lowest C/N ratio (Table 1) and applied the highest amount of NH_4^+-N (Supplemental Table S4). This was then confirmed in 2022, as N_2O emissions were negatively correlated with C/N of the manures ($r = -0.65$, $p < 0.01$) and positively correlated with the amount of NH_4^+ applied ($r = 0.69$, $p < 0.01$).

However, these correlations were not observed during the 2021 campaign. Instead, a positive correlation was found between N_2O emissions in this year and C/N ratio of the manures ($r = 0.73$, $p < 0.01$, over the entire measurement period). Despite PM applying the highest amount of NH_4^+-N after the slurries (Supplemental Table S4), no correlation was found between applied NH_4^+ and N_2O emissions. The high content of uric acid-N in PM could explain the high emissions observed in this treatment in 2021, as this N component converts to urea and NH_4^+ given the optimal conditions (Ruiz Diaz et al., 2008). Subsequently, NH_4^+-N nitrifies under aerobic and warm conditions, releasing NO_3^- (Ruiz Diaz et al., 2008), which can denitrify and produce N_2O. The highest N_2O emissions in this treatment occurred during a period with warmer temperatures and lower soil moisture, which could have provided optimal conditions for nitrification and consequently increased NO_3^- availability for denitrification. However, this relationship between soil conditions and N_2O emission peaks was not confirmed by a linear relationship.

PM emitted more N_2O than CsM in both the 2021 and 2022 campaigns, which is consistent with previous literature findings (Akiyama et al., 2004; Shakoor et al., 2021). According to Shakoor et al. (2021), this disparity could also be attributed to higher levels of easily decomposable organic carbon and increased rates of nitrification and denitrification in the PM treatment. The analysis of soil N levels in 2022 showed higher soil NH_4^+ levels in the PM treatment on several occasions compared with CsM, indicating higher rates of N mineralisation in the former treatment. However, soil NO_3^- levels were higher in the CsM treatment on different dates, contradicting the expectations of higher nitrification rates in PM. It could be argued that lower NO_3^- content in PM might have resulted from losses through N_2O emissions, however, the lack of N_2O peaks or significant emissions during this period undermines this justification. Further assessments are required to completely understand the differences between these two treatments.

Application of manure C also alleviates C limitation for denitrification processes, potentially favouring NO_3^- reduction rather than N_2O reduction (Leytem et al., 2019). Consequently, this might have contributed to the higher N_2O emissions observed in the manure-amended treatments compared to the CTRL, which only received mineral fertilisers without addition of a C source. Similar findings were reported by Leytem et al. (2019), observing higher N_2O emissions with increased application rates of dairy manure compared with mineral fertilisers in a cropping system with cereals. This effect was then confirmed in 2021 by a positive correlation between the amount of organic matter (OM) applied though manure and N_2O emissions during the orchard's dormant stage ($r = 0.56$, $p < 0.05$). However, in 2022, cumulative N_2O emissions in the CsM treatment did not significantly differ from those observed in the CTRL treatment. Interestingly, in this year, N_2O emissions showed a negative correlation with OM applied ($r = -0.60$, $p < 0.05$).

Compared to all other treatments, emissions in the IR treatment during the 2022 campaign showed significantly lower values during the active stage. This difference can be attributed to the absence of irrigation and N fertilisation in the IR treatment (Smart et al., 2011). Regarding our fertilised control (CTRL), N_2O emissions reached 3.31 mg N m^{-2} d^{-1} in 2021 and 2.46 mg N m^{-2} d^{-1} in 2022. Contrastingly, in the manure-amended treatments, N_2O fluxes reached 151.41 mg N m^{-2} d^{-1} (in PM) and 19.20 mg N m^{-2} d^{-1} (in ACS) in 2021 and 2022, respectively. In a peach orchard, N_2O emissions in the mineral fertiliser treatment reached 131.34 mg N m^{-2} d^{-1}, while supplementation with organic manure alongside mineral fertilisers resulted in emissions reaching 161.21 mg N m^{-2} d^{-1} (Cheng et al., 2017). Despite slight differences in absolute values, the overall trend remains consistent, with higher N_2O emissions associated with the application of manure.

4.1.4. Relationship between N_2O emissions and soil conditions

The relationship between soil N dynamics and N_2O emissions remains unclear in the literature. For example, Alsina et al. (2013) found no correlation between emitted N_2O and soil mineral N in an almond orchard, while Yang et al. (2022) observed a correlation in a Chinese citrus orchard. In our study, the correlation was found to be weak. While the peak of soil NH_4^+-N in the CS and ACS treatments could potentially explain the initial N_2O emission peaks in these treatments, we did not find a significant correlation between these parameters. However, a negative correlation was found between soil NO_3^--N and N_2O emissions in the CS treatment which, along with low NO_3^--N levels in this treatment, suggest a direct loss of NO_3^--N as N_2O. Concurrently, the lower N values in the CTRL, CsM and IR treatments could also potentially explain the lower N_2O emissions and absence of N_2O peaks in these treatments, however, no correlations were found. Therefore, under the conditions of this study, it was not possible to establish a direct and consistent correlation between soil N and N_2O emissions. This is further supported by the high NO_3^--N content in the PM treatment, which did not lead to increased N_2O emissions or peaks.

In the context of soil conditions, previous studies have observed that high temperatures (Viguria et al., 2015) and soil moisture, often indicated by higher water-filled pore space, promote denitrification processes (Fangueiro et al., 2015b) and consequently N_2O emissions (Chadwick et al., 2000a; Akiyama et al., 2004; Alsina et al., 2013). While this experiment provides several indications that soil moisture and temperatures influenced N_2O emissions, their impact was inconsistent across the study years and treatments. Specifically, in 2021, N_2O emissions in the CS and ACS treatments demonstrated a positive correlation with soil moisture and a negative correlation with soil temperature (Supplemental Fig. S8). In contrast, in 2022, N_2O emissions in the CTRL treatment showed a negative correlation with soil moisture and a positive correlation with soil temperature. In the IR treatment, only soil temperature seemed to impact N_2O, showing a positive correlation. These findings indicated that the relationship between soil conditions and N_2O emissions were not consistent, implying the presence of underlying mechanisms other than soil moisture and temperature, such as N availability for nitrification or denitrification reactions (Rochette et al., 2008).

4.2. Carbon dioxide

4.2.1. Comparison between growing seasons (impact of soil conditions)

The dynamics of CO_2 emissions in the two campaigns exhibited some similarities, with higher emissions observed at the beginning of the experiment, particularly in the manure-amended treatments. However, a notable difference between the two years was observed during the dormant stage: while CO_2 emissions remained at low values in the 2021 dormant stage, they increased again during the 2022 dormant stage. This increase in 2022 was observed across all treatments, including in the IR treatment, and was likely due to a rise in soil moisture following the December and January rains (Supplemental Fig. S2). This result aligns with existing literature, which shows that soil moisture levels positively influence CO_2 emissions (Leytem et al., 2019; Shakoor et al., 2021; Hou et al., 2021). In 2021, CO_2 emissions showed a strong correlation with soil moisture in all treatments except the CTRL (Supplemental Fig. S8). Surprisingly, in 2022, only the IR treatment was correlated with soil moisture. This finding supports the conclusion that CO_2 emissions in the IR treatment increased due to higher soil moisture levels in 2022. Conversely, the other treatments did not exhibit a correlation with soil moisture in this campaign, as the higher emissions in the manure-amended treatments occurred after manure application, coinciding with a drier period.

Other studies have shown that soil temperature in orchards is equally an important factor at increasing CO_2 emissions (Yang et al., 2022). However, in this case, it seems that CO_2 emissions decreased with increased soil temperature, due to very dry conditions observed during the warmer period that limited CO_2 emissions. A strong negative correlation between CO_2 emissions and soil temperature levels confirmed this finding in 2021 (Supplemental Fig. S8). In 2022, this negative correlation was only observed in the IR treatment. In conclusion, CO_2 emissions were highest under wet conditions and lower during the warm and dry conditions, which aligns with findings from Mediterranean regions (Steenwerth et al., 2010). Nevertheless, in 2022, CO_2 emissions in the CTRL treatment exhibited a positive correlation with soil temperature.

The magnitude of the CO_2 emissions also varied between 2021 and 2022, with higher values in 2021, mirroring the pattern observed with N_2O emissions. This discrepancy can also be attributed to differences in soil conditions, particularly moisture. For instance, during the initial 72 days when emissions were the highest, soil moisture was higher in 2021 compared to 2022, averaging 218 mm and 188 mm, respectively.

4.2.2. Impact of fertilisation treatments on CO_2 emissions

After the initial peak in CO_2 emissions in the manure-amended treatments, there was a subsequent period with elevated CO_2 emissions. This occurred between day 45 and 70 in 2021 and between day 34 and 64 in 2022. During this period, the emissions in the CTRL treatment also increased, indicating that the application of MF also impacted CO_2 emissions. This observation was further confirmed by the higher CO_2 emissions in the CTRL treatment compared with the IR treatment. A similar trend was observed in a Chinese peach orchard (Cheng et al., 2017). Nevertheless, consistent with our experiment, the authors also found that the combined application of manure and MF resulted in generally higher CO_2 emissions compared with the exclusive application of MF (Cheng et al., 2017). Higher CO_2 emissions in manure treatments has been documented in other experiments (Fangueiro et al., 2015a; Cheng et al., 2017; Leytem et al., 2019), attributed to the application of organic C, which stimulated microbial activity and consequently increased emissions. The impact of manure application was particularly noticeable during the 2021 campaign, which was further confirmed by a significant correlation found between CO_2 emissions during the orchard's active stage and the amount of OM applied ($r = 0.55$, $p < 0.05$). While in 2022, no correlation was found between CO_2 emissions and the quantity of OM applied.

PM and CsM treatments exhibited the highest CO_2 emissions, particularly during the 2021 active stage (Table 2). Conversely to 2021, PM resulted in higher CO_2 emissions than CsM in 2022. This difference can be attributed to several factors. Poultry manure contains greater amounts of readily decomposable carbon compounds, such as volatile fatty acids (VFA), compared to cattle manures (Zhou et al., 2017), which could have stimulated microbial activity and thus explain the higher CO_2 emissions in this treatment. Additionally, this difference between the two treatments can also be attributed to varying amounts and forms of applied N, which could have also stimulated microbial activity and consequently CO_2 emissions. Shakoor et al. (2021), in a meta-analysis, confirmed that poultry manure emits more CO_2 after soil application compared with the application of pig or cattle manure.

Root growth and exudation are also known to promote CO_2 emissions (Leytem et al., 2019), which could explain the emissions observed in the IR treatment, characterised by herbaceous vegetation. Considering the orchard's active stage, emissions from the IR treatment were lower compared with the other treatments (except for CS). However, considering the emissions from both the active and dormant stage, cumulative CO_2 emissions in the IR treatment were not significantly different from those of the fertilised treatments (CTRL, CS, ACS, CsM). This lack of significant differences considering the entire measurement period was attributed to emissions during the dormant season, which resulted from continuous growth of the vegetation, as the vegetation is not cut during this time. Additionally, the dormant stage coincides with the rainy season in this region, further promoting vegetation growth and CO_2 emissions, as previously confirmed by a significant correlation with soil moisture (Supplemental Fig. S8). The emissions from this stage were found to significantly contribute to the total CO_2 emissions, underscoring the importance of measuring greenhouse gas emissions throughout the crop cycle, not just during the actively growing stage.

4.3. Methane

4.3.1. Comparison between untreated slurry and acidified slurry

In both years, a peak of CH_4 emissions in the CS treatment was observed immediately after manure application. This peak was also observed in the ACS treatment in 2022, although it was significantly smaller than that of CS. These initial peaks might be attributed to the release of CH_4 produced during slurry storage rather than methanogenesis in the soil. This is due to the anaerobic conditions during storage, created by the slurry's liquid consistency, and the presence of carbon substrates that allow the formation of CH_4 (Chadwick et al., 2000a; Viguria et al., 2015; Fangueiro et al., 2015a, 2015b). Chadwick et al. (2000a) also suggested that CH_4 can be produced within the first hours after manure application due to the degradation of short-chain VFA which are also contained within the slurry, further contributing to high emissions during this period. The same authors mentioned that more than 90% of CH_4 was emitted during the first 24 h of the experiment. A similar proportion was observed in this experiment, as CH_4 was only emitted during the first six days in 2021 and during the first four days in 2022, while emissions were non-existent or negative for the remainder of the experiment.

The reduced CH_4 peak in the ACS treatment during both the 2021 and 2022 campaigns, compared with CS, can be attributed to the mitigation effect of acidifying the slurry. Methanogenic communities are highly sensitive to pH ranges outside the neutral range (Beeman and Suflita, 1990; Ye et al., 2012; Hou et al., 2017), thus the acidification process likely suppressed methanogenesis, leading to lower CH_4 emissions. Notably, this mitigation effect was much more effective in 2021, where no CH_4 peak was observed in ACS, and ACS resulted in significantly lower cumulative CH_4 emissions compared with the CS treatment. Whereas in 2022, ACS also showed an initial CH_4 peak and resulted in statistically the same cumulative CH_4 emissions as CS. However, the observed difference between 2021 and 2022 could be attributed to variations in the acidification process. For instance, greater agitation during acidification in 2021 may have facilitated the release of trapped CH_4 in the slurry, resulting in lower CH_4 emissions during the 2021 campaign.

Comparing with the literature, Viguria et al. (2015) reported higher daily CH_4 fluxes of up 1456.2 mg C m^{-2} day^{-1} with dairy cow slurry, due to a higher application rate in their study. In contrast, Chadwick et al. (2000a), also using dairy cow slurry, observed lower CH_4 peaks of around 144 mg C m^{-2} day^{-1}, which is notably lower than the peaks observed in this experiment.

4.3.2. CH_4 oxidation

The other treatments did not present the initial CH_4 peak and, throughout the trial, either showed no CH_4 emissions or oxidation of

CH_4, as indicated by the negative emissions. The absence of CH_4 emissions in the solid manures (PM and CsM) could be explained by their higher dry matter content, which likely prevented the formation of anaerobic conditions during manure storage and in the soil after application, thereby inhibiting CH_4 production. Simultaneously, CH_4 oxidation in these two treatments can be attributed to a higher availability of O_2 (Gao et al., 2014), likely promoted by the dry matter content of the manures. In fact, CH_4 emissions exhibited a negative correlation with the dry matter content of manure and slurry in 2021 ($r = -0.52$, $p < 0.05$) and 2022 ($r = -0.51$, $p < 0.05$). Additionally, the presence of methanotrophic communities, which metabolise CH_4, could contribute to the observed CH_4 oxidation (Praeg et al., 2014).

These negative emissions were also observed in other experiments, mostly during the dry and hot period of the experiment, where soil acted as a CH_4 sink rather than a source (Chadwick et al., 2000a). For instance, in a citrus orchard with mineral fertilisation, CH_4 uptake was promoted by higher soil temperature and higher SOC content (Yang et al., 2022), which could have also influenced CH_4 oxidation in the present study. The experimental site experienced warm temperatures and had relatively high SOC values compared to standard Mediterranean values, suggesting favourable conditions for CH_4 oxidation. However, only CH_4 emissions in the CS treatment during the 2021 campaign showed a negative correlation with soil temperature (Supplemental Fig. S8). Additionally, during the 2022 campaign, CH_4 emissions were negatively correlated with SOC values ($r = -0.57$, $p < 0.01$), aligning with the findings of Yang et al. (2022). Furthermore, the amount of OM applied through the manures and slurries was negatively correlated with CH_4 emissions in both the 2021 ($r = -0.51$, $p < 0.05$) and 2022 ($r = -0.89$, $p < 0.001$) campaigns. These correlations help explain the consistently higher CH_4 oxidation observed in the PM and CsM treatments compared with CTRL, as the application rate of OM was higher in these two treatments (Supplemental Table S4).

CH_4 oxidation was notably higher in 2021 during both active and dormant stages ($p < 0.001$) compared to 2022. This difference may be attributed to an increase in soil N content in 2022 (Esteves et al., 2023), likely resulting from the third consecutive year of manure application. The increase in soil N levels could have led to competition between NH_4^+-N and CH_4 for oxidizing bacteria or even inhibited enzyme activity due to soil NO_3^--N, thereby impacting CH_4 oxidation (Bodelier and Laanbroek, 2004; Yang et al., 2022). However, further assessments are required to fully understand the underlying causes for this difference.

4.4. Yield-scaled GHG emissions

Reporting GHG emissions relative to crop productivity is important for assessing the environmental impact of agricultural practices on a per-unit basis. Yield-scaled emissions provide insights into the efficiency of different fertilisers and their overall contribution to GHG emissions per unit of crop produced. The results from the yield-scaled emissions did not substantially deviated from the previously made observations. Specifically, replacement of MF with animal manures in 2021 resulted in higher GWP per kilogram of fruit produced (Table 3). This was mostly due to higher CO_2 and N_2O emissions in the manure-amended treatments, along with higher CH_4 emissions in the CS treatment. In contrast, in 2022, there were no significant differences in yield-scaled GWP between treatments. Partial replacement of MF with animal manures in this year did not lead to an increase in the orchard's yield-scaled GWP compared to the conventional practice (CTRL).

In a citrus orchard, Zhou et al. (2022) reported yield-scaled N_2O emissions ranging from 39.82 to 232.9 mg N kg^{-1} fruit, observing a decrease of 38.9% and 50% when mineral fertilisers were replaced by 30 or 25% with organic manure, respectively. This contrasts with our results, where the replacement of MF with manure led to increased yield-scaled N_2O emissions, although only in 2021. In 2022 there was an exception with the CsM treatment, which resulted in a yield-scaled N_2O emission of 59.49 mg N kg^{-1} of fruit, representing a 6% decrease compared with CTRL, albeit not significantly lower. Furthermore, in an apple orchard, Sompouviset et al. (2023) demonstrated yield-scaled N_2O emissions of 50 mg N kg^{-1} fruit in NPK + goat manure treatment, and 70 mg N kg^{-1} fruit in the NPK treatment. These findings are more aligned with our results, particularly considering the CsM and CTRL scenarios.

In their study, Sompouviset et al. (2023) also investigated CH_4 emissions in the orchard and reported yield-scaled CH_4 emissions of -100 g C kg^{-1} fruit and -110 mg C kg^{-1} fruit in the manure + NPK and just NPK treatments, respectively. Our experiment yielded contrasting results, with significantly higher oxidation potential for CH_4 emissions observed in the manure-amended treatments, specifically in the PM and CsM treatments, compared with the CTRL treatment. However, the replacement with ACS and CS increased yield-scaled CH_4 emissions compared with CTRL, resulting in positive emissions, while CTRL resulted in neutral yield-scaled CH_4 emissions.

4.5. Emissions factors for N_2O emissions

The EF in the CS (1.77%) and CsM (0.68%) treatments did not differ significantly from the EF in the CTRL (0.79%) treatment. However, the EF in PM (4.40%) and ACS (3.97%) treatments were significantly higher, indicating that the application of these two materials presented high potential for N losses through N_2O emissions. The new updated IPCC emissions factors for N_2O emissions consequent from cattle, poultry and pig application is 0.4% (IPCC, 2019), which is substantially smaller than the values presented here. Indeed, emission factors for N_2O emissions can vary widely depending on various factors such as the type of manure applied, application rate and technique, as well as climate and soil characteristics (Velthof et al., 2003; van Groenigen et al., 2004; Viguria et al., 2015). For example, Viguria et al. (2015) reported an EF of 4.4% with dairy slurry application on rapeseed, whereas van Groenigen et al. (2004) found EFs ranging from 0.51% to 1.21% with cattle slurry application on sandy and clay soils, respectively, during silage maize cultivation. The authors also studied the effects of MF combined with slurry application, reporting EF of 0.26% and 1.69% in sandy and clay soils, respectively (van Groenigen et al., 2004). Similarly, in a peach orchard study by Cheng et al. (2017), which investigated the combined application of MF and organic manure, it was found that 1.32% of applied N was emitted in the treatment with only MF, while 1.86% was emitted in the treatment with the combined use of fertilisers. In a Mediterranean apple orchard, Fentabil et al. (2016) obtained an EF for N_2O emissions ranging from 0.39% to 0.80% of applied N consequent from calcium nitrate fertilisation. Such variations underscore the importance of considering multiple factors when estimating emission factors for N_2O and emphasise the need for site-specific assessments to accurately estimate emission factors.

However, comparing our results with other orchards studies proved to be challenging. Gu et al. (2019) conducted a review on N_2O emissions in orchards and found that these agricultural systems are underrepresented in EF estimations, due to multiple reasons like insufficient measurement data. This highlights the importance of conducting field trials to provide empirical evidence for emission factor determination, facilitating more precise estimations of global budgets for N_2O emissions and enabling target-specific mitigation measures (Gu et al., 2019). Moreover, Mediterranean regions are often overlooked in reviews of N_2O emissions (Cayuela et al., 2017). To the best of our knowledge, there are no defined EF for Portuguese orchards. Hence, the data presented here represents an initial step toward establishing more accurate standardised N_2O emissions on a regional scale for apple orchards. It is also important to consider N_2O emissions during the dormant stage, as included in our study, when determining emission factors (Fentabil et al., 2016).

4.6. GWP of each fertilisation scenario

In the 2022 campaign, the additional measurement taken in the orchard's interrow provided a more precise estimation of the orchard's GWP, by considering both the fertilised areas, represented by the row of trees, and the unfertilised area, situated between the rows.

The results showed that during the active stage of the orchard, there were no significant differences in the orchard's GWP between the fertilisation scenarios established in this study (Table 4). During this period, the CTRL treatment was receiving mineral fertilisers through the fertigation system which impacted GHG emissions comparing with the unfertilised treatment (IR), as previously observed (Table 2). This may explain the lack of differences between the CTRL and the manure-amended scenarios, as all were receiving fertilisers to match crop requirements. Overall, these findings are promising, suggesting that replacing mineral fertilisers did not have a significant impact on the orchard's GWP during the active period.

However, considering both the active and the dormant stage of the orchard, replacing MF with manures resulted in higher GWP compared with the CTRL, especially PM. This could be attributed to the long-term availability of N and to high C contents in these organic materials (Ginting et al., 2003). Under optimal conditions, these factors can promote soil biochemical reactions, resulting in increased GHG emissions. Nevertheless, replacement with CS or CsM lead to statistically the same GWP as using only mineral fertilisers, highlighting the potential of these two animal manures as MF replacements.

Although replacement with manures may potentially increase the orchard's GWP, depending on the type of manure used as replacement, it is important to note that manure application also increased soil organic carbon, as previously reported (Esteves et al., 2023). This effect can enhance C sequestration, potentially offsetting the increased GWP (Leytem et al., 2019). The authors found that, considering the increased SOC, manure treatments resulted in a net negative GWP, whereas mineral fertiliser treatments resulted in a neutral GWP. It is worth noting that the emissions associated with the production and mining of mineral fertilisers were not considered in this study, which would likely increase the GWP associated with their use. Moreover, manure has a long-lasting effect, leading to increases in soil microbial biomass and mineralisable N even four years after application (Ginting et al., 2003). This long-term impact is particularly important in Mediterranean soils, which are high in carbonate and low in organic matter and are prone to soil erosion due to weather conditions (Calleja-Cervantes et al., 2017). Therefore, from an holistic perspective, there is considerable potential for using manures to replace mineral fertilisers in a Mediterranean context, with minimal impacts on the orchard's GWP.

5. Conclusions

The results showed that the application of manures as replacement for MF increased GHG emissions, however, the magnitude of these effects depended on the manure's characteristics, like dry matter content and C to N ratio, and on the amount of nutrients and organic matter applied. Overall, the application of moisture-rich slurries was associated with higher N_2O and CH_4 emissions, while manures with higher dry matter content and OM, such as PM and CsM, increased the CH_4 oxidation potential (negative CH_4 emissions) and CO_2 emissions compared with CTRL. Additionally, the quality of N present in PM seemed to stimulate N_2O and CO_2 emissions in this treatment, although further assessments are required to confirm such association.

Moreover, soil conditions were found to influence GHG emissions, although inconsistently throughout the two years of measurement. Acidification of slurry proved effective in reducing CH_4 emissions; however, this efficacy varied between the years, likely due to differences in the acidification processes. Also, in the second year, acidification led to an emission trade-off by increasing N_2O emissions compared to the CS treatment.

The application of mineral fertilisers also impacted GHG emissions, which was observed by the higher emissions in the CTRL treatment compared with the unfertilised interrow. Replacement with CS and CsM resulted in comparable GWP in relation to the CTRL, indicating high suitability of these manures as replacements for mineral fertilisers, using a replacement rate of almost 60%. About 1.8% and 0.7% of applied nitrogen are lost as N_2O in the CS and CsM treatments, respectively, which was comparable to that of the CTRL treatment.

The replacement of mineral fertilisation with manure holds significant potential to promote nutrient cycling in agriculture, reduce reliance on synthetic nutrient production, and sustain high crop productivity. Furthermore, this experiment provided important field data, which included emissions from different fertilisation scenarios, interrow spaces, and the complete plant growth cycle. Such information is important for regional N budgets and the implementation of region-specific mitigation measures to support agricultural sustainability.

CRediT authorship contribution statement

Catarina Esteves: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Eva Costa:** Writing – original draft, Investigation, Data curation. **Miguel Mata:** Investigation, Data curation. **Mariana Mota:** Formal analysis. **Miguel Martins:** Resources. **Henrique Ribeiro:** Supervision, Methodology, Conceptualization. **David Fangueiro:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

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

Data will be made available on request.

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Appendix A. Supplementary data

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