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Direct and Residual Impacts of Olive-Mill Waste Application to Rice Soil on Greenhouse Gas Emission and Global Warming Potential under Mediterranean Conditions

Damián Fernández-Rodríguez [1,*], David Paulo Fangueiro [2], David Peña Abades [3], Ángel Albarrán [1], Jose Manuel Rato-Nunes [4] and Antonio López-Piñeiro [5]

1 Área de Producción Vegetal, Escuela de Ingenierías Agrarias—IACYS, Universidad de Extremadura, 06071 Badajoz, Spain; angliso@unex.es
2 LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal; dfangueiro@isa.ulisboa.pt
3 Área de Edafología y Química Agrícola, Escuela de Ingenierías Agrarias—IACYS, Universidad de Extremadura, Ctra de Cáceres, 06071 Badajoz, Spain; davidpa@unex.es
4 Instituto Politécnico de Portalegre, Escola Superior Agrária de Elvas, 7300-110 Portalegre, Portugal; ratonunes@ipportalegre.pt
5 Área de Edafología y Química Agrícola, Facultad de Ciencias—IACYS, Universidad de Extremadura, Avda de Elvas s/n, 06071 Badajoz, Spain; pineiro@unex.es
* Correspondence: dfernandez@unex.es

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Abstract: The olive oil industry produces high amounts of waste, which need to be valorized in a more sustainable way as an alternative to its traditional use as an energy source, with high associated CO_2 emissions. Rice (*Oryza sativa* L.) is one of the most important crops for global food security; however, the traditional cropping systems under flooding lead to an important decrease of soil quality, as well as relevant emissions of greenhouse gases (GHG). The aim of this study was to assess the GHG emission from rice fields amended with composted two-phase olive mill waste (C-TPOW), in Mediterranean conditions. A field experiment was carried in rice cultivated by the traditional system, either unamended (Control) or amended with C-TPOW (Compost). GHG emissions were measured over three years following a single C-TPOW application (80 Mg ha^{-1} only in the first year of study), so that the results found in the first and third years correspond to its direct and residual effects, respectively. Compost decreased CO_2 emissions relative to Control by 13% and 20% in the first and third year after C-TPOW application, respectively. However, in the case of CH_4 and N_2O, increases in the total cumulative emission were recorded in Compost relative to Control throughout the study, in agreement with the highest β-glucosidase and urease activity observed in the amended soil. The values of global warming potential (GWP) and yield-scaled GWP increased by 14% and 11%, respectively, in Compost relative to Control in the first year, but no significant differences between treatments were observed three years after application for GWP and yield-scaled GWP. Therefore, the use of C-TPOW as soil amendment in rice fields could be a good option since its impact on GHG emissions seems to decrease over time, while the benefit for soil remained clear even after 3 years.

Keywords: carbon dioxide; methane; nitrous oxide; organic amendment

1. Introduction

Olive groves represent a high economic and social relevance in the Mediterranean region. The modernization of the olive oil production has led to the development of more intensive planting schemes, such as the Super High Density (SHD) [1]. However, this system of olive oil production also increased the amount of two-phase olive mill waste (TPOW) produced the main sub-product of this activity. Every year, around 9.5 million Mg of TPOW are produced in the Mediterranean region, thereby constituting a serious problem for the olive oil industry [2]. Indeed, TPOW discharge in ecosystems is a serious environmental issue in many countries of the Mediterranean basin [3]. Traditionally, TPOW has mainly been used as fuel for small boilers. However, this practice has been restricted due to consequent environmental impacts as smoke emission from burning of this waste [4]. It is also important to stress that many olive oil industries pointed out TPOW as the main limitation for production intensification since there is low capacity of TPOW treatment. Thus, new alternatives are required for appropriate disposal or recycling of TPOW [5].

The Mediterranean region is also the largest rice-producer in Europe, but production is mainly carried out under conventional tillage methods and flooding irrigation system, which lead to well-known negative environmental impacts such as methane emissions and soil degradation [6]. According to Patra et al. [7] the intensive tillage operations can reduce soil quality and productivity related with decreases of the organic matter and nutrients contents in soil, as well as modification of the soil structure. Hence, agronomic practices contributing to increase the soil organic matter content are strongly recommended for Mediterranean agro-ecosystems [8]. Given that TPOW contains a high amount of organic matter (>85%), the use of compost of TPOW (C-TPOW) as an organic amendment for rice production in fields located near olive mills could be a good strategy to recycle this abundant sub-product and simultaneously improve soil properties [9].

Rice cultivation accounts for about 10% of global CH_4 emissions, being one of the main contributors to greenhouse gas emissions (GHG) [10]. In fact, positive and significant correlations had been observed in paddy soils between the amounts of CH_4 produced and the soil content in terms of total organic carbon and water-soluble organic carbon [11]. Therefore, the application of organic amendments such as C-TPOW on paddy soil could enhance CH_4 production by supplying available organic substrates [12].

However, a recent study hypothesized that short-term aerobic pre-digestion of green manured soils under dry soil conditions can lead to a reduction of methane emissions during flooded rice cultivation [13]. Hence, any amendment to rice fields should not increase CH_4 emissions but, on the contrary, should stimulate C sequestration. This could be achieved by the application of compost as C-TPOW, rich in carbon, that could improve soil organic carbon stock and lead to sequestration of atmospheric CO_2 [14]. However, the effect of C-TPOW application in rice fields on other gases as nitrous oxide (N_2O) or CO_2 is not known. Indeed, little is known regarding the effects of organic amendments' application on GHG emissions in rice fields [15]. In fact, to our knowledge, no studies provide information on GHG emissions in traditional rice fields of Mediterranean climate with application of C-TPOW. Therefore, the aim of this field experiment was to assess the effects of C-TPOW application in traditional rice fields on N_2O, CH_4, and CO_2 emissions under Mediterranean climatic conditions. Given that these effects could be time-dependent, we considered that the measurements of GHG emissions performed on the first and third years after C-TPOW application correspond to direct and residual impacts, respectively. Besides, since GHG emissions relies biological soils properties, the activity of different enzymes was also studied to assess their effects on GHG emissions and GWP.

2. Materials and Methods

The experimental field was located in the Vegas Bajas of Guadiana—Spain (38°55′ N, 6°57′ W). This region has a Mediterranean climate with <445 mm of precipitation, a maximum average temperature of 24 °C, and a minimum average temperature of 17 °C [16]. The experiment was carried out from spring 2015 to autumn 2017. The rice fields, under flooding practice for more than 11 years, had a total area of 5.35 ha and the experimental plots occupied 1520 m^2 (40 m length × 38 m width), more precisely 1080 m^2 without the protective buffer zone. Conventional rice cultivation techniques (deep plowing through hand rotavator and flooding) were followed over the three years of the study. The variety of rice (*Oryza sativa* L.) used was Gladio. Conventional tillage of the soil was carried out incorporating the crop residues of the previous year's harvest, taking advantage of the autumn rains to incorporate rice straw. The soil was classified as Hydragic anthrosol [17].

The granulometric analysis carried out showed a value of 20.9% clay, 28.8% silt, and 50.3% sand, leading to a classification of loam texture according to [18]. The main characteristics of the 0–20 cm soil layer are presented in Table 1.

Table 1. Effects of different management systems on soil physicochemical properties (0–20 cm depth). Mean value and standard error of 3 replicates.

	2015		2016		2017		Y	T	Y × T
	Control	**Compost**	**Control**	**Compost**	**Control**	**Compost**	**F-Values**	**F-Values**	**F-Values**
TOC (g kg^{-1})	10.9 aA (0.186)	20.3b AB (0.058)	13.4 aB (0.641)	22 bB (1.28)	13.9 aB (0.034)	19.1 aB (0.171)	6.84 *	218 ***	NS
WSOC (mg kg^{-1})	72.7 aA (4.57)	490 bC (6.24)	113 aB (3.28)	401 bB (19.8)	220 aC (2.88)	325 bA (7.68)	7.21 *	4389 ***	102 ***
FA (g kg^{-1})	0.848 aB (0.012)	1.02 bB (0.002)	0.773 aA (0.024)	0.784 aA (0.033)	1.03 aC (0.019)	1.19 bC (0.020)	139 ***	37.7 **	NS
HA (g kg^{-1})	1.49 abB (0.053)	1.60 bB (0.007)	0.659 aA (0.091)	1.25 bA (0.036)	1.48 aB (0.020)	1.88 bC (0.030)	108 ***	155 ***	NS
HI	13.7 bC (0.250)	7.89 aB (0.056)	4.91 aA (0.587)	5.70 aA (0.274)	10.7 bB (0.172)	9.84 aC (0.243)	204 ***	53.1 **	65.8 ***
pH	4.93 aA (0.026)	6.06 bB (0.052)	5.27 aB (0.009)	5.76 bA (0.003)	5.65 aC (0.012)	5.80 bA (0.023)	38.2 ***	1710 ***	142 ***
N (g kg^{-1})	0.770 aA (0.008)	1.61 bA (0.059)	1.47 aB (0.010)	2.31 bB (0.014)	1.85 aC (0.046)	2.48 bC (0.012)	661 ***	581 ***	NS
Eh (mV)	−110 aA (5.42)	−178 bA (10.9)	−143aB (10.7)	−200bA (7.31)	−173 aB (9.60)	−188 aA (3.19)	47.8 ***	18.4 *	24.7 **

TOC: Total Organic Carbon; WSOC: Water Soluble Organic Carbon; FA: Fulvic Acid; HA: Humic Acid; HI: Humification index; N: Total Nitrogen; Eh: Redox Potential. Rice cultivated by traditional tillage techniques (Control) and with application of C-TPOW (Compost). ANOVA factors are Y: Year; T: Treatment; Y × T: Interaction Year × Treatment. F-values indicate the significance levels * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, respectively, and NS: not significant. Different letters indicate differences ($p < 0.05$) between treatments in the same year (lower case letters) and between years within the same treatment (upper case letters).

2.1. Experimental Design and Agricultural Management Practices

The field was split into six plots of 18 m × 10 m, where a sowing rate of 180 kg ha^{-1} of rice was applied. Three repetitions of the following treatments were considered: 1. Rice cultivated by traditional tillage techniques (Control), and 2. Rice cultivated by traditional tillage techniques with application of C-TPOW (Compost). The plots were separated by ridges (35 cm high) with a 4 m wide protective buffer zone set up between adjacent plots. A C-TPOW from a national oil mill was used as organic amendments at an application rate of 80 Mg ha^{-1}, an application dose, which can be considered normal when organic amendment is applied from time to time. This application was carried out manually only once throughout the study and one month before the start of cultivation (April 2015), the C-TPOW was incorporated at a depth of 15–20 cm using disc harrow. The main properties of C-TPOW were as follows: Total Organic Carbon (TOC) content of 384 g kg^{-1}, Humic Acid (HA) 47.9 g kg^{-1}, Fulvic Acid (FA) 18.9 g kg^{-1}, Humification index (HI) 12.5, pH 7.71, Electrical Conductivity (EC) 2.32 dS m^{-1}, and Total Nitrogen (N) 21.7 g kg^{-1}. Therefore, the C-TPOW application rate of 80 Mg ha^{-1}, supplied approximately 31 Mg C ha^{-1} to soil.

In both treatments, regional management tasks were carried out, such as the permanent flooding of the rice crop throughout its biological cycle and deep plowing prior to sowing. The edges of the treatment plots, were created with a height of 40 cm to favor the containment of water in the treatments; the water level maintained in the treatments was about 10 cm. Each year, after harvest of the crop (October), four subsamples of soil were taken from each of the plots for soil properties determinations. The subsamples from each plot were mixed and homogenized to get a composite sample for every plot (i.e., total of 6 composite samples each year).

The fertilizers used were the same in both treatments and in all years of the study. Thus, composite fertilizer at a dose of 49.5 kg N ha^{-1}; 99 kg P_2O_5 ha^{-1}, and 148.5 kg K_2O ha^{-1} was applied before sowing as basal fertilization at rate of 550 kg ha^{-1}, and then two applications of urea as cover N fertilizer at a rate of 92 kg N ha^{-1} and 69 kg N ha^{-1} at tillering and at panicle initiation stages, respectively.

2.2. Analytical Methods

The granulometric analysis of soil was performed by destruction of the organic matter of the samples by means of hydrogen peroxide (6%). A sodium hexametaphosphate solution was used as a dispersant. The fine fractions (clay and silt) were determined by sedimentation following the Robinson pipette method [19]. The coarse fractions (sands) were obtained by sedimentation to subsequently determine the different sub-fractions by dry sieving. TOC of soil was measured by the wet oxidation method, with potassium dichromate and subsequent evaluation of the excess of ferrous ammonium sulfate [20]. Water Soluble Organic Carbon (WSOC) of soil, was determined by extraction with distilled water in a ratio of 1/100 (*w*/*v*) and then a partial oxidation of the carbon was carried out with 1N potassium dichromate in a sulfuric acid medium followed by quantification by spectrophotometry at =590 nm [21]. Redox potential (Eh) was measured in a saturated solution (1:2 (*w*/*v*) soil/water ratio) using a platinum electrode. Measurements of the soil pH, FA, and HA contents were also performed, as described by López-Piñeiro et al. [22]. N (Kjeldahl) was also measured as described by Sánchez-Llerena et al. [23]. Determinations of soil enzyme activities were carried out according to López-Piñeiro et al. [5] (Table 2). Briefly, for Dehydrogenase (DH) activity, 1 g of soil was incubated for 20 h at 20 °C in darkness with 0.20 mL of 0.4% 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium (INT) chloride as substrate. The activity of β-glucosidase (GL) was determined by incubating 1 g of soil with 4 mL of 25 mM 4-nitrophenyl-β-d-glucopyranoside in 0.1 M modified universal buffer (MUB) pH 6.0. To assay the urease (UR) activity, 2 mL of 0.1 M pH 7.0 phosphate buffer and 0.50 mL of 1.07 M urea were added to 0.50 g of soil and incubated for 1.5 h at 30 °C.

Table 2. Effects of different management systems on soil enzyme activities (0–10 cm depth). Mean value and standard error of 3 replicates.

	2015		2016		2017		Y	T	Y × T
	Control	Compost	Control	Compost	Control	Compost	F-Values	F-Values	F-Values
DH (μg INTF g^{-1} h^{-1})	0.560 aA (0.015)	1.42 bB (0.058)	0.487 aA (0.033)	0.810 bA (0.165)	0.820 aB (0.064)	0.963 aA (0.018)	NS	44.1 *	11.9 *
GL (μmol pNP g^{-1} h^{-1})	0.123 aA (0.012)	0.780 bB (0.160)	0.110 aA (0.015)	0.390 bA (0.012)	0.077 aA (0.011)	0.267 bA (0.021)	NS	43.8 *	NS
UR (μg NH_4^+ g^{-1} h^{-1})	4.62 aA (1.15)	14.1 bA (2.12)	6.84 aA (1.36)	24.4 bB (1.31)	8.79 aA (2.47)	26.8 bB (1.85)	12.1 *	161 ***	NS

DH: Dehydrogenase Activity; GL: β-glucosidase Activity; UR: Urease Activity. Rice cultivated by traditional tillage techniques (Control) and with application of C-TPOW (Compost). ANOVA factors are Y: Year; T: Treatment; Y × T: Interaction Year × Treatment. F-values indicate the significance levels * $p < 0.05$; *** $p < 0.001$, respectively, and NS: not significant. Different letters indicate differences ($p < 0.05$) between treatments in the same year (lower case letters) and between years within the same treatment (upper case letters).

2.3. Greenhouse Gas Emission Measurements

Greenhouse gas emissions were manually assessed throughout the rice growing cycle (May to October) using the static chamber techniques following the procedure described by Fangueiro et al. [10]. In each treatment, six high-density polyethylene chambers (40 cm diameter and 30 cm height) were placed in the soil, immediately after tillage operations. Each chamber was buried at a depth of 10 cm in the soil. The upper end of the chambers was open to the air and was closed at the time of each sampling with an airtight lid. Inside each chamber, a fan was introduced with a wiring system that would allow them to be turned on from outside the chamber, with the aim of homogenizing the air before each measurement.

Furthermore, it is important to note that there were no plants in the chambers. Air samples were collected from the chamber headspace immediately after closure and 30 min after setting up the chamber. Gas sampling in each chamber was performed using a 60 mL syringe; 20 mL of each gas sample were then transferred to a glass vial sealed with a Teflon septum. At each sampling, the temperature inside the chamber was recorded. Air samples were stored in the dark and kept at a temperature of 20 °C, until their subsequent analysis, which was performed as soon as possible without exceeding 48 h of storage in any case. The gas samples were analysed according to Ekeberg et al. [24] using a mass selective detector (MS), model 5973 (Agilent, Santa Clara, CA, USA), connected via an interface to an Agilent 6890 N gas chromatograph (GC). The column used was a CP Pora Plot Q capillary column (Variant Inc., Palo Alto, CA, USA) with dimensions of 27.5 m, 0.32 mm, and 10 mm. Helium (Praxair, Madrid, Spain) was used as the carrier gas at a flow rate of 2 mL min^{-1}. The volume injected into the GC was 1 mL through direct injections (splitless mode). The MS was used in selected ion monitoring (SIM) mode. The selected ions were m/z 22 for CO_2, m/z 15 for CH_4, and m/z 44 for N_2O. The retention times for CO_2, CH_4, and N_2O were 1.46, 1.19, and 1.61 min, respectively. Thus, the total time for one sample was set to 3.3 min. This method was tested against certified gas references; the analytical data gave a precision of ±3%.

The standards gases, which were representative of gas concentration expected, were analysed alongside field samples. Five standard gases were purchased from BOC (British Oxygen Company, Woking), and their compositions were: Standard 1: 0.329 ppm N_2O, 2.05 ppm CH_4, and 297 ppm CO_2; Standard 2: 0.948 ppm N_20, 5.18 ppm CH_4, and 593 ppm CO_2; Standard 3: 1.59 ppm N_2O, 10.18 ppm CH_4, and 1198 ppm CO_2; Standard 4: 5.23 ppm N_2O, 50.1 ppm CH_4, and 2467 ppm CO_2; Standard 5: 40.2 ppm N_2O, 101.2 ppm CH_4, and 5022 ppm CO_2.

2.4. Calculations and Global Warming Potential

The N_2O, CH_4, and CO_2 emission fluxes (F, ppm min^{-1}) were first calculated based on the difference in their respective concentrations at 0 and 30 min using Equation (1):

$$F = \frac{(Ct30 - Ct0)}{30} \quad (1)$$

where C (ppm) is the gas concentration at time 0 or 30 min [25,26]. CO_2, CH_4, and N_2O emission rates (ER, g C or N ha^{-1} d^{-1}) for each sampling period were calculated using Equation (2).

$$ER = \frac{F \times M}{V \times \left(\frac{273+T}{273}\right)} \times h \times k \times 10000 \quad (2)$$

where F is the gas emission flux calculated above (ppm min^{-1}), M is the gas molecular weight (44 g mol^{-1} for CO_2 or N_2O and 16 g mol^{-1} for CH_4), V is the volume of an ideal gas (0.022 m^3 mol^{-1}), T is the temperature during the sampling period (in °C), h is the height of the chamber (m), and k is the time corrected for a 1-day duration (1440 min). The cumulative emission was estimated by averaging the flux between two samplings and multiplying by the time interval. The linearity of the gas concentration increase was assessed at the beginning of the experiment and then checked regularly, especially after each action described in Figures 1–3. For this, the procedure described below for gas fluxes determination was followed and air samples from each chamber were taken at 0, 15, 30, 45, and 60 min. This procedure was performed for one replicate of each treatment. Based on the results obtained on the first day, an enclosure time of 30 min was chosen for all measurements.

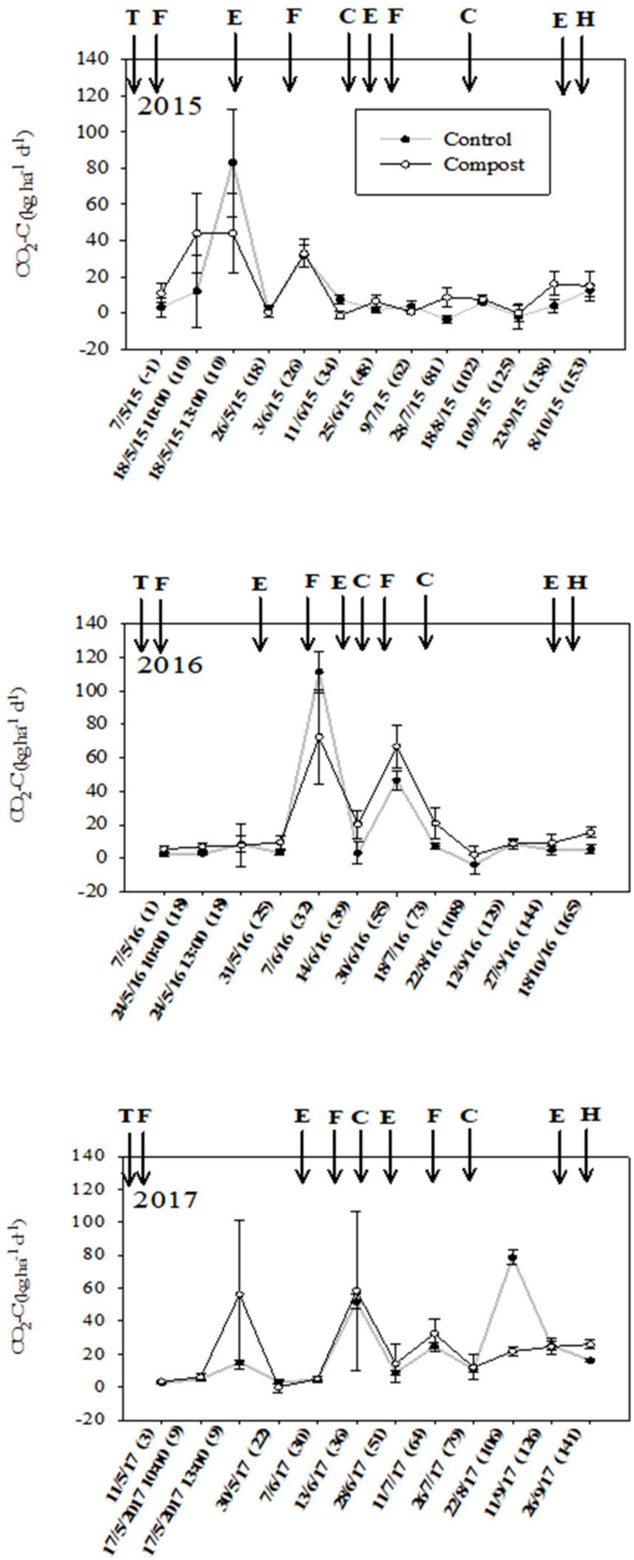

Figure 1. Effect of different management systems on CO_2 emission rates throughout rice growing cycle. The error bars represent the standard error of the mean (n = 3). T: Tillage and fertilizer, C: Cover fertilizer, F: Flood, E: Emptying of terraces, H: Harvest. The numbers in parentheses on the *x*-axis indicate days after sowing (DAS).

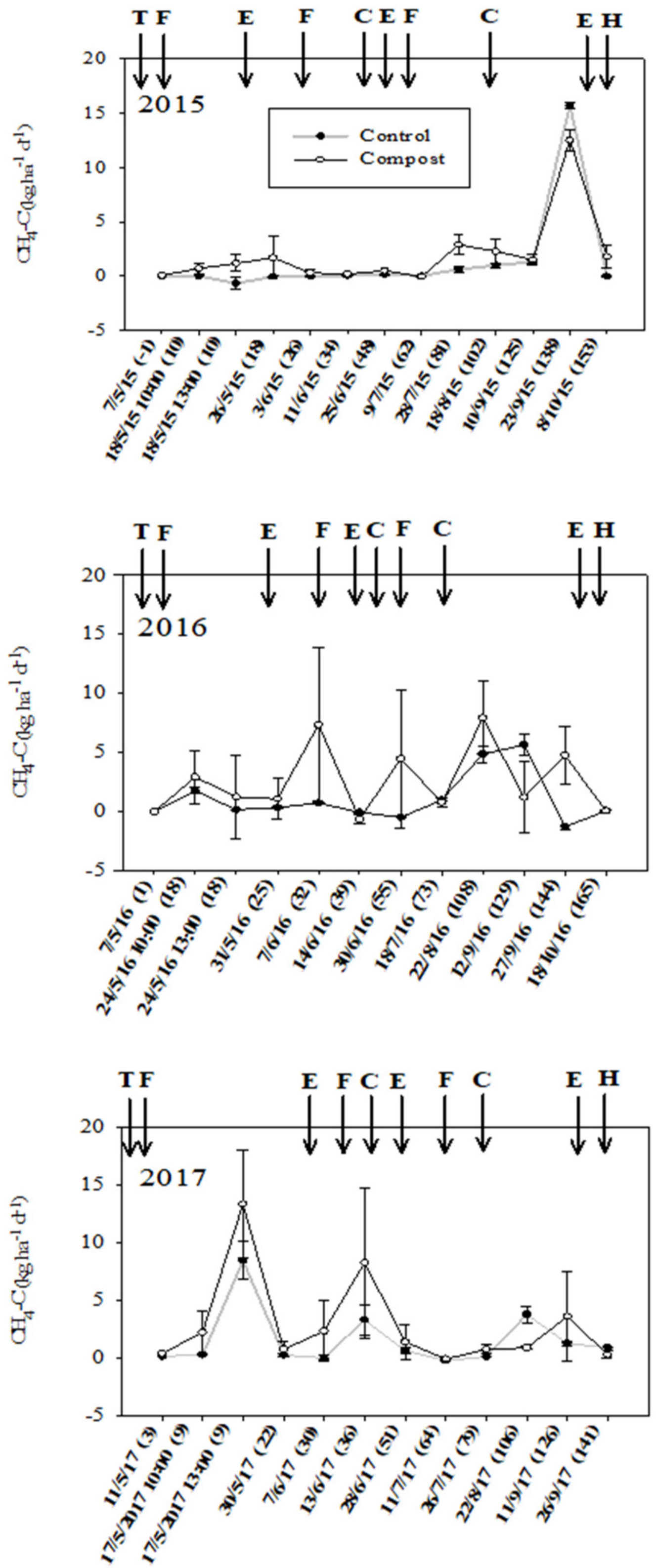

Figure 2. Effect of different management systems on CH_4 emission throughout the rice growing cycle. The error bars represent the standard error of the mean (n = 3). T: Tillage and fertilizer, C: Cover fertilizer, F: Flood, E: Emptying of terraces, H: Harvest. The numbers in parentheses on the *x*-axis indicate days after sowing (DAS).

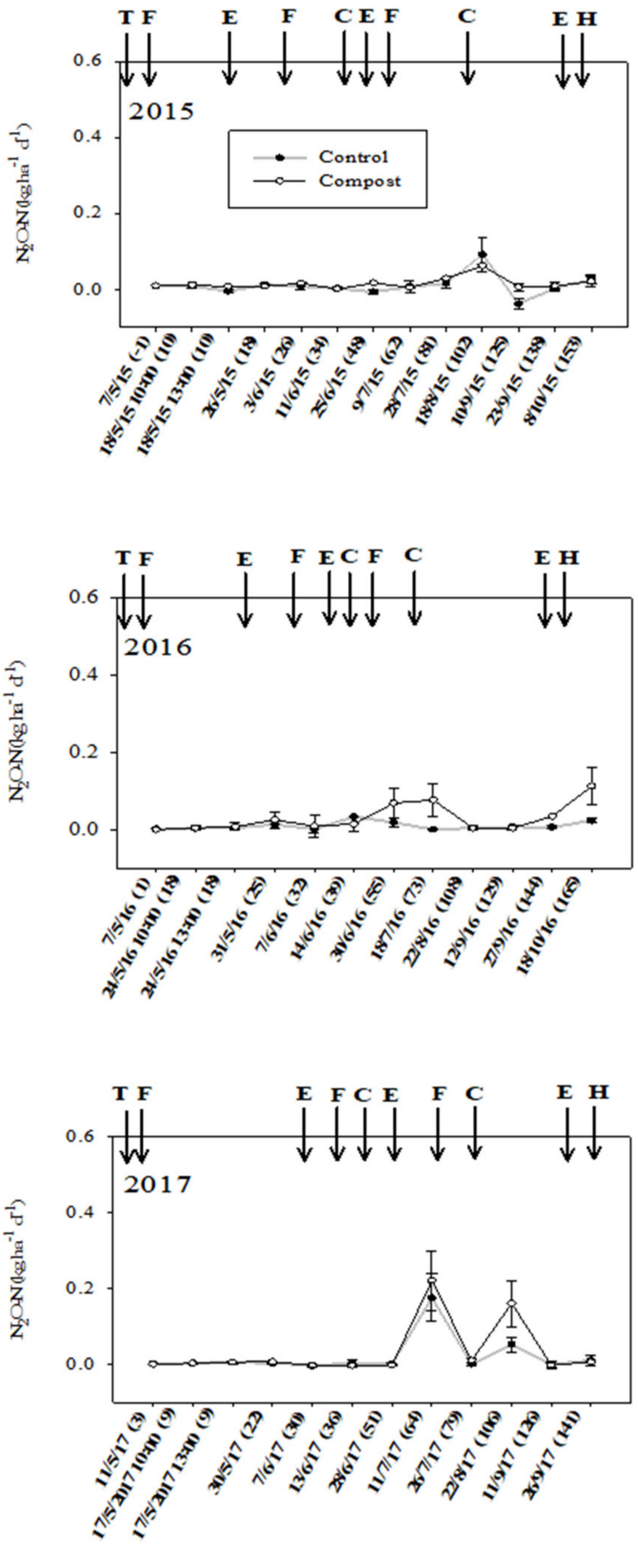

Figure 3. Effect of different management systems on N_2O emission throughout the rice growing cycle. The error bars represent the standard error of the mean. T: Tillage and fertilizer, C: Cover fertilizer, F: Flood, E: Emptying of terraces, H: Harvest. The numbers in parentheses on the *x*-axis indicate days after sowing (DAS).

The net global warming potential (GWP, Mg CO_2 eq ha^{-1}) for both treatments were calculated using the GWP coefficients based on a 100-year time frame [27], using Equation (3).

$$\text{GWP}\left(\text{Mg CO}_2\text{eq ha}^{-1}\right) = (\text{CO}_2) + (\text{CH}_4 \times 28) + (\text{N}_2\text{O} \times 265) \quad (3)$$

where CO_2 is the cumulated amount of CO_2 emitted in kg CO_2 ha^{-1}, CH_4 is the cumulated amount of CH_4 emitted in kg CH_4 ha^{-1}, and N_2O is the cumulated amount of N_2O emitted in kg N_2O ha^{-1}.

The GWP was also expressed per grain yield unit (yield-scaled GWP) as described by Fangueiro et al. [10]. The yield data have been analysed and extensively discussed by Peña et al. [28].

2.5. Statistical Analysis

Statistical analysis was performed using SPSS software package version 22.0. A two-way ANOVA with repeated measures on the factor "year" was used to analyse the greenhouse gas parameters, and the Student's t-test was used to test the significance of the differences between treatments. The Pearson correlation coefficient was used to study possible correlations between cumulative GHG emission rates and different soil properties (n = 18). All the statistical analyses were carried out with a significance level of 0.05.

3. Results and Discussion

3.1. CO_2 Emissions

The effects of C-TPOW application to soil on CO_2 emission rates over the rice growing cycle during the years 2015, 2016, and 2017 are shown in Figure 1. The most significant emission occurred in the drainage intervals of the fields, a situation that could be caused by a greater microbial activity during these intervals. Thus, Sahrawat [29] indicated that, due to the lack of oxygen under flooding conditions, the decomposition of soil organic matter is carried out at a slower rate by facultative and obligate anaerobes that operate at a much lower energy level than aerobic microorganisms, thus emitting less CO_2 with flooding irrigation. Therefore, the higher CO_2 emission fluxes could be associated with the formation of cracks in the soil during dry periods, a situation that can facilitate the release of this gas, since during flooding, a large proportion of the soil pores are filled with water, so aeration is very restricted and consequently the flow of CO_2 decreases [30]. In general, the highest CO_2 fluxes were observed between 10–15 and 70–80 days after sowing (DAS) (Figure 1), in agreement with results reported by Das et al. [31]. Similar results were reported by several authors [32,33], who observed a low flow of CO_2 between the beginning of rice growth and the three-leaf stage (10–15 DAS), with increases from this stage until the panicle emergence one (70–80 DAS) and then decreases in the reproductive stage. As the plant grows, the rate of photosynthesis increases and part of the photosynthates are released as root exudates, composed mainly of easily degradable C substrates that contribute to the labile C pool of soil and stimulates microbial activity. Furthermore, an increase in CO_2 emissions is observed after topdressing fertilization, probably due to the fact that the microorganisms that processed urea (during the nitrification-denitrification processes) consumed carbon to later emit it in the form of CO_2.

Generally, both treatments follow similar CO_2 emissions pattern with differences in the intensity of the emissions peaks. It indicates that the observed CO_2 emissions rely mostly on climatic conditions, namely temperature and soil moisture as well as on the rice management practices, as previously referred. The application of organic amendments together with fertilizers improved the available organic carbon stock and increased CO_2 emissions by promoting the utilization of organic carbon by microbes [32]. Furthermore, it is important to refer the differences observed in CO_2 emission peaks throughout the growing cycle between years. Thus, in 2015 and 2017 years, CO_2 emissions higher than 54.5 kg CO_2-C ha^{-1} d^{-1} were found during the first month of the study (May), while in the same period of 2016, residual CO_2 emissions were observed (Figure 1). This could be attributed to the different temperatures observed in May between the three years of the study, since there is evidence that higher temperatures lead to increased CO_2 emissions (e.g., [10,34]). Indeed, whereas in May 2016 the mean value of temperature registered was 16.5 °C, in the same period for 2015 and 2017 it was close to 20 °C. Similarly, at the end of August 2017, a large CO_2 emission peak was observed after the second cover application of fertilizer, namely under Control treatment (Figure 1) while in 2016 and 2015, such a peak was not observed. As above, this could be attributed to differences observed in the temperature values between the different years of study (23.8, 25.3, and 27.9 °C in 2015, 2016, and 2017, respectively). Nevertheless, in this specific case, the CO_2 emission peak was observed only from Control treatment while in Compost treatment, CO_2 emissions remained residual. The reason for this is not clear since during this period, some C release was expected to happen mainly through CH_4 since the field was flooded.

The effect of the two management systems tested here on the total amount of CO_2 released is shown in Table 3. In the first year of the study (direct effect), there were no significant differences between Control and Compost treatment with a cumulative emission value of 1665 kg of CO_2-C ha^{-1} (Table 3). Similar CO_2 losses were observed by Harada et al. [35] in Japanese rice fields. Likewise, Pandey et al. [36], who evaluate the effects on GHG emissions of organic fertilizer application in flooded rice, reported 1840 kg CO_2-C ha^{-1} of total CO_2 emissions. During the second year, Compost treatment emits a higher concentration of CO_2 than Control management, although the differences were not significant. This finding could be due to mineralization of organic amendment after one year since its application. In fact, the level of WSOC in Compost treatment decreased significantly in 2016 compared to 2015 (Table 1), given that this fraction of organic matter one of the main C sources for microorganisms and a vital environmental factor that determines soil CO_2 emission process (e.g., [34]). Similar results have been observed by Wang et al. [37], who found that amended soils increased CO_2 emission relative to unamended soil under traditional rice management, probably due to higher soil organic carbon decomposition in amended soils. However, in the third year, the highest value of CO_2 emission was found under T treatment (Table 3). In fact, over the three years of study, higher values of cumulated CO_2 emissions were observed in T treatment than in TC treatment, although the differences were not statistically significant (Table 3). These results could be related to a greater availability of N in the amended soils (fertilizers + C-TPOW), which decreases the need for soil microorganisms to mineralize soil organic matter as a source of N needed for growth and reproduction [38]. Indeed, under laboratory conditions, Al-Kaisi et al. [39] found that cumulative CO_2 emissions were higher without N applications and decreased when the N rate application increased, which could be attributed to suppression of microbial respiration under high rates of N fertilizer application. Moreover, it is important to highlight that the cumulative CO_2 emission significantly increased over the study year under Control management. Thus, in 2017 the cumulated CO_2 emission in Control treatment increased by a factor of 2.20 relative to 2015 (Table 3). This result could be due to the high increasing number of weeds under Control management, since weed density in Control was four times higher in the first year compared to the third year [28], with species of the genus *Echinoclhoa* and *Leptoclhoa* as the most abundant. In fact, a significant and positive correlation was observed between WSOC levels and cumulative CO_2 emissions (r = 0.971, $p < 0.01$) under Control management. Due to the incorporation of biomass through weeds, the immediate production of CO_2 could be increased due to the oxidation of labile organic C fraction, as referred by Conrad et al. [40]. Additionally, the significant increase in pH values under Control treatment over the three years (Table 1) could explain the significant increase in cumulated CO_2 emissions in the last year. Khan et al. [41] found that soil acidification decreases microbial activity by suppressing the decomposition of organic carbon that microbes use as a source of energy. In fact, the enzymatic activities analysed in our study showed increases in 2017 relative to 2015 in both treatments (Table 2).

Table 3. Effect of the different management systems on the cumulative emission of CO_2, CH_4, and N_2O (kg ha^{-1}) during the rice cultivation cycle. Mean and standard error of 3 replicates.

	2015		2016		2017		Y	T	Y × T
	Control	**Compost**	**Comtrol**	**Compost**	**Control**	**Compost**	**F-Values**	**F-Values**	**F-Values**
CO_2-C (kg ha^{-1})	1780 aA (144)	1552 Aa (379)	2086 aA (206)	3189 aA (544)	3994 aB (86)	3208 aA (603)	46.1 ***	NS	11.6 *
CH_4-C (kg ha^{-1})	279 aA (48.9)	360 aA (57.9)	258 aA (22.3)	463 bA (58.8)	229 aA (2.92)	337 bA (48.6)	NS	9.60 **	NS
N_2O-N (kg ha^{-1})	2.19 aA (0.500)	2.89 aA (0.194)	1.51 aA (0.269)	5.31 bAB (0.865)	4.11 aB (0.227)	7.45 bB (1.305)	25.4 ***	10.4 **	6.28 **

Rice cultivated by traditional tillage techniques (Control) and with application of CW (Compost). ANOVA factors are Y: Year; T: Treatment; Y × T: Interaction Year × Treatment. F-values indicate the significance levels * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, respectively, and NS: not significant. Different letters indicate differences ($p < 0.05$) between treatments in the same year (lower case letters) and between years within the same treatment (upper case letters).

3.2. Emissions CH_4

Methane is the most abundant organic trace gas in the atmosphere, with a warming potential 20 to 30 times higher than CO_2 [42]. The production of CH_4 in the soil is a strictly anaerobic microbial process known as methanogenesis that requires a low redox potential (Eh < −150 mV, [43]).

The effect of C-TPOW application to rice soils on CH_4 emission rates over rice growing cycles during 2015, 2016, and 2017 are shown in Figure 2. After the beginning of irrigation (about 7 days), under low concentrations of oxygen in soil and when the redox potentials (Eh) are lower than −165 mV (Table 1), emission of CH_4 started due to the reduction of carbon dioxide [44]. Other authors indicate that CH_4 production is greatly favored by pH values between 6 and 8 and the supply of low molecular weight fatty acids derived from easily degradable organic matter [45]. However, there are several other biotic and abiotic factors that influence the seasonal dynamics of CH_4 flux in paddy field. It is still noteworthy that punctual CH_4 emission peaks were observed, coinciding with specific tasks, as topdressing fertilization. In fact, a positive correlation of CH_4 emission with the activity of UR was observed (r = 0.681, $p < 0.01$). The process starts as urea processing by microorganisms imply C consumption, thus causing the emission of CH_4 [46]. Such a process was strongly enhanced when the application of N fertilizer was preceded by the organic amendment (C-TPOW). Indeed, the values of accumulated CH_4 emissions were 1.29, 1.80, and 1.48 greater in Compost than Control for 2015, 2016, and 2017, respectively (Table 2). In addition, it is reported that NH_4^+-N inhibits the oxidation of CH_4 at the biochemical level [47]. Moreover, the increases of CH_4 emission after C-TPOW observed in the short and long term, could also be attributed to the stimulation of soil microorganism [48]. In fact, cumulative CH_4 emission was positively correlated with GL (r = 0.568, $p < 0.01$) indicating the importance of enzymatic activities involved in important processes such as organic matter decomposition in rice soils. It is also important to highlight that a strong decrease of CH_4 emissions was observed at the end of the crop cycle, after the drainage of the water for the subsequent harvest. Similar results had been found by Wang et al. [49] who observed high CH_4 emissions at the tillering stage (basal fertilizer and tillering fertilizer at 20 DAS) and low CH_4 emissions at the other growth stages. Nevertheless, it is important to note that during 2016 and 2017, similar CH_4 emission patterns under both treatments were found. However, in 2015, no significant peak of CH_4 emission was observed during the first half of the growing cycle, regardless of the treatment (Figure 2). Due to inverse relationship between CH_4 and CO_2 in C emission from flooding rice soils [50], these results could be explained by high peaks of CO_2 found in 2015 during the initial stages of crop. In fact, in 2015 the highest peak of CH_4 emission is in line with residual emission for CO_2.

Table 3 shows the effect of C-TPOW application on the cumulated CH_4 released during the rice cultivation cycle throughout the 3 years of the experiment. The total amount of CH_4 released over the 3 years in both treatments ranged from 229 to 463 kg of CH_4-C ha^{-1} (Table 3). Similar values of CH_4 losses have been reported by different authors, in soils similar to the one used here in terms of total N concentration and TOC (2.10 g kg^{-1}, 17.9 g kg^{-1}, respectively) [51]. Ishfaq et al. [52] showed cumulated emission of 255–317 kg CH_4-C ha^{-1} in flooded rice fields throughout the crop cycle. In addition, a recent report from FAO [53] indicated a CH_4 release of 378 kg of CH_4-C ha^{-1} in rice cultivation for the year 2017.

The application of C-TPOW increased significantly the cumulated CH_4 emission by a factor of ~1.62 (average value for 2016 and 2017) relative to the Control treatment. Such a result can be attributed to the increase of the C substrate for methanogenic bacteria and a decrease in the redox potential [54], after the application of organic amendments to flooded soils such as those dedicated to rice cultivation [55]. In fact, in our study we found significant correlations between cumulative CH_4 emission and TOC (r = 0.651, $p < 0.01$) as well as cumulative CH_4 emission and WSOC (r = 0.579, $p < 0.01$). Furthermore, values of Eh significantly decrease after C-TPOW application, especially in the first year (Table 1). These results indicated that organic matter not only provides substrates for methanogens but also accelerates the reduction process [12]. Thus, the Compost treatment showed higher CH_4 emission fluxes than Control treatment in the initial stages of the crop cycle, over the three years of the study, since Eh values necessary for methanogenesis were reached earlier (Figure 2).

3.3. Emissions N_2O

Nitrous oxide is generated in the soil from microbial processes known as nitrification and denitrification, being a gas with a high global warming potential, 265 times higher than CO_2 [56]. Therefore, reducing N_2O emissions is a priority in order to avoid global warming.

Figure 3 shows the effects of C-TPOW application to rice soils on N_2O emission rates over rice growing cycles during 2015, 2016, and 2017. It is commonly assumed that anaerobic conditions greatly reduce the production of nitrates [57] and may, consequently, lead to lower N_2O emissions. Overall, the N_2O emissions observed here are fundamentally influenced by fertilization practices, especially topdressing fertilization (Figure 3). Similarly, Sander et al. [58] reported that most N_2O emissions occurred after nitrogen fertilization. In fact, a positive and significant correlation has also been observed here between N application and N_2O emission rate (r = 0.477, $p < 0.05$), as reported by other authors [59].

It is important to highlight that, in the first and last year of the study, the N_2O emission observed in both treatments followed a similar pattern throughout the rice growing cycle (Figure 3). However, in the second year, higher N_2O fluxes were observed from Compost in relation to Control for several days (from 40 until 100 DAS). This situation could be attributed to highest value of temperature registered in 2016. Thus, during the months of June, July, and August, the average maximum temperatures reached a value of over 35 °C, leading to an increase of 4.6% compared to same months of 2015 and 2017. Different authors have suggested that an increase of 2 °C of temperature could significantly increase the nitrification and denitrification rates [60] and hence also the N_2O emissions. A higher temperature can be expected in the Compost amended soil compared to the Control due to a high temperature retention capacity provided by the C-TPOW. Additionally, as mentioned above, the highest emission peaks were due to the application of topdressing fertilization, with a clear increase in N_2O emissions in the last year of the study (Figure 3).

Previous studies showed high N losses through the denitrification process in rice soils where organic amendments had been applied in relation to soils without amendment or fertilized with chemical fertilization [61]. Köster et al. [62] indicated that the application of organic amendments provides energy for soil microorganisms, increasing the microbial biomass as well as the denitrification rate due to a decrease in redox potentials [30]. However, unlike what was observed in the first year after C-TPOW where no significant increases of N_2O emissions were observed, a significant increase of N_2O emission rates (1.8 times higher in Compost compared to Control) was observed in third year (Table 2). In the present study, the topdressing fertilizer used was urea, which has a higher leaching potential. In fact, authors such as Jiang et al. [63] also reported a 14.9% loss of N by leaching using urea as fertilizer. However, once the urea is converted to ammonium, leaching may be reduced since ammonium is relatively immobile [63], especially in soils with high soil organic matter content. Therefore, in Compost treatment, the N lost by leaching process should have been reduced, leading to higher N_2O emission rate [64]. The increases of N_2O emission in C-TPOW amended soil were significant only from the second year onward probably due to a slower amendment mineralization under anaerobic conditions [65].

On the other hand, it is important to highlight the effect of UR activity on the emission of N_2O, since this enzyme contributes to the conversion of urea to NH_4^+, which is the substrate for nitrification process. Thus, the cumulative N_2O emissions were positively correlated with UR activity (r = 0.785, $p < 0.01$), reflecting that N_2O emissions depend greatly on soil enzyme activities and especially UR. Therefore, the higher activity of UR observed in Compost relative to Control, regardless of the year considered (Table 2), could also explain the increases in N_2O emission fluxes in the amended treatment (Table 3).

3.4. Global Warming Potential

Table 4 shows the effects of the treatments on Global Warming Potential (GWP) and yield based GWP (GWPr) over the three years study, as well as the level of significance of the variable years (Y), treatments (T), and the interaction between both (Y × T).

Table 4. Effect of the different management systems for the global warming potential and the global warming potential based on the agronomic yield of rice during the rice cultivation cycle. Mean value and standard error of 3 replicates.

	2015		2016		2017		Y	T	Y × T
	Control	**Compost**	**Control**	**Compost**	**Control**	**Compost**	**F-Values**	**F-Values**	**F-Values**
GWP (Mg CO_2 eq ha^{-1})	17.8 aA (0.207)	20.3 bA (0.821)	17.9 aA (1.59)	31.2 bB (3.30)	24.9 aB (0.287)	27.5 aAB (3.33)	8.56 **	9.11 **	5.96 **
GWPr (kg CO_2 eq kg^{-1})	1.62 aA (0.019)	1.80 bA (0.073)	2.86 aB (0.254)	6.03 bC (0.636)	2.94 aB (0.034)	3.52 aB (0.426)	45.5 ***	15.7 **	15.9 ***

Rice cultivated by traditional tillage techniques (Control) and with application of C-TPOW (Compost). ANOVA factors are Y: Year; T: Treatment; Y × T: Interaction Year × Treatment. F-values indicate the significance levels ** $p < 0.01$; *** $p < 0.001$, respectively, and NS: not significant. Different letters indicate differences ($p < 0.05$) between treatments in the same year (lower case letters) and between years within the same treatment (upper case letters).

The mean value of GWP for Control treatment did not vary significantly between the three years and was equal to 20.3 Mg CO_2 eq ha^{-1}. Similarly, Win et al. [66], in a field study with rice, reported a value of 20 Mg CO_2 eq ha^{-1}. The application of the C-TPOW caused a significant increase of the GWP value. However, on the third year of the experiment, no significant differences were observed between treatments (Table 4). These results indicated that the effects of C-TPOW on GWP were time-dependent, probably as a result of the amendment stabilization over the years [67]. In fact, the values of FA and HA in Compost treatment increased significantly in the third year compared to the first year as a result of the humification process (Table 1). Thus, some of the GHG mitigation strategies from agricultural soils are based on using management systems that reduce the mineralization rate of organic matter in addition to increasing C sequestration in the soil [30].

Considering the contribution of each gas to GWP value, in both treatments N_2O represented less than 9% and CH_4 was the most significant contributor to GWP for Compost treatment with 53.6%, while in Control it represented 49.3%. However, the contribution of CO_2 in treatment Control represented 43.3%, whereas in Compost it was 36.6%. An analysis of the effect of new rice management systems on the GWP may be incomplete if it does not consider the impact of these systems on agronomic performance. In fact, it is important to assess if the proposed systems are sustainable, environmentally and economically, being essential to keep agronomic yields at acceptable levels. Therefore, to globally evaluate the effect of the different production systems on the GWP, the yield based

GWP was also calculated based on the agronomic performance, as recommended by Van Groenigen et al. [68].

Similarly to GWP results, the effects of C-TPOW on GWPr were timing-dependent (Table 4). During the first year, a significant increase of GWPr in Compost relative to Control was observed. This result is in agreement with the meta-analysis conducted by Zhao et al. [69] who, after reviewing 230 publications, indicated that the application of organic amendments in rice cultivation could cause an average increase of 37.3% in GWPr values. However, in the third year, there were no significant differences between both treatments (Table 4) with values of GWPr similar to those reported by Fangueiro et al. [10] under edaphoclimatic conditions similar to those of the present study. It is important to highlight the increases observed in the GWPr values in 2016 compared to 2015, regardless of the treatment selected (Table 4). Indeed, the values of GWPr for Control and Compost treatment in 2016 were 2.86 and 6.03 kg CO_2 eq kg^{-1}, respectively. These results could be attributed to the decrease in yield observed in 2016 compared with 2015 due to temperatures higher than usual, as has been mentioned previously, giving rise to high levels of spikelet sterility [25]. Therefore, based on these findings, to reduce the adverse effects of climate change such as global warming and ensure food security, there is a need to develop alternative rice farming systems [70]. Furthermore, the increases of GWPr values in 2017 relative to 2015 in both treatments could have been motivated by the decline in rice yield after different years of monoculture under tillage and flood irrigation [71].

4. Conclusions

The application of C-TPOW in rice fields induces changes in the soil properties such as quantity and quality of organic matter, pH, as well as the enzymatic activities, which, in turn, modify the emissions of GHG, a key aspect in rice crop. Indeed, the results presented here shown that application of C-TPOW has significant effects on GHG emissions throughout the rice growing cycle. CH_4 emissions increased significantly with C-TPOW application, especially during the first year after application, while CO_2 emissions, observed mainly during drainage of the field, were slightly reduced in the amended soil. In the case of N_2O, a clear effect was observed throughout the study, with an important increase of N_2O emission with C-TPOW application, due to high UR activity when soil received this amendment.

Although the GWP and yield-scaled GWP may increase immediately after C-TPOW application (direct effect), no significant differences between treatments were found three years after application (residual effect), probably due to the amendment stabilization. Nevertheless, further studies are needed to clarify the long-term effects of C-TPOW on GHG emissions, since adoption of effective strategies for mitigation of GHG emission is crucial to achieve a sustainable rice production.

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