Carbon input management in temperate rice paddies: Implications for methane emissions and crop response

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Abstract

Agriculture contributes to over 20% of global anthropogenic greenhouse gas emissions and irrigated paddy fields account for 5-10% of CH_4 emissions. Main organic input providing methanogenesis substrate is straw. We hypothesized that removing rice straw can mitigate CH_4 emissions, and that replacing its carbon (C) input with raw or solid digestate can be a valuable alternative both for crop, soil and emission responses. A mesocosm study was setup to follow crop growth, changes in soil pore water chemistry (dissolved Fe(II) and dissolved Organic C), and CH_4 emissions over one cropping season on soil treated with the combination of two straw managements (removal or incorporation) and three fertilizations (mineral, raw digestate, solid digestate). Soils not receiving straw on average emitted 38 % less than soils after straw incorporation, while the two organic fertilizers did not increase emissions with respect to mineral N application. Furthermore, straw incorporation induced a yield depression independently from the fertilization strategy, probably as a result of N immobilization, especially in early stages. This was evidenced by early SPAD observations and flag leaf length, and both grain and straw final production. Moreover, the two organic fertilizers were not fully able to sustain crop N requirements with respect to the mineral fertilizer. Straw management was therefore decisive for determining both rice yield and CH_4 emissions, while the impact of fertilization treatments was crucial only for crop productivity.

Introduction

Global warming driven by increasing greenhouse gas (GHG) concentrations in the atmosphere is a matter of great environmental concern throughout the 21st century. Methane (CH_4) is one of the most important GHGs with the second-largest radiative forcing (~20%) after CO_2 (~60%) (IPCC, 2013). Its concentration in the atmosphere has more than doubled, from approximately 700 ppb in 1720-1800 period to 1875 ppb in December 2019 (Hawkins *et al*. 2017; ESRL-NOAA, 2020). Current estimates of the total global CH_4 budget are between 500 and 600 Tg CH_4 yr^{-1} (Dlugokencky *et al*., 2011; FAOSTAT, 2013; Tubiello *et al*., 2013). The residence time of CH_4 in the atmosphere is relatively short (9 yrs) compared to CO_2 (100 yrs) and N_2O (170 yrs). Therefore, reduction of the global CH_4 sources offers possibilities for decreasing the growing trend of global warming on a short time scale (Dlugokencky *et al*., 2011). Agriculture contributes to over 20% of global anthropogenic GHG emissions (IPCC, 2013). Irrigated paddy fields are one of the main human-induced sources of CH_4, accounting for roughly 5-10% of the global CH_4 source strength (Matthews *et al*., 2000; Kirk, 2004; Bertora *et al*., 2010), and therefore represent a promising target for mitigating CH_4 emissions (Wassmann *et al*., 2004).

Over 75% of the world rice is cropped in continuously flooded paddies (Van der Hoek *et al*., 2001). Waterlogging has several agronomic advantages: it mainly limits variations in soil moisture and temperature, and depresses soil-borne diseases and aerobic weed growth. Nevertheless, flooding causes prevalent anaerobic soil conditions. Consequently, organic matter decomposition is coupled to the reduction of inorganic electron donors that once consumed lead to the production of CH_4 by methanogenic microorganisms through the disproportionation of acetate to CO_2 and CH_4 or by reduction of CO_2 with H_2. Soil CH_4 emission encloses a series of complex processes involving methanogenic and methanotrophic microorganisms (Le Mer and Roger, 2001), and is dependent on soil dissolved organic carbon (DOC) availability (Bossio *et al*., 1999). In fact, as much as 60 to 90% of the CH_4 produced is oxidized by aerobic methanotrophic bacteria predominantly at the oxic soil-water and soil-root interfaces (Holzapfel-Pschorn *et al*., 1985; Sass *et al*., 1990). Some of the CH_4 is also leached as dissolved CH_4 in floodwater that percolates from the field (Neue *et al*., 1995). The remaining un-oxidized CH_4 is transported from the soil to the atmosphere primarily by diffusive transport through the rice aerenchyma with minor amounts of

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CH_4 also escaping from the soil via diffusion and ebullition through floodwaters.

Straw returning practices typically influence topsoil DOC concentrations under flooded conditions that could drive CH_4 emissions (Schütz *et al*., 1989; Zou *et al*., 2005; Bhattacharyya *et al*., 2012). Temporal variations in CH_4 emissions from paddy soils over the cropping season were shown to follow the pattern of DOC in the topsoil (Lu *et al*., 2000; Said-Pullicino *et al*., 2016), suggesting that this labile C pool may serve as a major C source for methanogenic microorganisms. Agricultural production generates approximately 4 billion metric tons of crop residue per year globally (Lal, 2005), of which 800 to 1000 million tons per year is rice straw [International Rice Research (IRRI); 2019, available at: irri.org] that often represents the main paddy soil C input (Zhu *et al*., 2014). Returning crop residue to the soil helps maintain fertility, improve crop yields by providing nutrients, and promotes soil C sequestration (Mandal *et al*., 2004; Yadvinder *et al*., 2004; Tirol-Padre *et al*., 2005; Huang *et al*., 2013; Turmel *et al*., 2015).

However, incorporation of fresh straw (up to 12 t ha^{-1} annually) prior to field flooding leads to an acceleration in the establishment of reducing soil conditions (Yuan *et al*., 2014), the accumulation of phytotoxic substances (like low molecular weight aliphatic acids) derived from straw fermentation (Pramaink *et al*., 2001), and an increase in straw and soil-derived substrate availability for methanogenesis (Liou *et al*., 2003; Naser *et al*., 2007; Zou *et al*., 2005). Rice straw is, in fact, the primary source of C for CH_4 production during the early growth period of rice plants (Watanabe *et al*., 1999), and straw incorporation significantly enhances CH_4 emission from paddy fields as it can selectively stimulate the growth of particular methanogenic archaea populations (Glissmann *et al*., 2001) and provide acetate and H_2 substrates (Conrad and Klose, 2006; Bhattacharyya *et al*., 2012). It is therefore pivotal to find efficient methods to allow maintaining or increasing soil organic C stocks while mitigating GHG emissions.

In order to ensure sustainable outcomes, straw residues must be properly managed (Yadvinder *et al*., 2004), and over the years several options have been identified. These include early-incorporation of rice straw or removing and collecting it for compost or biochar production or generation of energy (Roca-Pérez *et al*., 2009; Parameswaran *et al*., 2010; Bertora *et al*., 2018a).

The collection and treatment of rice straw through anaerobic digestion (transforming the most labile C fractions into biogas) is a viable option for producing clean, renewable energy, and eliminating a major source of GHG emissions (Mussoline *et al*., 2016). Subsequently, returning the digestate to the paddy soil from where the straw was previously removed contribute to preserve the C content in the soil while mitigating the GHG emissions.

Based on these considerations we hypothesize that post-harvest removal of rice straw in temperate rice cropping systems can effectively mitigate CH_4 emissions but concurrently reduce soil C input that would lead to a loss of soil C stocks in the long-term. On the other hand, replacing this C input with raw digestate or the solid fraction of digestate can be a win-win environmental and agronomic strategy. In order to test this hypothesis we conducted a mesocosm experiment with the objectives of: i) assessing if straw removal can effectively reduce DOC pore water concentrations and mitigate CH_4 emissions; ii) evaluating alternative C returning strategies on DOC concentrations and CH_4 emissions; and iii) exploring the impact of straw and alternative C input on the rice agronomic performances.

Materials and methods

Experimental design

The experiment was performed at the Department of Agricultural, Forest and Food Sciences of the University of Turin, in Grugliasco (NW, Italy). The experiment was designed in order to compare two rice straw management practices (incorporation *versus* removal) and three different pre-seeding N fertilization strategies (mineral, raw digestate, solid fraction of digestate), totalling 2 × 3 treatments:

i) straw removal and mineral fertilization, SR-M; ii) straw incorporation and mineral fertilization, SI-M; iii) straw removal and raw digestate fertilization, SR-RD; iv) straw incorporation and raw digestate fertilization, SI-RD; v) straw removal and digestate solid fraction fertilization, SR-SD; vi) straw incorporation and digestate solid fraction fertilization, SI-SD.

Non-fertilized control treatments with straw removal (SR) or incorporated (SI) were also set up for comparison of yield parameters.

The experiment was organized as a completely randomized design with four replicates.

Soil and organic materials properties

In November 2014, soil was collected from the top layer (0-0.4 m) of a commercial paddy farm in Crescentino, within the Italian rice district (Vercelli province, NW Italy). The soil is classified as an Alfisol (USDA, 2014), with a loam texture in the 0-40 cm Ap horizon (7.0% clay, 45.2% sand), sub-acid pH (6.1), low cation exchange capacity (8.5 meq 100 g^{-1}), high organic C content (15.4 g kg^{-1}), medium total N (1.4 g kg^{-1}), low exchangeable K (0.09 mg kg^{-1}) and medium Olsen P (17.3 mg kg^{-1}). All analyses were performed following national official standards (Mipaaf, 1999).

The collected topsoil was air-dried and then sieved at 5 mm. Rice straw was also collected from the same field after harvest.

Digestate material used in the experiment originated from a biogas plant fed with maize silage (68% of annual feedstock), triticale silage (30%), and extruded rice straw (2%). After production, a solid/liquid separation was performed trough a screw press separator. Main properties of raw digestate and its solid fraction are shown in Table 1.

Separation resulted in a moderate decrease in the amount of total N and a marked decrease in NH_4^+ content of the solid fraction. On the other hand, the contents of dry matter, organic C, and total P were higher in SD than in RD.

Table 1. Main chemical properties of the raw digestate (RD) and solid fraction of raw digestate (SD) used in the experiment.

		Raw digestate	Solid fraction of digestate
pH		8.0	8.2a
total N	% ww	0.47	0.33
NH4+-N	% ww	0.24	0.01
organic C	% ww	3.8	5.7
total P	% ww	0.1	0.2
total K	% ww	0.5	0.5
moisture	% ww	91.6	87.1

Mesocosms design and management

Mesocosms, consisting of 28-litre cylindrical plastic pots (30 cm diameter, 40 cm height), were first equipped with a bottom drainage system consisting of a 5 cm thick gravel (0.5-1 cm diameter) layer covered by a mulching black polypropylene fabric (105 g m^{-2}) that allowed for water percolation without the loss of soil material (Figure 1). Within this drainage layer, a 7 mm drainage tube equipped with a stopcock for outflow adjustment controlled the water percolation rate. The mesocosms were filled with 25 kg of air-dried and sieved (at 1 cm) paddy soil. Soil bulk density was adjusted to 1.3 Mg ha^{-1}, representing a mean value from a paddy soil of the area throughout a cropping cycle (Sacco *et al*., 2012). Half of the pots received 50 g of rice straw, equivalent to 7 t ha^{-1} representing typical straw yields in the area, that were thoroughly mixed with the soil (SI treatment), while the other half did not (SR treatment). Rhizon-samplers (Rhizosphere Research Products, The Netherlands) were installed horizontally in each mesocosm at a depth of 15 cm from the soil surface to allow for pore water sampling. The experiment was carried out for about 6 months (from April 28th to September 22nd of 2014) covering the typical duration of an entire rice growing season in Italy. During this period, the pots were kept under an open tunnel greenhouse covered by a net that prevented seed predation by birds and possible damage by hail, and provided a modest shading effect (maximum 10%).

On April 28th, all fertilized mesocosms received 100 kg N ha^{-1} pre-seeding fertilization as urea for M treatment, raw digestate for RD treatment, and solid fraction of digestate for SD treatment.

Considering their respective C contents determined by elemental analysis (NA1500 Nitrogen Analyser, Carlo Erba Instruments), the amount of C supplied with the different organic sources were estimated at 2800 kg C ha^{-1} for straw, 800 for RD, and 1700 for SD. After pre-seeding fertilization, all pots were flooded and maintained under 10 cm of ponding water. On April 30th each mesocosm was water seeded at rate of 40 seeds per mesocosm with rice variety Loto (Long A, following Regulation CE 1234/2007). Plant density (25 plant per mesocosm) was standardized just before tillering stage by thinning or using transplanted rice grown in a nursery bed. This value corresponded approximately to 350 plants m^{-2}, similar than final plant density normally set in field after tillering stage (Moretti *et al*., 2019). During the seedling stage, soil was drained for one week for the radicle to penetrate the soil and anchor the seedling. At the end of this period, flooding was re-established. At tillering (June 17th), and panicle initiation stages (July 22nd), the pots were drained and, two days later, 30 kg N ha^{-1} of top-dressed urea were distributed for all treatments except the controls. Immediately after fertilization, flooding was restored and a permanent ponding water depth of 10 cm was maintained. All pots were drained on 1st September, approximately one month prior to harvest. Although measurements were performed on mesocosm having a soil surface of 706.5 cm^2, results were expressed per hectare to allow for field-scale agronomic discussion.

Gas sampling and methane flux measurements

Methane emissions were measured over the rice-cropping season by the non-steady-state closed chamber technique (Livingston and Hutchinson, 1995; Peyron *et al*., 2016) on all treatments except the non-fertilized controls. Measurements covered the entire growing season (40 measurements events), subdivided into four main phenological stages: the early vegetative stage (EVEG, from germination to tillering, 16 measurement events), the late vegetative stage (LVEG, from tillering to panicle initiation, 10 measurement events), the reproductive stage (REP, from panicle initiation to flowering, 4 measurement events), and the ripening stage (RIP, from flowering to senescence, 10 measurement events) (Meijide *et al*., 2011). On sampling dates, gas fluxes were measured around midday (11:00-14:00 h) to minimize variability due to diurnal variations in gaseous fluxes (Pittelkow *et al*., 2013). Cylindrical PVC chamber covers perfectly fitting the mesocosm diameter were closed during each measurement events. Chamber volume followed rice growth, ranging from 31 to 59 litres. The chambers were equipped with a battery driven 12-V circulating fan to ensure complete gas mixing and were wrapped with a layer of polystyrene and aluminium foil to minimize air temperature changes inside the chamber during the gas sampling period. A pressure vent was installed to prevent the effect of pressure changes outside the chamber and to equilibrate internal and external pressure (Hutchinson and Mosier, 1981). During gas sampling, the chamber cover was placed over the vegetation with the rim of the chamber fitting over the mesocosm, ensuring perfect airtightness by means of a rubber seal. Proper extensions were interposed to increase chamber height in order to accommodate the growing rice plants. During this study, CH_4 emissions were usually measured once a week, except during drainage and fertilization periods, when a higher sampling frequency was adopted. Gas samples (30 mL) were withdrawn using airtight syringes at 0, 15 and 30 min after chamber closure, and transferred into 12 ml pre-evacuated vials (Exetainer®, Labco Limited, UK). We recorded the temperature inside the chamber during gas sampling in order to calculate the volume to mass ratio of gas mixture, necessary for proper estimate of fluxes. Gas samples were analysed by gas chromatography with flame ionization detection (Agilent 7890A, Santa Clara CA, USA). Methane emission flux (expressed in g C m^{-2} d^{-1}) was calculated from the linear resolution of the rate of increase in gas concentration in the chamber during closure (Bertora *et al*, 2018b). When the rate of gas concentration decreased over the sampling period suggesting a deviation from non-steady state conditions, fluxes were calculated by applying the nonlinear Hutchinson and Mosier (1981) model. The MDF (Minimum Detectable Flux) varies in relation to the detection limit of the gas chromatograph and the chamber volume. The latter changed in time during the cropping season to accommodate for rice growth. Values for MDF ranged between 12 and 48 g C ha^{-1} d^{-1} for CH_4. Fluxes were set to

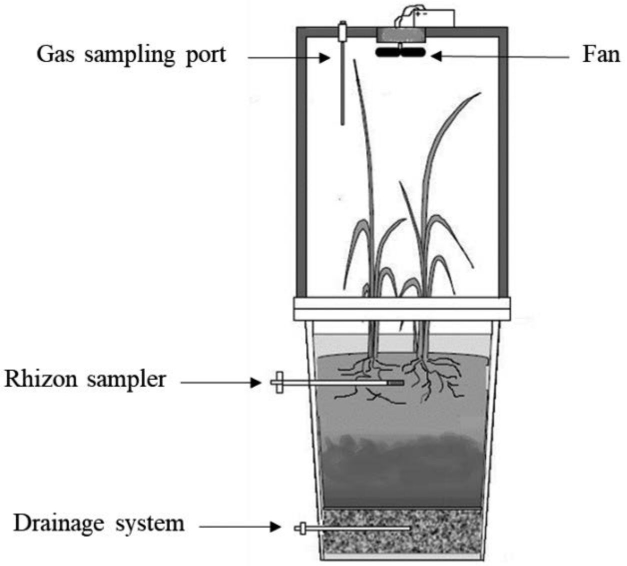

Figure 1. Scheme of experimental unit, mesocosm and chamber design.

zero if the change in gas concentration during chamber enclosure fell below the MDF. Estimates of cumulative CH_4 emissions for each plot were based on linear interpolation across sampling days (Zou *et al*., 2005; Peyron *et al*., 2016).

Soil pore water analyses

Samples were collected on a weekly basis from all the treatments except the non- fertilized controls. Samples were immediately filtered through a 0.45 μm nylon membrane filter, and subsequently analysed for DOC concentration, specific ultraviolet absorbance at 254 nm (SUVA), and Fe(II) concentration. Dissolved organic carbon was determined using Pt-catalysed, high-temperature combustion (850°C) followed by infrared detection of CO_2 (VarioTOC, Elementar, Hanau, Germany), after removing inorganic C by acidifying to pH 2 and purging with CO_2-free synthetic air. UV absorption at 254 nm was measured (Helios Gamma Spectrophotometer, Thermo Electron, Waltham, MA) after appropriate dilution to DOC <50 mg L^{-1}. The SUVA values, calculated by normalizing measured absorbance values to the concentration of DOC, were used as an estimate for the aromatic content of water samples (Weishaar *et al*., 2003). Dissolved Fe(II) concentrations were determined colorimetrically immediately after sampling, using the 1,10-phenanthroline method (Loeppert and Inskeep, 1996).

Plant sampling and measurement of nutritional status and yield parameters

As from the tillering stage, plant nutritional status was assessed by means of a vegetation index measured with SPAD 502 Minolta, with a variable frequency (every 1-3 weeks) for a total of six measurement events. Plant vigour was also assessed measuring the length of the flag leaf twice, during the reproductive and ripening stages. At the end of the cropping cycle, grain and straw yield, and root biomass were determined for each mesocosm. Total biomass was harvested at 146 days after seeding (DAS). Grain and straw were manually separated and weighted independently. Dry matter (DM) was evaluated by drying biomass at 60°C for 72 h, when a constant weight was reached. Grain and straw total N contents was determined utilizing by elemental analysis (Flash EA 1112, Thermoquest, MIPAAF, 2000). Moreover, yield components (*i.e*. filled and unfilled grain per panicle) were measured and Harvest Index was calculated.

Statistical analysis

Methane emissions and pore water data were analysed by a linear mixed model utilizing nlme R package (Pinheiro *et al*., 2019). Fixed effects *between subjects* were straw management, fertilization treatments and their interaction, while *within subjects* effect was the different phenological stage. Random effect was represented by each mesocosm. Nutritional status parameters were analogously analysed applying a linear mixed model, where *within subject* effect was the different date.

Cumulative CH_4 emissions for the whole cropping cycle and yield parameters were analysed by a linear model including straw management, fertilization treatments and their interaction as fixed effects. Data were checked for normality through Shapiro Wilk test and for homoscedasticity through Levene test. When data was not normally distributed, logarithm transformation was applied and distribution checked again. When Levene test demonstrated heteroscedasticity, internal variances of each group were modelled through VarIdent function. Groups considered for modelling variances were both straw management and fertilization strategy. Best fitting solution was chosen using smaller AIC (*Akaike information criterion*). When significant, treatment averages were separated through Bonferroni post hoc test including Satterthwaite correction for degrees of freedom in case of modelled heteroscedasticity. Correlations between cumulative CH_4 emissions, mean Fe(II), DOC and SUVA for the different phenological stages were analysed for all treatments together, as well as separating different straw and fertilizer treatments, by means of Pearson correlation.

All statistical analyses were performed using R version 3.6.0 (R Core Team, 2019).

Results

Methane emissions

Methane fluxes in the SI-M treatment (Figure 2A) started around 20 DAS and rapidly increased reaching a peak of 11.74 kg CH_4-C ha^{-1} d^{-1} at 37 DAS. Subsequently, CH_4 flux decreased during the drainage period corresponding to the second fertilization and ranged between 2 and 10 kg CH_4-C $ha^{-1}d^{-1}$ until flowering,

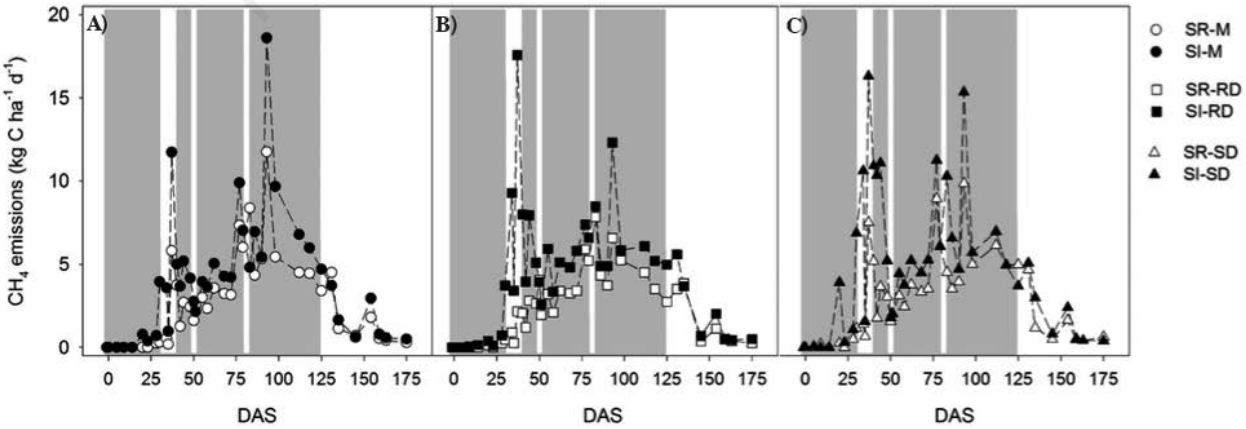

Figure 2. Seasonal of variations of CH_4 emissions over the cropping season as a function of fertilization and straw management in soils receiving (A) mineral fertilization, (B) raw digestate, and (C) solid fraction for the digestate (M, RD and SD, respectively), with (closed symbols) or without (open symbols) straw incorporation (SI and SR, respectively). Shaded areas represent the presence of floodwater.

when the highest peak of the season across treatments was observed (18.6 kg CH_4-C ha-^{1}d^{-1} at 93 DAS). That peak was followed by a sharp reduction of fluxes to almost zero due to final drainage in preparation for harvest. Fluxes in the SR-M treatment followed a similar trend to that observed for SI-M but with a lower magnitude. Mean fluxes were generally lower (2.68 kg CH_4-C ha^{-1}d^{-1} in SR-M and 3.92 kg CH_4-C ha^{-1}d^{-1} in SI-M). The maximum daily flux (11.8 kg CH_4-C ha^{-1}d^{-1}) was registered at the same date as the SI-M treatment (93 DAS) at the beginning of flowering.

In treatment SI-RD (Figure 2B), CH_4 emissions did not start immediately after the initial flooding but were delayed by approximately one month later, and sharply increased (within only 10 days) to reach the highest flux of the season (17.6 kg CH_4-C ha^{-1}d^{-1}) corresponding with the beginning of tillering (37 DAS). Afterwards the flux decreased for the late vegetative stage with a mean flux of 5.3 kg CH_4-C ha^{-1}d^{-1}. A second peak was observed during the reproductive stage (12.3 kg CH_4-C ha^{-1}d^{-1} at 93 DAS). During the subsequent ripening stage and following the final drainage, the flux gradually decreased to zero. The SR-RD treatment showed a different emissions pattern with fluxes starting late in the season (around 30-40 DAS), and gradually increased during the vegetative stage, producing a significant peak (7.8 kg CH_4-C ha^{-1}d^{-1}) around the third fertilization (83 DAS). The following reproductive and ripening stages showed a constant decrease in the emissions fluxes. The SI-SD treatment (Figure 2C) showed a pulse-like pattern of emissions as from the beginning and then during the entire growing season with many significant peaks. Emissions of CH_4 started 20 DAS (with a peak of 3.9 kg CH_4-C ha^{-1}d^{-1}, higher than that of the other treatments) and rapidly increased over the early vegetative stage until the maximum peak of the season at 37 DAS (16.3 kg CH_4-C ha^{-1}d^{-1}). The emissions subsequently dropped almost to zero due to drainage around second fertilization. In the late vegetative stage, the fluxes augmented with two significant peaks of approximately 10-11 kg CH_4-C ha^{-1}d^{-1}. The second highest peak of the season was recorded at 93 DAS (15.3 kg CH_4-C ha^{-1}d^{-1}) during the panicle emergence. After this peak, the flux decreased gradually down to zero in the ripening stage. The SR-SD treatment showed a pattern similar to SI-SD but with a lower intensity: CH_4 emissions started around one month after seeding and the first peak was recorded at 37 DAS (7.5 kg CH_4-C ha^{-1}d^{-1}). During the late vegetative stage, the emissions were relatively low, with the exception of a peak (8.9 kg CH_4-C ha^{-1}d^{-1} at 77 DAS) in correspondence of the second drainage period. The reproductive stage was characterized by the highest peak of the entire season (9.9 kg CH_4-C ha^{-1}d^{-1} at 77 DAS) as observed for most of the other treatments. When comparing cumulative fluxes over the different phenological stages for the different treatments (Table 2), significant interactions between straw and stage, and between fertilization and

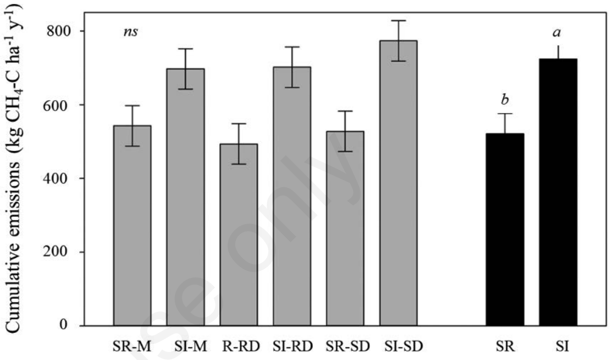

Figure 3. Cumulative CH_4 emissions for over the whole cropping season for the different straw residue and fertilization management practices involving straw removal and mineral fertilization (SR-M), straw incorporation and mineral fertilization (SI-M), straw removal, pre-seeding raw digestate incorporation (SR-RD), straw incorporation, pre-seeding raw digestate incorporation (SI-RD), straw removal, pre-seeding solid fraction digestate incorporation (SR-SD) and straw incorporation pre-seeding solid fraction digestate incorporation (SI-SD). Different letters indicate a significant difference between mean values ($P<0.05$). Error bars represent standard error of the mean.

Table 2. Cumulative CH_4 emissions (kg CH_4-C ha^{-1}) over the cropping season and at different phenological stages for the straw and fertilization treatments. Values presented are logarithmic estimated marginal means. while in bracket are back-transformed values.

	Early vegetative stage	Late vegetative stage	Reproductive stage	Ripening stage			
	(kg CH_4-C ha^{-1})				Average for Straw		
Straw							
SI	5.0 (142.3)^a	5.3(203.2)^a	5.3 (198.1)	5.1 (160.0)	5.2 (174.0)		
SR	3.8 (43.6)^b	5.0 (152.1)^b	5.1 (162.7)	4.9 (131.6)	4.7 (109.3)		
					Average for for Fertilization*	Average for Fertilization*Straw	
						SI	SR
M	4.1 (62.6)^b	5.1(171.9)	5.3 (207.9)	5.0 (144.2)	4.9 (134.0)	5.1 (161.9)	4.7 (111.1)
RD	4.2 (68.5)^{ab}	5.2(176.6)	5.1 (164.0)	5.0 (141.5)	4.9 (129.5)	5.1 (167.3)	4.6 (100.2)
SD	4.7 (114.0)^a	5.2(180.4)	5.1 (169.5)	5.0 (149.8)	5.0 (151.1)	5.3 (194.4)	4.8 (117.6)
Average for Phenological Stage	4.4 (78.8)	5.2 (176.3)	5.2 (179.5)	5.0 (145.0)			
P(f) Straw		0.000					
P(f) Fertilization		ns					
P(f) Phenological stage		0.000					
P(f) Straw*Fertilization		ns					
P(f) Fertilization*Phenological stage		0.020					
P(f) Straw*Phenological stage		0.000					

stage were evidenced. Effects were observable only in EVEG, when SI showed higher fluxes than SR, and SD emissions were higher than M, while throughout the rest of the season no difference were observed.

Cumulative emissions over the entire cropping season, from seeding to harvest (Figure 3) ranged between 773 and 493 kg CH_4-C ha^{-1} with highest and lowest emissions measured for SI-SD and SR-RD treatments respectively.

Although it was possible to identify a significant effect of straw incorporation that induced higher fluxes than straw removal, no significant effect of fertilization or straw×fertilization interaction was detectable.

Soil pore water and soil analyses

With the onset of flooding, reductive soil conditions led to an immediate increase in pore water Fe(II) concentrations reaching values of 20-25 mg L^{-1} across treatments (Figure 4). High Fe(II) concentrations were maintained for most of the cropping season, except for short periods concomitant with drainage events during which lower concentrations were measured, and after final drainage, when Fe(II) concentrations decline to low but nonetheless detectable levels.

When analysing mean values for the different phenological stages, significant effects of straw×phenological stage and fertilization×phenological stage interactions were found (Table 3). In

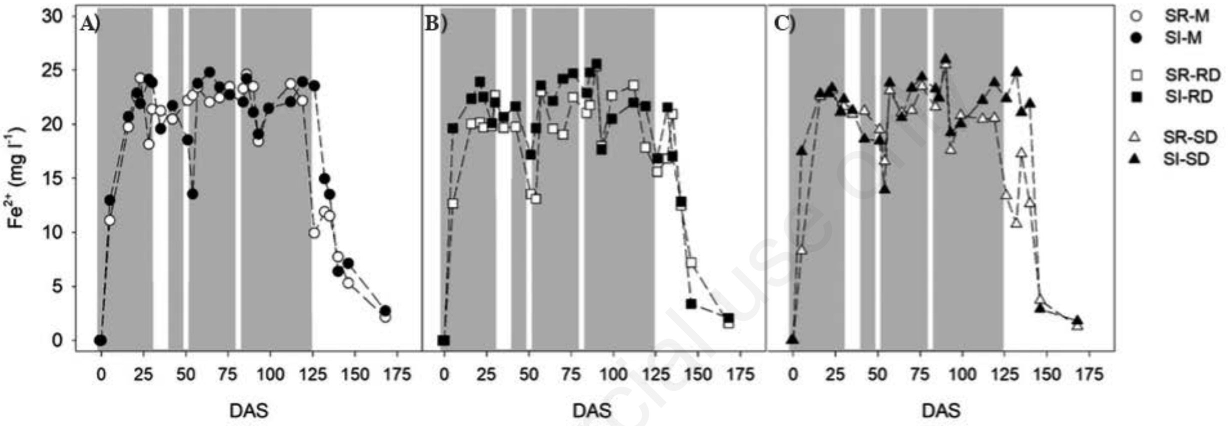

Figure 4. Variations in pore water Fe(II) concentrations over the cropping season as a function of fertilization and straw management in soils receiving (A) mineral fertilization, (B) raw digestate, and (C) solid fraction for the digestate (M, RD and SD, respectively), with (closed symbols) or without (open symbols) straw incorporation (SI and SR, respectively). Shaded areas represent the presence of floodwater.

Table 3. Mean Fe(II) concentration (mg L^{-1}) in soil pore water over the cropping season and at different phenological stages for the straw and fertilization treatments. Values presented are logarithmic estimated marginal means. while in bracket are back-transformed values.

	Early vegetative stage	Late vegetative stage	Reproductive stage	Ripening stage			
	mg L^{-1}				Average for Straw		
Straw							
SI	2.81 (16.7)^a	3.07 (21.5)	3.06 (21.2)	2.70 (14.9)^a	2.91 (18.3)		
SR	2.73 (15.4)^b	3.05 (21.1)	3.07 (21.6)	2.44 (11.5)^b	2.82 (16.8)		
					Average for for Fertilization*	Average for Fertilization*Straw	
						SI	SR
Fertilization							
M	2.77 (15.9)	3.10 (22.3)^a	3.05 (21.2)	2.40 (11.0)	2.83 (17.0)	2.84 (17.1)	2.82 (16.8)
RD	2.76 (15.7)	3.01 (20.3)^b	3.08 (21.6)	2.65 (14.2)	2.87 (17.7)	2.95 (19.0)	2.84 (17.2)
SD	2.80 (16.4)	3.06 (21.2)^{ab}	3.07 (21.4)	2.66 (14.4)	2.90 (18.1)	2.94 (18.9)	2.81 (16.5)
Average for Phenological Stage	2.77 (16.0)	3.06 (21.3)	3.06 (21.4)				
P(f) Straw		ns					
P(f) Fertilization		ns					
P(f) Phenological stage		0.000					
P(f) Straw*Fertilization		ns					
P(f) Fertilization*Phenological stage		0.005					
P(f)Straw*Phenological stage		0.005					

detail, straw incorporation induced higher Fe(II) concentrations during EVEG and RIP stages, while M showed higher values than RD only in the LVEG stage.

Across treatments pore water DOC concentrations tended to increase with the onset of flooding, reaching maximum concentrations in excess of 125 mg C L^{-1} during the LVEG stage that were highest in the organically amended soils that also received straw (Figure 5). Concentrations tended to decrease with time during the later stages of the cropping season. When analysing mean concentrations for the different phenological stages, a significant effect of straw and fertilization×phenological stages was detected (Table 4). Straw incorporation always enhanced DOC concentrations irrespective of fertilization treatments and phenological stage, while a significant fertilization effect was only observed in EVEG and LVEG, stages during which mean DOC concentrations in RD were higher than M and SD. SUVA values generally increased rapidly with the onset of flooding, reaching highest values within 21 DAS (Figure 6). However, whereas these high values were maintained throughout most of the cropping season in the M treatments, SUVA values in RD and SD treatments showed a bimodal trend with high values observed even towards the later REP stage of the cropping season.

In fact, statistical analyses evidenced a significant effect of the phenological stage and fertilization treatment, with highest mean DOC concentrations during EVEG with respect to all other stages, and higher values for SD with respect to M, irrespective of phenological stage and straw treatment (Table 5).

Table 4. Mean DOC concentration (mg C L^{-1}) in soil pore water over the cropping season and at different phenological stages for the straw and fertilization treatments.

	Early vegetative stage	Late vegetative stage	Reproductive stage	Ripening stage			
	(mg C L^{-1})				Average for Straw		
Straw							
SI	88.0	126.9	100.7	70.0	96.4^{a}		
SR	77.2	116.3	91.6	63.4	87.1^{b}		
					Average for for Fertilization*	Average for Fertilization*Straw	
						SI	SR
M	80.1^{b}	113.8^{b}	105.0	69.0	91.9	85.5	98.4
RD	89.8^{a}	131.7^{a}	88.9	63.5	93.5	88.5	98.5
SD	77.8^{b}	119.2^{b}	94.5	67.6	89.8	87.3	92.3
Average for Phenological Stage	82,6	121.6	96.1	66.7			
P(f) Straw		0.000					
P(f) Fertilization		0.000					
P(f) Phenological stage		0.000					
P(f) Straw*Fertilization		ns					
P(f) Fertilization*Phenological stage		0.032					
P(f) Straw*Phenological stage		ns					

Table 5. Mean soil pore water specific UV absorbance (SUVA) over the cropping season and at different phenological stages for the straw and fertilization treatments.

	Early vegetative stage	Late vegetative stage	Reproductive stage	Ripening stage			
					Average for Straw		
Straw							
SI	3.654	3.852	4.58	4.066	4.038		
SR	3.536	3.845	4.704	3.788	3.968		
					Average for for Fertilization*	Average for Fertilization*Straw	
						SI	SR
M	3.379	3.867	4.355	3.356	3.739^{b}	3.801	3.677
RD	3.64	3.531	4.809	4.331	4.078^{ab}	4.338	4.047
SD	3.766	4.148	4.763	4.094	4.193^{a}	3.974	4.181
Average for Phenological Stage	3.60^{b}	3.849^{b}	4.642^{a}	3.927^{b}			
P(f) Straw		ns					
P(f) Fertilization		0.011					
P(f) Phenological stage		0.000					
P(f) Straw*Fertilization		ns					
P(f) Fertilization*Phenological stage		ns					
P(f) Straw*Phenological stage		ns					

Correlation analysis

When analysing all data together, CH_4 emissions and Fe(II) were significantly and positively correlated with all other considered variables, while only DOC and SUVA were not correlated each other (Table 6). When separating analysis per phenological stage, observed significant correlations are: CH_4 with Fe(II) in EVEG, CH_4 and SUVA in EVEG, Fe(II) and DOC in EVEG and REP, DOC and SUVA negatively correlated in LVEG and REP, Fe(II) and SUVA in RIP. When distinguishing the different fertilization managements, significant correlations are: in M, CH_4 and Fe(II), CH_4 and SUVA, Fe(II) and DOC, Fe(II) and SUVA, DOC and SUVA, in RD, CH_4 and Fe(II), Fe(II) and DOC, DOC and SUVA, in SD, CH_4 and SUVA, Fe(II) and DOC. For both SI and SR treatments, significant correlations are: CH_4 and Fe(II), CH_4 and SUVA, Fe(II) and DOC, Fe(II) and SUVA.

Rice nutritional status and grain yield

SPAD index revealed a significant difference of straw×date and fertilization×date. In detail, SR (30.4) showed higher value than SI (28.7) only at tillering stage (BBCH 24), while no effect was observed throughout the rest of the cropping cycle. Fertilization effect was as well detected in two dates of tillering stage (BBCH 24 and 26) when M (30.8) was higher than SD (27.8) and control (26.5), and this last was also lower than RD (29.3). The flag leaf showed a fertilization effect, with M (13.9 cm) higher than control (12.1 cm), and RD (12.8 cm) and SD (12.4 cm) with intermediate values.

The interaction between straw×fertilization never showed significant effects on different yield parameters (Table 7). On the other hand, single factors affected all parameters differently. Straw removal induced significant increase in grain and straw yield independently from fertilisation management. However, straw management did not determine any other significant effect on roots,

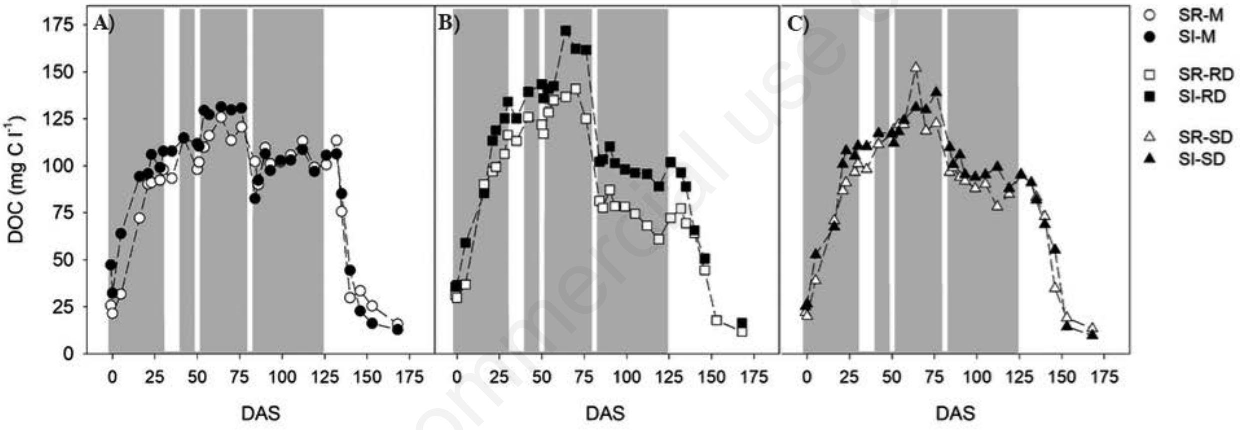

Figure 5. Variations in pore water DOC concentrations over the cropping season as a function of fertilization and straw management in soils receiving (A) mineral fertilization, (B) raw digestate, and (C) solid fraction for the digestate (M, RD and SD, respectively), with (closed symbols) or without (open symbols) straw incorporation (SI and SR, respectively). Shaded areas represent the presence of floodwater.

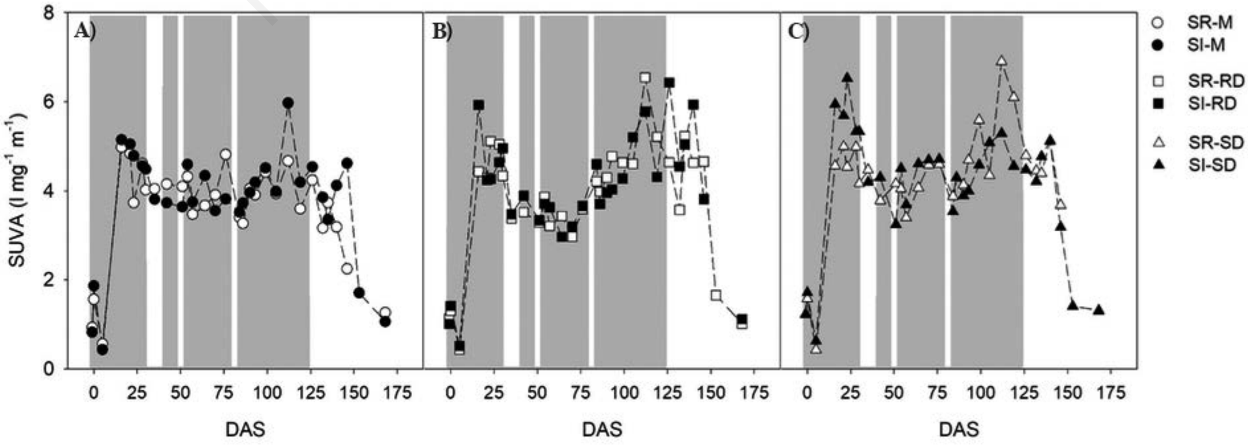

Figure 6. Variations in pore water SUVA values over the cropping season as a function of fertilization and straw management in soils receiving (A) mineral fertilization, (B) raw digestate, and (C) solid fraction for the digestate (M, RD and SD, respectively), with (closed symbols) or without (open symbols) straw incorporation (SI and SR, respectively). Shaded areas represent the presence of floodwater.

Harvest Index, crop density and unfilled grain for panicle. When the fertilisation management effect was significant, M had in general given the best results for all the parameters considered. Straw and grain yield with organic fertilisation supply were 19% lower than mineral for both measurements. However, compared with non-fertilized treatment, the increase in grain and straw production was 51 for RD and 46% for SD. The ability of the two organic fertilizers to support a crop density similar to those of M was demonstrated, while, even if not statistically significant, the percentage of unfilled grain for panicle was 16% greater than in control.

Discussion

In flooded rice, the major driver of CH_4 emissions was straw management. In fact, rice straw removal mitigated CH_4 emission by 38% on average with respect to straw incorporation. On the other hand, addition of raw or the solid fraction of digestate did not increase emissions with respect to mineral fertilization. These findings were probably due to the combined effects of different C inputs and quality of the added organic matter. Organic C input with straw addition was equivalent to 2800 kg C ha^{-1} while the application of organic fertilizers alone led to lower C inputs of 800 and 1700 kg C ha^{-1} with the addition of raw digestate and solid fraction, respectively, primarily due to the lower total N content of the latter. Moreover, rice straw represents a greater input of labile, easily decomposable, fresh organic matter with respect to the organic fertilizers originating from an industrial anaerobic decomposition process during which most of the more labile organic matter deriving from the vegetal feedstock was presumably lost during digestion. Separation of the liquid phase from the raw digestate to obtain the solid fraction further deprived the material from the

Table 6. Correlation analysis.

ALL TREATMENTS				EVEG				SI			
	Mean Fe(II)	Mean DOC	Mean SUVA		Mean Fe(II)	Mean DOC	Mean SUVA		Mean Fe(II)	Mean DOC	Mean SUVA
Cumulative CH_4	0.366 ***	0.281 **	0.364 ***	Cumulative CH_4	0.463 *	ns	0.506 *	Cumulative CH_4	0.297 *	ns	0.460 **
Mean Fe(II)		0.665 ***	0.420 ***	Mean Fe(II)		0.407 *	ns	Mean Fe(II)		0.717 ***	0.376 **
Mean DOC			ns	Mean DOC			ns	Mean DOC			ns
M				LVEG				SR			
	Mean Fe(II)	Mean DOC	Mean SUVA		Mean Fe(II)	Mean DOC	Mean SUVA		Mean Fe(II)	Mean DOC	Mean SUVA
Cumulative CH_4	0.368 *	ns	0.544 **	Cumulative CH_4	ns	ns	ns	Cumulative CH_4	0.375 **	ns	0.314 *
Mean Fe(II)		0.863 ***	0.646 ***	Mean Fe(II)		ns	ns	Mean Fe(II)	0.624 ***	0.448 **	
Mean DOC			0.431 *	Mean DOC			–0.513 *	Mean DOC			ns
RD				REP							
	Mean Fe(II)	Mean DOC	Mean SUVA		Mean Fe(II)	Mean DOC	Mean SUVA				
Cumulative CH_4	0.411 *	ns	ns	Cumulative CH_4	ns	ns	ns				
Mean Fe(II)		0.531 **	ns	Mean Fe(II)		0.413 *	ns				
Mean DOC			–0.394 *	Mean DOC			–0.801 ***				
Mean SUVA											
SD				RIP							
	Mean Fe(II)	Mean DOC	Mean SUVA		Mean Fe(II)	Mean DOC	Mean SUVA				
Cumulative CH_4	ns	ns	0.422 *	Cumulative CH_4	ns	ns	ns				
Mean Fe(II)		0.642 ***	ns	Mean Fe(II)		ns	0.554 **				
Mean DOC			ns	Mean DOC			ns				

Table 7. Main yield parameters of rice at harvest.

		Grain	Straw	Root	Harvest Index	Density		Unfilled grain per panicle
		(t DM ha^{-1})			(%)	(n plant^{-1})	(n m^{-2})	(%)
Straw	SI	6.72^{b}	6.77^{b}	5.66	49.79	1.86	556.16	9.0
	SR	7.16^{a}	7.12^{a}	5.59	50.03	1.94	580.03	9.2
Fertilization								
	M	8.78^{a}	8.73^{a}	6.27	50.11	2.13^{a}	650.77^{a}	8.6
	RD	7.23^{b}	7.21^{b}	5.66	50.03	2.02^{a}	597.72^{a}	11.1
	SD	7.03^{b}	6.98^{b}	5.62	50.16	1.96^{a}	583.57^{a}	9.5
	Control	4.72^{c}	4.85^{c}	4.94	49.33	1.48^{b}	440.33^{b}	7.9
P(f) Straw		0.044	0.046	ns	ns	ns	ns	ns
P(f) Fertilization		0.000	0.000	ns	ns	0.000	0.000	ns
P(f) Straw*Fertilization		ns	ns	ns	ns	ns	ns	ns

more labile, soluble organic constituents. Nonetheless, the slightly higher CH_4 emissions observed after application of the solid fraction with respect the raw digestate (+11%) and mineral fertilization (+14%) was probably more ascribable to the higher amounts of C supplied. The respective contributions of amount and degradability of C supplied via the two organic fertilizers remain however difficult to disentangle with the current experimental setup.

The order of magnitude of cumulative CH_4 emissions over the cropping cycle were in good agreement with results obtained from field studies measured in the Italian rice area (Peyron *et al*., 2016; Bertora *et al*., 2018a), supporting the validity of mesocosm studies in simulating real paddy field conditions. Moreover, Bertora *et al*. (2018a) also evidenced a significant reduction in CH_4 emissions with straw removal practices, that almost halved (–46%) annual cumulative fluxes with respect to incorporation in spring. With respect to the temporal dynamics, our results confirmed the peculiar behaviour of fluxes during the pinpoint flooding technique adopted at seedling stage, during which the soil is partially drained to allow for root anchoring (Peyron *et al*., 2016; Bertora *et al*., 2018a). This was responsible for the early season intense emission peaks, over and above the typical trend of CH_4 fluxes with crop growth and subsequent potential of CH_4 transportation through the rice aerenchyma (Pittelkow *et al*., 2013). Other temperate and tropical studies of CH_4 emissions from rice paddies typically reported major fluxes during the reproductive stages (Gogoi *et al*., 2005; Meijide *et al*., 2011; Bayer *et al*., 2015). Seedling-stage peaks represented major emission fluxes for treatments with straw incorporation (Figure 2). In fact, the contribution of straw incorporation to the higher measured cumulative emissions was only significant at the beginning of the cropping season, particularly during the early and late vegetative stages, with no significant effect during the subsequent reproductive stages (Table 2). It can be hypothesized that straw decomposition supplied important amounts of labile compounds with a high methanogenic potential predominantly during vegetative stages (Katoh *et al*., 2005; Said-Pullicino *et al*., 2016), while during the later stages organic substrates were mainly soil-derived. One hand, in fact, pore water DOC concentrations were generally higher in treatments receiving straw with respect to those without straw irrespective of the phenological stage; however, on the other hand, higher SUVA values were observed during the reproductive stages suggesting an increase in the contribution of more aromatic, soil-derived organic C.

Only for the mineral fertilized treatment, CH_4 fluxes at seedling-stage were not the highest in presence of straw incorporation, and major CH_4 emissions were measured in the reproductive stage. Although not statistically significant, during this stage, the latter treatment emitted on average 22% more CH_4 than those treatments receiving organic fertilizers irrespective of straw management, and this was probably linked to a more effective CH_4 aerenchyma transportation due to higher crop vigour. As expected, reducing conditions resulting from flooding led to an increase in pore water DOC concentrations with time across all treatments (Figure 5) known to be a direct consequence of a slower microbial mineralization with respect to early stages of organic matter decomposition, together with the release of soluble organic constituents during the reductive dissolution of Fe (hydr)oxides under anoxic conditions (Said-Pullicino *et al*., 2016). The latter process was responsible for the concomitant increase in pore water Fe(II) concentrations with the establishment of anaerobic conditions (Figure 4). The overall highly significant correlation between DOC and Fe(II) concentrations suggests a strongly link between DOC and Fe cycling with redox fluctuations over the cropping season. Nonetheless, the similar trends and maximum pore water Fe(II) concentrations across treatments, irrespective of organic matter inputs, suggest that Fe(III) (hydr)oxide reduction was hardly limited by organic matter availability in this relatively young temperate paddy soil. In fact, minor but significant difference in Fe(II) concentrations between soils with or without straw were only noted at the beginning of the cropping season (Table 3), in line with our previous findings in the field (Bertora *et al*., 2018a). The relatively high SUVA values throughout the flooded period of the cropping season suggests a relative enrichment of more aromatic organic constituents of DOC under anoxic conditions. Although we do not have an explanation for the bimodal trend in SUVA values observed for soils receiving organic fertilizers but not for those receiving mineral fertilizer, we speculate that this could be due to the contribution of different C sources (*i.e*. native and added organic C) in the organically amended soils. Both soil organic matter and exogenous organic matter added with the digestates can contribute more aromatic constituents to the DOC pool under anoxic conditions, albeit through different mechanisms. In contrast to our previous findings from field-scale studies (Said-Pullicino *et al*., 2016; Bertora *et al*., 2018a), in this study correlation analysis showed that DOC is a weak descriptor of CH_4 emissions where a significant correlation between the two variables with a modest Pearson coefficient was only obtained when data were analysed globally (Table 6). However, this confirms previous insights that the source and consequently the quality of DOC in rice paddies may determine the link between topsoil DOC concentrations and substrate availability for CH_4 production (Ye and Horwath, 2017; Bertora *et al*., 2018a). Conversely, Fe(II) concentrations represented a good proxy of CH_4 emissions, probably as an indicator of reducing soil conditions necessary for methanogenic processes. Straw incorporation not only enhanced CH_4 emissions, but also caused a yield depression, independently from the fertilization strategy. As largely acknowledged, this is probably the result of a reduction in plant N availability due to enhanced microbial N immobilization, particularly during the early stages, preventing optimal crop vigour and proper rice N uptake (Said-Pullicino *et al*., 2014; Cucu *et al*., 2017). This was supported by early season SPAD observations and flag leaf length, and, affected both grain and straw final production. Both organic fertilizers were not fully able to sustain crop N requirements with respect to mineral fertilizer.

Although total N supply was equal, approximately 50 and 97% of the applied N with the raw and solid fraction of the digestate, respectively, was in organic form available for rice nutrition only after microbial decomposition and mineralization. Organic fertilizers are less N efficient than mineral fertilizers in aerobic conditions (Moretti *et al*., 2020), and this effect is further magnified under anoxic conditions (Ishii *et al*., 2011). This yield-depression effect was reported to be evident in the first additions, but repeated applications can reduce differences with mineral fertilizers (Zhang *et al*., 2018). When combining emissions and yield in the eco-efficiency indicator (*i.e*. the amount of grain obtained for each unit of emitted CH_4), a significant effect of straw management was evidenced, with values of 10.5 and 15.7 Mg of grain per Mg of emitted CH_4-C with and without straw incorporation, respectively. Fertilization management, on the contrary, showed statistically similar mean values (14.9, 13.1, and 11.4 Mg grain Mg CH_4-C^{-1} for mineral, raw digestate and solid fraction, respectively). The environmental costs of rice grain in terms of CH_4 emitted at field level depends on the type of C input, with a negative effect of straw incorporation that was not observed for the utilization of digestate and its solid fraction.

Conclusions

Straw management was decisive for determining both rice yield and CH_4 emissions, while the impact of fertilization treatments was crucial only for crop productivity. Results obtained suggest a detrimental effect of straw incorporation when incorporated in temporal proximity to rice seeding and field flooding, for both agronomic and environmental aspects, with lower grain yields and enhanced CH_4 emissions. The alternative use of straw as part of the feedstock in biogas plants for the production of organic fertilizers (digested or its solid fraction) and subsequent return to the soil can mitigate CH_4 emissions at field scale, partially substitute mineral N fertilization, but can also lead to a reduction in crop yields over the short-term that warrants attention. Further investigations are required to evaluate the beneficial effects of repeated additions of these organic materials on the accrual of organic matter pools guaranteeing a yield stabilization, and enhancing SOC stocks without increasing CH_4 emissions.

Highlights

Rice straw incorporation into the soil increased methane emissions with respect to its removal

No significant differences in methane emissions were observed between mineral and organic fertilization

Both straw incorporation and organic fertilization decreased rice productivity

Pore water Fe(II) concentrations represented a good proxy for CH_4 emissions especially during the initial stages of the cropping cycle.

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