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Dissolved organic carbon cycling, methane emissions and related microbial populations in temperate rice paddies with contrasting straw and water management.

Chiara Bertora[1], Maria Alexandra Cucu[2,3], Cristina Lerda[2], Matteo Peyron[1], Laura Bardi[3], Roberta Gorra[2] Dario Sacco[1], Luisella Celi[2], Daniel Said-Pullicino[2]

[1]*Environmental Agronomy, Dept. of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco, Italy.*
[2]*Soil Biogeochemistry, Dept. of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco, Italy.*
[3]*CREA-IT, Council for Agricultural Research and Economics, Research Centre for Engineering and Agro-Food Processing, Torino, Italy.*

*Corresponding author: Dr. Chiara Bertora, Dept. of Agricultural, Forest and Food Sciences, University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy (Email: chiara.bertora@unito.it; Tel: +39.011.670.8905)

Abstract

Rice cultivation is recognised as a pivotal source of atmospheric methane (CH_4), accounting for 11% of global emissions. The main drivers of CH_4 production are redox conditions of soil, substrate availability, and abundance of methanogenic archaea, all potentially governed by management practices for straw and water management. In the present study, we combined crop residue and water management practices aimed at limiting substrate availability and reducing soil conditions required for methanogenesis, and tested their efficiency for mitigating CH_4 emission in a field trial conducted on a long-term experimental platform. Combined straw and water management practices (i.e. the early crop residue incorporation, AUT, the adoption of dry with respect to water seeding, DRY, and the straw removal, REM) were effective in reducing dissolved organic carbon (DOC) concentrations, methanogenic abundances and overall CH_4 fluxes, with respect to the typical technique adopted in the Italian rice district that involves spring incorporation of straw and water seeding (SPR). The latter treatment enhanced substrate availability as well as favoured methanogenic archaea abundances and resulted in the highest CH_4 fluxes and cumulative emissions. Treatments AUT and REM showed similar behaviours, reducing emissions of SPR by 48% and 46%, respectively. The highest mitigation efficiency was obtained by DRY that reduced emissions by 69% as a result of the oxic soil conditions during the early vegetative stage, the decreased substrate availability with the onset of field flooding, and the lower abundance of methanogenic communities.

Keywords: Rice straw incorporation; Water or dry seeding; Methanogenic and methanotrophic communities; Straw decomposition; Reductive dissolution; Methane eco-efficiency.

1. Introduction

Rice cultivation represents the major source (11%) of global methane (CH_4) emissions, one of the principal greenhouse gases, with annual emissions estimated to range between 493 and 723 Mt CO_2-eq yr^{-1} in 2010 (Kimura et al. 2004; Smith et al. 2014). Several field studies in the past two decades have been dedicated to the identification of agricultural practices that could substantially reduce CH_4 emissions from rice paddies, while maintaining or increasing rice yields. Most of these mitigation strategies involve irrigation water management practices alternative to continuous flooding or a reduction in organic matter (OM) inputs into the soil (Peyron et al. 2016; Tyagi et al., 2010; Wang et al. 2012; Xu et al., 2015; Yang et al., 2012; Zou et al. 2005), although their effectiveness often also depends on specific pedoclimatic conditions (Wassmann et al., 2004). Peyron et al. (2016) recently showed that dry seeding and delayed flooding can reduce annual CH_4 emissions from temperate rice paddies by 60 % with respect to the conventional water seeding and continuous flooding, while adoption of intermittent irrigation regimes can totally prevent CH_4 emissions. Similarly, rice straw removal was shown to mitigate CH_4 emissions by 46 % with respect to incorporation prior to continuously flooded rice cultivation (Wang et al. 2012). Nonetheless, the influence of combined straw and water management practices on the underlying soil processes controlling CH_4 emissions (e.g. availability of electron donors and acceptors, methanogenic and methanotrophic abundances) are still not well understood and warrant further investigation.

Temporal variations in CH_4 emissions from paddy soils over the cropping season generally follow the pattern of dissolved organic carbon (DOC) concentrations in the topsoil (Lu et al. 2000; Said-Pullicino et al. 2016). Under anoxic conditions, DOC may provide C substrates, i.e. electron donors, for anaerobic microorganisms including methanogenic archaea, recognized as key players in CH_4 production in rice paddies (Conrad 2007; Thauer et al. 2008; Watanabe et al. 2007). Liu et al. (2012a; 2014a) reported that water-extractable organic C contents and composition may influence substrate availability and substrate-driven changes in the abundance and community structure of methanogenic archaea in natural wetlands. Moreover, net CH_4 emissions are also influenced by CH_4 oxidation due to methanotrophs, the abundance and activity of which was shown to be strongly influenced by management practices adopted in rice paddies (Dubey 2005; Ma et al. 2013; Zhang et al. 2013).

Rice paddy soils are generally characterized by large concentrations and fluxes of DOC in comparison to other ecosystems (Kögel-Knabner et al. 2010; Krupa et al. 2012). Crop residues incorporated into the soil after harvest represent the main input of organic C into paddy soils, returning about 2-3 Mg C ha^{-1} yr^{-1} in single-cropped rice paddies (Kimura et al. 2004). The anaerobic decomposition of these residues may supply important amounts of DOC to soil porewaters (Katoh et al. 2005; Ruark et al. 2010) and also affect its chemical composition due to the different biodegradability of its constituents as a function of soil redox conditions (Chen et al. 2010). Moreover, the supply of electron donors with the input of residue-derived labile OM may further increase DOC contents by stimulating the microbially-catalyzed reductive dissolution of Fe and Mn oxyhydroxides under anoxic conditions, and release of soil organic carbon (SOC) derived DOC previously stabilized on the mineral matrix (i.e. positive feedback; Grybos et al. 2009; Said-Pullicino et al. 2016).

DOC cycling in rice paddies is also known to be strongly influenced by water management practices, not only by altering soil moisture and redox conditions, and associated microbial processes, but also by affecting hydrological flow regimes and DOC transport along the soil profile. Said-Pullicino et al. (2016) have shown that the combination of relatively high porewater DOC concentrations under anoxic soil conditions (> 10-20 mg C l^{-1}) and important percolation fluxes of DOC during field flooding (18-51 g C m^{-2}) may influence C availability for microbial processes in the topsoil over the cropping season.

These considerations suggest that straw and water management practices that influence the input, turnover and transport of DOC in rice paddies, are expected to have important implications on CH_4 emissions, and consequently affect the agro-ecosystem C balance (Kindler et al. 2011; Ye and Horwath 2017). In fact, the combination of crop residue and water management practices adopted in temperate rice paddies may play an important but still not well understood role controlling DOC cycling, and therefore the C source/sink functions of paddy soil agro-ecosystems (Kimura et al. 2004; Kögel-Knabner et al. 2010). Moreover, our understanding of how variations in substrate availability and specific redox conditions influence the relative abundance of methanogens and methanotrophs in a rice paddy soil during the cropping season is still limited. Based on these considerations we hypothesize that a combination of crop residue and water management practices that bring about a reduction in the amount of labile organic C present in the soil during field flooding may mitigate CH_4 emissions by limiting substrate availability for methanogens. We tested this hypothesis by evaluating the effects of (i) the timing of crop residue incorporation (spring or autumn), (ii) the adoption of dry seeding rather than water seeding, and (iii) the removal of rice straw, on variations in the quantity and quality of DOC at different soil depths, related microbial abundances and resultant CH_4 emissions, over the cropping season within a long-term temperate paddy field experimental platform (NW Italy).

2. Materials and Methods

2.1 Experimental site description

The field experiment was carried out within a long-term experimental platform located in the plains of the river Po (45°17′47″N, 8°25′51″E; Vercelli, NW Italy) in the western Po River basin (132 m a.s.l.) dedicated to single-cropped rice cultivation for the last 30 years. In 2003, the 2-ha platform was divided into hydrologically isolated plots for comparing different rice cultivation techniques. Climate within this area is temperate, sub-continental, including rainy periods in spring (April and May) and autumn (September–November). Mean annual temperature is 12.8 °C and annual precipitation 936 mm. Mean daily air temperatures and cumulative precipitation over the experimental period are shown in Fig. 1. The soil of the experimental field was classified as a Typic Endoaquept, coarse-silty, mixed, non-acidic, mesic soil (Sacco et al., 2012; Soil Survey Staff, 2010). The topsoil above the plough pan (0-30 cm) had a sandy loam texture (65 and 411 g kg^{-1} clay and silt, respectively) a pH of 6.3 and mean organic C of 11.5 g kg^{-1}. The contents of oxalate and dithionite-citrate-bicarbonate extractable Fe were 3.2 and 7.0 g kg^{-1}, respectively.

2.2 Experimental design and management

The study was conducted from May to October 2014. Field treatments involved the comparison of four long-term crop residue and water management practices (Fig. 2), including: (a) tillage and crop residue incorporation in spring followed by water seeding (SPR); (b) tillage and crop residue incorporation in spring in combination with dry seeding and 1-month delayed flooding (DRY); (c) tillage and crop residue incorporation in autumn with water seeding in the following spring (AUT); (d) post-harvest straw removal, tillage in spring and water seeding (REM).

Details of the experimental design and agricultural practices were reported previously in Sacco et al. (2012) and Zhao et al. (2015), while a comprehensive list of the field operations adopted during the 2014 cropping season is provided in Table A1 of Appendix A in Supplementary material. Briefly, all plots were hydrologically isolated by 80-cm embankments, had an area of 1840 m^2 (23×80 m), and received water from a common inflow canal. Soil was tilled in spring for all treatments, except AUT (tilled during previous autumn) with a moldboard plough, then laser levelled, seedbeds prepared by means of a rotovator, and finally seeded with rice (*Oryza sativa* L. cv. Loto; 180 kg ha^{-1}). For the REM treatment, straw was removed from field before tillage.

In the water seeded treatments, pinpoint flooding method was applied (Hardke and Scott, 2013) during the seedling stage (mid-May), following the typical practices of the region. During this period, flooding was gradually stopped for ten days to allow the radicle to penetrate the soil and anchor the seedling. Although no ponding water was present, the soil was almost saturated throughout this phase. Flooding was subsequently re-established and a permanent ponding water depth of 5-20 cm was continuously maintained until the field was drained approximately one month prior to harvest (beginning October), except for two short mid-season drainage events (3–4 days) in correspondence with the two top-dressing fertilizations at tillering and panicle initiation stage, respectively. In the DRY treatment, after dry seeding the field was kept drained for 40 days until tillering, after which water management followed the same technique described for the other treatments.

For each field treatment, 130 kg N ha^{-1} of urea were applied and split between a pre-seeding basal fertilization and two top-dressed fertilizations at tillering and panicle differentiation stages, as follows: 60-40-30 kg N ha^{-1} for SPR, AUT and REM, and 40-60-30 kg N ha^{-1} for DRY. A different fertilizer N split rate was adopted for DRY with respect to the other treatments to limit N losses via nitrification/denitrification and NH_3 volatilization during the beginning of the cropping season. All plots also received 22 kg P ha^{-1} as basal fertilization and 83 kg K ha^{-1} split half as basal fertilization and half at panicle differentiation. Weeds and pests were controlled as needed, following recommended practices for the region.

2.3 Water sampling and analyses

Ceramic suction cups were installed vertically at 20 and 40 cm depths to collect soil solutions above and below the plough pan respectively, with three replicates per plot. All water samples were collected on a weekly basis, filtered through a 0.45 µm nylon membrane filter, and subsequently analyzed for DOC, specific ultraviolet absorbance at 254 nm (SUVA), Fe^{2+} and Mn. Dissolved organic carbon was determined using Pt-catalyzed, 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 concentration of aromatic ring structures in dissolved OM (Weishaar et al. 2003). This allowed to evaluate changes in DOC quality during the cropping season due to desorption of soil-derived DOC or the relative enrichment of more aromatic organic constituents, as a result of the selective mineralization of DOC. Dissolved Fe^{2+} was fixed by addition of 1,10-phenanthroline in the field immediately after sampling and concentrations measured colorimetrically (Loeppert and Inskeep, 1996), while Mn was determined by atomic absorption spectroscopy in acidified aliquots (AAnalyst 400, Perkin Elmer, USA). The initial rates of Fe^{2+} and Mn release in the topsoil porewaters as a result of the biotic reductive dissolution of Fe and Mn oxyhydroxides, were calculated from the linear regression of the respective concentration data points over the first 20 days after the onset of field flooding. These rates were used to evaluate the influence of management practices on the availability of organic substrates for the anaerobic microbial biomass.

2.4 Methane flux measurements

Methane emissions were measured over the whole rice cropping season by the non-steady-state closed chamber technique as described in detail by Peyron et al. (2016). During emission measurement, rectangular stainless steel flux chambers (0.27 m^2) were place over the vegetation and onto anchors installed into the soil prior to seeding. Plant density within the chamber was equal to that of the surrounding field (i.e. 630 stems m^{-2}) Gas samples were collected at 0, 15 and 30 min after chamber closure, transferred into pre-evacuated vials (Exetainer®, Labco Limited, UK), and subsequently analyzed by gas chromatography with flame ionization detection (Agilent 7890A, Santa Clara CA, USA). Methane emission fluxes (expressed in g C m^{-2} d^{-1}) were calculated from the rate of increase in gas concentration in the chamber expressed as the slope of the linear regression of CH_4 against time. When the slope decreased over the sampling period due to a deviation from non-steady state conditions, fluxes were calculated by applying the nonlinear Hutchinson and Mosier (1981) model. Cumulative emissions for the whole season were then calculated assuming linearity between subsequent sampling events.

CH_4 Eco-efficiency for the different treatments was then estimated as the ratio between produced grain and emitted CH_4, based on grain yield data obtained for the same year of experimental activity (namely, 7.1, 5.8, 6.3 and 7.0 Mg d.m. ha^{-1} for SPR, DRY, AUT, and REM, respectively), and expressed as Mg grain Mg^{-1} CO_2-equivalents of emitted CH_4.

2.5 DNA extraction and quantification of marker genes

Topsoil samples were collected for microbiological analysis at three times during the cropping season in correspondence with the early vegetative (20 June 2014), late vegetative (4 July 2014) and reproductive stages (24 July 2014). All soil samples were collected from plots that were under flooded conditions, except for those from the DRY treatment during the early vegetative stage.

DNA was extracted from triplicate homogenized fresh soil subsamples (approximate 500 mg each) using the FastDNA® SPIN Kit for Soil (MPiomedicals, Solon, OH, USA) following the manufacturer's protocol. Concentrations of DNA extracted were measured on a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The following microbial groups were measured by qPCR: abundance of methanogens harbouring the *mcrA* gene encoding the α-subunit of the methyl coenzyme M reductase, and the abundance of methanotrophs harbouring the *pmoA* gene encoding the α subunit of membrane-bound particulate methane monooxygenase (pMMO). According to Kolb et al. (2003), assays targeting for the subgroups of aerobic methanotrophs, characterized by different ecological strategies (Ho et al., 2013), type Ia and Ib (MBAC and MCOC) and type II (meth II), were chosen. Amplification of the qPCR products was carried out on a Chromo 4TM Continuous Fluorescence Detector associated with PTC-200 termocycler (MJ Research, St. Bruno, Quebec, Canada) with the thermal profiles and primers as described by Steinberg and Regan (2009) for methanogens and by Kolb et al. (2003) for methanotrophs. The 20 µl reaction mixture was composed of 10 µl SsoAdvanced Universal SYBR Green Supermix (Bio Rad), 0.3 µM of forward and reverse primers, and 2 µl. To control the specificity of qPCR products and their correct fragment size, a melt curve analysis (dissociation stage) and/or a gel electrophoresis on 1 % agarose gel were performed after each run. Standard curves were obtained with serial plasmid dilutions of the respective genes (Kolb et al., 2003; Steinberg and Regan, 2009).

2.6 Data analyses

Data were analysed by means of a linear mixed model. Single ceramic cup for soil solution extraction or chamber for CH_4 emission measurement or sampling area for microbial abundances were considered random subjects. Data collected over the whole cropping season were divided into four phenological stages namely early vegetative stage from seeding to tillering (EVEG), late vegetative stage from tillering to panicle initiation (LVEG), reproductive stage from panicle initiation to flowering (REP), and ripening stage from flowering to harvest (RIP). These phenological stages were considered as repeated measures. Variance-covariance matrix was modelled using a compound symmetry type. Residuals were checked for normality using the Shapiro-Wilk test and when not normally distributed, data were log-transformed and normality checked again. Statistical analysis included Treatment, Stage and Treatment × Stage effects.

As DRY was not flooded during EVEG, ceramic cups were not always able to sample soil solution from the topsoil for chemical analysis, and consequently data was often missing. For parameters related to topsoil ceramic cups, statistical analysis was therefore run independently for EVEG and other stages.

Correlations between daily CH_4 emissions, and chemical composition of topsoil and subsoil porewaters were analysed by means of Pearson correlation both for all treatments together and for each treatment separately, while when microbial abundances were also considered, correlations were analysed only for all treatments together.

3. Results

3.1 Soil solution dissolved organic carbon

Variations in DOC concentrations in the topsoil over the cropping season were strongly influenced by the management practice adopted. Generally, DOC accumulation was observed under flooded soil conditions, towards the beginning of the cropping season, and concentrations tended to decrease with time (Fig. 3). In all water seeded treatments (SPR, AUT and REM), DOC concentrations at 20 cm tended to increase rapidly with the onset of field flooding during the early vegetative stage, while relatively low concentrations were maintained during the same period under dry seeding (DRY). Highest topsoil DOC contents during the early vegetative stage decreased in the order SPR ≥ AUT > REM > DRY with maximum concentrations of 38, 32, 19 and 11 mg C l^{-1}, respectively (Fig. 3). Mean DOC concentrations over this period were significantly higher in SPR and AUT with respect to REM ($P < 0.05$; Table 1).

Trends in topsoil DOC after the early vegetative stage varied among treatments. In SPR and AUT, DOC concentrations tended to decrease with time with lowest mean values observed during reproductive and ripening stages (Table 1). However, in both treatments a slight and temporary increase in DOC contents was observed towards the end of the cropping season, though mean concentrations in the reproductive and ripening states were not significantly different. REM generally showed lower DOC concentrations with respect to the other treatments throughout the cropping season, with highest maximum and mean concentrations observed during the late vegetative stage. In the DRY treatment, topsoil DOC concentrations increased rapidly with the onset of field flooding at tillering, and remained relatively high (on average 28 mg C l^{-1}) during both the late vegetative and reproductive stages, with the exception of a temporary drop in DOC in correspondence with mid-season drainage. As for the other treatments, concentrations tended to decrease to initial values after final field drainage during the ripening stage.

The initial increase in topsoil DOC contents with field flooding in the water seeded treatments also corresponded to an increase in DOC at 40 cm, particularly for SPR where concentrations as high as 24 mg C l^{-1} were recorded (Fig. 3). These higher concentrations were however limited to the early vegetative stage when mean DOC concentrations between treatments ranged between 11-19 mg C l^{-1} (except for SPR where this increase was maintained throughout the late vegetative stage too). Lower mean subsoil DOC concentrations (7-12 mg C l^{-1}) were observed for all water seeded treatments during the other phenological stages, with lowest concentrations generally observed for REM. Subsoil DOC contents in DRY remained relatively low and constant throughout the cropping season with a mean concentration of 9 mg C l^{-1}.

The increase in topsoil DOC concentrations with field flooding was generally accompanied by an increase in its aromatic character evidenced by increasing SUVA values (Table 1). Relatively low mean SUVA values were observed for all treatments during the early vegetative stage (3.9-4.7 l mg^{-1} m^{-1}) that tended to increase to highest values towards the reproductive stage (5.3-7.1 l mg^{-1} m^{-1}), only to decrease again at ripening (4.0-5.6 l mg^{-1} m^{-1}). Mean SUVA values over the whole cropping season for the different treatments did not show significant differences ($P > 0.05$), though a significant treatment × phenological stage interaction was observed ($P < 0.05$). In fact, during the late vegetative stage DRY showed a lower mean SUVA with respect to the other treatments, while during the ripening stage SPR showed the highest mean SUVA value.

3.2 Dissolved iron (II) and manganese concentrations

Soil solution Fe^{2+} and Mn concentrations generally depended on soil redox conditions and therefore resulted in a significant distinction between the trends observed for water seeded treatments (SPR, AUT and REM) with respect to the dry seeded (DRY) treatment (Fig. 4 and 5). In general, Fe^{2+} and Mn concentrations in the topsoil increased rapidly with field flooding reaching maximum values of 26-32 mg Fe l^{-1} and 4-16 mg Mn l^{-1} respectively, and decreased rapidly with final field drainage.

Mean values over the different phenological stages differed significantly between treatments (*P(F)* treatment × stage = 0.001). During the early vegetative stage higher mean topsoil Fe^{2+} concentrations were observed in SPR and AUT (15.3 and 12.9 mg Fe l^{-1}, respectively) with respect to REM (6.2 mg Fe l^{-1}), while Fe^{2+} was not detected in DRY. In the late vegetative stage mean Fe^{2+} concentrations were significantly lower in DRY (10.6 mg Fe l^{-1}) than the other treatments (23.4, 22.7 and 19.7 mg Fe l^{-1} for SPR, AUT and REM, respectively). No differences between treatments were observed during the reproductive stage, while during ripening highest mean concentrations were obtained for SPR (12.9 mg Fe l^{-1}) that were significantly higher than those obtained for REM and DRY (7.8 and 8.1 mg Fe l^{-1}).

Mean topsoil Mn concentrations also differed for treatments in the different stages (*P(F)*= treatment × stage = 0.009). During the early vegetative stage, mean concentrations decreased in the order SPR > AUT > REM with values of 7.4, 3.7 and 1.4 mg Mn l^{-1}, respectively, while Mn was not detected in DRY. Similarly in the late vegetative stage highest and lowest mean Mn concentrations were observed in SPR (10.1 mg Mn l^{-1}) and REM (1.4 mg Mn l^{-1}), respectively. In both the reproductive and ripening stages, REM continued to show lowest mean Mn concentrations that were significantly lower with respect to the other treatments that showed similar concentrations.

The crop residue management practices studied also influenced the initial rates of increase in soil solution Fe^{2+} and Mn concentrations (at 20 cm) after the onset of field flooding (Table 2). In fact, SPR showed the highest rates of increase for both Fe^{2+} and Mn that were 5 to 6 times faster than those observed for REM. DRY and AUT showed intermediate rates of increase that were nonetheless 2.5 to 4.3 times faster with respect to REM.

3.3 Methane emissions

Crop residue management significantly influenced the intensity and seasonal pattern of CH_4 emissions during the cropping season (Fig. 6). SPR showed the earliest and highest emission peaks. In detail, emissions started immediately after the onset of flooding and increased nearly continuously towards a maximum flux observed in correspondence with the drainage event for root anchoring during the early vegetative seedling stage. This peak of 0.94 g C m^{-2} d^{-1} represents the earliest and highest daily flux observed over the whole experimental period among all treatments studied. Fluxes tended to decrease afterwards and were generally stable (on average 0.44 g C m^{-2} d^{-1}) for approximately one month. A second significant peak in CH_4 emissions was observed around panicle differentiation (0.82 g C m^{-2} d^{-1}), after which fluxes were again rather constant (on average 0.48 g C m^{-2} d^{-1}) during the following reproductive and ripening stages, until a sharp drop in emissions after final drainage in preparation to harvest.

In AUT, CH_4 emissions showed a gradual increase after the onset of flooding with fluxes generally remaining below 0.26 g C m^{-2} d^{-1} throughout the flooded period except for three peaks, around panicle differentiation (0.43 g C m^{-2} d^{-1}), a second more intense peak at booting stage (0.55 g C m^{-2} d^{-1}), and a third immediately after final drainage (0.36 g C m^{-2} d^{-1}).

In REM, CH_4 emissions did not start immediately after the initial onset of flooding but approximately one month later, when flooding was re-established after the root anchoring drainage. Methane fluxes were generally stable thereafter, with a mean value of 0.28 g C m^{-2} d^{-1} except for a late season peak (0.59 g C m^{-2} d^{-1}) in correspondence with final drainage.

Methane emissions in DRY were absent during the early vegetative stage and increased gradually approximately 10 days after the onset of flooding. Fluxes were relatively low throughout the cropping season except for a first peak (0.38 g C m^{-2} d^{-1}) at late vegetative stage and a second, more intense peak (0.65 g C m^{-2} d^{-1}), at flowering stage.

When comparing mean cumulative fluxes produced by the different treatments at the different phenological stages (Table 3), a significant interaction between treatment and stage effects was clearly identified. Major differences among treatments were observed during the early vegetative stage, with SPR showing the highest fluxes, DRY the lowest, while AUT and REM showed intermediate values. However, SPR maintained its higher cumulative fluxes even during the late vegetative and reproductive stages. In contrast, lowest cumulative emissions were observed for DRY in the late vegetative and ripening stages, and for REM in the reproductive stage.

Cumulative emissions over the whole cropping season, from seeding to harvest, decreased in the order SPR > REM > DRY, with AUT in intermediate position between REM and DRY, but not statistically different from them (Fig. 7). Values for methane Eco-efficiency showed an inverted rank among treatments with respect to cumulative emissions. In fact, DRY showed the highest value (0.93 Mg grain Mg^{-1} CO_2-eq), followed by AUT and REM (0.68 and 0.66 Mg grain Mg^{-1} CO_2-eq, respectively), while the lowest value was observed for SPR (0.37 Mg grain Mg^{-1} CO_2-eq).

3.5 Methanogenic and methanotrophic community abundances

The influence of management practices was more evident on the methanogenic rather than on the sum of methanotrophic types (TOTmeth), as shown in Table 4. In particular, the greatest difference in methanogens *mcrA* gene abundance among treatments was observed in the early vegetative stage, when SPR showed the highest abundance, DRY the lowest, while AUT and REM showed intermediate values. During the late vegetative and reproductive stages no significant differences were observed in the abundance of methanogens in the water seeded treatments (i.e. SPR, AUT and REM), whereas DRY always showed the lowest abundance. Across all treatments *mcrA* abundance was generally lower in the reproductive stage with respect to the early and late vegetative ones.

The abundance of total methanotrophs did not show any significant differences among treatments, except for the late vegetative stage when REM showed a significantly higher abundance with respect to SPR. In general, the abundances of TOTmeth were generally higher in the early and late vegetative stages with respect to the reproductive stage.

When evaluating the different methanotrophic communities, the highest abundances were shown by MBAC, followed by meth II, and MCOC (Table 5). The abundance of MBAC was not significantly influenced by treatment although a significant interaction of treatment with stage was observed. In fact, significantly higher abundances were observed for REM with respect to SPR only in the late vegetative stage (Table 5). As for TOTmeth, MBAC were generally higher in the early and late vegetative stages with respect to the reproductive stage. On the other hand, treatments significantly influenced MCOC abundance only in the early vegetative stage, when higher values for SPR and DRY with respect to AUT and REM were observed (Table 5). In contrast to MBAC, MCOC abundance tended to increase with time over the phenological stages. Management did not significantly influence the abundance of the meth II methanotrophs although a significant effect of phenological stage was observed. In fact, higher meth II abundances were observed in the late vegetative with respect to the early vegetative and reproductive stages.

3.4 Correlations analysis

Methane emissions were positively correlated with topsoil concentration of DOC, Fe^{2+} and Mn, both considering all treatments together and separating them, with the exception of AUT, where CH_4 emissions were not correlated with DOC (Table 6). Correlations between DOC concentrations at the two soil depths showed that DOC concentrations above (20cm) and below (40 cm) the plough pan were positively correlated in all treatments when considered together, and only in SPR and AUT when considered separately. SUVA was positively correlated with CH_4 and DOC in the topsoil, but this correlation was weak (CH_4) or even absent (DOC) in SPR. Among microbial abundances, mcrA were positively correlated with CH_4 emissions.

4. Discussion

4.1 Dissolved organic carbon cycling

Soil DOC may have different sources including root exudates, plant residue decomposition by-products, and SOC (Bolan et al. 2011). Root-derived organic C is rapidly mineralized and hardly contributes to DOC concentrations (He et al. 2015), although this C pool was shown to contribute to CH_4 emission during the later stages of the rice cropping season (Kimura et al., 2004). Soil organic C often represents the dominant source of DOC in paddy soils, although the presence of plant residues may lead to a temporary contribution of residue-derived DOC, and provide substrate for CH_4 production (Ye and Horwath, 2017). Moreover, apart from accelerating the creation of reduced conditions, straw addition can prime the release of soil-derived DOC and further enhance CH_4 emissions from paddy soils (Ye and Horwath, 2017), although the mechanisms involved and the substrate functions of different DOC sources for methanogens remain elusive. Based on these concepts we hypothesized that in temperate rice paddies, management-driven changes in DOC concentrations, particularly during the early stages of the cropping season, may be linked to substrate availability for anaerobic microorganisms including methanogens.

Porewater DOC concentrations during the cropping season were strongly affected by combined straw and water management practices, clearly showing that DOC cycling in paddy soils depends on both the input of organic C and soil redox conditions. The incorporation of crop residues generally represents the main input of organic C into paddy soils (Kimura et al. 2004). Considering that residue decomposition is one of the main active processes contributing DOC to paddy topsoil water, predominantly during the first stages of the cropping season (Katoh et al. 2005), management practices that affect the return of crop residues to the topsoil, and/or the rates of OM decomposition and mineralization are expected to influence DOC cycling.

Among the water seeded treatments (i.e. SPR, AUT and REM), the management practice that involved post-harvest straw removal also resulted in lowest maximum and mean topsoil DOC concentrations. This can be potentially attributed to a smaller input of plant residues with respect to the practices that involved straw incorporation. In contrast, the timing of crop residue incorporation (i.e. spring vs. autumn) did not result in significant differences in the mean DOC concentrations over the different phenological stages, and variations in the temporal trends were similar in the two treatments. Spring incorporation did however result in slightly higher maximum concentrations immediately after field flooding. In this respect, incorporation of residues in proximity of field flooding with spring tillage could have contributed important amounts of relatively labile DOC having a fast turnover even under anaerobic soil conditions. On the other hand, autumn incorporation probably allowed for the partial oxic degradation and mineralization of the more labile fraction of the plant residues during the warmer months of the intercropping period before the beginning of the cropping season (i.e. from March to mid-May). This could have influenced the quality of straw-derived OM present in the soil with the onset of flooding, leading to the release of less biodegradable DOC with a slower turnover under anoxic conditions.

Adoption of dry seeding and delayed flooding (DRY) with respect to water seeding and continuous flooding (SPR, AUT and REM) affected the soil moisture status during the early vegetative stage. This was expected to influence soil redox conditions and consequently the rates of OM degradation and mineralization. The higher topsoil DOC concentrations in the water seeded (13-38, 14-32 and 10-19 mg C l^{-1} for SPR, AUT and REM, respectively) with respect to the dry seeded (8-11 mg C l^{-1} for DRY) treatments during the early vegetative stage was attributed to the limited or incomplete decomposition of OM and reduced DOC mineralization rates under anoxic soil conditions in the former (Devêvre and Horwáth, 2000; Sahrawat 2004). These results are in line with the typically high DOC concentrations that generally characterize flooded rice paddies (He et al. 2015; Kögel-Knabner et al. 2010; Maie et al. 2004), and confirm previous findings by Said-Pullicino et al. (2016) who also observed elevated topsoil DOC concentrations in excess of 10-20 mg C l^{-1} under reducing conditions resulting from field flooding in temperate rice paddies. He et al. (2015; 2017) also reported a similar increase in DOC over the cropping season under continuous paddy rice cropping, although maximum concentrations were smaller. The authors argue that the influence of management practices on DOC accrual under anoxic conditions may strongly depend on soil properties (e.g. clay content, SOC contents) and porewater sampling depth.

On the other hand, the fast OM turnover and rapid mineralization of DOC under oxic soil conditions present in the early stages of the dry seeded treatment resulted in relatively low DOC concentrations. Nonetheless, with the onset of field flooding at tillering, topsoil DOC concentrations in this treatment increased to values around 30 mg C l^{-1}, such that no significant differences in mean concentrations were observed between dry and water seeded treatments (i.e. DRY vs. SPR) during the late vegetative, reproductive and ripening stages showing that one month of oxic soil conditions does not reduce the potential for DOC accrual.

The influence of combined straw and water management practices on substrate availability for the anaerobic microbial biomass under flooded conditions was evaluated by determining Fe^{2+} and Mn concentrations in porewaters. Under water saturated conditions, available O_2 is rapidly depleted and the soil microorganisms use alternative electron acceptors for the anaerobic mineralization of OM. The dissimilatory reduction and dissolution of Fe(III) and Mn(IV) (hydr)oxides coupled with OM decomposition leads to release of Fe^{2+} and Mn^{2+} in solution, the rate of which depends on the amount of labile OM available for microbial utilization (Reddy and DeLaune, 2008). The initial rates of Fe^{2+} and Mn release in the topsoil porewaters reflected the relative availability of labile OM under anoxic conditions. Crop residue incorporation in spring close to field flooding favoured the reductive dissolution of Fe and Mn (hydr)oxides leading to the fastest rates and highest concentrations of Fe^{2+} and Mn in solution. In contrast, early residue incorporation in autumn or straw removal led to a reduced availability of labile OM under anoxic conditions, and consequently slower rates of release and lower concentrations of Fe^{2+} and Mn in solution. In the dry seeded treatment, although Fe^{2+} and

Mn concentrations were not detectable when the field was initially drained, concentrations increased steadily with the onset of field flooding at tillering, reaching values only slightly lower than those obtained for water seeded treatments. Nonetheless, slower initial rates of release of Fe^{2+}, but particularly of Mn, with respect to the water seeded treatment (i.e. DRY vs. SPR) were observed. This confirmed the partial degradation of incorporated crop residues under oxic conditions during the early stages of the cropping season, and a reduced availability of labile OM for the anaerobic microbial biomass. Similar results were reported by Said-Pullicino et al. (2016) who also observed a slower and more contained increase in topsoil porewater Fe^{2+} concentrations with field flooding in dry seeded with respect to water seeded treatments.

Fe and Mn (hydr)oxides are also known to stabilize important amounts of soil OM (Kaiser and Guggenberger, 2000). The reductive dissolution of these (hydr)oxides under anoxic conditions could lead to the abiotic release of soil-derived DOC previously stabilized by these minerals (Grybos et al. 2009). This process could partly explain the observed increasing trend in SUVA values with time, suggesting a shift from residue-derived DOC with a relatively low aromatic character at the beginning of the cropping season to more aromatic, soil-derived DOC towards the later stages. However, the expected differences in the positive feedback of straw incorporation on the release of DOC with the reductive dissolution of Fe and Mn (hydr)oxides (Said-Pullicino et al. 2016; Ye and Horwath, 2017) among treatments were not evidenced by spectroscopic analysis alone. In fact, crop residue incorporation in spring, which favoured the dissolution of Fe and Mn (hydr)oxides, did not show significantly higher SUVA values as did the other continuously flooded treatments in which mineral dissolution was less marked. Most probably the greater contribution of fresh, straw-derived C with crop residue incorporation in proximity of field flooding could have made the relative increase in aromatic character of DOC upon mineral dissolution difficult to detect. Nonetheless, the overall highly significant inter-correlations obtained between topsoil DOC, SUVA, Fe^{2+} and Mn values all point to a strong link between DOC and Fe/Mn cycling in these soils (Table 6). Considering the treatments separately, strong correlations between these variables were obtained for all treatments except SPR. In the latter, the quantity and quality of DOC during the cropping season was probably influenced by the important contribution of rice straw decomposition to the DOC pool that somewhat masked the contribution of soil-derived C to the DOC pool.

Combined straw and water management also influenced DOC concentrations in the subsoil, although to a lesser extent with respect to the topsoil. Sorption of soluble organic constituents during water percolation results in an exponential decrease in DOC fluxes with increasing soil depth (He et al. 2017), and may explain the smaller influence of management practices on the subsoil porewater DOC pool. Nonetheless, DOC concentrations below the plough pan did reflect differences in OM input with decomposing residues and hydrological flows between treatments. In fact, among the water seeded treatments, crop residue incorporation in spring that resulted in highest DOC concentrations in the topsoil, also led to highest DOC concentration in the subsoil (up to 23.5 mg C l^{-1}), particularly during the earlier stages of the cropping season. On the other hand, straw removal resulted in significantly lower mean DOC concentrations in the subsoil with respect to the other treatments. Dry seeding that was previously shown to bring about lower percolation fluxes of DOC at the beginning of the cropping season when the field was still drained (Said-Pullicino et al. 2016), led to slightly lower DOC concentrations in the subsoil during this period with respect to the water seeded analogue, although mean DOC concentrations over the cropping season were not significantly different. Moreover, the significant correlation between DOC concentrations at the two soil depths analyzed (Table 6) suggests a strong coupling between the topsoil and subsoil DOC pool in these paddy soils where the plough pan did not act as a transport barrier for DOC (c.f. Wissing et al. 2011). However, this correlation was not significant in those treatments where low OM input or reduced water percolation fluxes (i.e. REM and DRY, respectively) negatively affected DOC mobility along the soil profile.

4.2 Methanogenic and methanotrophic community abundances

The influence of combined straw and water management practices on changes in the methanogenic and methanotropic communities was evaluated as potential functionality drivers controlling CH_4 emissions. The observed abundance of the methanogenic community (Table 4) was lowest in the dry seeded treatment over all vegetative stages with respect to the other water seeded treatments, suggesting that aerobic soil conditions at the beginning of the cropping season negatively affected the abundance of methanogenic archaea throughout the cropping season. In agreement with these results, lower CH_4 emissions were observed in the dry seeded treatment. Previous studies have however acknowledged that a significant methanogenic population may survive in paddy soils even under dry conditions, without any significant changes in their abundance and structure, but with important modifications at transcriptional level (Breidenbach and Conrad, 2014; Ma et al. 2012). On the other hand, rice straw incorporation shortly before field flooding with spring tillage resulted in the largest *mcrA* abundance during the early vegetative stage. In this treatment, the combination of greater inputs of straw-derived OM (i.e. greater substrate availability) and anoxic soil conditions represented a suitable environment for the methanogenic archaea. Nevertheless, the influence of straw management practices on substrate availability was limited to the early stages of the cropping season as no significant differences in the *mcrA* gene copy numbers were observed in the water seeded treatments during the late vegetative and reproductive stages. This was probably due to the more relevant role, in these later stages, of the rice root exudation, that may provide more suitable substrates for methanogenesis than OM derived from soil and from straw decomposition (Lu et al., 2004; Pump and Conrad, 2014).

The relationship between methanogen abundances and CH_4 flux data was highlighted by a significant positive correlation between *mcrA* gene and CH_4 emissions, showing a functional connection between their presence and the net result of their catabolism. This was also suggested by the ranking among treatments that is generally the same for both *mcrA* and CH_4 emissions. This positive correlation is in agreement with results reported from flooded rice ecosystems experiments (Lee et al. 2014; Liu et al. 2012b; Ma et al. 2012), and suggest that the *mcrA* gene abundance could serve as a proxy for predicting CH_4 emissions based on different management strategies.

Methane emissions from paddy fields are a result of the net balance between CH_4 production by methanogens and CH_4 oxidation by methanotrophs (Minamikawa and Sakai 2006; Sheng et al. 2016). No significant differences in the total *pmoA* abundance were however observed among treatments, except for the late vegetative stage, when rice straw removal resulted in maximum abundance, probably increasing the methanotrophic function, while crop residue incorporation in spring caused the smallest abundances of *pmoA* genes.

While no clear overall effect of treatments on total methanotrophs was evidenced, different trends were detected among the considered groups. Categorization of methanotrophs types not only represents a phylogenetic distinction but also corresponds to different ecological distributions and life strategies (Ho et al., 2013), different substrate utilization, growth rates and methane affinity (Chowdhury et al., 2013; Shukla et al., 2013), with opposite or diversified responses to water management (Ho et al., 2016). In this sense, although the complexity of factors controlling methanotrophy in this site has not been fully explained, and no significant correlation was obtained between CH_4 fluxes and the abundance of DNA-based methanotroph population sizes, our results suggest that management practices could differentiate distinct ecological niches.

Overall, our data suggest that, from a microbiological point of view, the effects of different crop residue and water management practices on net CH_4 emissions are mainly dictated by their influence on methanogens, rather than on aerobic methanotrophic microrganisms. This, together with the determination of a less predictable influence of treatments on methanotrophs itself, reflects how a possible CH_4 mitigation strategy should be addressed at inhibiting methanogens rather than stimulating methanotrophs.

4.3 Methane emissions

Apart from a direct influence on soil redox conditions, the significant differences in CH_4 fluxes and cumulative emissions over the cropping season among treatments could be interpreted in terms of substrate availability and microbial populations. Ye and Howarth (2017) evidenced a soil-dependent link between straw addition, surface water DOC concentrations and substrate availability for CH_4 production. By means of stable isotope tracing in a water-saturated soil microcosm laboratory incubation, they observed that, in the 30 d period following addition of ^{13}C-labeled rice straw to a paddy soil having a low SOC content (20 g C kg^{-1}), 25-38% of DOC and 52-67% of total CH_4 production were residue-derived. They also showed that the contribution of residue-derived C to the DOC and CH_4 pools decreased with increasing SOC contents due to a relative increase in the priming efficiency of rice residues. In our field experiment, we observed a significant positive correlation between CH_4 emissions, and both topsoil DOC concentrations and the abundance of methanogenic *mcrA* communities across treatments (Table 6). This is consistent with our hypothesis that, in temperate rice paddies having relatively low SOC contents (here 11.5 g C kg^{-1}), management-induced changes in DOC concentrations during the cropping season may be linked to substrate availability for CH_4 production. However, considering the high temporal and spatial variability in the contribution of different organic C sources to the DOC pool in rice paddies, the significance of DOC as a substrate for methanogenic microorganisms still needs to be confirmed through further research, possibly involving *in situ* stable isotope tracing studies.

Crop residue incorporation in spring, which was shown to enhance substrate availability as well as favour the presence of methanogenic archaea, resulted in the highest CH_4 fluxes and cumulative emissions, not only during the early vegetative stage but also during the late vegetative and reproductive stages. This was in line with the significant positive correlations observed between CH_4 fluxes and topsoil DOC concentrations.Adoption of management practices that involved crop residue incorporation in autumn or straw removal resulted in lower peak CH_4 emissions, as well as significantly lower cumulative emissions during the early vegetative stage with respect to spring incorporation, with both treatments showing similar behaviours (Hussain et al. 2015, Liu et al. 2014b). Although autumn incorporation resulted in slightly higher mean topsoil DOC concentrations and substrate availability for anaerobic microorganisms in the early stages of the cropping season with respect to rice straw removal, this did not seem to influence CH_4 emissions. A plausible explanation for this could be that with autumn incorporation the partially degraded plant residues contributed to a greater release of SOC-derived DOC after field flooding with respect to straw removal, explaining the higher DOC concentrations in the former. However, the primed DOC production did not result in a proportional increase of CH_4 production due to the presumably relatively low degradability of SOC-derived DOC (Ye and Horwath, 2017). DOC concentrations alone may therefore not always explain CH_4 fluxes, as evidenced by the significant correlation between topsoil DOC concentrations and CH_4 fluxes obtained for straw removal but not for autumn incorporation. These observations suggest that the influence management practices may have on the source partitioning and/or quality of DOC during the cropping season may also affect the relevance of this C pool in providing organic substrates for CH_4 production.Delaying the beginning of field flooding through the adoption of dry seeding contributed to maintain the soil under aerobic conditions during the early vegetative phase, thus preventing methanogenesis and CH_4 emissions. Moreover, dry seeding also resulted in lowest CH_4 emissions during the later stages of the cropping season, when relatively high topsoil DOC concentrations were observed. The aerobic soil conditions during the initial phase of the cropping season favoured the decomposition and mineralization of the more labile fractions of residue-derived C, possibly limiting substrate availability for methanogens when the field was subsequently flooded. Apart from the influence of dry seeding on substrate availability, the lower CH_4 emissions observed could be also ascribed to a lower abundance of methanogenic archaea throughout the cropping season with respect to the other treatments (Table 4). This effect was probably enhanced by the long-term nature of the experimental field where these management practices were compared.

Evaluating CH_4 fluxes over the cropping season also allowed to understand which phenological stages or field operations contributed most to the total cumulative emissions of each management practice. Based on a previous experiment (Peyron et al. 2016) and similar studies on temperate rice cropping systems (Bayer et al., 2015; Gogoi et al., 2005; Pittelkow et al., 2013) three main peaks of CH_4 emissions were expected: a first intensification of fluxes during pinpoint drainage, a second in correspondence with maximum biomass growth during the reproductive stage, and a third with final field drainage before harvest. Although the pinpoint drainage peak was recorded in all water seeded treatments, only residue incorporation in spring produced important fluxes, suggesting that water management during seed establishment in the presence of recently incorporated straw is critical for total CH_4 emissions. Methane emissions were frequently reported to increase with crop growth, leading to an intensification of fluxes during the reproductive stage (Pittelkow et al. 2013). In the current study, this was observed at the flowering stage for management practices involving dry seeding and residue incorporation in autumn, and anticipated to panicle initiation for residue incorporation in spring. Final drainage resulted in an important emission peak in all water seeded treatments but not in the dry seeded one. This final contribution to total CH_4 emissions was particularly pronounced in the treatment involving straw removal.

4.4 Environmental implications

Management practices that involved the removal or early incorporation of crop residues as well as the adoption of dry seeding were all effective, to various extents, in mitigating CH_4 emissions from temperate paddy fields with respect to the most widespread technique involving incorporation of crop residues in spring followed by continuous flooding. The latter treatment resulted in highest cumulative CH_4 emissions with up to 548 kg C ha^{-1} over the cropping season. This value was in line with that measured by Sander et al. (2014) in the Philippines, and generally higher than those measured in temperate climate conditions (Peyron et al., 2016; Meijide et al. 2011, Pittelkow et al. 2013).

Rice straw removal or autumn incorporation of crop residues decreased cumulative CH_4 emissions by 46 and 48%, respectively. These results confirm our hypothesis that crop residue management practices that bring about a reduction in the amount of soil labile organic C during field flooding may mitigate CH_4 emissions by limiting substrate availability for methanogens. Considering the higher energy and labour costs required for straw removal, as well as the consequent reduction in the return of plant nutrients to the soil, the similar behaviour of these two management practices in mitigating CH_4 emissions suggests that early incorporation of crop residues can represent a more sustainable option for temperate rice paddies.

The adoption of dry seeding resulted in the lowest cumulative CH_4 emissions with a reduction of 69% in the total CH_4 emitted compared to the water seeded analogue. Similar results were obtained by Pittelkow et al. (2014) and Peyron et al. (2016) who observed that avoiding anoxic conditions during the early stages of the growing season strongly limited methanogenesis and reduced the overall seasonal CH_4 emissions by 35–70% in rice paddies. Our results show that delaying field flooding until tillering stage can be very effective in mitigating CH_4 emissions, not only by avoiding the establishment of anoxic soil conditions necessary for CH_4 production, but also by affecting the abundance of methanogenic communities, and favouring the degradation of incorporated crop residues thereby reducing substrate availability for methanogens when field flooding is initiated at tillering.

Proper evaluation of the influence of management practices on the mitigation of CH_4 emissions requires considerations on the grain yields of the different treatments (equal to 7.1, 7.0, 6.0 and 5.8 Mg of grain ha^{-1} for SPR, REM, AUT, and DRY, respectively; unpublished data). The CH_4 Eco-efficiency, i.e. the amount of grain yield obtained per unit CH_4 emitted, expressed in Mg grain Mg^{-1} CO_2-equivalent units, showed that, among the treatments, crop residue incorporation in spring emitted the most CH_4 to reach its elevated productive performance. On the other hand, despite producing the lowest yields, dry seeding resulted in the highest amount of grain per unit CH_4 emitted confirming the high environmental sustainability of this management practice with respect to CH_4 emissions.

Conclusions

Results from this study suggest that combined straw and water management practices can represent excellent strategies to significantly mitigate CH_4 emissions from rice paddies. Increasing the temporal distance between straw incorporation and the establishment of flooding was always successful in reducing CH_4 fluxes. In fact, practices that promote the turnover of incorporated residues under oxic soil conditions affect both the quantity and quality of the DOC pool during the cropping season, and contribute to reduce CH_4 emissions possibly by affecting C availability for anaerobic microbial populations. The substrate function of different sources of DOC for methanogenic microorganisms however remains an open question. Although straw removal led to relatively low early season DOC concentrations, this did not favor CH_4 mitigation more than crop residue incorporation in autumn, which is surely an operationally simpler practice and is agronomically more appropriate in terms of soil fertility. The highest mitigation efficiency was obtained by dry seeding. This practice, which maintains the field drained during the early vegetative stage, resulted in lowest cumulative CH_4 emissions by both allowing for the partial decomposition of incorporated crop residues under oxic conditions and reducing the abundance of methanogenic communities with respect to water seeded treatments.

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Table 1: Mean log-transformed soil solution DOC concentrations (20 and 40 cm) and SUVA values (20 cm) over the cropping season at different phenological stages for the different crop residue management practices.

Treatment	Early vegetative stage	Late vegetative stage	Reproductive stage	Ripening stage	Average for treatment
	DOC at 20 cm (mg C l^{-1})				
SPR	3,4 (31.0) a	3,4 (30.3)	3,0 (20.1)	3,1 (21.6) a	3,2 (23.6)
DRY	nd	3,3 (27.7)	3,3 (27.7)	2,9 (18.4) a	3,2 (24.2)
AUT	3,3 (26.6) a	3,3 (26.7)	3,1 (22.7)	2,7 (15.3) ab	3,0 (21.0)
REM	2,7 (14.5) b	2,9 (18.3)	2,7 (15.0)	2,2 (8.7) b	2,6 (13.4)
Average for stage	3,1 (22.8)	3,2 (25.3)	3,0 (20.9)	2,7 (15.1)	
P(F) Treat	*0,003*	*ns*			
P (F) Stage	-	*0,000*			
*P(F) Treat*Stage*	-	*0,013*			
	DOC at 40 cm (mg C l^{-1})				
SPR	2,9 (18.8)	2,7 (14.6)	2,5 (12.2)	2,4 (11.2)	2,6 (13.9) a
DRY	2,4 (10.6)	2,0 (7.6)	2,2 (9.1)	2,2 (9.2)	2,2 (9.1) ab
AUT	2,6 (13.3)	2,3 (10.2)	2,2 (9.3)	2,2 (9.1)	2,3 (10.3) ab
REM	2,4 (10.6)	2,0 (7.4)	2,0 (7.5)	2,0 (7.3)	2,1 (8.1) b
Average for stage	2,6 (13.0) a	2,3 (9.5) b	2,2 (9.4) b	2,2 (9.1) b	
P(F) Treat	*0,037*				
P (F) Stage	*0,000*				
*P(F) Treat*Stage*	*ns*				
	SUVA at 20 cm (l mg^{-1} m^{-1})				
SPR	1,55 (4.69)	1,75 (5.74) ab	1,67 (5.34)	1,73 (5.61) a	1,72 (5.56)
DRY	nd	1,45 (4.25) b	1,79 (6.02)	1,40 (4.06) b	1,55 (4.70)
AUT	1,40 (4.07)	1,71 (5.53) ab	1,86 (6.41)	1,45 (4.26) ab	1,67 (5.33)
REM	1,36 (3.91)	1,81 (6.12) a	1,96 (7.09)	1,39 (4.00) b	1,72 (5.58)
Average for stage	1,44 (4.21)	1,68 (5.36)	1,82 (6.18)	1,49 (4.44)	
P(F) Treat	*ns*	*ns*			
P (F) Stage	-	*0,000*			
*P(F) Treat*Stage*	-	*0,003*			

Values represent logarithmic estimated marginal means, while back-transformed values are shown in parenthesis. Natural logarithm was used for data transformation. Phenological stages: Early vegetative stage from seeding to tillering; Late vegetative stage from tillering to panicle initiation; Reproductive stage from panicle initiation to flowering; Ripening stage from flowering to harvest.

Table 2: Initial rates of increase in porewater Fe^{2+} and Mn determined from the linear regression of soil solution concentrations (at 20 cm) over the first 20 days after the onset of flooding for the different crop residue management practices.

Treatment	Initial rate of Fe^{2+} increase ($mg\ l^{-1}\ d^{-1}$)	*r*	Initial rate of Mn increase ($mg\ l^{-1}\ d^{-1}$)	*r*
SPR	1.31 ± 0.11	0.9626	0.45 ± 0.07	0.8678
DRY	1.11 ± 0.10	0.9519	0.28 ± 0.05	0.8438
AUT	0.71 ± 0.07	0.9583	0.20 ± 0.04	0.8467
REM	0.26 ± 0.09	0.7404	0.08 ± 0.02	0.7475

Values represent linear coefficients ± standard error; *r* is the linear correlation coefficient.

Table 3: Log-transformed cumulative CH_4 emissions (kg C ha^{-1}) over the cropping season and at different phenological stages for the different crop residue management practices.

Treatment	Early vegetative stage	Late vegetative stage	Reproductive stage	Ripening stage	Average for treatment
	Cumulative CH_4 fluxes (kg C ha^{-1})				
SPR	5,12 (168.16) a	4,72 (111.74) a	5,10 (163.90) a	4,30 (73.91) a	4,81 (122.83)
DRY	-0,55 (0.58) c	3,52 (33.64) b	4,62 (101.41) ab	3,34 (28.23) b	2,73 (15.36)
AUT	3,61 (36.88) b	4,32 (74.99) ab	4,69 (108.70) ab	3,98 (53.59) a	4,15 (63.35)
REM	3,94 (51.52) b	4,35 (77.85) ab	4,51 (91.34) b	4,13 (62.01) a	4,23 (69.04)
Average for stage	3,03 (20.72)	4,23 (68.44)	4,73 (113.34)	3,94 (51.31)	
P(F) Treat	*0,000*				
P (F) Stage	*0,000*				
*P(F) Treat*Stage*	*0,000*				

Values represent logarithmic estimated marginal means, while back-transformed values shown in parenthesis. Natural logarithm was used for data transformation. Phenological stages: Early vegetative stage from seeding to tillering (duration: 32 days for all treatments, except for DRY, with 35 days); Late vegetative stage from tillering to panicle initiation (duration: 28 days); Reproductive stage from panicle initiation to flowering (duration: 31 days); Ripening stage from flowering to harvest (duration: 47 days).

Table 4: Abundance of methanogenic (mcrA) and sum of abundances of methanotrophic groups (TOTmeth) over the cropping season and at different phenological stages for the different crop residue management practices.

Treatment	Early vegetative stage	Late vegetative stage	Reproductive stage	Average for treatment
	mcrA (log-copy $\mu gDNA^{-1}$)			
SPR	7,054 a	6,658 a	6,548 a	6,754
DRY	6,191 c	6,254 b	6,070 b	6,172
AUT	6,642 b	6,897 a	6,265 ab	6,601
REM	6,486 b	6,499 ab	6,265 ab	6,417
Average for flooding	6,593	6,577	6,287	
P(F) Treat	*0,000*			
P (F) Stage	*0,000*			
*P(F) Treat*Stage*	*0,008*			
	TOTmeth (log-copy $\mu gDNA^{-1}$)			
SPR	7,569	7,374 b	7,146	7,363
DRY	7,439	7,457 ab	7,337	7,411
AUT	7,459	7,598 ab	7,125	7,394
REM	7,466	7,697 a	7,171	7,445
Average for flooding	7,483	7,531	7,195	
P(F) Treat	*ns*			
P (F) Stage	*0,000*			
*P(F) Treat*Stage*	*0,016*			

Values represent estimated marginal means. Logarithm to base 10 was used for data transformation.

Table 5: Abundance of different methanotrophic groups over the cropping season and at different phenological stages for the different crop residue management practices.

Treatment	Early vegetative stage	Late vegetative stage	Reproductive stage	Average for treatment
	MBAC (log-copy $\mu gDNA^{-1}$)			
SPR	7,538	7,248 b	7,032	7,273
DRY	7,390	7,345 ab	7,224	7,319
AUT	7,420	7,478 ab	7,058	7,319
REM	7,418	7,561 a	7,056	7,345
Average for flooding	7,442	7,408	7,092	
P(F) Treat	*ns*			
P (F) Stage	*0,000*			
*P(F) Treat*Stage*	*0,022*			
	MCOC (log-copy $\mu gDNA^{-1}$)			
SPR	5,508 a	5,326	5,539	5,457
DRY	5,588 a	5,432	5,623	5,547
AUT	4,595 b	5,359	5,477	5,144
REM	4,532 b	5,470	5,324	5,109
Average for flooding	5,056	5,397	5,491	
P(F) Treat	*0,004*			
P (F) Flooding	*0,000*			
*P(F) Treat*Flooding*	*0,001*			
	meth II (log-copy $\mu gDNA^{-1}$)			
SPR	6,344	6,748	6,430	6,507
DRY	6,390	6,784	6,619	6,598
AUT	6,375	6,934	6,208	6,506
REM	6,476	7,110	6,501	6,696
Average for flooding	6,396 b	6,894 a	6,439 b	
P(F) Treat	*ns*			
P (F) Stage	*0,000*			
*P(F) Treat*Stage*	*ns*			

Values represent estimated marginal means. Logarithm to base 10 was used for data transformation.

Table 6. Correlations between CH_4 emissions (g C m^{-2} d^{-1}), dissolved organic carbon (DOC) concentrations (mg C l^{-1}) at 20 and 40 cm depth, Fe^{2+} and Mn concentrations (mg Fe l^{-1}) at 20 cm, SUVA at 20 cm, Mn concentrations (mg l^{-1}) at 20 cm, abundance of methanogenic (mcrA, logcopy DNA-1) and methanotrophic (TOTmeth, logcopy DNA^{-1}) communities.

Treatment		n	DOC at 20 cm	n	SUVA at 20 cm	n	Fe(II) at 20 cm	n	Mn at 20 cm	n	DOC at 40 cm	n	mcrA	n	TOTmeth
SPR	CH_4	29	0.530**	27	0.385*	27	0.706***	26	0.609**	28	0.471*				
	DOC at 20 cm			27	0,232	27	0,243	26	-0,123	28	0.703***				
	SUVA at 20 cm					26	0.448*	25	0,262	26	0,057				
	Fe(II) at 20 cm							26	0.808***	26	0,089				
	Mn at 20 cm									25	-0,254				
DRY	CH_4	22	0.542**	20	0.669**	18	0.664**	20	0.679**	27	-0,172				
	DOC at 20 cm			20	0.608**	18	0.600**	18	0.712**	22	-0,033				
	SUVA at 20 cm					18	0.622**	17	0.608**	20	0,196				
	Fe(II) at 20 cm							17	0.871***	18	0,272				
	Mn at 20 cm									20	-0,194				
AUT	CH_4	29	0,198	29	0.590**	27	0.776***	26	0.829***	29	0,137				
	DOC at 20 cm			29	0.567**	27	0.517**	26	0,286	29	0.642***				
	SUVA at 20 cm					27	0.715***	26	0.660***	29	0,327				
	Fe(II) at 20 cm							26	0.929***	27	0,166				
	Mn at 20 cm									26	0,031				
REM	CH_4	29	0.589**	28	0.624***	27	0.780***	26	0.717***	29	-0,252				
	DOC at 20 cm			28	0.554**	27	0.640***	26	0.537**	29	-0,035				
	SUVA at 20 cm					26	0.717***	25	0.694***	28	-0,306				
	Fe(II) at 20 cm							26	0.843***	27	-0.515**				
	Mn at 20 cm									26	-0.587**				
All	CH_4	109	0.438***	104	0.507***	99	0.694***	98	0.693***	113	0.381***	100	0.615*	11	-0,060
	DOC at 20 cm			104	0.389***	99	0.449***	96	0.427***	108	0.532***	98	0,438	12	0,493
	SUVA at 20 cm					97	0.623***	93	0.336**	103	0,079	95	-0,152	11	-0,405
	Fe(II) at 20 cm							95	0.681***	98	0,154	97	0,173	11	-0,313
	Mn at 20 cm									97	0.343**	95	0,190	11	-0,471
	mcrA													12	0,477

* = $p< 0.05$; ** = $p< 0.01$; *** = $p< 0.001$; n represents the number of matching data pairs.

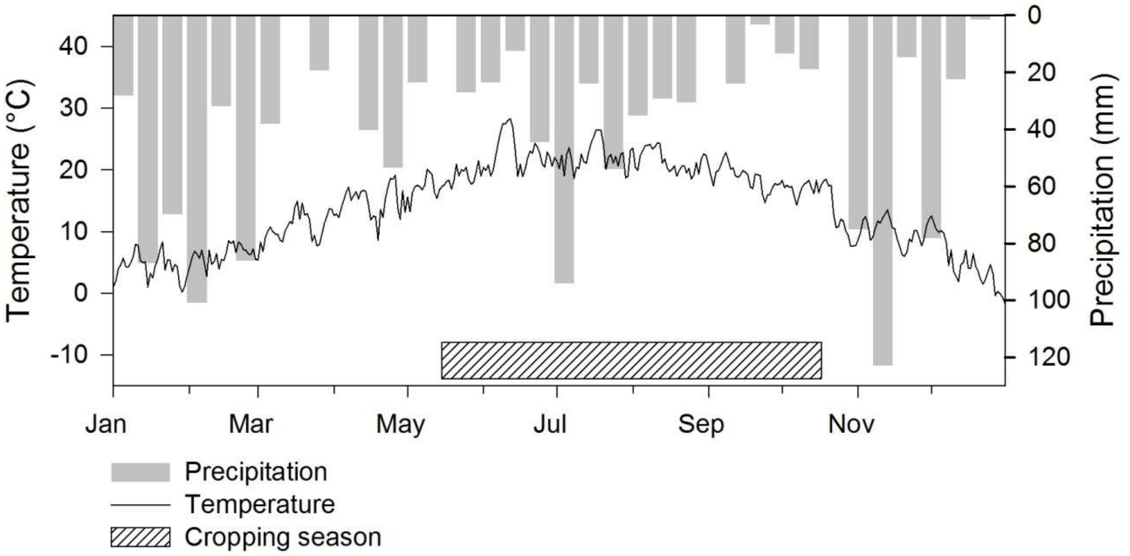

Figure 1. Variations in mean daily air temperatures and cumulative precipitation (over 10 consecutive day periods) during the experimental period (2014).

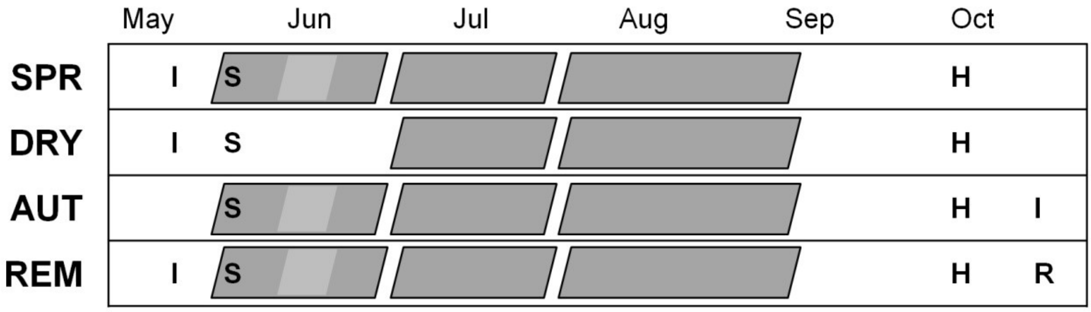

Figure 2. Schematic diagram of the agricultural practices in the four treatments in the experimental period, the cropping season (from May to October 2014), indicating the approximate times of straw incorporation (I), rice straw removal (R), seeding (S), harvest (H), and field flooding (shaded areas). SPR, tillage in spring and water seeding; DRY, tillage in spring, dry seeding and delayed flooding; AUT, tillage in autumn and water seeding; REM, post-harvest removal of straw, tillage in spring and water seeding.

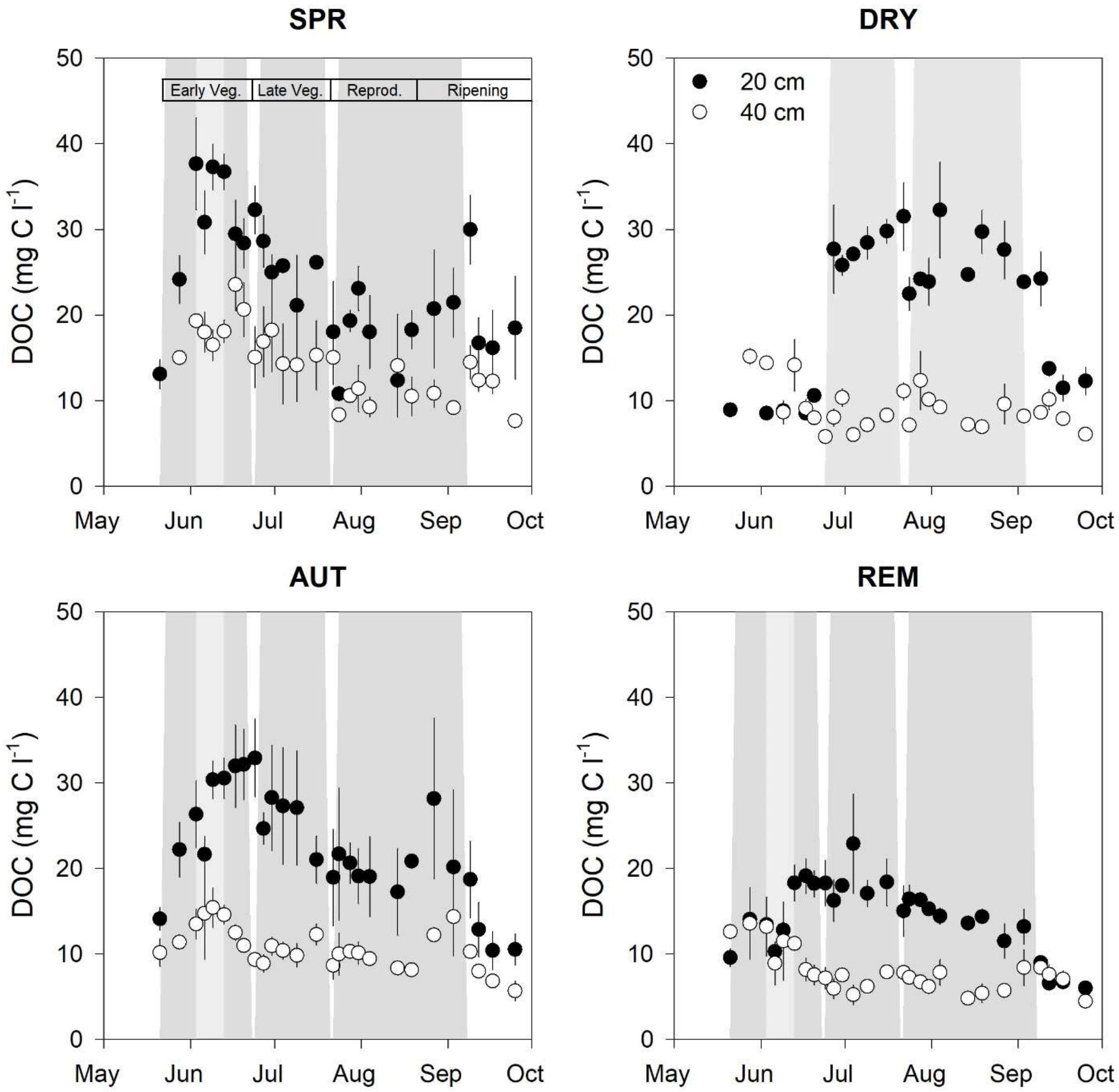

Figure 3. Variations in DOC concentrations at different depths over the cropping season as a function of crop residue management practices involving spring incorporation (SPR), spring incorporation and dry seeding (DRY), autumn incorporation (AUT), and straw removal (REM). Error bars represent standard error of the mean while shaded areas represent pin-point flooding after seeding (light grey) or the presence of ponding water (dark grey).

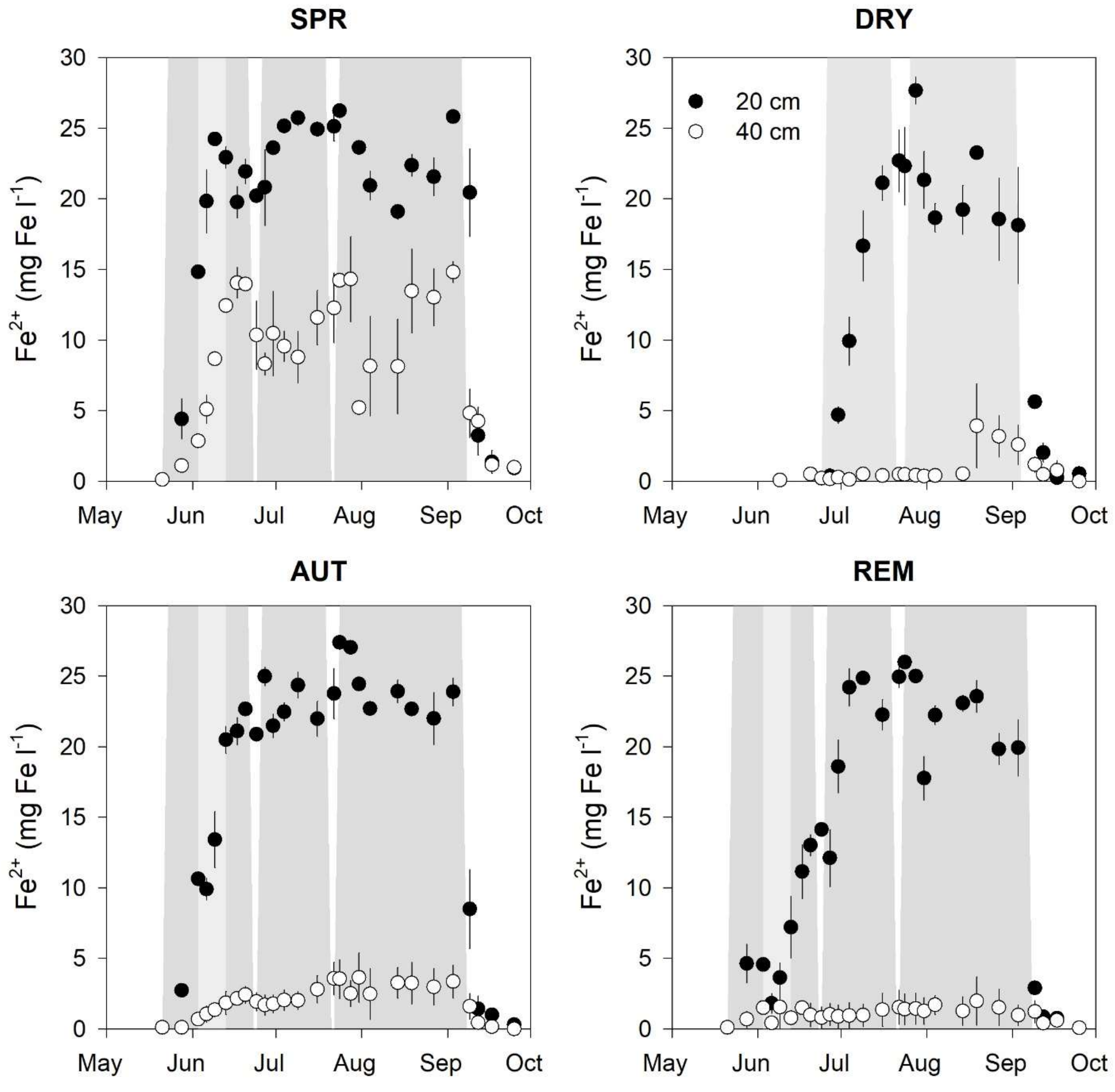

Figure 4. Variations in soil solution Fe^{2+} concentrations at different depths over the cropping season as a function of crop residue management practices involving spring incorporation (SPR), spring incorporation and dry seeding (DRY), autumn incorporation (AUT), and straw removal (REM). Error bars represent standard error of the mean while shaded areas represent pin-point flooding after seeding (light grey) or the presence of ponding water (dark grey).

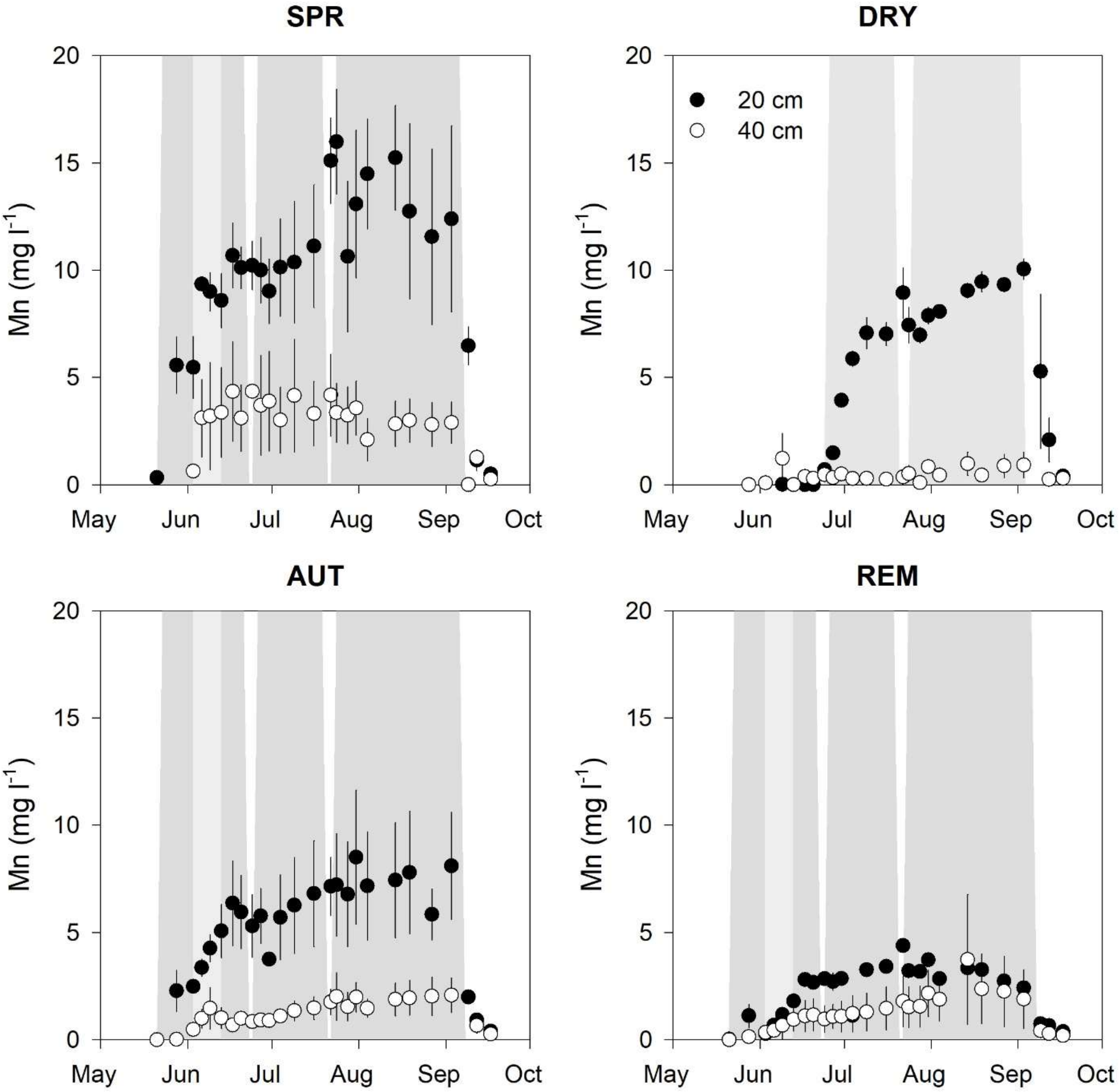

Figure 5. Variations in soil solution Mn concentrations at different depths over the cropping season as a function of crop residue management practices involving spring incorporation (SPR), spring incorporation and dry seeding (DRY), autumn incorporation (AUT), and straw removal (REM). Error bars represent standard error of the mean while shaded areas represent pin-point flooding after seeding (light grey) or the presence of ponding water (dark grey).

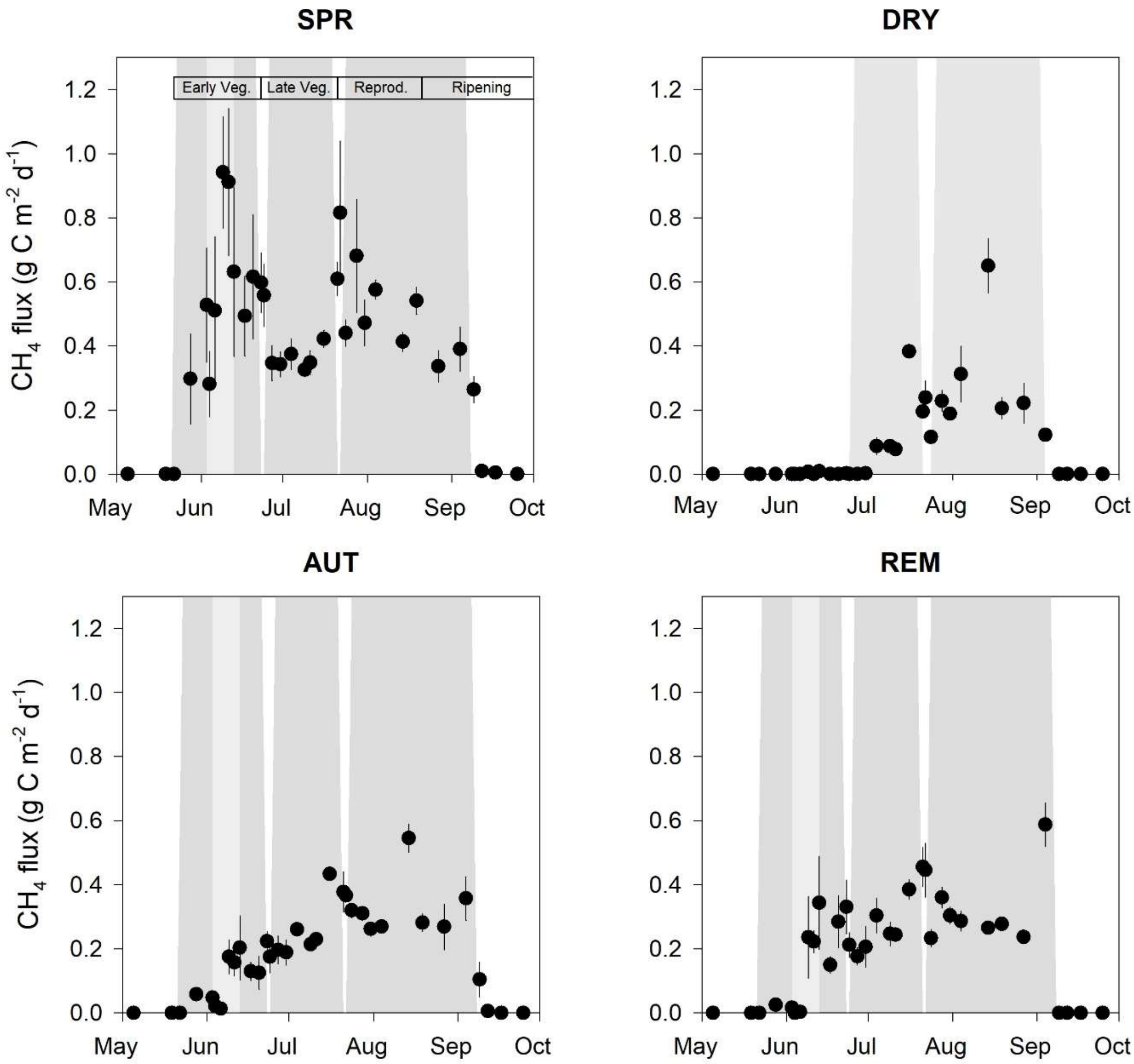

Figure 6. Variations in CH_4 emission fluxes over the cropping seasons as a function of crop residue management practices involving spring incorporation (SPR), spring incorporation and dry seeding (DRY), autumn incorporation (AUT), and straw removal (REM). Error bars represent standard error of the mean while shaded areas represent pin-point flooding after seeding (light grey) or the presence of ponding water (dark grey).

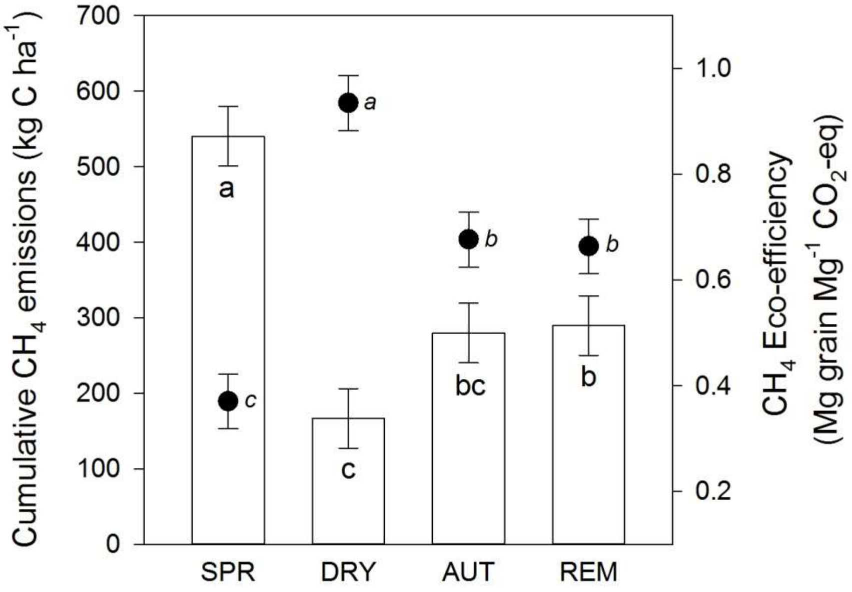

Figure 7. Cumulative CH_4 emissions over the cropping season (bars) and CH_4 Eco-efficiency (symbols) for the different crop residue management practices involving spring incorporation (SPR), spring incorporation and dry seeding (DRY), autumn incorporation (AUT), and straw removal (REM). Different letters indicate a significant difference between mean values ($P<0.05$).