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Effects of Different Water Management and Fertilizer Applications on CO_2 Fluxes from a Selected Myanmar Rice (*Oryza sativa* L.) Cultivar

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Authors' contributions

This work was carried out in collaboration between both authors. Authors SM and MR conceived the study design. Author SM implemented the greenhouse experiment, collected and analyzed the statistical data. Author SM wrote the first draft of the manuscript with the help author MR. Author MR commented on and edited the manuscript. Both authors have read and agreed to final version of the manuscript.

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ABSTRACT

The application of nitrogen fertilizer and the water management practices are important to optimize potential yields in rice cultivation. Moreover, they may affect the emissions patterns of methane (CH_4) and carbon dioxide (CO_2) emission. Compared to methane, knowledge about the combined effects of different fertilizer rates together with different water management practices on CO_2 fluxes are scarce. Therefore, this study aims to assess CO_2 fluxes of a selected rice cultivar in response to different fertilizer applications and water management practices. The treatments included two different applications of inorganic fertilizer (recommended rate and farmer's practice), organic manure application and water management practices; continuous flooding (CF) and alternate wetting and drying (AWD). Mean total CO_2 flux in CF was -30.82 g CO_2 m^{-2} d^{-1} during daytime and 29.64 g CO_2 m^{-2} d^{-1} during nighttime. Surprisingly, the average net CO_2 fluxes were negative under both CF (-49 mg CO_2 $m^{-2}h^{-1}$) and AWD practices (-127 mg CO_2 $m^{-2}h^{-1}$), indicating a net CO_2 uptake by the rice plants. Inorganic fertilizer applications led to considerably higher net CO_2 emissions compared to the control under both CF and AWD. Conversely, CO_2 emission fluxes in the treatment with organic manure showed negative net CO_2 fluxes under both water management practices and

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while revealing the same fresh biomass as observed in other treatments (inorganic fertilizer and control). Taken together, modifications of current cultivation systems toward using organic manure, that emit less CO_2, could effectively mitigate CO_2 impacts regardless of the selected water management practice.

Keywords: Alternate wetting and drying; CO_2 fluxes; continuous flooding; inorganic fertilizer; organic manure; rice.

1. INTRODUCTION

Climate warming is caused by unprecedented emissions of greenhouse gases (GHGs), such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). GHGs emissions are produced to a large extent by anthropogenic activities with CO_2 and CH_4 being the most important gases that contributing 60% and 15%, respectively, to the anthropogenic GHG effect [1]. Agricultural activities are estimated to account for 39% of the global methane emissions and for 1% of the global CO_2 emissions [2]. The main CO_2 emitters are fossil fuel and industrial processes (65%) and forestry and other land uses (11%) [3]. Among the agricultural activities, the Greenhouse Gas Management Organization [4] (2000) reported that paddy fields, most commonly farmed with rice, were the major contributor of GHGs and contribute 57.7% of the emitted greenhouse gases. GHGs from rice cultivation comprise CO_2, CH_4 and N_2O. Under anaerobic conditions typical for paddy fields, CH_4 is released at substantial rates during organic matter decomposition [5], rendering the rice ecosystem a considerable source of CH_4 emission on a global scale. The CO_2 balance of rice fields is also subject to variation and depends on parameters such as rate of photosynthesis and respiration of rice plants, and the metabolic activities of soil microbes. Depending on these parameters, rice fields may both represent a source or a sink for CO_2 [6] and there is still no clear conviction about how important role play rice fields in CO_2 world emissions.

Rice is a main staple food crop for a large part of the world's population. About 80% of the rice fields are grown under flooded condition, i.e. paddy fields, in Asia [7–9]. Amongst the major rice producers, Myanmar holds 7th place in the world ranking by rice growing area and production[10]. The total area of paddy fields in Myanmar amounts to 7.6 million ha, comprising 6.42 million ha cultivated under monsoon and 1.19 million ha under summer paddy conditions with an overall average yield of 4.19 metric ton per hectare [11]. Most of the major rice growing areas belong to irrigated lowland rice fields.

Irrigated lowland paddy fields play an important role in the world food security by providing major source of rice supply. On the other hand, lowland rice fields are the largest consumer of water in the agriculture [12]. Due to the need of relatively high water amounts in lowland rice fields, water shortage is becoming a major challenge nowadays in rice production [13]. The development of water-saving management systems and alternative rice production systems is urgently needed to maintain production during ongoing and future periods of increasing water scarcity [14]. In addition, water management is one of the most promising options to mitigate GHGs emissions [15].

Alternate wetting and drying (AWD) is the most widely adopted water-saving approach and also known as alternating between submergence and non-submergence [16]. AWD represents the alternating irrigation of rice fields with periods of standing water and damp or dry soil conditions which is approximately 30 days after transplanting or planting of rice plants up to harvesting [17]. Wassmann et al. [18] concluded that changing alternate pattern of aerobic and anaerobic condition is the best option compared to normal flooding condition for reducing CH_4 emission by rice fields. This is supported by an independent experiment revealing lower CH_4 emissions in wetting-drying alteration patterns than in continuous flooding [19]. However, water-saving irrigation approaches may increase CO_2 emissions as aerobic conditions favour complete oxidation of carbon compounds to CO_2 rather than CH_4. Simultaneously, increased oxygen availability can increase total microbial activity and as such the decomposition of soil organic matter. Consequently, changes in water management in rice agriculture were reported to alter the soil organic carbon (SOC) balance and soil fertility [20].

Alternate wetting and drying (AWD) causes a reduction of water inputs by about 15–30% and as such is commonly applied as a water-saving approach, but as well to increase rice yield [17,21–23]. AWD causes dramatic changes between aerobic and anaerobic state of the soil environment that could directly influence nitrogen content in soil and plant growth [24]. For instance, higher biomass production as well as greater nitrogen content under aerobic conditions compared to flooded ones was observed by Katsura et al. [25]. Alternate wetting and drying cycle also provides enough oxygen to the root system and creates better conditions for the organic matter mineralization and retards soil nitrogen immobilization, all these factors should increase soil fertility status and enhance rice growth by utilizing essential plant-available nutrients [24, 26-27].

Application of nitrogen fertilizer may increase crop yield as well as the SOC stock [28]. Higher SOC supports higher crop biomass and the microbial decomposition of the crop residues [29]. Although the application of nitrogen fertilizer typically increases the crop biomass, its impact on soil carbon content may vary with the soil type [30]. The application of nitrogen fertilizer also increased CH_4 emissions from paddy fields due to the raising of rice biomass and providing additional C source, but showed no effect on total CO_2 emissions [31]. This process was explained by Stevens et al. [32], due to the fact that CO_2 is reduced to CH_4 under anaerobic environment where oxygen is unavailable. Therefore, microbes metabolizing without oxygen (anaerobes) decompose the organic matter to methane. On the contrary, CO_2 emission increases with increasing plant biomass by the application of nitrogen fertilizer [33]. Salehi et al. [34] observed that the combined effect of cattle manure and chemical fertilizer application increased soil CO_2 flux compared to a single urea application. Chicken manure significantly increases ecosystem respiration (CO_2), however pig slurry has no effect on CO_2 emission in Mediterranean paddies [35]. In contrast to CH_4, the effects of different fertilizing practices on CO_2 seems less consistent and requires further investigations.

Until now, only a few studies have evaluated the combined effects of different water and nitrogen fertilizing regimes on the level of CO_2 emission in rice fields. As such, we sought to investigate these combined effects by monitoring CO_2 dynamics in rice cultures grown under greenhouse conditions subjected to different treatments. This research included different fertilizing regimes as typically applied by farmers in Myanmar. Inorganic fertilizer rates were based on recommended rates according to the extension service from the Ministry of Agriculture, Livestock and Irrigation (MOALI)[36], inorganic fertilizer rate by farmer's practice [37,38] and cow manure application by Moe et al.[39]. Our aims were (1) to quantify CO_2 fluxes under different water management practices and fertilizer application during common Myanmar's rice cultivar cultivation; and (2) to elucidate the combined effect of water management practices and fertilizer application on the biomass yield of rice.

2. MATERIALS AND METHODS

The pot experiment was performed from June to November 2017 under glass greenhouse condition at the Department of Botany, Palacky University in Olomouc, Czech Republic. The tested rice cultivar was Manawthukha (*Oryza sativa* L.) (*indica*) type variety abundantly grown in Myanmar. After soaking in water for 24 h, 3 seeds were transferred per pot (26.5 x 25 cm) filled with 5 kg of alluvial soil with a sandy clay loam texture. After 14 days, seedlings were thinned out to one healthy seedling per pot.

The experiment was laid out by factorial design with 3 replications, including two main treatments factors. For water management treatments, the main factors were CF - continuous flooding, and AWD - alternate wetting and drying. Flooding in the CF was maintained to keep a 5-cm water layer above the soil surface during entire experimental period. For the AWD treatment, the pot was submerged for one day and drying periods of 3 days without standing water were maintained before each new irrigation [40]. After 3 days, treated pots were re-flooded to a 5-cm water layer above the soil surface until the next drying cycle. Subplot factor included different fertilizer treatments with recommended rate of inorganic fertilizer- F1 [Nitrogen (N): 50 kg ha^{-1} (Ammonium Sulphate), Phosphorus (P): 30 kg ha^{-1} (Triple superphosphate) and Potassium (K): 20 kg ha^{-1} (Muriate of potash)], inorganic fertilizer application by farmer's practice- F2 [N: 21 kg ha^{-1}, P: 5 kg ha^{-1} and K: 6 kg ha^{-1}], organic manure application-F3 [cow manure: 5 ton ha^{-1} (1.5% N: 2.5% P_2O_5 and 1.5% K_2O)] and a control [no fertilizer] – F4 (Fig. 1). All treatments were replicated 3 time, yielding a total of 30 pots (4 fertilizer treatments + bare control soil* 2 water management practices (CF/AWD) *3 replicates).

The plexiglass tube chamber (25 cm x 25 cm x 100 cm) was used as mobile gas chamber (Fig. 2), while CO_2 gas fluxes inside the chamber were recorded using the Sense Air® CO_2 sensor Module K33 ELG, designed to measure and store records of environmental parameters such as temperature (T), relative humidity (RH) and carbon dioxide (CO_2) concentration (up to 5000 ppm range) [41]. The sensor device was installed inside the mobile gas chamber together with a fan to enhance CO_2 circulation inside the chamber. The tube chamber was airtight sealed and carefully placed over the rice plant for 10 min in each treatment. Instantaneous CO_2 concentrations (ppmv) inside of the chamber was measured every 30 s during both the day and nighttime. CO_2 fluxes were measured every week, starts from 30 days after planting until the end of the experiment, i.e. 30, 37, 45, 59, 66, 73, 80, 87, 94, 101, 108, 115 and 122 days after planting (DAP) under continuous flooding (CF) and alternate wetting and drying (AWD).

Growth of rice plants was performed with no artificial light, the plants used only light penetrated inside. As we did not measure intensity of irradiation inside of the greenhouse, we used sunlight global radiation data (Wm^{-2}) provided by Czech Meteorological Institute and converted them to Photosynthetic Photon Flux Density (PPFD) values inside the greenhouse by a formula suggested by Nederhoff & Marcelis [42] (Fig. 4a & b). Due to the high variability of CO_2 fluxes by photosynthetic activities during daytime [43], CO_2 flux measurements were performed consistently at a fixed time during entire experimental period: between 9:00 and 12:00 for the day (Fig. 4b), and between 21:00 and 24:00 for the night.

Flux calculation for CO_2 was performed by using the following equation [44]:

$$F_{CO2} = \left({}^{V}/_{A}\right)\left({}^{dc}/_{dt}\right) \qquad (1)$$

Where F_{CO2} is the Total CO_2 flux density (mg CO_2 m^{-2} h^{-1}), *V* and *A* are the volume (litres) and base area (m^2) of the chamber, *dc/dt* describes the CO_2 concentration change in the chamber over time.

The *net CO_2 flux* refers to a difference in total CO_2 fluxes measured during the night and day, while negative and positive values represent uptake or emissions of CO_2 by the rice plants and soil. Daily temperature and humidity changes was automatically recorded every 30 min using portable data logger.

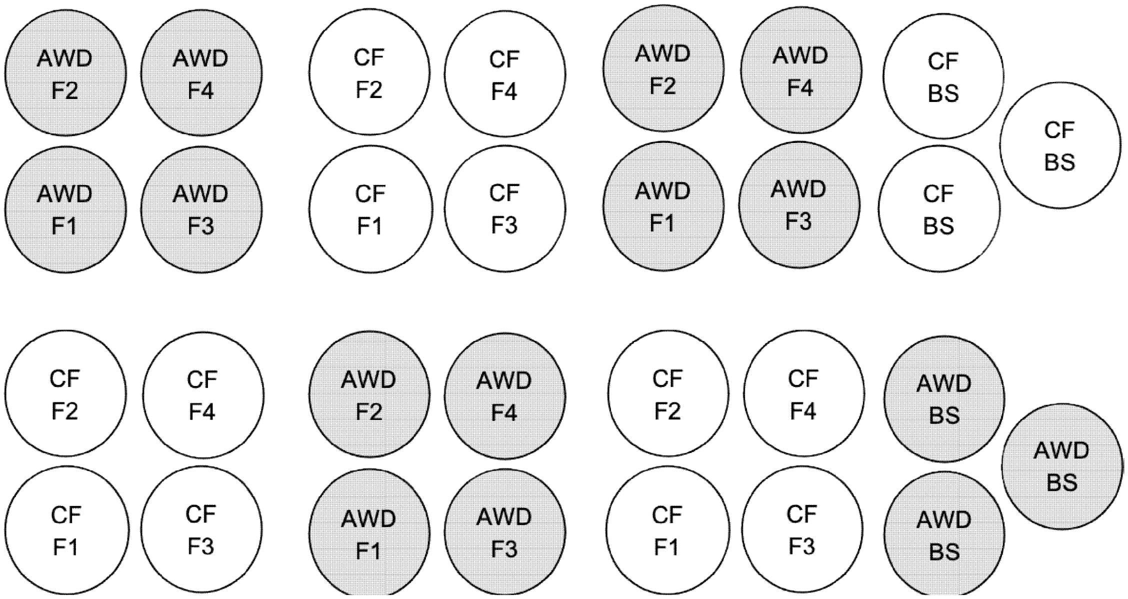

Fig. 1. Layout for experimental pots in the greenhouse

CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha^{-1}, Phosphorus (P) 30 kg ha^{-1} and Potassium (K) 20 kg ha^{-1}]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha^{-1}, Phosphorus (P) 5 kg ha^{-1} and Potassium (K) 6 kg ha^{-1}]; F3 = Organic manure application (cow manure - 5 t ha^{-1}); F4 = Control; BS = Bare Soil

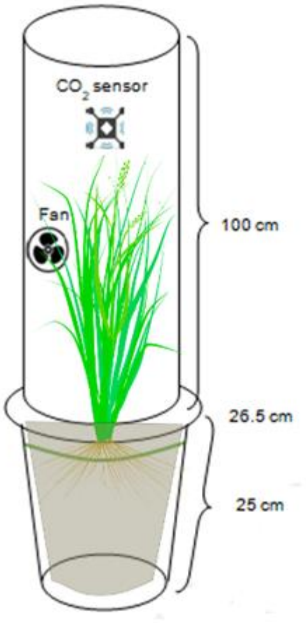

Fig. 2. Plexiglass gas tube chamber for measurement of CO_2 fluxes from rice plant

2.1 Plant Parameters

Mean value for the above and below ground biomass were taken by separating above and below ground portions of rice plants during the biomass harvest. After recording the fresh weight of the biomass, both parts were dried at 80°C for 48 hours to determine the corresponding dry weight. Root were immediately washed by tilting pots and carefully spraying with water until the attached soil and sand particles were removed. Subsequently, the roots volume was analysed by gravimetric apparatus for volume displacement method [45]. Leaf area per plant (cm^2) from individual pots was recorded at 42, 56, 70, 112 and 122 DAP. Leaf area (cm^2) was analysed by using a digital camera and Easy Leaf Area Software based on digital images recorded from the plants [46]. Plant height (cm) was recorded every week starting 14 days after planting (DAP) until harvest. Additionally, plant height (cm), number of tillers per plant, below and above ground biomass (g $plant^{-1}$) were recorded at harvest.

2.2 Data Analysis

Data were subjected to analysis of variance (ANOVA) to assess the effect of water management levels, level of fertilizer application and their interaction with the measured values. To analyse the effects of treatments and fertilizer application on Total and Net CO_2 fluxes, other plant characteristics, above-ground and belowground biomass yields, a two-way ANOVA and Tukey's Honest Significant Different (HSD) post hoc test ($P < 0.05$) were conducted using water treatment and fertilizer application as independent variables and, response variables as Total and Net CO_2 fluxes, other plant characteristics, above-ground and belowground biomass yields, to examine statistically significant differences between means. The statistical analysis was performed using R.

3. RESULTS

3.1 Temperature and Relative Humidity

Mean weekly ambient temperature ranged from 23 to 33 °C with an average to be 29 °C during daytime, while temperature during the night ranged from 22 to 39 °C with an averaged 26 °C (Fig. 3). Relative humidity (RH%) recorded during day and nighttime showed similar patterns, averaged 46% and 50% of daytime and nighttime respectively. Average temperature and relative humidity (%) in the chamber tube showed slight fluctuations during day and night. The temperature ranged between 20-50°C during the day inside the chamber, the minimal and maximal temperature at night measured showed 15 and 30°C, respectively. The relative humidity (%) inside the chamber ranged between 23-91% during the day and 41-94% during the night (Fig. S1; where “S1” denotes supplementary material).

3.2 Photosynthetically Active Radiation (PPFD) Inside the Greenhouse

Based on the data from natural light intensity of the outside environment, the amount of PAR (PPFD) inside the greenhouse ranged from 103-1196 µmol photons m^{-2} s^{-1}, while the average amount of PAR was 641 µmol photons m^{-2} s^{-1} during experimental period (from July to October 2017, Fig. 4a). During the day (5:00-19:00), mean PAR value was 404 µmol photons m^{-2} s^{-1} and ranged from 10-836 µmol photons m^{-2} s^{-1} (Fig. 4b).

3.3 Total CO_2 Fluxes (Day and Night)

Generally, CO_2 emission fluxes measured during daytime were negative for all treatments except 45, 52 and 122 DAP in CF and 59, 115 DAP in AWD treatment (Figs. 5a, b). In contrast, CO_2 emission fluxes were positive during nighttime in all treatments (Figs. 5c, d). Weekly CO_2 fluxes fluctuated considerably under both CF and AWD practices, with a range from -3341 mg CO_2 $m^{-2}h^{-1}$ to 1035 mg CO_2 $m^{-2}h^{-1}$ in CF treatment (Fig. 5a) and from 2015 mg CO_2 $m^{-2}h^{-1}$ to -3729 mg CO_2 $m^{-2}h^{-1}$ in AWD treatment (Fig. 5b). However, differences between various fertilizer subfactors (F1-F4) were less apparent in the AWD treatment compared to those from CF treatment, i.e. CO_2 fluxes remained indifferent in response to different water and fertilizer treatments. Nighttime CO_2 fluxes revealed less fluctuation compared to those from daytime (Figs. 5c, d). CO_2 fluxes ranged from 278 mg CO_2 $m^{-2}h^{-1}$ to 1923 mg CO_2 $m^{-2}h^{-1}$ (Fig. 5c) and from 634 mg CO_2 $m^{-2}h^{-1}$ to 2140 mg CO_2 $m^{-2}h^{-1}$ (Fig. 5d) under CF and AWD treatments, respectively. No interaction effects by different water management practices and fertilizer treatments were observed on average total CO_2 fluxes during daytime and nighttime. Mean CO_2 fluxes during daytime ranged from -2061 to -719 mg CO_2 $m^{-2}h^{-1}$ and nighttime ranged from 938 to 1558 mg CO_2 $m^{-2}h^{-1}$.

3.4 Net CO_2 Fluxes

According to the two-way ANOVA, Net CO_2 flux data averaged per week were not significantly altered by any of the treatments or their interactions. Net CO_2 fluxes varied from -1723 to 2308 mg CO_2 $m^{-2}h^{-1}$ in CF treatment and from -2778 to 3854 mg CO_2 $m^{-2}h^{-1}$ in AWD treatment, respectively (Fig. 6). Generally, mean uptake of net CO_2 flux from AWD (-68 mg CO_2 $m^{-2}h^{-1}$) treatment was higher than the CF (-49 mg CO_2 $m^{-2}h^{-1}$) ones (Table 1). Soil respiration showed significant differences among water management practices during daytime, while no significant differences were found during nighttime. Net soil respiration (388 mg CO_2 $m^{-2}h^{-1}$) was higher in CF compared to AWD (254 mg CO_2 $m^{-2}h^{-1}$) (Table 1).

With respect to inorganic and organic fertilizer applications (F1-F3 treatments) used, only the F3 treatment with organic manure application showed negative net CO_2 fluxes under both water management practices, while remaining treatments applied with inorganic fertilizer (F1 and F2) showed either positive or negative values (Fig. 7). The application of inorganic fertilizer (F1) resulted in a pronounced negative CO_2 fluxes, i.e., CO_2 uptake, under AWD compared to F2 and F3 treatments.

3.5 Plant Characteristics and Biomass Yields

Plant height increased sharply at the beginning of the growing periods from 14 DAP to 42 DAP and reached a plateau approximately 70 DAP for both CF and AWD treatments (Fig. 8). Interestingly, plant height differed significantly during late growth period (105 and 112 DAP) in CF, while in AWD during the early periods in AWD (14, 21, 28, 42 and 49 DAP) depending on the water management practice. However, the difference was compensated over time and mean plant height was indistinguishable at the time of harvest. The plant height ranged from 73 to 82 cm under CF and, while for AWD ranged from 71 to 87 cm.

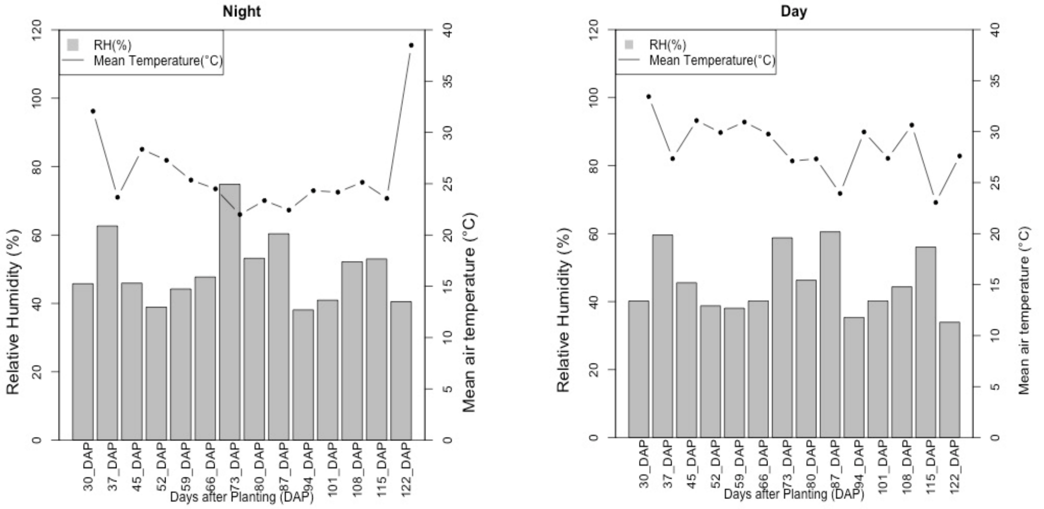

Fig. 3. Mean weekly ambient air temperature (°C) and relative humidity (%) recorded during day and night.

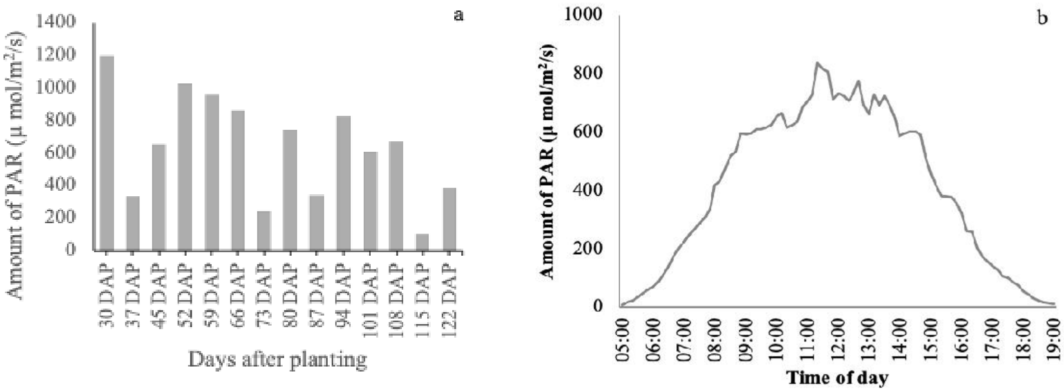

Fig. 4. (a) Amount of photosynthetically active radiation (PAR) of sunlight inside the greenhouse at Olomouc, Czech Republic, July-October 2017 and (b) course of average PAR during the period from 5 a.m. to 7 p.m.

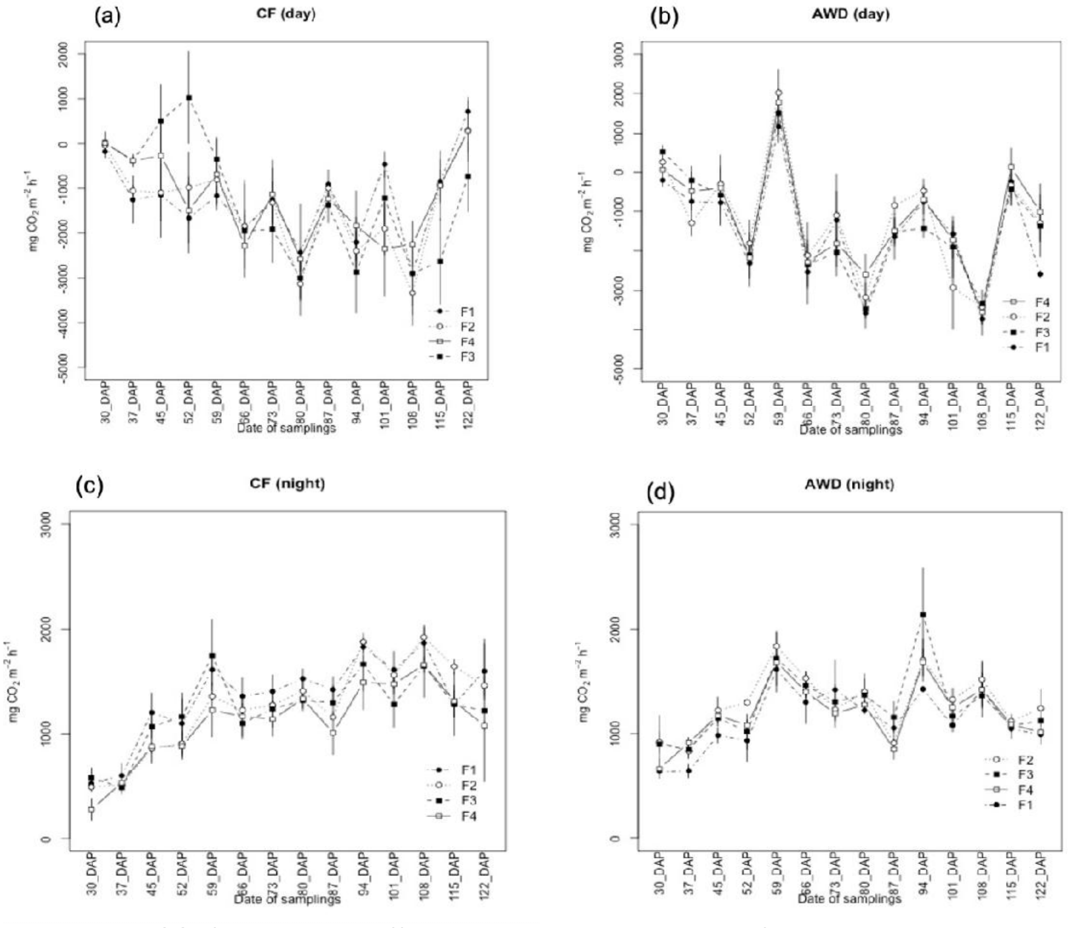

Fig. 5. Total CO_2 fluxes under different water management and fertilizer applications during day and night measurement. DAP = Days after planting

CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha^{-1}, Phosphorus (P) 30 kg ha^{-1} and Potassium (K) 20 kg ha^{-1}]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha^{-1}, Phosphorus (P) 5 kg ha^{-1} and Potassium (K) 6 kg ha^{-1}]; F3 = Organic manure application (cow manure - 5 t ha^{-1}); F4 = Control

Vertical bar indicates the standard error (+/-) of mean.

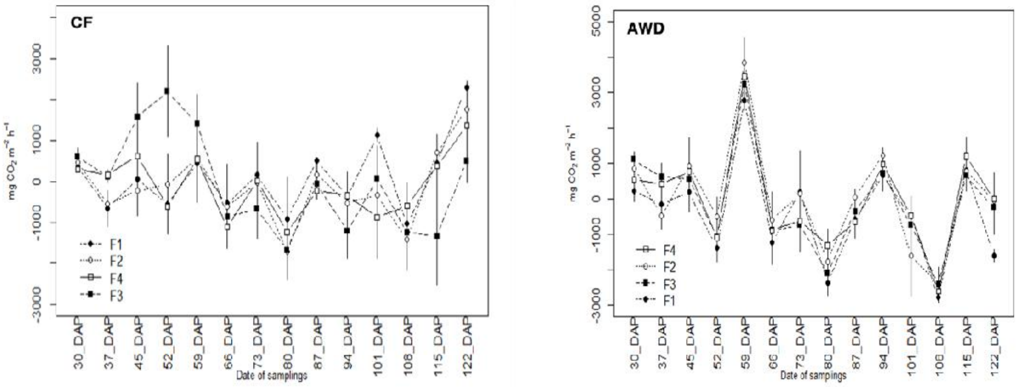

Fig. 6. Net CO_2 fluxes under continuous flooding (CF) and alternate wetting and drying (AWD) with different fertilizer applications during study period

CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha^{-1}*, Phosphorus (P) 30 kg ha*$^{-1}$ *and Potassium (K) 20 kg ha*$^{-1}$*]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha*$^{-1}$*, Phosphorus (P) 5 kg ha*$^{-1}$ *and Potassium (K) 6 kg ha*$^{-1}$*]; F3 = Organic manure application (cow manure - 5 t ha*$^{-1}$*); F4 = Control*
Vertical bar indicates the standard error (+/-) of mean.

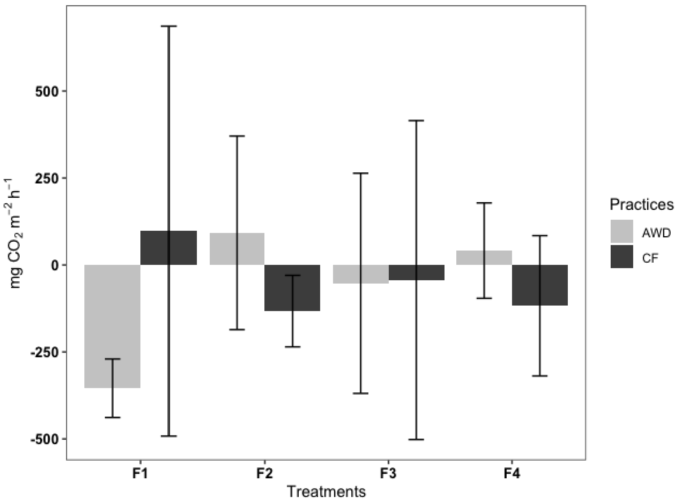

Fig. 7. Comparison of net CO_2 fluxes in treatments with different water management practices and various fertilizer applications

CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha^{-1}*, Phosphorus (P) 30 kg ha*$^{-1}$ *and Potassium (K) 20 kg ha*$^{-1}$*] ; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha*$^{-1}$*, Phosphorus (P) 5 kg ha*$^{-1}$ *and Potassium (K) 6 kg ha*$^{-1}$*]; F3 = Organic manure application (cow manure - 5 t ha*$^{-1}$*); F4 = Control*
Vertical bar indicates the standard error (+/-) of mean.

Mean leaf area per plant (hill) was neither significant differences under water management practices nor under fertilizer applications (Fig. 9). The largest leaf area per hill was observed in the F2 treatment under AWD practice (1413 cm^2 per hill), while the lowest leaf area was found in control under CF (1042 cm^2).

The highest above-ground biomass weight was recorded in F1 treatment under both CF (236 g plant^{-1}) and AWD practices (266 g plant^{-1}) (Table 2). The highest below-ground biomass was found in F1 treatment under CF (197 g plant^{-1}) and AWD practices (160 g plant^{-1}). Root lengths (cm) were not affected by the different treatments, however root volume (cm^3) and number of tillers/plant differed significantly in response to the different treatments (Table 2). Regarding water management practices, CF resulted in a significantly higher root volume (cm^3) compared to the AWD practice. In contrast, AWD resulted in significantly more tillers per plant compared to the CF practice (Table 2).

Table 1. Total CO_2 fluxes as affected by the different water managements practices and fertilizer applications during day and night measurements

Treatments	**Total CO_2 fluxes (mg CO_2 m^{-2}h^{-1})**		
	Day	**Night**	**Net**
CF x F1	-1256	1353	97
CF x F2	-1394	1262	-133
CF x F3	-1265	1221	-43
CF x F4	-1222	1105	-117
AWD x F1	-1481	1126	-354
AWD x F2	-1199	1292	92
AWD x F3	-1336	1283	-53
AWD x F4	-1160	1201	41
Pr (>F)	0.679	0.337	0.287
Water management (W)	**Day**	**Night**	**Net**
CF	-1284	1235	-49
AWD	-1294	1226	-68
Fertilizer application (F)	**Day**	**Night**	**Net**
F1	-1368	1239	-129
F2	-1297	1277	-20
F3	-1300	1252	-48
F4	-1191	1153	-38
Soil respiration	**Day**	**Night**	**Net**
CF	123	265	388
AWD	-96	350	254
Pr (>F)	0.052*	0.51	0.056

CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha^{-1}, Phosphorus (P) 30 kg ha^{-1} and Potassium (K) 20 kg ha^{-1}]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha^{-1}, Phosphorus (P) 5 kg ha^{-1} and Potassium (K) 6 kg ha^{-1}]; F3 = Organic manure application (cow manure - 5 t ha^{-1}); F4 = Control

**Significant at 5% level of significance*

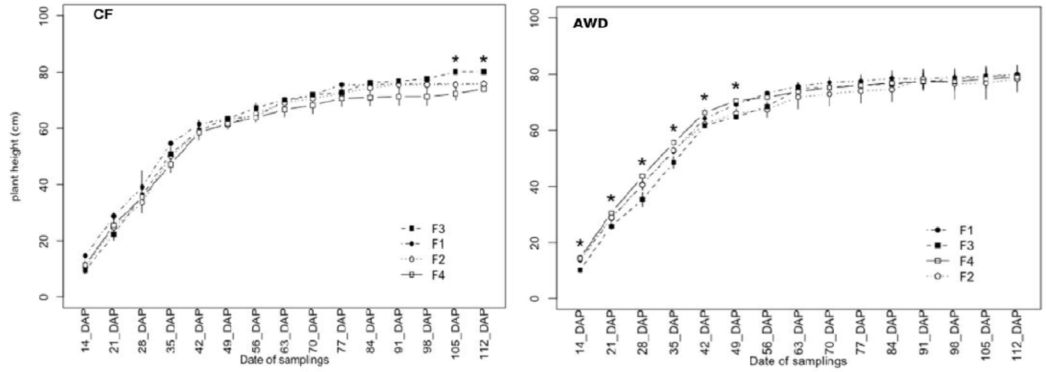

Fig. 8. Effects of different water management and fertilizer applications on plant height (cm) of rice

CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha^{-1}, Phosphorus (P) 30 kg ha^{-1} and Potassium (K) 20 kg ha^{-1}]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha^{-1}, Phosphorus (P) 5 kg ha^{-1} and Potassium (K) 6 kg ha^{-1}]; F3 = Organic manure application (cow manure - 5 t ha^{-1}); F4 = Control

Vertical bar indicates the standard error (+/-) of mean.

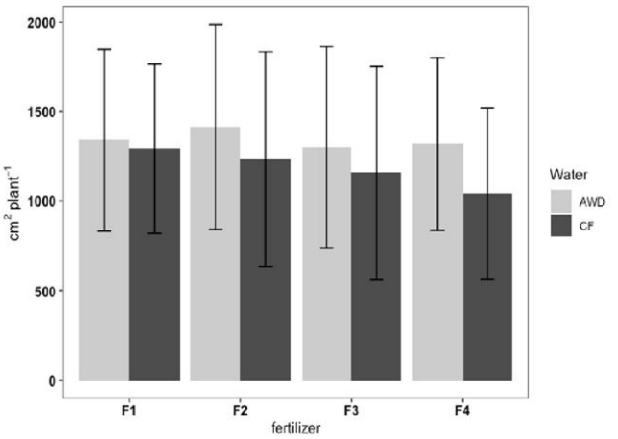

Fig. 9. Effects of different water management and fertilizer applications on mean leaf area per hill (cm^2) of rice

CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha^{-1}, Phosphorus (P) 30 kg ha^{-1} and Potassium (K) 20 kg ha^{-1}]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha^{-1}, Phosphorus (P) 5 kg ha^{-1} and Potassium (K) 6 kg ha^{-1}]; F3 = Organic manure application (cow manure - 5 t ha^{-1}); F4 = Control
Vertical bar indicates the standard error (+/-) of mean.

Table 2. Effects of different water management practices and fertilizer applications on biomass yield of rice plants

Treatments	**Above-ground biomass (g plant^{-1})**		**Below-ground biomass (g plant^{-1})**		**Root length (cm)**	**Root volume (cm^3)**	**No. of tillers plant^{-1}**
	Fresh	**Dry**	**Fresh**	**Dry**			
Water management (W)							
CF	205	51.2	169.9	41.0	39.83	176.7	35.1
AWD	242	55.2	137.4	33.4	39.92	124.2	47.9
Pr (>F)	0.131	0.062	0.388	0.258	0.965	0.003**	0.002**
Fertilizer application (F)							
F1	251	58.9	178.3	45.8	40.42	178.3	45.8
F2	206	53.2	147.6	37.3	37.08	146.7	42.5
F3	223	51.3	139.5	24.7	40.50	141.7	39.0
F4	214	49.8	149.3	41.4	41.50	135.0	38.7
Pr (>F)	0.561	0.374	0.465	0.163	0.389	0.206	0.464
Interaction (W x F)							
CF x F1	236	58.7	196.6	48.2	42.0	213.3	43.3
CF x F2	190	49.6	150.7	33.8	37.8	163.3	33.7
CF x F3	200	59.4	162.0	33.8	39.0	166.7	30.7
CF x F4	193	53.2	170.3	48.2	40.5	163.3	32.7
AWD x F1	266	59.2	160.0	43.3	38.8	143.3	48.3
AWD x F2	222	57.0	144.4	40.8	36.3	130.0	51.3
AWD x F3	247	43.2	117.0	15.6	42.0	116.7	47.3
AWD x F4	234	46.5	128.3	34.0	42.5	106.7	44.7
Pr (>F)	0.993	0.819	0.270	0.537	0.618	0.844	0.588
CV (%)	25.8	32.1	24.9	48.5	11.9	24.8	20.8

*CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha^{-1}, Phosphorus (P) 30 kg ha^{-1} and Potassium (K) 20 kg ha^{-1}]; F2 = inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha^{-1}, Phosphorus (P) 5 kg ha^{-1} and Potassium (K) 6 kg ha^{-1}]; F3 = Organic manure application (cow manure - 5 t ha^{-1}); F4 = Control. **Significant at 1% level of significance*

4. DISCUSSION

4.1 Effects of Water Management Practices and Fertilizer Applications on the CO_2 Fluxes

Studies investigating CO_2 balances of paddy fields and the atmosphere have focused mostly on flood irrigation [35,47,48]. It was concluded that the flooded rice ecosystem function as a CO_2 sink during the day but act as the CO_2 source during the night [6]. Similar tendencies were found also in our present study. Declining CO_2 concentrations, hence negative CO_2 fluxes, are most likely caused by photosynthesis-driven carbon-assimilation by the rice plant and algae in overlying water or at the soil surface. During the night, increase in CO_2 concentration and positive fluxes might indicate prevalent respiration of the rice plant together with soil and water biota [49]. Lower CO_2 uptakes with lower photosynthetic activities can be attributed to relatively higher net CO_2 fluxes during an early period of plants (Fig. 5). In this study, we focused mainly on CO_2 fluxes under different fertilizer applications and water management in paddy rice cultivation, thus, no light use efficiency (LUE) data were applied as a parameter that allows to compare the CO_2 uptake effectiveness among presented treatments. As all rice plants inside the greenhouse were exposed to the equal light intensity coming from outside space, we expected that any fluctuation of PAR outside should be reflected by the plants photosynthesis as well (Fig. 4 a & b). Moreover, it is known that under varying light conditions rice leaves do not use light efficiently [50], hence any standardization of the light intensity in this greenhouse experiment was unrealistic.

Noteworthy, total CO_2 fluxes did not differ significantly between different water management practices in our experiment which seems contradictive to previously published data, which suggested functioning as net sinks of atmospheric C. Weekly mean for total CO_2 fluxes under CF treatment was -30.82 g CO_2 m^{-2} d^{-1} (-8.3 g C m^{-2} d^{-1}) during daytime and 29.64 g CO_2 m^{-2} d^{-1} (8 g C m^{-2} d^{-1}) during nighttime (Table 1), and are in concordance with former published data obtained by eddy covariance techniques from flooded paddy fields in East Asia, India and the USA which reported CO_2 fluxes in a range between 5 and -39 g C m^{-2} d^{-1} [51-55]. While in AWD treatment, weekly mean for total CO_2 fluxes were similar to CF -31.06 g CO_2 m^{-2} d^{-1} (8.3 g C m^{-2} d^{-1}) during daytime and 29.42 g CO_2 m^{-2} d^{-1} (7.94 g C m^{-2} d^{-1}) during nighttime (Table 1). However, reported data from intermittently flooded systems showed more positive values of the CO_2 fluxes [7,47,56]. These finding suggest that slightly effects on total CO_2 fluxes by water management practices, but were more affected by the specific growth stages of the plant development and soil temperature [57].

Similar to the observed different CO_2 fluxes in soil respiration during the daytime in response to the different water management practices, Hossain [58] also observed that alternate wetting and drying (AWD) increased CO_2 emission by 16% compared to continuous flooding (CF). This is congruent with our data (Table 1) on night soil respiration under AWD treatment when average total CO_2 flux was higher compared to flux under CF treatment. For decomposition processes, good aeration is an essential factor by enhancing microbial activity. As such, the AWD practice favors oxidation processes of organic residues than CF [59]. Our experiment revealed that net soil CO_2 emission flux was by CF 35% higher than under AWD. Positive CO_2 fluxes (emissions) found during both day and nighttime indicate that anoxic respiration and/or fermentation performed by anaerobic microorganisms release CO_2, which quantity is comparable with the CO_2 fluxes produced under aerobic conditions. Thus, soil moisture could greatly contribute to the decomposition process of organic residues and CO_2 flux [60]. It remains therefore arguable whether AWD practice is really appropriate method to mitigate GHGs. Nevertheless, contribution of soil respiration to overall CO_2 fluxes seems to be negligible (9.0% of the total fluxes) even when comparing bare soils with F4 treatments (i.e. control without fertilization).

Input of nitrogen, either as commercial fertilizers or organic manure applications, increases biomass production and C input from enhanced crop growth, effects on mineralization rates of soil organic matter [61] and consequently increase CO_2 flux [62]. The major CO_2 emission sources in rice fields are plant and soil respiration [63], with the total CO_2 flux varying depending on the applied fertilizing regime. Indeed, the highest net CO_2 fluxes, i.e. emissions, were found under inorganic fertilizer treatments F1 and F2, however, in contrast to our expectation both results were obtained under different water management practices (Fig. 7). The discrepancy is likely attributed to the limited sample sizes and heterogeneity of the growth conditions in the greenhouse, yet it supports the notion that the factor nitrogen fertilization has a dominant impact over the watering regime on CO_2 fluxes. As expected, the F1 treatment with the highest nitrogen content showed the highest above-ground and below-ground biomass alongside the highest average total CO_2 flux during the night [62,63]. Organic manure treated pots (F3) showed negative net CO_2 fluxes under both CF and AWD practices when compared with inorganic fertilizer treatments: F1 and F2 (Fig. 7). This finding is congruent with results by Sampanpanish [5] who also found reduced CO_2 emissions after organic fertilizer (cow manure) addition.

4.2 Effects of Water Management Practices and Fertilizer Application on Plant Height and Biomass Yields

In this study, above-ground and below-ground biomass of the rice plants grown under AWD was higher than under CF in all fertilizer treatments, accounted for an increase of 7.84% of dry above-ground biomass under AWD compared with CF (Table 2). This is supported by an independent study, where the maximum dry matter accumulation was also higher under AWD compared to continuous submergence [25].

In our study, plant height was significantly taller in AWD than under CF treatment at an early growth of the rice plant and equivalent result found by Pascual and Wang [64]. However, the effects of the two watering regimes seemed similar when comparing the average plant height. Mean leaf area per hill (cm^2) and number of tillers per plant (hill) were also significantly higher in AWD when compared to those under CF in this experiment. Similar results were presented by Shukla [65] and Pascual & Wang [64]. These findings suggest that rice plant does not need to be continuously flooded if there is adequate amount of water during the critical growth stage. Aerobic conditions created by AWD in soil led to better plant growth and ultimately produced a greater number of tillers per hill than when the soil was continually flooded.

In this experiment, higher tillers number were detected under AWD when compared to those practice under CF and equivalent results also confirmed by Howell et al. [66]. As a consequence, one might expect to get a higher grain yield as well. However, the present experiment was limited by controlled glass greenhouse conditions and harvest of biomass ended just before flowering without data for grain yields. Therefore, biomass yield, yield related traits and other agronomic traits are not directly comparable to the actual field situations. Typically, biomass accumulation is important for grain yield formation. The increased biomass production is directly linked to the improvement of potential rice yield [67]. Moreover, plants grown under controlled environmental conditions have a tendency to differ in the morphological characters and biomass yields compared with those grown under natural conditions [49,68,69]. In accordance with Thakur et al. [70], continuous flooding supported significantly higher root volume (cm^3), although, there was found no significant difference among fertilizer treatments.

5. CONCLUSION

Surprisingly, net CO_2 fluxes were negative under both CF and AWD water management practices, indicating that CO_2 uptake by the rice plants was prevalent during the entire growing period. These results are seemingly in contrast to our previous fields measurements [49] and might be attributed to the different environmental conditions (field versus greenhouse) in the two studies. Number of tiller per plant was 36.5% higher in AWD than CF conditions. Along that line, the plants' CO_2 uptake was 28% higher under AWD conditions. This might be due to better soil aeration leading to an enhanced growth of the rice plants and consequently more CO_2 fixation.

Net CO_2 emissions from inorganic fertilizer application (F1 and F2) were considerably higher than the control (F4) with no fertilizer under both drained and flooded situations. This may be due to an increased availability of nitrogen that can promote crop growth and as such more CO_2 fixation. Our findings also revealed that applying the recommended rates of inorganic fertilizer (F1) increase the dry biomass weight by 18.3% compared to the control. On the other hand, CO_2 emission fluxes in the treatment with farmyard manure application were negative under both water management practices, while the fresh biomass weight remained indistinguishable to those in other inorganic and organic manure treatments (F2, F3 and control).

In conclusion, modifications of current cultivation systems toward using farmyard manure, that emits less CO_2, could effectively mitigate GHGs impacts from lowland rice ecosystems regardless of flooding or drying out practices. We need more measurements for multiple years to assess the long-term effect of alternate flooding and draining practice on the exchanges of CO_2 in rice paddy fields.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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