

PALACKÝ UNIVERSITY OLMOUC

Faculty of Science

Department of Ecology and Environmental Sciences



**Effects of Different Agricultural Management Systems  
on CO<sub>2</sub> Fluxes from Irrigated Rice Cultivation**

Ph.D. Thesis

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Olomouc 2020

I, Saw Min, thereby declare that I wrote the Ph.D. thesis myself using results of my own work or collaborative work of me and colleagues and with help of other publication resources which are properly cited.

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### Abstract

Rice (*Oryza sativa* L.) is a major food crop around the world, especially in Asia. Rice production plays an important role in the global budget of emissions of greenhouse gases (GHGs) such as methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). The majority of research has been focussing on CH<sub>4</sub>. As for CO<sub>2</sub> fluxes, it has been established that lowland rice paddy acts as a net sink of CO<sub>2</sub>, however, knowledge on CO<sub>2</sub> fluxes from paddy rice cultivation is overall scarce. Therefore, this thesis investigated the potential changes of CO<sub>2</sub> fluxes between plant ecosystem–atmosphere by altering irrigation practices and different agricultural management systems in paddy rice cultivation.

Two experiments were performed to quantify CO<sub>2</sub> fluxes between atmosphere and rice plants. The first experiment involved different irrigation practices; Continuous Flooding (CF) vs Alternate Wetting and Drying (AWD) combined with different fertilizer (organic and inorganic) applications under greenhouse condition at Palacký University Olomouc, Czech Republic, 2017. The second experiment was conducted in an irrigated rice field, an area with widespread rice cultivation at the Yezin Agricultural University, Myanmar. It included two different agricultural tillage systems; Conventional (Conv) vs Conservation (Cons). We found that the effects of different agricultural management systems altered various patterns of CO<sub>2</sub> fluxes from irrigated paddy rice cultivation.

Results from the first experiment show that daytime CO<sub>2</sub> fluxes were almost always negative for all treatments, while positive fluxes were observed consistently for all treatments during nighttime. Noteworthy, total CO<sub>2</sub> fluxes did not significantly differ between different water management practices in our experiments. The average net CO<sub>2</sub> fluxes were negative under both CF (-49 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>) and AWD water management practices (-127 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>), indicating a net uptake of CO<sub>2</sub> by the rice plants during the growing period. The 28% higher uptake of the CO<sub>2</sub> under AWD conditions than under CF management was likely caused by better soil aeration leading to an enhanced growth of the rice plants and consequently more CO<sub>2</sub> fixation. Indeed, above-ground fresh biomass weight from plants grown under AWD management was 15% higher than under CF conditions. The application of recommended inorganic fertilizer rates led to considerably higher net CO<sub>2</sub> emissions compared to no fertilizer application (control) under both CF and AWD practices. Importantly, application of inorganic fertilizers also increased fresh biomass by 14.7% compared to the control.

Results from the second study indicate that total CO<sub>2</sub> emissions from Conv practices were significantly higher than Cons practices during nighttime; however, no significant difference of net CO<sub>2</sub> was observed between those management practices. Total net CO<sub>2</sub> fluxes ranged from -59 to 1614 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> and from -282 to 1082 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> in Conv and Cons practices, respectively. Significantly higher above-ground rice biomass and yield were observed in Conv practices, causing a significant rise in both the uptake and emissions of the CO<sub>2</sub> during day and night, respectively.

Our study revealed that modification of agricultural management systems could contribute to lower carbon emissions from irrigated rice paddy fields. Conclusively, our results may provide as a basic to encourage a deeper understanding of the CO<sub>2</sub> fluxes from rice paddy research.

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## **Content**

### **List of publications**

## **1. INTRODUCTION**

### **1.1 Rice Production and Global Climate Change**

- 1.1.1 Rice (*Oryza sativa* L.)
- 1.1.2 Paddy soil and fertilization
- 1.1.3 Impacts of climate change on rice production

### **1.2 Rice Paddy and Carbon Cycle**

- 1.2.1 Greenhouse Gases (GHGs) emission from rice cultivation
- 1.2.2 Carbon cycling in rice paddy systems
- 1.2.3 Impacts of agricultural tillage practices on CO<sub>2</sub> emission

### **1.3 Rice and Water Use**

- 1.3.1 Global rice water use
- 1.3.2 The plants-soil-water system
- 1.3.3 Water conservation in paddy rice production

## **2. OBJECTIVES OF DISSERTATION THESIS**

## **3. MATERIAL AND METHODS**

## **4. MAIN RESULTS AND DISCUSSION**

- 4.1 Effects of Different Water Management and Fertilizer Applications on CO<sub>2</sub> Fluxes from a Selected Myanmar Rice (*Oryza sativa* L.) Cultivar
- 4.2 Comparison of Carbon Dioxide (CO<sub>2</sub>) Fluxes between Conventional and Conserved Irrigated Rice Paddy Fields in Myanmar

## **5. CONCLUSIONS**

## **6. REFERENCES**

## **7. ATTACHED PUBLICATIONS**

## **8. CURRICULUM VITAE**

## **List of publications**

The thesis is based on the following papers:

Paper I:

**Min, S.;** Rulík, M. Effects of Different Water Management and Fertilizer Applications on CO<sub>2</sub> Fluxes from a Selected Myanmar Rice (*Oryza sativa* L.) Cultivar. International Journal of Plant and Soil Science (Accepted in January 2021)

Paper II:

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# 1. INTRODUCTION

## 1.1 Rice Production and Global Climate Change

### 1.1.1 Rice (*Oryza sativa* L.)

Rice is the central food crop for over 3 billion people, accounting for nearly half of the world's population (FAO, 2018). Most of the people relying on rice as main food, predominately live in less developed countries. However, due to the globalisation, rice consumption is also increasing in other parts of the world. Rice belongs to the genus *Oryza*, family: Poaceae (Gramineae) and tribe: *Oryzae*, had domesticated in Asia. According to the archaeological and historical evidence, the origin of the cultivated rice is from South-east Asia for *Oryza sativa* and Africa for *Oryza glaberrima*. However, *O.glaberrima* was domesticated after Asian rice and not as popular as *O. sativa* due to its low grain yields (Linares, 2002) and poor milling quality (IRRI, 2013). The two importance species of *O. sativa*, namely *japonica* and *indica*, originating from two different regions of domestication: *japonica* in China and *indica* in India (Gross and Zhao, 2014). A third subspecies, called *javanica* which was identified based on morphology with broad grained and known as *tropical japonica* (Prasad et al., 2017).

Rice can be grown in a wide range of locations across over the globe, from cool temperate regions of Northeast Asia, through low-lying river deltas in the tropics, to high mountainous region with an altitude of over 2 kilometres (Bouman, 2009). Rice consumes as a major staple food crop in Southeast Asia fully accounted for 60% and about 35% in East Asia and South Asia. Rice is an excellent food source of carbohydrates and the highest rice consumption per capita level is 130-180 kg per year takes place in Bangladesh, Cambodia, Indonesia, Laos, Myanmar (Burma), Thailand, and Vietnam (Kenneth and Kriemhild, 2000).

Globally, rice paddies amount to an area of nearly 167 million hectares (M ha), producing more than 503 million tons (Mts) of milled rice in 2017. Nearly 90% of the world's rice is obtained from Asia (approximately 680 million tons) (FAO Stat, 2018). China and India are the major rice producers and simultaneously also the major consumers, producing over 350 million tonnes of rice per year (FAO, 2018), which amounts to more than half of the global harvest (Fig.1). China is the world's leading rice producer with nearly 125 million tonnes production. Second is India, which possesses the largest rice area (45 M ha) and produces nearly a quarter of Asia's production (Moya et al., 2004). India is together with Thailand leading as the top rice

exporters on the global rice market. Associated import markets are the European Union and the US (Reay, 2019).

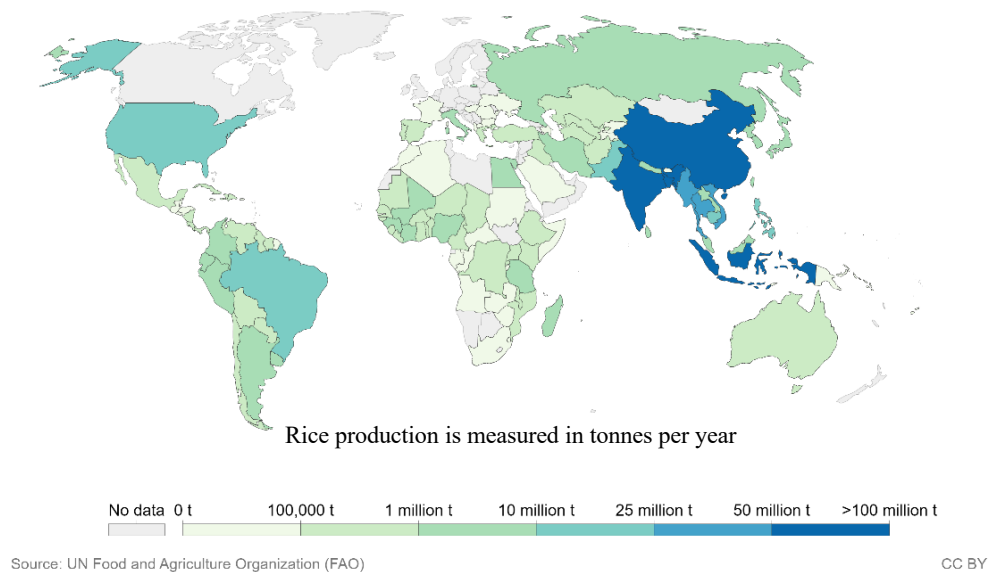


Fig. 1. Global rice production in 2018 by the country of origin (Source: Reay, (2019))

Rice is grown under temperate, subtropical, and tropical climatic conditions with the weather varying from arid and semiarid to sub-humid and humid. Therefore, rice is cultivated in many diverse environments and in many growing schemes. Rice production may differ from country to country as well as from location to location due to the variation of environmental and socioeconomic conditions which affected rice productivity in the past and influences the potential of improving future rice production. Rice environments are classified based on altitude (upland, lowland, deep water) and water source (irrigated or rainfed) (Fig. 2) (IRRI, 2009). According to soil water conditions, rice production ecosystems can be categorized into irrigated lowland, rainfed lowland and rainfed upland, and deep water ecosystems (Chauhan et al., 2017).

Myanmar is the world's six-largest rice producing country (IRRI, 2013) and therefore rice production play a crucial sector for economic development of the country. Rice is main staple food in Myanmar and consumption per capita is approximately 154 kg per year (IRRI, 2017). Total sown area for rice paddy is approximately 7.6 million hectares and production was reached at 28.21 million metric tons (MoALI, 2016). The rice ecosystems of Myanmar include irrigated lowland, rainfed lowland, deepwater and upland. Rainfed lowland paddy are largest ecosystems except for the middle part of Myanmar with dry zone mainly practice irrigated cultivation system. The total irrigated areas for rice had also increased progressively and reached to 2 million ha during 2005-



06 and taking approximately 33% of the overall rice production area and increased to 7.186 millions ha in 2007-2011(CSO, 2011).

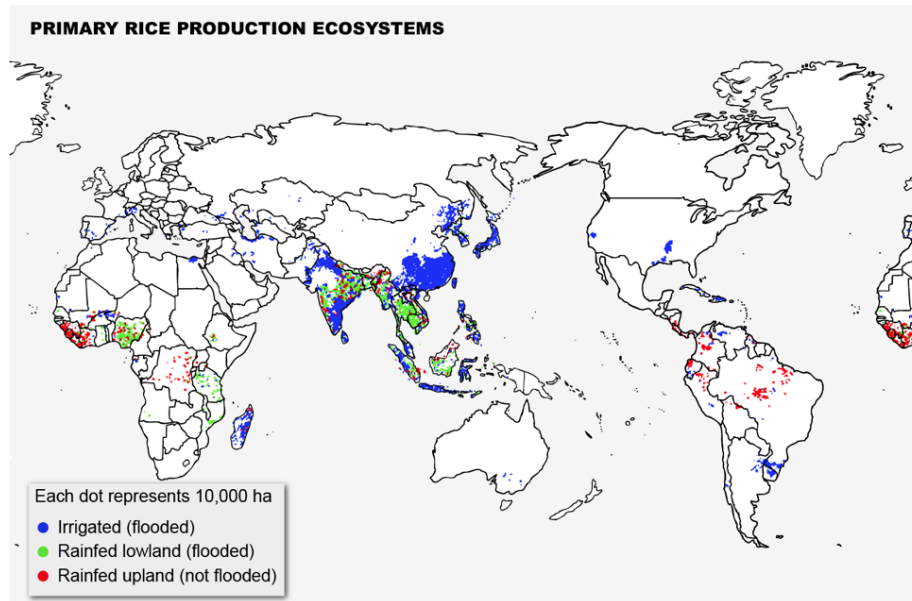


Fig. 2. Map of different rice production systems globally, showing the considerable extent of irrigated rice (blue). Source: IRRI, 2009

### 1.1.2 Paddy Soil and Fertilization

#### Biogeochemistry of paddy soils

Paddy fields are typically submerged soil condition for growing of rice and other semi-aquatic crops. Paddy soils represent the major portion of the world's anthropogenic wetlands (Kögel-Knabner et al., 2010). In pedological terms, paddies may originate from any type of soil, but are highly altered by anthropogenic activities. The development of a paddy soil is driven by specific soil management practices that modify the soil's original character (Kirk, 2004). These practices are artificial submergence and drainage, ploughing and puddling (ploughing and levelling the surface layer of a submerged soil), organic manuring (animal manure, rice straw and other crop residues) and fertilization. The management induced change of aerobic (oxic) and anaerobic (anoxic) conditions result in temporal and spatial variations in oxidation and reduction reactions. These in turn affect the dynamics of organic and mineral soil constituents (Cheng et al., 2009). Moreover, these cultural management system leads to the development of pedogenic horizons that are specific to paddy soils (Fig.3). These pedogenic horizons are: (1) a thin layer of standing water (*W*): This layer of standing water is the habitat of bacteria, phytoplankton, macrophytes and small fauna and is mainly oxic; (2) an oxic and partly oxic zone (*Ap*): The thickness of the oxic zone

may range from several millimetres after flooding, to several centimetres when the rice plants are completely grown and start to deliver oxygen from their roots. The range of the oxic zone is affected by pedoturbation, evapotranspiration and percolation. (3) the upper portion of an anthraquic horizon (Arp) (IUSS Working Group, 2006): this is the reduced puddled layer, distinguished by the absence of free oxygen in the soil solution. (4) the lower part of an anthraquic horizon or plough pan (Ardp): This horizon (>7 cm) is comparatively compact, has a platy structure, high mechanical strength and low hydraulic conductivity (0.34 to 0.83 mm day<sup>-1</sup>). Hydraulic properties of the plough pan mainly control the water regime of the underlying B or C horizons, which may have either oxic or reducing conditions (Kögel-Knabner et al., 2010).

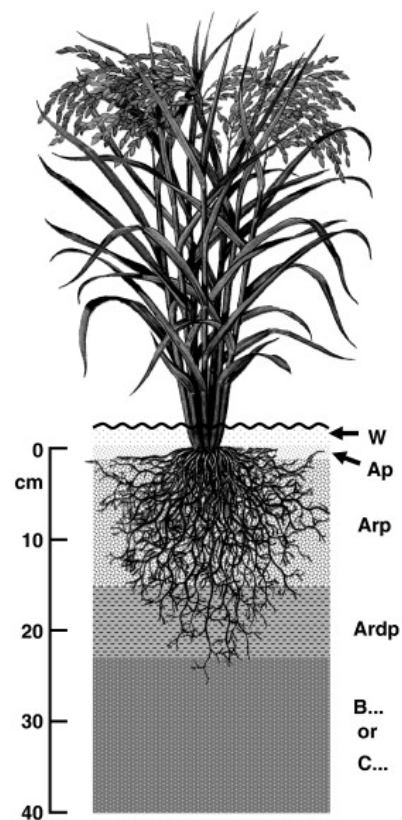


Fig. 3. Typical horizon sequence of a rice paddy soil (FAO, 2006)

When the supply of oxygen cut off from the atmosphere, microbial activities from aerobic (i.e. oxic condition) change to facultative (i.e. hypoxic condition) and to anaerobic (i.e. anoxic condition) fermentation of organic matter, where alternative electron acceptors are used. The series is resolved by thermodynamics. This includes, in terms of oxidation-reduction potential, from high to low: aerobic respiration, nitrification, denitrification, Manganese (Mn<sup>4+</sup>) reduction, Ferric (Fe<sup>3+</sup>) reduction, Sulfate (SO<sub>4</sub><sup>2-</sup>) reduction, and methanogenesis (Reddy et al., 1986) (Fig. 4). In terms

of quantity,  $\text{Fe}^{3+}$  is by far the most important oxidant in rice soils and thus determines the period during which organic matter is oxidized to  $\text{CO}_2$  (Krüger et al., 2001).

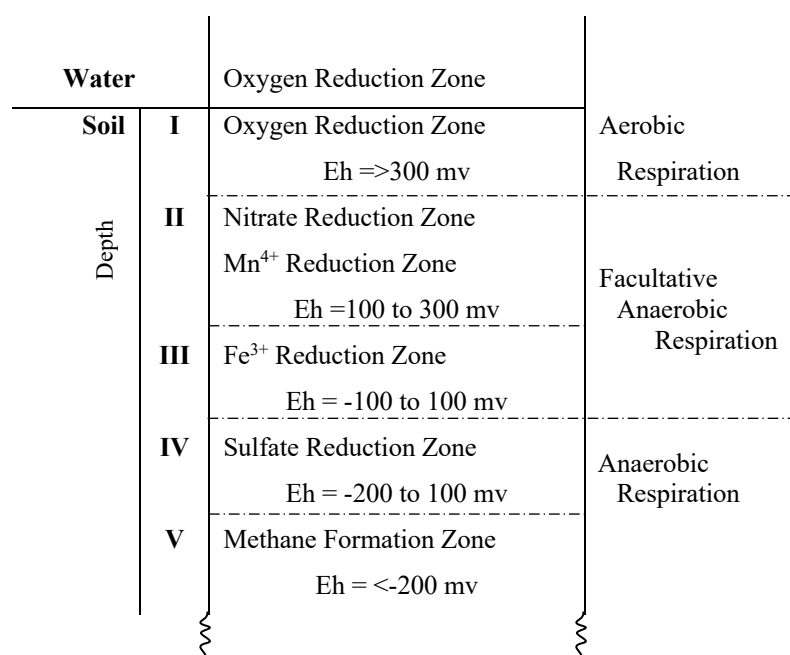


Fig. 4. Schematic presentation of a flooded soil showing the zones with different microbial metabolism (Adapted from Reddy et al. 1986)

### Fertilizer management in paddy rice cultivation

Fertilizer is the major input and one of the most important factors for paddy rice production. Proper fertilizer management can increase rice yield and reduce production cost. However, it is necessary to provide adequate amount of nutrient to attain high performance in the rice plant (Slaton et al., 2001). Proper management strategies such as appropriate rate and timing of fertilizer application can increase rice yield and optimize production cost. Nitrogen (N), phosphorus (P), and potassium (K) are applied as essential fertilizers in large quantities to rice fields, and a deficiency of either of the nutrient leads to yield losses. Nutrient absorption rates mainly depend on many factors such as cultivar, soil type, fertilizer type, fertilization technology, and environmental factors (Liu et al., 2005; Zhang et al., 2006). To produce potential rice yield levels, modern rice cultivars require adequate quantities of essential nutrients. Of the total 172.2 M t fertilizer (N + P<sub>2</sub>O<sub>5</sub> + K<sub>2</sub>O) consumed globally during 2010–2011, 14.3 % (24.7 Mt) was used in rice production. Percentages for N, P and K were 15.4, 12.8, and

12.6, respectively (Heffer, 2013). With balanced fertilization (N, P and K), yield was increased primarily due to an increase in recovery and agronomic efficiency. Imbalanced use of fertilizers not only increases the deficiency of K as well as micro-nutrients in the soils (Ladha et al., 2003), but also manifests to be uneconomic and environmentally unsafe.

Globally, 90% of the rice is produced by Asia, which experiences high soil degradation as a result of the negative nutrient balance, poor soil and crop management (Von Uexkull and Beaton, 1992). Farmers in most developing countries in Asia mainly depends on N fertilizers in order to maximize potential rice yields. As a result of higher amount of N fertilizer without appropriate balance with P and K, causes negative effects on rice yields, the soil, and the environment. On the other hand, insufficient quantities of fertilizer application by the rice farmers with limited resource, decline in rice yield. Despite a large potential exists for increasing rice yield, inefficient nutrient use is one of the most limiting factors to achieve target yields. To achieve higher rice yield, adequate nutrient management practices have become an important factor for the modern rice production technology. Nutrient use efficiency in rice paddy can be increased by using appropriate rate and timing of inorganic and organic nutrient sources, water management, soil pH management, and the use of high yielding cultivars adapted to a specific rice growing environment. Moreover, knowledge-intensive strategies will be essential for intensive rice production including the efficient use of fertilizer nutrients. Therefore, efficient nutrient management in rice has primary important not only for improving yield and profitability in short term, but also better ecological management services in the long-term perspective (Singh and Singh, 2017).

Fertilizer application may influence on methane (CH<sub>4</sub>) emission by a two-way effect. It can either enhance emissions through the provision of methanogen substrate or otherwise through the promotion of CH<sub>4</sub> oxidation. Nitrogen (N) fertilizers have also been reported to encourage the growth of rice plants and hence provide more carbon substrates to methanogens for CH<sub>4</sub> production (Inubushi et al., 1990). Nitrogen fertilizers also change the activities of methanotrophs in soils (Bodelier and Laanbroek, 2004). Furthermore, the application of organic fertilizers has also an important influence on the carbon exchange of agro-ecosystems. Many studies show that organic fertilizers can enhance soil fertility (Li et al., 2017; Penha et al., 2015), increase crop yield (Wei et al., 2016) and quality (Zhou, 2012), and improve fertilizer utilization rate

(Cho et al., 2009; Liu et al., 2012). The application of organic fertilizers can also significantly affect the carbon exchange of farmland. Most of the existing research has focused on the effect of organic fertilizers on the CO<sub>2</sub> flux from soil respiration. Salehi et al. (2017) found that the integrated application of cattle manure and chemical fertilizer significantly increased soil CO<sub>2</sub> flux by 9% over solitary urea application. On the other hand, the addition of organic fertilizer can also modify the physical and chemical properties of soil, including its bulk density and porosity (Bassouny and Chen, 2016). However, only few studies have reached conclusions about the effect of organic fertilizer application on the CO<sub>2</sub> flux of plant–soil ecosystems. Therefore, it is also necessary to understand the effect of organic manure application on CO<sub>2</sub> fluxes from rice paddy cultivation.

### **1.1.3 Impacts of climate change on paddy rice production**

Climate change threatening the stability of global food security due to warming, changing precipitation patterns and greater frequency of some extreme events that disrupt food chains increase. Global climate projection by Intergovernmental Panel on Climate Change (IPCC), atmospheric CO<sub>2</sub> concentration will increase between 730 and 1020 ppm by 2100 (Meehl et al., 2007). The increasing concentration of greenhouse gases in the air will affect climate and global mean air temperature, which are estimated to increase by 1.4-5.8°C (Fahad et al., 2019). Other impacts of global warming are expected to be sea level rise and an increase in climate-related extreme events such as floods, droughts, and storms. Sea level may rise by 9-88 cm in different location of the world between 1990 and 2100 (IPCC, 2001). Recent studies predict that sea level may rise by 1 m or more in the 21<sup>st</sup> century, which would adversely affect one billion people by 2050 (Brecht et al., 2012; Hansen and Sato, 2012). Sea level rising will make an impact on rice production where especially in larger deltas areas of Vietnam, Myanmar, and Bangladesh (Wassmann et al., 2009). The above changes will affect crop production systems because increased heat and changes in precipitation amounts will negatively affect rice yields (Kong et al., 2012).

Rice is typically well-adapted to a broad range of different climatic conditions. However, climate change will aggravate a variety of stresses that can affect rice production, namely, heat, drought, salinity, and submergence (Wassmann et al., 2004). The increased temperature may also influence on sea level rise, a rise of 1000 mm sea

level due to thermal expansion is estimated for 3.58 °C increase in temperature, thus causing increased salinity in coastal area and additional yield decline (Manabe and Stouffer, 1994; Wassmann et al., 2004). Peng et al. (2004) stated that rice yield decreased by 15% for dry season rice when the average growing season temperature increase in every 1°C. Temperature schemes significantly influence not only growth duration, but also the growth pattern and the productivity of rice crops. Extremely high temperature cause damage to the rice plants especially in tropical region (Nguyen, 2002). Reiner and Dobermann (2007) observed that increasing temperatures or hotter night temperatures can cause increased spikelet sterility and reduce grain yield in rice. Wassmann et al. (2009) observed that current temperatures are already approaching critical levels during the susceptible stages of the rice plant in many Asian countries and drought stress is expected to affect rice growth and production. Drought stress is the largest threat to rice production especially in rainfed rice system, more than 13 million ha of rainfed lowland rice and 10 million ha of upland rice fields were affected by drought stress only in Asia (Pandey et al., 2007). Typically, soil water deficit is an important environmental constraint directly affected to plant physiological processes such as plant growth and development (Wassmann et al., 2009).

Climate change considerably increases not only seawater level but also increase in frequencies and intensities of flooding caused by extreme weather events (Bates et al., 2008). Although rice plant is a semi-aquatic, the plants die after few days when the field was continuously submerged. Submergence is an important abiotic stress affecting about 10–15 million ha of rice fields causing yield losses every year in South and South East Asia (Anderson, 1997). Many studies indicate that the global climate change could lead to significant changes in land and water resources for rice production as well as the productivity of rice crops grown in various regions of the world.

## **1.2 Rice paddy and Carbon Cycle**

### **1.2.1 Greenhouse gases (GHGs) emissions from paddy rice cultivation**

Greenhouse gases (GHGs) are any gases in the atmosphere such as water vapor, carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) that can absorb infrared radiation, trapping heat in the atmosphere (IPCC Press Release, 2019). Well documented scientific evidence suggests that among these GHGs, CO<sub>2</sub> is recognized as a significant contributor accounts for 60 percent of global warming (IPCC, 2001;

Rodhe, 1990). Greenhouse gases occur naturally in the earth's atmosphere, but especially by human activities, such as the intensive rice cultivation, are increasing the levels of GHG's in the atmosphere, causing global warming and climate change. These GHGs are also referred to as the "Kyoto gases" and are listed in Table 1 (IPCC, 2007). The "global warming potential" (GWP) of a GHG indicates the amount of warming a gas causes over a given period of time (normally 100 years). GWP is a unit value, with CO<sub>2</sub> having baseline unit of 1 and the GWP for all other GHGs is the number of times more warming they cause compared to CO<sub>2</sub> (IPCC, 2007).

Table1. Kyoto gases and their global warming potentials (GWPs) (adapted from IPCC, 2007)

Greenhouse gas	GWP
CO <sub>2</sub>	1
CH <sub>4</sub>	25
N <sub>2</sub> O	298
Hydrofluorocarbons (HFCs)	124-14800
Perfluorocarbons (PFCs)	7390-12200
Nitrogen trifluoride (NF <sub>3</sub> ) <sub>3</sub>	17200

Agricultural activities contribute to 10-14% of global anthropogenic GHG emission (Smith et al., 2007; Tubiello et al., 2013), mainly from enteric fermentation (CH<sub>4</sub>), application of synthetic fertilizers (N<sub>2</sub>O), and tillage (CO<sub>2</sub>) (Allen et al., 2012). Approximately 20% of the present concentrations of atmospheric GHGs due to the agricultural activities (Hütsch, 2001), especially the emissions of CH<sub>4</sub> and N<sub>2</sub>O from paddy fields (Huang et al., 2013).

Rice cultivation is one of the major emitters of Greenhouse gases (GHGs) contribute to global climate change, mainly by CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O gases to the atmosphere and simultaneously are affected by variation of the climatic factors (Ali, 2019). Rice is grown in different environmental conditions ranging from tropical to temperate regions and various emission patterns of these gases (CH<sub>4</sub> and CO<sub>2</sub>) may naturally occurs under different agricultural management practices (Bhattacharyya et al., 2013). Typically, rice paddy fields were grown under flooded conditions, leads to anaerobic decomposition and associated CH<sub>4</sub> flux. Flooded paddy fields in tropical region play an important role in global budget of greenhouse gases such as CH<sub>4</sub> and

CO<sub>2</sub> (IPCC, 2007). In 2012, global rice production covered 163 million ha of cropland, where nearly 80 million ha of rice paddy fields were managed under continuous flooded irrigation and contributed to 75% of the world's rice production (IRRI, 2013). Carlson et al. (2016) estimated that total global GHGs emissions from croplands range from 2–3 Gt CO<sub>2</sub>-eq yr<sup>-1</sup>, including about 48% of CH<sub>4</sub> emissions are from flooded rice, 32 % of CO<sub>2</sub> emission from peatland cultivation and 20% of N<sub>2</sub>O emission from N fertilizer application (Fig.5) (Dinu, 2019). Moreover, CO<sub>2</sub> flux by soil respiration is also important for accurately evaluating the effects of land use changes on global warming and carbon cycling (Liu et al., 2013).

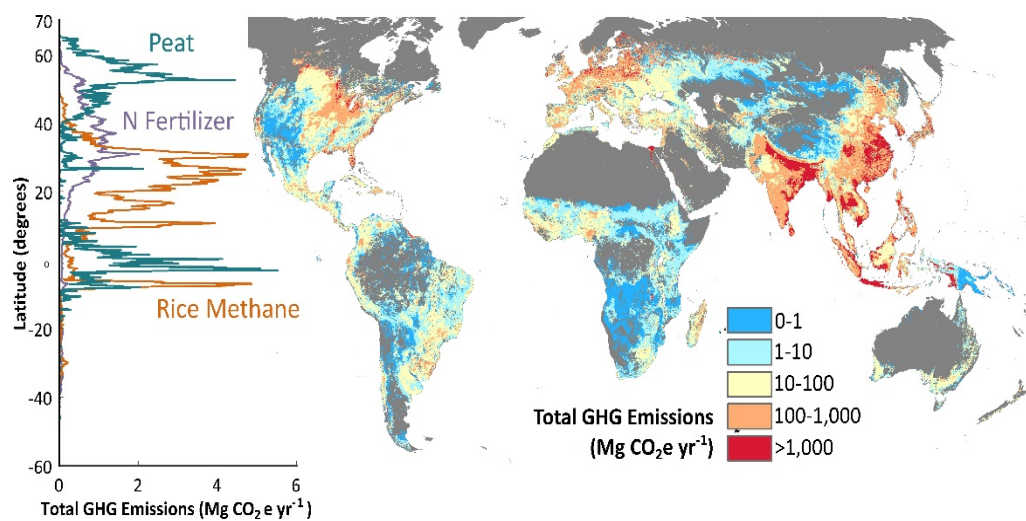


Fig. 5. Global distribution of Greenhouse Gas (GHG) emissions from total cropland, Source: (Carlson et al., 2016) <https://www.nature.com/articles/nclimate3158>

The amount of GHG emissions by paddy rice cultivation not only depends on the amount and types of farm inputs, but also varies with irrigation systems and water managements practices (Maraseni et al., 2009). Carlson et al. (2016) projected by using spatial data from irrigation, organic amendment, and crop calendar data, flooding generates 92% of total rice emissions. Irrigated areas are the major source of rice CH<sub>4</sub>, which make up 60% of total rice harvested area and produce 78% of emission.

### 1.2.2 Carbon cycling in rice paddy ecosystem

Rice paddies in tropical flooded lowland soil play an vital role within the global budget of greenhouse gases like CO<sub>2</sub> and CH<sub>4</sub> (IPCC, 2007) and naturally alter the rates of emissions under different agricultural management practices like tillage and fertilization. In line with the flooded nature of the rice paddy fields, gas exchange



between paddy fields and therefore the atmosphere is different from the opposite dryland ecosystems and this mechanism is regulated by many factors (Miyata et al., 2000). CO<sub>2</sub> exchange in paddy fields is driven by photosynthesis and autotrophic (plant) and heterotrophic (mainly microbial) respiration (Minamikawa et al., 2005). During the photosynthesis activities by the plants, atmospheric carbon is converted from inorganic carbon (CO<sub>2</sub>) to organic carbon by standing vegetation also as algae. When the plants die, it become detritus which then undergoes decomposition and therefore the plants becomes the major source of carbon (C) in most wetlands (Reddy and DeLaune, 2008) (Fig.6). Many biological and physical processes regulate the exchange of CO<sub>2</sub> and CH<sub>4</sub> between paddy fields and the atmosphere. Previous studies indicated that a big amount of CO<sub>2</sub> is stored by the plants in paddy fields and therefore huge amount of GHGs are released from paddy fields (Ball et al., 1999; Liou et al., 2003; Tang et al., 2016; Zou et al., 2003). Plants utilize atmospheric CO<sub>2</sub> and respired CO<sub>2</sub> released by the soil and floodwater for photosynthesis process (Wang et al., 2017).

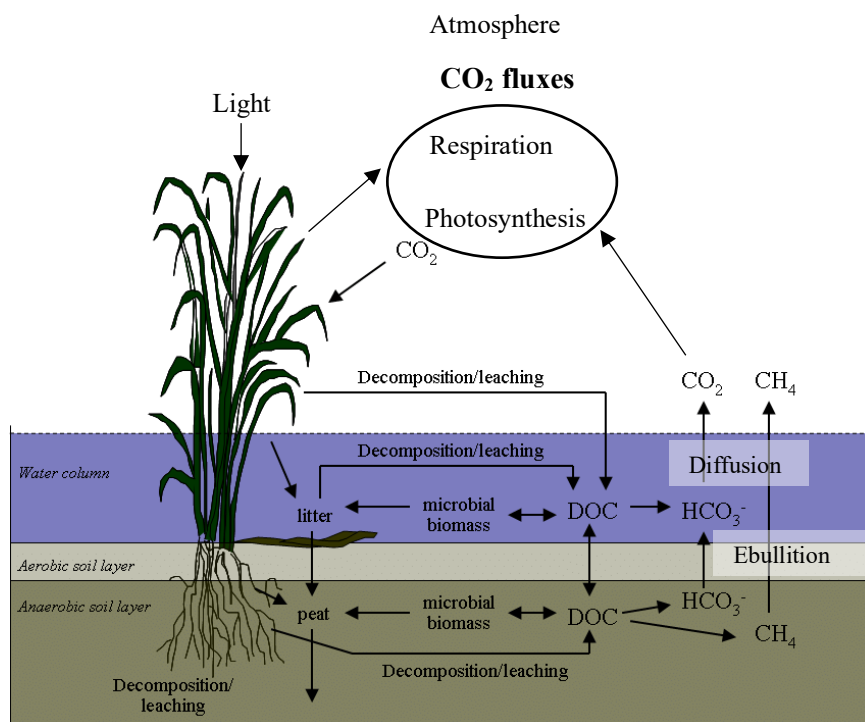


Fig. 6. Carbon cycling in wetland rice ecosystem. Adapted from (Debusk et al., 2001)

Rice fields are covered with flooded water during most parts of rice growth periods. Flooding the field results in reductive soil conditions, under which decomposition of organic materials proceeds during the period of rice cultivation.

Therefore, the decomposition of plant residues in rice field ecosystems goes on anaerobically. A large type of organic materials from plant residues are incorporated into rice soils in line with field managements. Plant residues from rice, weeds and algae are the main sources of organic materials which will vary in quantity and quality because of the various field management practices and plant growth. Final products of organic materials are CO<sub>2</sub> and CH<sub>4</sub> within the rice field ecosystem, and that they escape the system by water percolation to the subsoil layer and by flux to the atmosphere (Kimura et al., 2004).

Decomposition of plant residues is shown to be the active process in carbon cycling in rice fields. Rice releases photosynthates into the rhizosphere (rhizodeposition), then decomposition of plant residues occur in the soil by different processes (Kimura et al., 2004). Not only CO<sub>2</sub> but also CH<sub>4</sub> are produced within the decomposition process of organic materials in flooded rice fields. CO<sub>2</sub>, the end product of organic matter decomposition, is present in the form of bicarbonate ion (HCO<sub>3</sub><sup>-</sup>) occupying the biggest portion of anions in percolating water in rice fields. CO<sub>2</sub> in the floodwater is assimilated by algae and aquatic weeds through photosynthesis, which is successively produced by their respiration. Yamagishi et al. (1980) found that the absorption of CO<sub>2</sub> by floodwater phototrophs for a period after sunset in the early stage of rice cultivation. Koizumi et al. (2001) stated that CO<sub>2</sub> fluxes exchange in rice field is especially controlled by photosynthesis of aquatic plants and respiration of both the plants and also the soil microorganisms. Moreover, emission due to soil respiration is suppressed by paddy water during flood irrigation (Koizumi et al., 2001; Nishimura et al., 2015). Komiya et al. (2015) investigated that emission of CO<sub>2</sub> between flooded water and the atmosphere was mainly by diffusion, whereas low CO<sub>2</sub> ebullition was due to CO<sub>2</sub> photosynthesis and respiration by aquatic plants throughout the day. Additionally, CO<sub>2</sub> produced in the upper layer of soil may diffuse upward into the floodwater.

### **1.2.3 Impacts of agricultural tillage practices on CO<sub>2</sub> emission**

Agricultural ecosystems play an important role in the storage and release of carbon (C) within the terrestrial C cycle (Lal, 2004). Varieties of agricultural practices may affect the production and emission of carbon dioxide (CO<sub>2</sub>) from paddy soils. Due to the changes in land management practices, are increasingly thought to affect soil

carbon levels and may partially boost CO<sub>2</sub> emissions and global climate change (DeLuca and Zabinski, 2011; Lal, 2004). Due to the current global change, it is necessary to use alternative approach to land use and land management to adapt dynamic global climate change (Rulík and White, 2020). The C cycle in these systems is sensitive to management practices such as tillage and N fertilization (Ding et al., 2007; West and Post, 2002). Furthermore, the effect of N fertilization on soil CO<sub>2</sub> fluxes from agricultural ecosystems remains uncertain (Alluvione et al., 2009). CO<sub>2</sub> fluxes from agricultural soils are as the result of complex interactions between climate and soil biological, chemical and physical properties (Oorts et al., 2007). Thus, it is necessary to understand the effects of tillage and N fertilization on soil CO<sub>2</sub> flux and its influencing factors for a better comprehension of carbon dynamics in rice paddy ecosystems (Li et al., 2010).

Tillage is a fundamental practice in agricultural management, and it has a significant influence on soil C emissions by reducing soil organic carbon (SOC) (Abdalla et al., 2013). Tillage systems may affect several soil properties such as biological, chemical and physical, and therefore influence the release of CO<sub>2</sub> gas (Oorts et al., 2007; Robertson et al., 2000). Reicosky and Archer (2007) found that the CO<sub>2</sub> released immediately following tillage increased with ploughing depth was substantially higher than that from the no-tillage treatment. The relationship between tillage, soil structure, and soil organic matter dynamics is crucial to C sequestration ability of agricultural soils (Tanveer et al., 2019). Intensive soil cultivation breaks down soil organic matter (SOM), producing CO<sub>2</sub>, and consequently reduces the total C content. There are many reports suggesting that soil tillage accelerates organic C oxidation, releasing large amounts of CO<sub>2</sub> to the atmosphere over a few weeks (La Scala et al., 2008, 2006). Tillage methods in world vary depending upon the soil, climate, crop management, and availability of technology.

**Conventional tillage (Conv)** embraces not only primary cultivation practices, based on ploughing or soil inversion, but also secondary operations directed at land preparation and sowing or planting. Conv practice typically involves inversion tillage which agitate the soil to a depth of 200–300 mm, reallocate soil layers and exposes subsurface horizons to oxidation (Shepherd et al., 2001). Generally, this practice includes ploughing (soil inversion), followed by one or two harrowing actions to produce a suitable layer for plant establishment, as well as the removal of most of the

plant residues derived from the previous crop. **Conservation tillage (Cons)** systems, in contrast, are primarily based on reducing soil disturbance by restricting any land preparation activities to a shallow depth and eliminating soil inversion, while conserving and managing crop residues (Cunningham et al., 2004). They include non-inversion tillage, eco-tillage, minimum tillage, mulch tillage, reduced tillage, zone tillage or no-tillage. Cons aims to leave at least 30% of the previous crop residues remaining on the soil surface, whereas Conv leaves less than 15% (Gebhardt et al., 1985).

Grace et al. (2012) reported that by following no tillage practice and increasing cropping intensity can enhance the accumulation rate of soil organic carbon (SOC), thereby sequestering CO<sub>2</sub> from the atmosphere. However, Hassink and Whitmore (1997), Thomson et al., (2006) and Gulde et al., (2008) have reported that the increase in soil organic C in response to C input and other management practices depends on the initial C content of the soil. Variation in soil CO<sub>2</sub> emission occurs due to the different effects of tillage from rice fields. Liang et al., (2007) have observed that Conv releases higher CO<sub>2</sub> emission than Cons, this was due to the full incorporation of crop residues and soils (Lal, 2004). Moreover, greater surface crop residues for Cons probably served as a barrier for CO<sub>2</sub> emissions from soil to the atmosphere; surface residues may also reduce crop residue decomposition rate because of reduced soil temperature and minimum soil-residue contact. However, lower CO<sub>2</sub> emission from Conv practice was reported by Cheng-Fang et al. (2012) and Pandey et al. (2012) compared with Conserved rice paddy fields. Furthermore, similar CO<sub>2</sub> emissions between Conv and Cons practices was observed by Harada et al. (2007). The differences in soil CO<sub>2</sub> emissions between tillage systems may depend on the effect of short- and long-term tillage effects (Oorts et al., 2007). Cons will improve environmental quality by lowering GHG emissions (less air pollution) through decreasing the use of diesel fuel and nonburning of rice residues (Adhikari et al., 2007). Although many studies have revealed potential impact on CH<sub>4</sub> emission associated with Cons (Ball et al., 1997; Bavin et al., 2009; Hütsch, 2001; Li et al., 2011; Venterea et al., 2005), but its direct impacts on CO<sub>2</sub> emissions is still unclear. Therefore, a deeper understanding of the effects of Cons and Conv practice on the CO<sub>2</sub> fluxes exchange from the irrigated rice paddy field is required.

## **1.3 Rice and Water**

### **1.3.1 Global rice water use**

Depending on the hydrology of the where rice is cultivated, the rice growing environment can be categorized into four types as irrigated lowland rice (79 million ha), rainfed lowland rice (54 million ha), flood-prone rice (11 million ha) and upland rice (14 million ha) (Bouman et al., 2007b). Lowland rice fields which require saturated soil (anaerobic) condition for at least 20% of crop's duration. In irrigated lowlands rice fields, irrigation water is necessary for 80% of crop's duration. In rainfed lowlands, source of water depends only on the availability of rainfall and not assure to obtain within certain duration of crop. In flood-prone environments, the fields typically experience by excess water and uncontrolled, deep flooding (e.g- deep water rice, floating rice). Upland rice fields are characterized by aerobic, well-drained, and non-saturated conditions without any irrigated water for more than 80 % of crop growth period (Bouman et al., 2007b).

Rice cultivation need more water than any other arable crops because of the submergence nature of the plant. Due to rapidly increasing water demand, the competition among household, industrial, environmental, and agricultural water uses have been escalating in Asia and in the World. Approximately 75 and 80 % of the total existing water resources of the world and Asia respectively, are consumed by rice cultivation (Bouman et al., 2007a). Because of the large rice surface and the particularly water- demanding regime, it is estimated that irrigated rice receives around 40% of the water globally used for irrigation purposes (Bouman et al., 2007b).

Irrigated rice receives 34-43% of the total world's irrigation water, or 24-30% of the total world's freshwater withdraws. Total worldwide withdraws of freshwater are estimated at 3600 km<sup>3</sup> annually, of which 2500 km<sup>3</sup> is used to irrigate crops (Falkenmark and Rockström, 2013) and less than 1000 km<sup>3</sup> is used for irrigated rice crops (Fig.7). The remaining fraction of water is used in industry and for domestic purpose. Irrigated area of all crops in Asia occupied 271 million ha, almost 56% of the world's irrigated area, where rice accounts for 40–46% of the net irrigated area of all crops (Dawe, 2005).

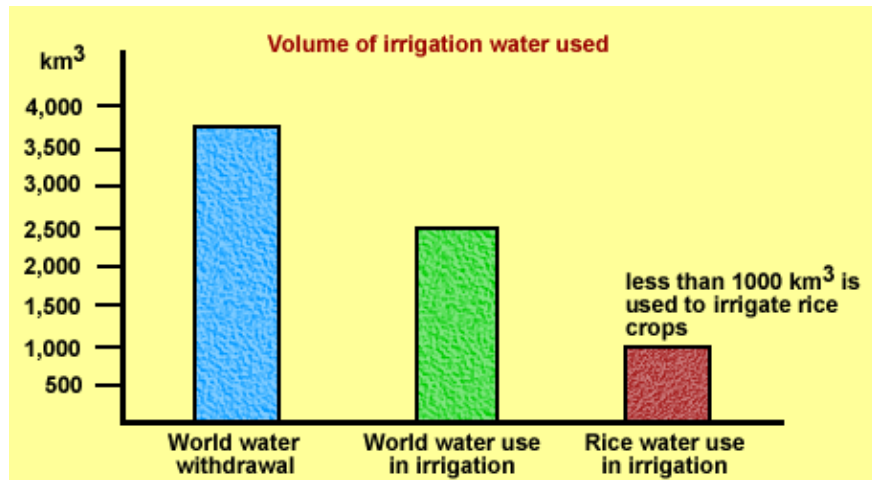


Fig. 7. Volume of irrigation water used in the world and in rice production (Adapted from <http://www.knowledgebank.irri.org/>)

### 1.3.2 The Plants-Soil-Water system

#### Water balance and management strategies in irrigated rice paddy field

Rice plants embrace water from the soil and transport it upward through the roots and stems and deliver it as vapor called transpiration through the leaves and stems to the atmosphere. The movement of water through the plant is driven by differences in water potential: water flows from a high potential to a low potential. In the soil-plant-atmosphere continuum, the water flows from the soil, with a relatively high potential, through the plant to the atmosphere just outside the leaves, which has a relatively low potential (Bouman et al., 2007b). The water tension in the atmosphere outside the leaves is determined by climatic factors: relative humidity, wind speed, temperature, and solar radiation. Besides, the water tension in the soil is determined by the amount of water in the soil and by soil physical properties such as texture and bulk density. The speed with which water moves through the plant is determined by the difference in water tension between the soil and the atmosphere (the higher the differences, the faster the water will flow) and by the resistance to water flow in the plant (Ehlers and Goss, 2003).

Flooded conditions are ideal for Irrigated lowland rice fields when the irrigation water is available. There are two major methods of rice establishment: direct seeding and transplanting. Direct seeding involve direct wet seeding (broadcasting pregerminated seeds onto wet soil) or direct dry seeding (broadcasting dry seeds onto dry or moist soil) in the paddy field (Bouman et al., 2007b). It was estimated that about

20% of Asia's farmers used direct seeded method in the late 1990s (Pandey and Velasco, 2002).

Another establishment method for raising rice plants is by transplanting the rice seedlings to well puddled soils. Predominantly, rice is initially raised in a separate seedbed and afterwards transplanted into the rice field when the seedlings are 2–3 weeks old. After crop establishment, the paddy field is typically maintained with flooded water to suppress weeds and pests. Before crop establishment, the paddy field is prepared under wet conditions. Land preparation for paddy field consists of soaking, plowing, and puddling (i.e., harrowing under shallow submerged conditions). Puddling is operated to manage weeds, to minimize soil permeability, and to soothe transplanting. Puddling leads to a complete or partial destruction of soil aggregates and macropore volume, and to a large increase in micropores (Moorman and Breemen, 1978). A typical vertical cross-section of a puddled rice field shows in Fig.8 which includes: a layer of 0–10 cm of irrigated water, a puddled layer, muddy topsoil of 10–20 cm, a plow pan that is developed by decades or centuries of puddling, and an undisturbed subsoil (Fig. 8).

Due to the flooded nature of the rice fields, water balance is different from that of the dryland arable crops such as wheat or maize. The water balance of a rice field consists of the inflows by irrigation, rainfall, and capillary rise, and the outflows by transpiration, evaporation, over bund flow, seepage, and percolation (Fig. 8). Capillary rise is the upward movement of water from the groundwater table. In non-flooded (aerobic) soil, this capillary rise may move into the root zone and provide a crop with extra water. However, there is a continuous downward flow of water in flooded rice fields from the puddled layer to below the plow pan called “**Percolation**”; that basically prevents capillary rise into the root zone. Therefore, capillary rise is usually neglected in the water balance of rice fields (Bouman et al., 2007b).

After establishment of rice crop, the soil is usually kept flooded with a 5–10-cm layer of water until 1–2 weeks before harvest. During flooded period before and after crop establishment, water outflows are by overbund (**O**) runoff, evaporation (**E**), seepage (**S**), and percolation (**P**). During crop growth period, water also leaves the rice field by *Transpiration*. *Evaporation* leaves the rice field directly from the flooded water layer. *Transpiration* by rice plants withdraws water from the puddled layer. Both processes are usually taken together as “*Evapotranspiration*.” During the crop growth

period, about 30–40% of evapotranspiration is evaporation (Bouman et al., 2005; Simpson et al., 1992). **Seepage** is the subsurface flow of water underneath the bunds of a rice field. Seepage rates are affected by the soil physical characteristics of the field and bunds, by the state of maintenance and length of the bunds, and by the depth of the water table in the field and in the surrounding drains, ditches, or creeks (Wickham and Singh, 1978). **Percolation** is the downward flow of water to below the root zone. The percolation rate of flooded rice fields is affected by a various factors of soil characteristics such as structure, texture, bulk density, mineralogy, organic matter content, and salt type and concentration (Wickham and Singh, 1978). Water losses by seepage and percolation account for about 25-50% of all water inputs in heavy soils with shallow groundwater tables of 20-50 cm depth (Cabangon et al., 2004; Dong et al., 2004), and 50-85% in coarse-textured soils with deep groundwater tables of 1.5 m depth or more (Sharma et al., 2002; Singh et al., 2002).

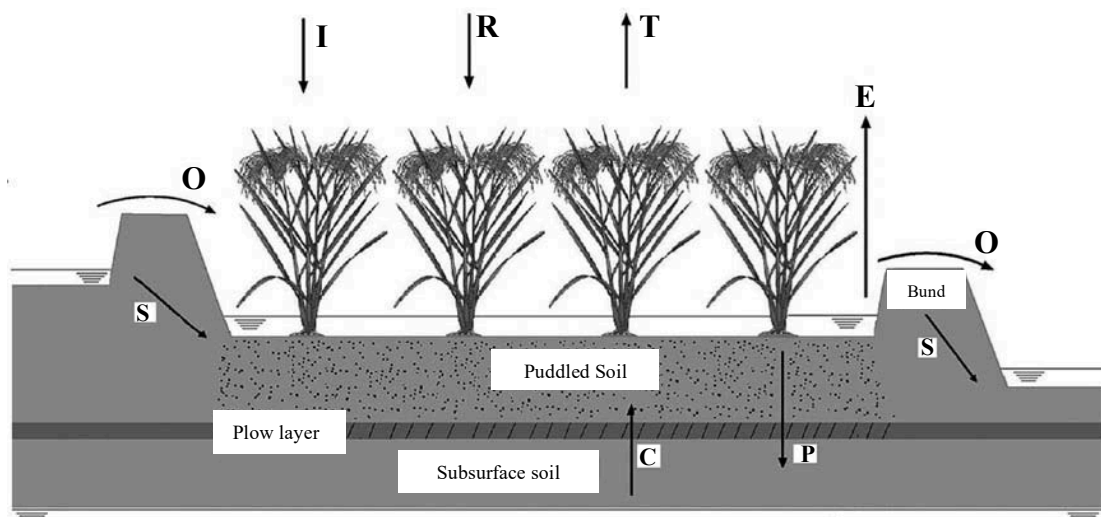


Fig. 8. Water balance of a lowland rice field. C = capillary rise, E = evaporation, I = irrigation, O = overbund flow, P = percolation, R = rainfall, S = seepage, T = transpiration.

As a consequence, new approaches known as “Water Saving Technologies” are being investigated in order to exploit the opportunity of reducing the water amounts required by traditional rice cropping systems (Belder et al., 2007; Dunn and Gaydon, 2011; Feng et al., 2007; Govindarajan et al., 2008; Sudhir-Yadav et al., 2011; Tabbal et al., 2002). Water fluxes in irrigated rice fields depend on the type of water management strategy that is adopted. Water management strategies that are currently most widely used across the world are listed as (1) Water seeding, continuous Flooding



(WFL): the rice field is submerged immediately after tillage operations; seeding is made directly in water, which is maintained for the whole crop cycle except for brief periods to allow treatments with herbicides or fertilizers; (2) Dry seeding and delayed Flooding (DFL): seeding is made before flooding, which takes place approximately when rice is around the 3-leaf stage; water management is then similar to WFL; (3) Dry seeding and intermittent Irrigation (DIR): no flooding takes place; the field is irrigated intermittently, either by border or sprinkler irrigation; this method is known as “aerobic rice” cultivation. Generally, the main fluxes are: irrigation supply and tailwater drainage, direct precipitation and evapotranspiration, percolation and capillary rise (Fig.9) (Chiaradia et al., 2015).

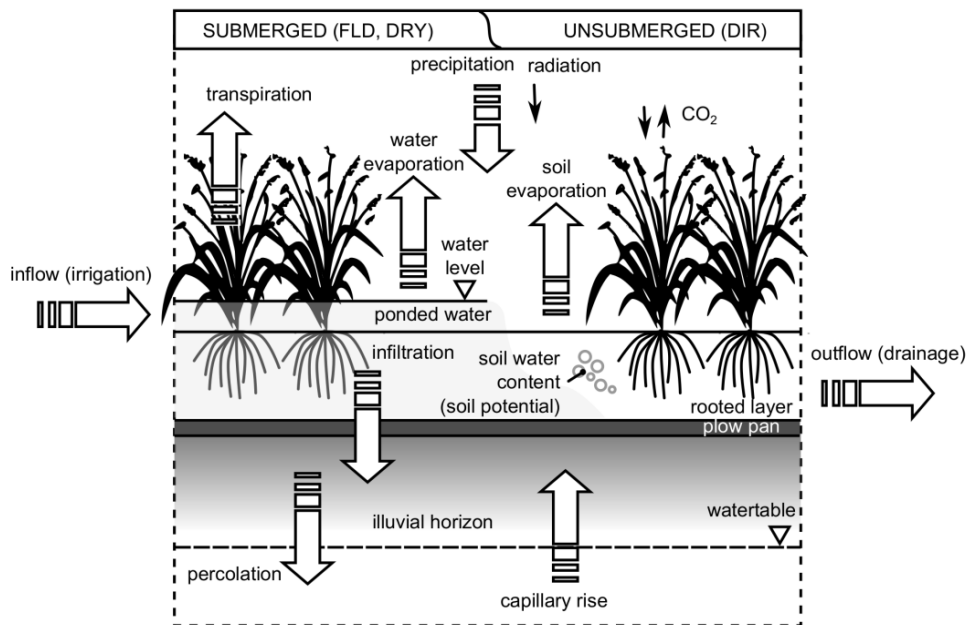


Fig. 9. Water fluxes and storage in flooded (Submerged) and non-flooded (Unsubmerged) rice fields (Adapted from Chiaradia et al., 2015)

### 1.3.3 Water saving technique in irrigated paddy rice cultivation

More than 75% of the global rice supply comes from lowland irrigated rice (Maclean et al., 2002), where rice has been conventionally grown under the continuous standing of a water depth ranging between 5 to 10 cm (Bouman et al., 2007b). It is estimated that irrigated rice receives around 40% of the water globally used for irrigation purposes as increasing area of irrigated paddy rice growing area and the particularly in the water demanding regime (Bouman et al., 2007b). According to the well projected scenarios, 15–20 million ha of irrigated rice paddy could suffer from certain degree of water scarcity by 2025 due to an increasing water competition among

users and therefore it is necessary for the improvements in the effectiveness of rice irrigation are becoming more and more urgent (Tuong and Bouman, 2003). Moreover, rice producers will have to face three major challenges: i) to save water, ii) to increase water productivity (i.e. grain yield over water input) and iii) to “produce more rice with less water”, were identified by Bouman and Tuong (2001).

The potential for water savings in irrigated rice culture is substantially important nowadays because of current global climate change (IPCC, 2007), Bouman and Tuong (2001) reported reduced water inputs and increased productivity of rice grown under saturated soil conditions, as compared with traditional flooded rice. Borrell et al. (1997) found that saturated soil culture with rice grown on raised beds reduced the amount of water use by approximately 32 percent as compared with conventional methods. Despite water saving techniques for rice culture has long been identified by many rice researchers, there are still questionable for the potential yield loss by the reduced use of water in rice cultivation. De Datta (1981) stated that rice grain yield was significantly associated with the amount of water use. Castillo et al. (1992) reported that draining rice fields at either vegetative or reproductive phases caused significant yield loss. The increasing scarcity and competition for water is occurring worldwide. Therefore, water conservation practices are the most priority task for increasing agricultural production, particularly rice production. Water saving irrigation practices change the situations from continuous anaerobic conditions to alternate anaerobic-aerobic and continuous aerobic conditions. The transformation from anaerobic to aerobic systems will have major consequences for weed, pest, and disease ecology, nutrient and soil organic matter dynamics, and greenhouse gas emissions and carbon sequestration (Singh et al., 2013).

Under anaerobic conditions, methane ( $\text{CH}_4$ ) is typically produced by methanogenic bacteria in the paddy soil. The contribution of rice paddies to the total emission of methane ( $530 \pm 20$  Tg per year) is considerable but not known precisely (Prather et al., 1995). The quantity of  $\text{CH}_4$  that reaches the atmosphere is, however, often substantially less than what is originally produced. This is because significant quantities of  $\text{CH}_4$  produced are oxidised before reaching the atmosphere (Kögel-Knabner et al., 2010). Methane produced by methanogenic bacteria in the soil is partly oxidized in the rhizosphere to  $\text{CO}_2$  by methanotrophic bacteria (Bilek et al., 1999; Butterbach-Bahl et al., 1997; Chanton et al., 1997; Conrad, 1993; Frenzel et al., 1992).

Methanogenesis in the rhizosphere itself is suppressed by oxygen (Fetzer and Conrad, 1993). This uncertainty is partially caused by the large variations in local rice growth conditions and by the complicated dynamics between the methane production and methane oxidation in the rice paddy soil (Van Bodegom et al., 2001). Therefore, a comprehensive understanding of methane oxidation in the rice rhizosphere is also necessary to quantify CO<sub>2</sub> fluxes from irrigated paddy rice cultivation.

One technique that has been developed by the International Rice Research Institute (IRRI) to reduce total water for irrigation in rice is Alternate Wetting and Drying (AWD). In AWD, the field is continuously flooded (CF), instead the soil is allowed to dry out for one or more days after the disappearance of ponded water, and after this drying phase the field is re-flooded (Lampayan et al., 2015). The irrigation water is applied for about 2–5 cm with an interval of 2–7 days followed by disappearance of ponded water from soil surface (Tuong and Bouman, 2003). AWD entails monitoring water levels above and below the soil surface and only irrigating when the water level drops to about 15 cm below the surface of the soil and allow the fields to dry at other time. The AWD water management regime starts two weeks after transplanting of seedlings. This cycle is repeated except during flowering stage since the rice plant is very sensitive to dry conditions. Hence from one week before to a week after flowering, the fields kept flooded. This reduces water use by up to 30% and methane emission by 48% with no yields loss (Richard and Sander, 2014). The advantage of AWD technique is to reduce water use by keeping field continuously flooded but allowing it to dry intermittently during the growing season. AWD practice not only saves water but also reduces greenhouse gas (GHG) emissions while maintaining yields. In the 2006 IPCC methodology, AWD is assumed to reduce methane (CH<sub>4</sub>) emissions by an average of 48% compared to continuous flooding. Nowadays, AWD has been field-tested and validated by rice farmers in Bangladesh, Indonesia, Laos, the Philippines, Myanmar and Vietnam, and is being mainstreamed in extension efforts by formal extension services and NGOs in Southeast Asia (Richard and Sander, 2014).

Due to the increasingly severe water scarcity and food security issues, water-saving irrigation (WSI) techniques are being broadly implemented in rice paddies (Bouman et al., 2007b; Mao, 2002). Moreover, rice WSI can also accelerate the decomposition of soil organic matter and increased soil organic carbon content (Yang

et al., 2018). Soil organic carbon is an important index for the sustainable utilization of soil. Therefore, rice WSI technologies should be applied in combination with organic carbon input technologies to reduce CO<sub>2</sub> emissions, increase soil organic matter content, and achieve a sustainable use of water and soil resources in paddy fields.

Although previous studies have demonstrated that alternative water management in lowland rice cultivation is an effective strategy for decreasing CH<sub>4</sub> emission (Gupta et al., 2002), it is however, not known the amount of CO<sub>2</sub> releases to atmosphere if reduced CH<sub>4</sub> emission under alternative irrigation management from enhanced CH<sub>4</sub> oxidation. Hence, a process-based understanding of diffusive quantity of CO<sub>2</sub>, escapes from CH<sub>4</sub> oxidation in paddy soil under fluctuating conditions of water management is necessary. Therefore, we also emphasize on alternate wetting and drying water management practice to envisage water use efficiently in quantifying CO<sub>2</sub> fluxes from paddy rice cultivation. In this thesis, two experiments were conducted to assess CO<sub>2</sub> fluxes under altering different agricultural management systems in irrigated rice paddy cultivation.

## **2. Objectives of dissertation thesis**

The main objectives of this thesis were:

1. To quantify carbon dioxide (CO<sub>2</sub>) fluxes under different water management practices and fertilizer applications from typical Myanmar's rice cultivation environment.
2. To elucidate the combined effects of water management practices and fertilizer applications on the rice biomass yields.
3. To investigate the effects of conventional and conservation tillage management practices on CO<sub>2</sub> fluxes in irrigated lowland rice paddies during the summer rice growing season including the fallow period.
4. To establish the effects of plant biomass on total CO<sub>2</sub> fluxes and compare the effects of different agricultural tillage management approaches on grain yield and quality of common Myanmar rice cultivar.

### 3. MATERIAL AND METHODS

The first trial for quantification of CO<sub>2</sub> have taken place from June to November 2017 under glass greenhouse condition at the Palacky University in Olomouc, Czech Republic. The aim was to quantify CO<sub>2</sub> fluxes in a representative rice cultivation system under different water managements and fertilizer applications. Further information of the experimental set up and treatments are detailed in Paper I. A schematic representation of the CO<sub>2</sub> fluxes measurement system from paddy rice cultivation under continuous flooding and alternate drainage water cycle management practice is shown in Fig. 10 a & b.

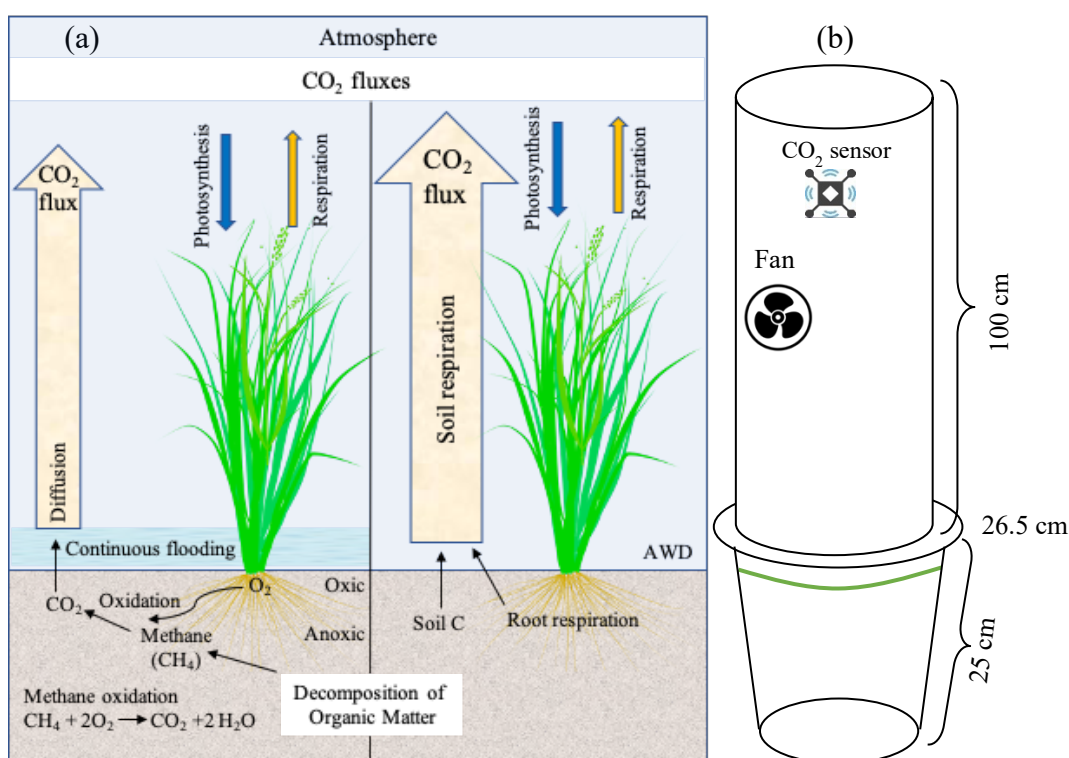


Fig. 10. (a) Schematic representation of CO<sub>2</sub> fluxes under continuous flooding (CF) and alternate wetting and drying (AWD) practices, and (b) CO<sub>2</sub> measurement chamber tube.

The pattern of CO<sub>2</sub> flux was analysed by recording CO<sub>2</sub> gas concentration from both plant and soil inside the gas chamber tube. Instantaneous CO<sub>2</sub> concentrations (ppmv) inside of the gas chamber tube was recorded every 30s for 10 minutes in each treatment. This measurement was conducted during day- and nighttime to assess the quantity of net CO<sub>2</sub> flux from the rice plants and paddy soil. The total CO<sub>2</sub> production was calculated from the slope of linear regression of the CO<sub>2</sub> concentration (ppm) over

time. The net CO<sub>2</sub> flux is the difference of total CO<sub>2</sub> fluxes recorded inside the gas chamber during day and night. The results of the net CO<sub>2</sub> fluxes indicates negative and positive values, i.e. uptake of CO<sub>2</sub> by the plants during photosynthesis and emission of the CO<sub>2</sub> by the plants and soil during respiration, respectively. The plant parameters such as above-ground and below-ground biomass, number of tiller per plant and plant height were measured at the time of harvest, exception for plant height which was measured weekly interval.

The second experiment to quantify CO<sub>2</sub> fluxes was conducted from mid-January through June of 2018 as a field experiment at the Department of Agronomy, Yezin Agricultural University, Myanmar. The experimental location with detail treatments and cultural practices are detailed in paper II. Figure 11. shows a schematic representation of the field measurement system to monitor CO<sub>2</sub> fluxes under conventional and conservation management practices from the irrigated rice paddy fields.

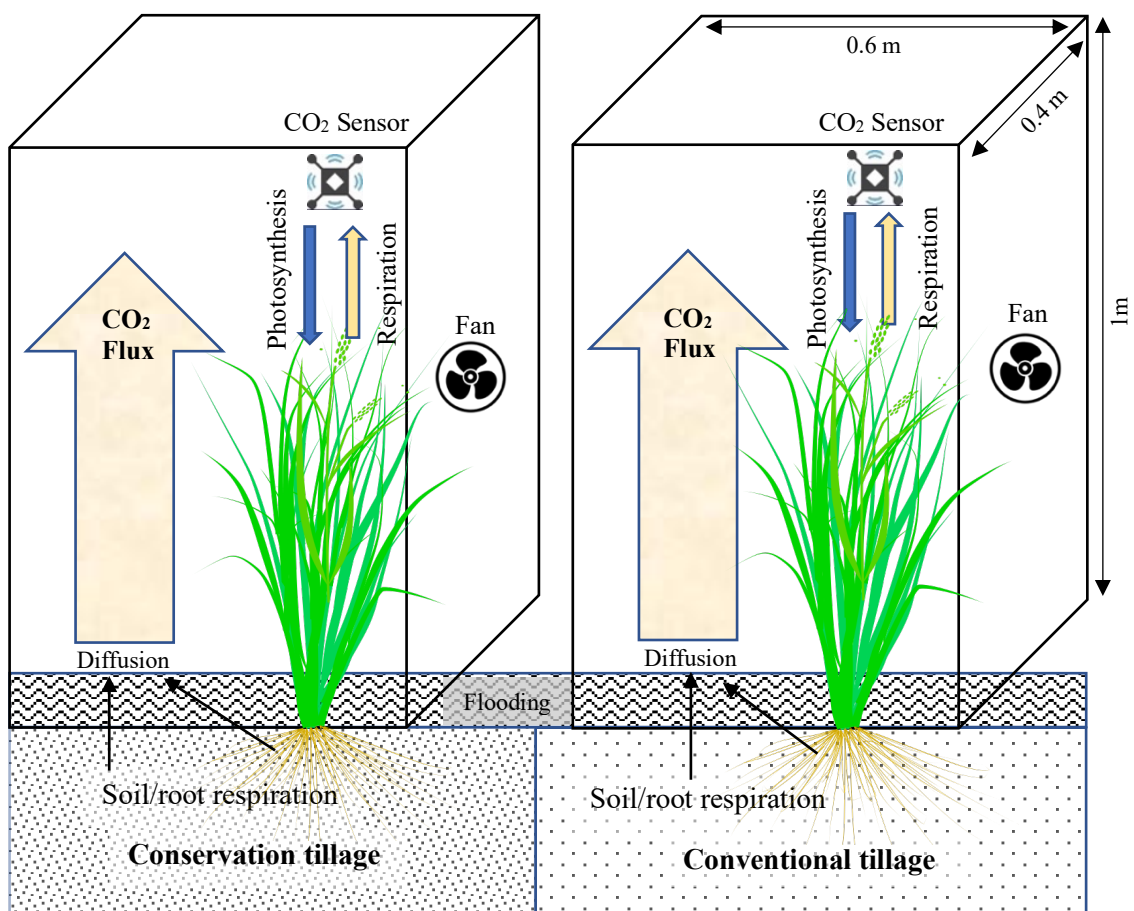


Fig. 11. Schematic of the closed chambers system to assess CO<sub>2</sub> fluxes under different agricultural tillage systems in an irrigated paddy rice field.

CO<sub>2</sub> fluxes were simultaneously recorded both in conventional and conservation management practices by using two separate gas chambers. This sampling procedure was done once a week for 30 minutes during day and nighttime throughout the entire summer rice growing season. Total CO<sub>2</sub> flux was calculated as the change of the CO<sub>2</sub> quantity in the space over the soil covered by a chamber per unit area, which represents the sum of CO<sub>2</sub> assimilated and respired by the rice plants and respired by the paddy soil. Net CO<sub>2</sub> emission or uptake by the rice plants was computed by subtracting soil respiration from total CO<sub>2</sub> flux. Based on the results from the measurement of CO<sub>2</sub> fluxes, we extrapolated CO<sub>2</sub> emissions from the cultivated summer rice fields in Myanmar considering additionally statistical data from the Department of Agriculture, Myanmar. Soil temperature from different depths (0–5 cm, 0–10 cm and 0–20 cm) were recorded separately to test for a putative relation of soil temperature with soil CO<sub>2</sub> fluxes. Daily ambient air temperature (°C) and relative humidity (RH%) were also recorded. Soil samples from the paddy rice field were collected before planting and after harvesting of the rice paddy for the analysis of soil organic content (SOC). Plant biomass weight (g plant<sup>-1</sup>) and other yield components were collected at harvesting time. After harvest, grain quality analysis was performed following standard methods that are detailed in Paper II.



## 4. MAIN RESULTS AND DISCUSSION

### Paper I

#### 4.1 Effects of different water management and fertilizer applications on CO<sub>2</sub> fluxes from a selected Myanmar rice (*Oryza sativa*. L) cultivar

The experiment aimed to quantify CO<sub>2</sub> fluxes between rice plants and atmosphere and to establish the effects of different agricultural management systems, focusing on different irrigation and fertilizer management practices. We compared the CO<sub>2</sub> fluxes exchange in conventional continuous flooding with that in alternate drainage condition and its responses to the application of inorganic and organic fertilizer. CO<sub>2</sub> fluxes showed almost always negative values during daytime due to the assimilation of CO<sub>2</sub> by the plants for photosynthesis activities, whereas positive flux values during nighttime resulted from respiration by the plants and paddy soil. Additionally, we found more subtle patterns of CO<sub>2</sub> fluxes caused by the effects of different fertilizer applications and water management practices.

Weekly mean values of the total CO<sub>2</sub> flux fluctuated considerably under both continuous flooding (CF) and alternate wetting and drying (AWD) practices. In contrast, the inorganic and organic fertilizer treatments revealed only minor impacts on both CF and AWD practices. Total CO<sub>2</sub> fluxes from nighttime were consistently positive and mostly stable under both CF and AWD practices. Moreover, no interaction effects of the different water management practices and fertilizer treatments on Total CO<sub>2</sub> fluxes were observed during day and night. Our findings from weekly Total CO<sub>2</sub> under CF were similar with previously reported findings from flooded rice paddy fields in East Asia, India and USA (Alberto et al., 2009; Bhattacharyya et al., 2013; Miyata et al., 2005; Saito et al., 2005; Swain et al., 2016). In contrast, previous studies on AWD (Alberto et al., 2014; Liu et al., 2013; Miyata et al., 2000) reported more positive values than detected in the here presented study. Surprisingly, net soil CO<sub>2</sub> emission from CF was 35% higher than AWD in our present study, despite the fact that contribution of soil respiration to the Total CO<sub>2</sub> fluxes seemed to be negligible and accounted for only 9% of the total flux. We found that Total CO<sub>2</sub> fluxes were elevated in the presence of inorganic fertilizers (recommended and farmer's practice), which also led to higher biomass yield and general enhanced crop growth (Paustian et al., 1997), and consequently increased CO<sub>2</sub> fluxes (Iqbal et al., 2009). The application of organic

manure shifted the CO<sub>2</sub> flux towards negative values under both CF and AWD practices when compared to inorganic fertilizer applications.

Taken together, we revealed a biomass yield increase of 7.8% under AWD compared to the CF practice. This finding is supported by Katsura et al. (2010), where the maximum dry matter accumulation was also higher under AWD compared to continuous submergence. Noteworthy, remaining plant characters such as plant height, leaf area and tillers number were also significantly higher under AWD than CF practice.

Paper II

#### **4.2 Comparison of carbon dioxide (CO<sub>2</sub>) fluxes between conventional and conserved irrigated rice paddy fields in Myanmar**

Results from the previous experiment demonstrated that the need for a better understanding of the variability of CO<sub>2</sub> fluxes in paddy rice cultivation. Accordingly, field investigation for CO<sub>2</sub> fluxes measurement in rice paddy fields were implemented at Yezin Agricultural University, Myanmar. The field was farmed separately under conventional (Conv) and conservation (Cons) agricultural tillage management practices. CO<sub>2</sub> fluxes were collected from both total (rice plants and paddy soil) and bare soil respiration during day and night along with soil temperature assessments in different soil depths.

The soil temperature at the three depths (0–5, 0–10 and 0–20 cm) was statistically indifferent both at day and night throughout entire experimental period. Weekly total CO<sub>2</sub> fluxes during daytime were consistently negative (i.e. uptake of CO<sub>2</sub> by the rice plants). Positive values of CO<sub>2</sub> fluxes from bare paddy soil were observed during day and night. The values were also positive for the net soil CO<sub>2</sub> flux throughout the entire rice growing period. Similar results were reported by Nishimura et al. (2015). They further mention that net soil CO<sub>2</sub> was close to zero under flooded conditions.

Correlating to the growth stage (details crop growth stages presented in paper II) of the rice plants, we found a peak of net CO<sub>2</sub> emission flux during grain filling period in both Conv and Cons practices. In contradiction to our findings, Dutta and Gokhale (2017) reported the peak slightly earlier in the plant development, during flowering. The discrepancy might be caused by different experimental setups such as cultivation and assessed rice cultivar. During the maturation of the leaves, both CO<sub>2</sub> uptake and emission during day and night gradually declined towards the late growth period

(Khatun, 2007). It is thus not surprising that the CO<sub>2</sub> emission peak is found in developmental stages before the plants' full maturation. Total CO<sub>2</sub> emission fluxes from Cons practice were significantly lower than Conv practice during nighttime, whereas they were statistically indifferent during daytime. Furthermore, higher CO<sub>2</sub> emissions were observed for Conv practice than Cons during the fallow period in our field investigation. This ties well with former findings(Liang et al., 2007).

Plant height is strongly associated with plant biomass. We observed that the mean plant height from Conv practice was higher than Cons practice, which was also true for biomass weight (g plant<sup>-1</sup>/hill<sup>-1</sup>) and leaf area (cm<sup>2</sup>). These findings are in line with previous findings by Tilly et al. (2013), describing that increased plant height is accompanied by higher plant biomass. Higher net CO<sub>2</sub> emission and increased biomass were observed in Conv compared to the Cons practice, indicating that aboveground biomass was a dominant factor in production of CO<sub>2</sub> in our study. Plant biomass is a primary source of organic matter and Conv tillage practice causes increase CO<sub>2</sub> emission by exposing soil organic matter to enhanced decomposition processes. Additionally, more effective tillers per plant (hill) were observed in the Conv compared to the Cons practice. Comparisons revealed that the hill was twice as high in the Conv compared to the Cons practice. It is likely that rice grain yield will also be affected by the different soil management practices.

We estimated the annual CO<sub>2</sub> emissions for the summer rice cultivated area in Myanmar by extrapolating our data from the field investigation to 2347 g CO<sub>2</sub> m<sup>-2</sup>yr<sup>-1</sup>. Comparing this estimation of the annual CO<sub>2</sub> flux to the previous findings from Asian rice fields (data comparison detailed in paper II), our findings were within the range of the reported emission fluxes, irrespective of the different cultivation practices applied, i.e. rice paddy under irrigated conditions.

## 5. CONCLUSIONS

Collectively, the presented study shows the effects of different agricultural management practices on net CO<sub>2</sub> flux patterns between rice plants and paddy soil. Furthermore, it highlights potential methods to reduce CO<sub>2</sub> emissions from rice paddy cultivation. Findings from the greenhouse and the field experiment revealed clear diurnal patterns of CO<sub>2</sub> fluxes throughout the crop growth period; a daytime uptake and nighttime release of CO<sub>2</sub> by the rice canopy. Based on the diurnal pattern of CO<sub>2</sub> fluxes in our study, it can be suggested that the flooded rice paddy ecosystem behaves either as a net CO<sub>2</sub> sink in the greenhouse study or a net CO<sub>2</sub> source in the field experiment.

Although continuously flooded rice system is sustainable in terms of yields and soil quality, AWD appears an effective approach to sustain or improve rice yields under water scarcity. The above-ground fresh biomass was 15% higher in the AWD than in the CF practice. The CO<sub>2</sub> uptake was increased by 28% under AWD, which might be due to the higher above-ground biomass compared to CF practice. This may effectively compensate the putatively increased soil CO<sub>2</sub> emission under AWD. A detailed assessment of additional site-specific parameters, such as climate, soil type, cultivars and crop rotation, will be required to enable a successful adoption of the AWD practice.

The conventional (Conv) practice of rice production in the field investigation revealed a higher above-ground biomass as well as an increased harvest grain yield compared to the Conservation (Cons) practice. However, the Conv practice also exhibited significantly higher CO<sub>2</sub> fluxes than the Cons practice, indicating that CO<sub>2</sub> fluxes were influenced by rice plant biomass. Considering current approaches to reduce the emission of GHGs from rice fields, it is noteworthy that modification of agricultural cultivation systems toward implementation of the Cons practice could contribute to lower CO<sub>2</sub> emissions from flooded rice fields. However, it will also require providing incentives to the farmers as this practice results in lower grain yields. Additional research is needed to optimize grain yield while minimising CO<sub>2</sub> emissions from rice paddy fields. Moreover, soil CO<sub>2</sub> emission is the result of complex interactions between various soil properties and climatic parameters, suggesting that quantifications of multi years/seasons rice fields are needed to generate robust CO<sub>2</sub> flux data.

Conclusively, I here reported results shall stimulate further research on this topic and may serve as a basis to foster a deeper understanding of the CO<sub>2</sub> fluxes in rice paddy ecosystems.

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## 7. ATTACHED PUBLICATIONS

### 7.1 Effects of Different Water Management and Fertilizer Applications on CO<sub>2</sub> Fluxes from a Selected Myanmar Rice (*Oryza sativa* L.) Cultivar

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## **Effects of Different Water Management and Fertilizer Applications on CO<sub>2</sub> Fluxes from a Selected Myanmar Rice (*Oryza sativa* L.) Cultivar**

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### **Authors' contributions**

*This work was carried out in collaboration among 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<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) emission. Compared to methane, knowledge about the combined effects of different fertilizer rates together with different water management practices on CO<sub>2</sub> fluxes are scarce. Therefore, this study aims to assess CO<sub>2</sub> 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<sub>2</sub> flux in CF was -30.82 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> during daytime and 29.64 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> during nighttime. Surprisingly, the average net CO<sub>2</sub> fluxes were negative under both CF (-49 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>) and AWD practices (-127 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>), indicating a net CO<sub>2</sub> uptake by the rice plants. Inorganic fertilizer applications led to considerably higher net CO<sub>2</sub> emissions compared to the control under both CF and AWD. Conversely, CO<sub>2</sub> emission fluxes in the treatment

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with organic manure showed negative net CO<sub>2</sub> fluxes under both water management practices and 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<sub>2</sub>, could effectively mitigate CO<sub>2</sub> impacts regardless of the selected water management practice.

*Keywords: Alternate wetting and drying; CO<sub>2</sub> 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<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O). GHGs emissions are produced to a large extent by anthropogenic activities with CO<sub>2</sub> and CH<sub>4</sub> 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<sub>2</sub> emissions [2]. The main CO<sub>2</sub> 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<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O. Under anaerobic conditions typical for paddy fields, CH<sub>4</sub> is released at substantial rates during organic matter decomposition [5], rendering the rice ecosystem a considerable source of CH<sub>4</sub> emission on a global scale. The CO<sub>2</sub> 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<sub>2</sub> [6] and there is still no clear conviction about how important role play rice fields in CO<sub>2</sub> 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 7<sup>th</sup> 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<sub>4</sub> emission by rice fields. This is supported by an independent experiment revealing lower CH<sub>4</sub> emissions in wetting-drying alteration patterns than in continuous flooding [19]. However, water-saving irrigation approaches may increase CO<sub>2</sub> emissions as aerobic conditions favour complete oxidation of carbon compounds to CO<sub>2</sub> rather than CH<sub>4</sub>. 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<sub>4</sub> emissions from paddy fields due to the raising of rice biomass and providing additional C source, but showed no effect on total CO<sub>2</sub> emissions [31]. This process was explained by Stevens et al. [32], due to the fact that CO<sub>2</sub> is reduced to CH<sub>4</sub> under anaerobic environment where oxygen is unavailable. Therefore, microbes metabolizing without oxygen (anaerobes) decompose the organic matter to methane. On the contrary, CO<sub>2</sub> 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<sub>2</sub> flux compared to a single urea application. Chicken manure significantly increases ecosystem respiration (CO<sub>2</sub>), however pig slurry has no effect on CO<sub>2</sub> emission in Mediterranean paddies [35]. In contrast to CH<sub>4</sub>, the effects of different fertilizing practices on CO<sub>2</sub> 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<sub>2</sub> emission in rice fields. As such, we sought to investigate these combined effects by monitoring CO<sub>2</sub> 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<sub>2</sub> 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. MATERIAL 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<sup>-1</sup> (Ammonium Sulphate), Phosphorus (P): 30 kg ha<sup>-1</sup> (Triple superphosphate) and Potassium (K): 20 kg ha<sup>-1</sup> (Muriate of potash)], inorganic fertilizer application by farmer's practice- F2 [ N: 21 kg ha<sup>-1</sup>, P: 5 kg ha<sup>-1</sup> and K: 6 kg ha<sup>-1</sup>], organic manure application-F3 [cow manure: 5 ton ha<sup>-1</sup> (1.5% N: 2.5% P<sub>2</sub>O<sub>5</sub> and 1.5% K<sub>2</sub>O)] 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<sub>2</sub> gas fluxes inside the chamber were recorded using the Sense Air® CO<sub>2</sub> sensor Module K33 ELG, designed to measure and store records of environmental parameters such as temperature (T), relative humidity (RH) and carbon dioxide (CO<sub>2</sub>) concentration (up to 5000 ppm range) [41]. The sensor device was installed inside the mobile gas chamber together with a fan to enhance CO<sub>2</sub> 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<sub>2</sub> concentrations (ppmv) inside of the chamber was measured every 30 s during both the day and nighttime. CO<sub>2</sub> 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<sup>-2</sup>) provided by Czech Meteorological Institute and converted them to Photosynthetic Photon Flux

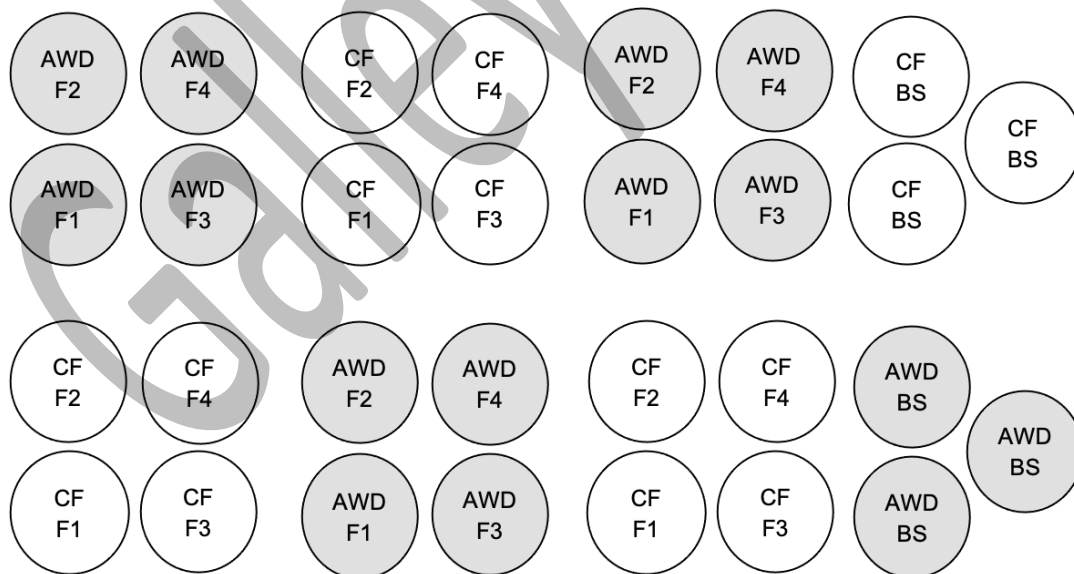
Density (PPFD) values inside the greenhouse by a formula suggested by Nederhoff & Marcelis [42] (Fig. 4a & b). Due to the high variability of CO<sub>2</sub> fluxes by photosynthetic activities during daytime [43], CO<sub>2</sub> flux measurements were performed consistently at a fixed time during entire 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<sub>2</sub> was performed by using the following equation [44]:

$$F_{CO_2} = (V/A)(dc/dt) \quad (1)$$

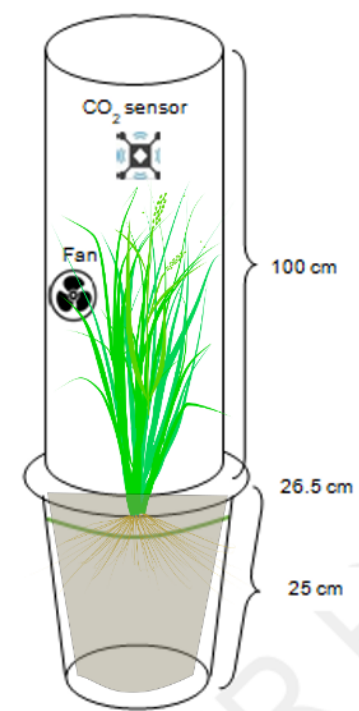
Where  $F_{CO_2}$  is the Total CO<sub>2</sub> flux density (mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>),  $V$  and  $A$  are the volume (litres) and base area (m<sup>2</sup>) of the chamber,  $dc/dt$  describes the CO<sub>2</sub> concentration change in the chamber over time.

The *net CO<sub>2</sub> flux* refers to a difference in total CO<sub>2</sub> fluxes measured during the night and day, while negative and positive values represent uptake or emissions of CO<sub>2</sub> 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<sup>-1</sup>, Phosphorus (P) 30 kg ha<sup>-1</sup> and Potassium (K) 20 kg ha<sup>-1</sup>]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha<sup>-1</sup>, Phosphorus (P) 5 kg ha<sup>-1</sup> and Potassium (K) 6 kg ha<sup>-1</sup>]; F3 = Organic manure application (cow manure - 5 t ha<sup>-1</sup>); F4 = Control; BS = Bare Soil



**Fig. 2. Plexiglass gas tube chamber for measurement of CO<sub>2</sub> 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<sup>2</sup>) from individual pots was recorded at 42, 56, 70, 112 and 122 DAP. Leaf area (cm<sup>2</sup>) 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<sup>-1</sup>) 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<sub>2</sub> 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<sub>2</sub> 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  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , while the average amount of PAR was 641  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  during experimental period (from July to October 2017, Fig. 4a). During the day (5:00-19:00), mean PAR value was 404  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and ranged from 10-836  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Fig. 4b).

### 3.3 Total CO<sub>2</sub> Fluxes (Day and Night)

Generally, CO<sub>2</sub> 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<sub>2</sub> emission fluxes were positive during nighttime in all treatments (Figs. 5c, d). Weekly CO<sub>2</sub> fluxes fluctuated considerably under both CF and AWD practices, with a range from -3341 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> to 1035 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> in CF treatment (Fig. 5a) and from 2015 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> to -3729 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> 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<sub>2</sub> fluxes remained indifferent in response to different water and fertilizer treatments. Nighttime CO<sub>2</sub> fluxes revealed less fluctuation compared to those from daytime (Figs. 5c, d). CO<sub>2</sub> fluxes ranged from 278 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> to 1923 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> (Fig. 5c) and from 634 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> to 2140 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> (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<sub>2</sub> fluxes during daytime and nighttime. Mean CO<sub>2</sub> fluxes during daytime ranged from -2061 to -719 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> and nighttime ranged from 938 to 1558 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>.

### 3.4 Net CO<sub>2</sub> Fluxes

According to the two-way ANOVA, Net CO<sub>2</sub> flux data averaged per week were not significantly altered by any of the treatments or their

interactions. Net CO<sub>2</sub> fluxes varied from -1723 to 2308 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> in CF treatment and from -2778 to 3854 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> in AWD treatment, respectively (Fig. 6). Generally, mean uptake of net CO<sub>2</sub> flux from AWD (-68 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>) treatment was higher than the CF (-49 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>) 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<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>) was higher in CF compared to AWD (254 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>) (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<sub>2</sub> 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<sub>2</sub> fluxes, i.e., CO<sub>2</sub> 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)

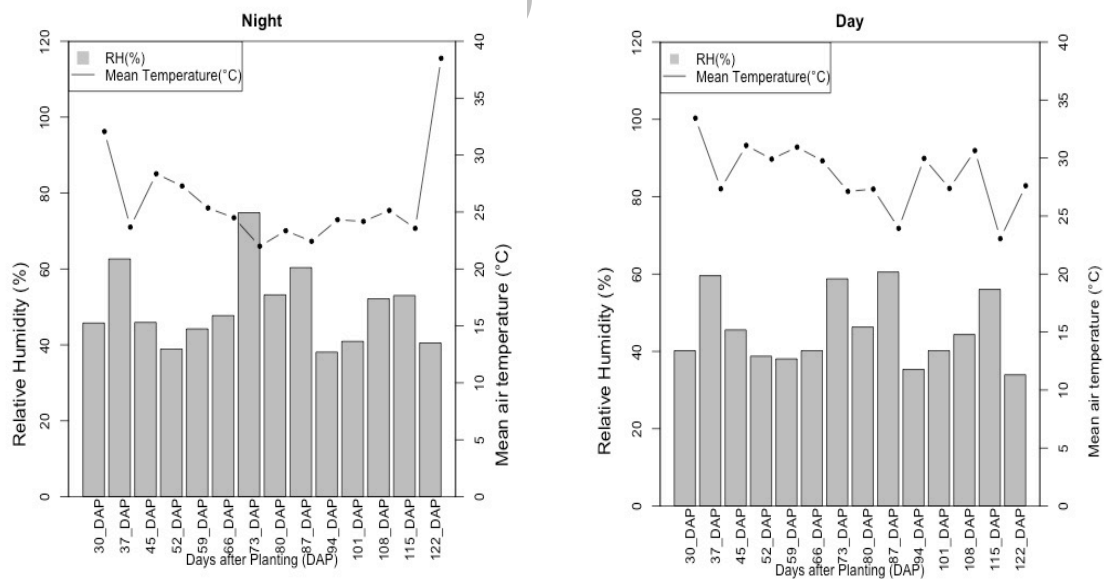
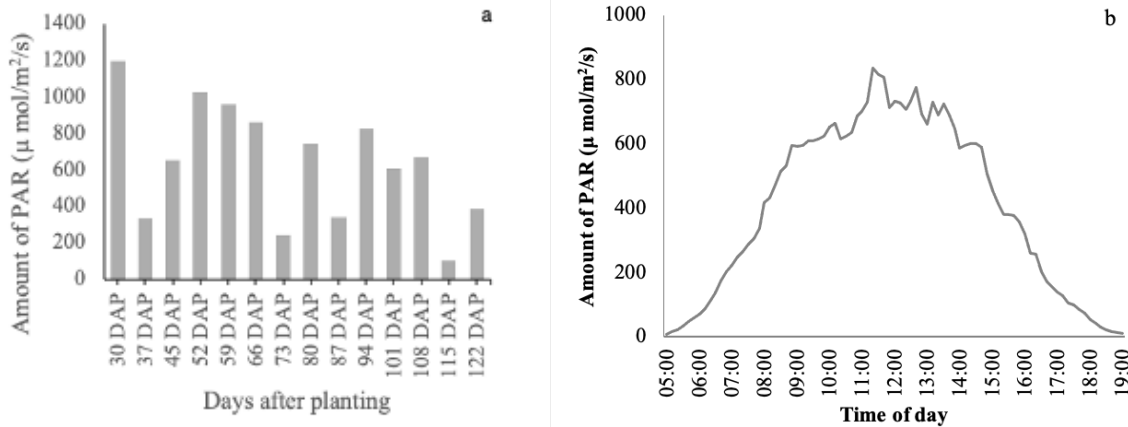


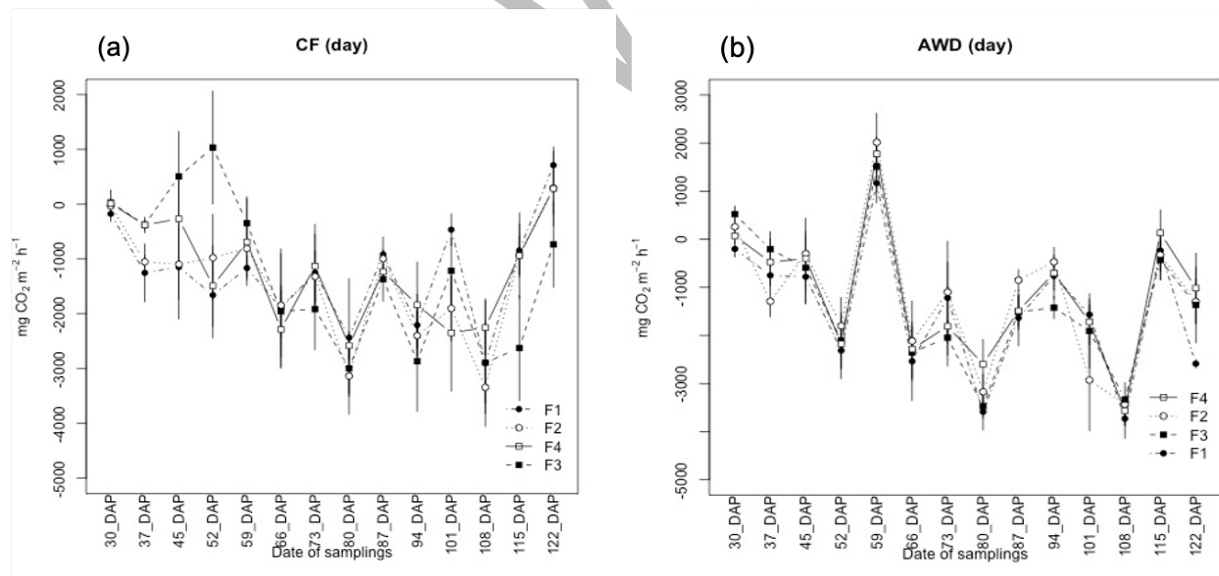
Fig. 3. Mean weekly ambient air temperature (°C) and relative humidity (%) recorded during day and night.

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

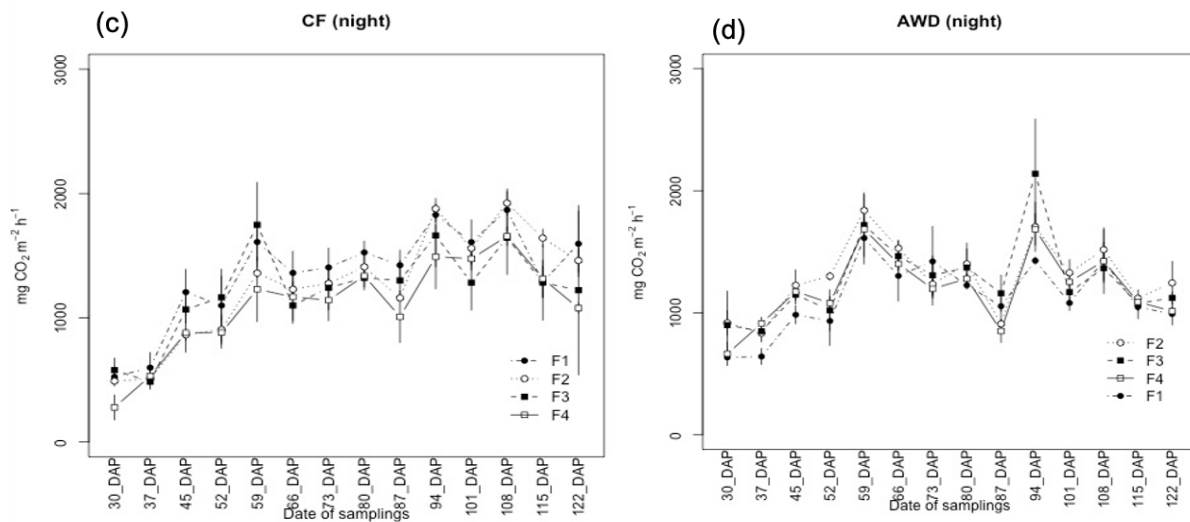
difference was compensated over time and mean plant height was indistinguishable at the time of harvest. The plant height ranged from 73 to 82 cm under CF and, while for AWD ranged from 71 to 87 cm.



**Fig. 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.**

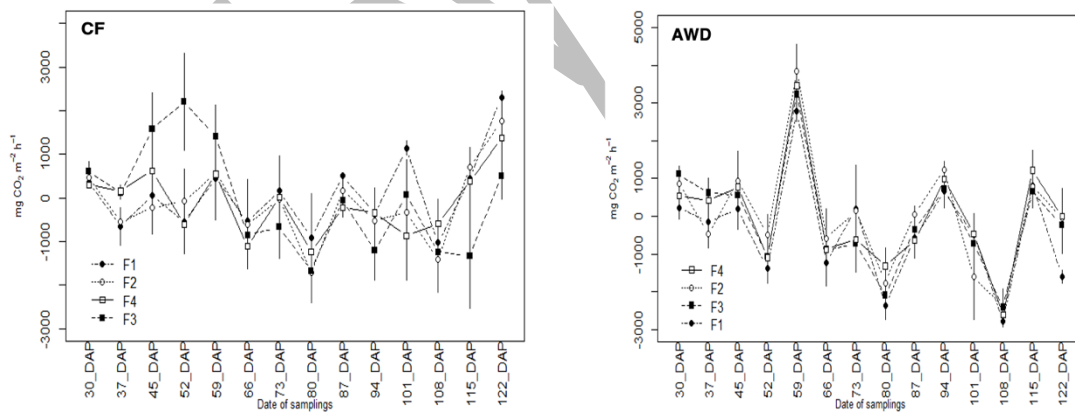






**Fig. 5. Total CO<sub>2</sub> fluxes under different water management and fertilizer applications during day and night measurement. DAP = Days after planting**

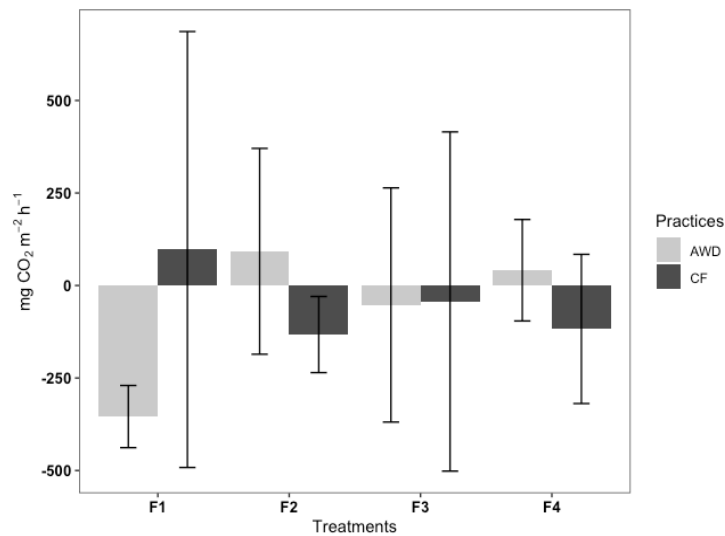
CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha<sup>-1</sup>, Phosphorus (P) 30 kg ha<sup>-1</sup> and Potassium (K) 20 kg ha<sup>-1</sup>]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N)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



**Fig. 6. Net CO<sub>2</sub> 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<sup>-1</sup>, Phosphorus (P) 30 kg ha<sup>-1</sup> and Potassium (K) 20 kg ha<sup>-1</sup>]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha<sup>-1</sup>, Phosphorus (P) 5 kg ha<sup>-1</sup> and Potassium (K) 6 kg ha<sup>-1</sup>]; F3 = Organic manure application (cow manure - 5 t ha<sup>-1</sup>); F4 = Control  
Vertical bar indicates the standard error (+/-) of mean.





**Fig. 7. Comparison of net CO<sub>2</sub> 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<sup>-1</sup>, Phosphorus (P) 30 kg ha<sup>-1</sup> and Potassium (K) 20 kg ha<sup>-1</sup>]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha<sup>-1</sup>, Phosphorus (P) 5 kg ha<sup>-1</sup> and Potassium (K) 6 kg ha<sup>-1</sup>]; F3 = Organic manure application (cow manure - 5 t ha<sup>-1</sup>); F4 = Control  
Vertical bar indicates the standard error (+/-) of mean.

**Table 1. Total CO<sub>2</sub> fluxes as affected by the different water managements practices and fertilizer applications during day and night measurements**

Treatments	Total CO <sub>2</sub> fluxes (mg CO <sub>2</sub> m <sup>-2</sup> h <sup>-1</sup> )		
	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
<b>Water management (W)</b>	<b>Day</b>	<b>Night</b>	<b>Net</b>
CF	-1284	1235	-49
AWD	-1294	1226	-68
<b>Fertilizer application (F)</b>	<b>Day</b>	<b>Night</b>	<b>Net</b>
F1	-1368	1239	-129
F2	-1297	1277	-20
F3	-1300	1252	-48
F4	-1191	1153	-38
<b>Soil respiration</b>	<b>Day</b>	<b>Night</b>	<b>Net</b>
CF	123	265	388
AWD	-96	350	254
Pr (>F)	0.052*	0.51	0.056

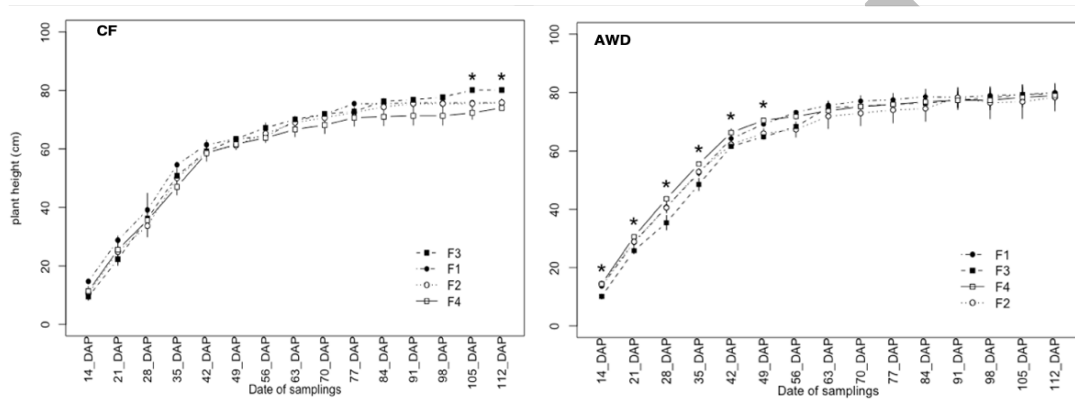
CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha<sup>-1</sup>, Phosphorus (P) 30 kg ha<sup>-1</sup> and Potassium (K) 20 kg ha<sup>-1</sup>]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N)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

\*Significant at 5% level of significance

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<sup>2</sup> per hill), while the lowest leaf area was found in control under CF (1042 cm<sup>2</sup>).

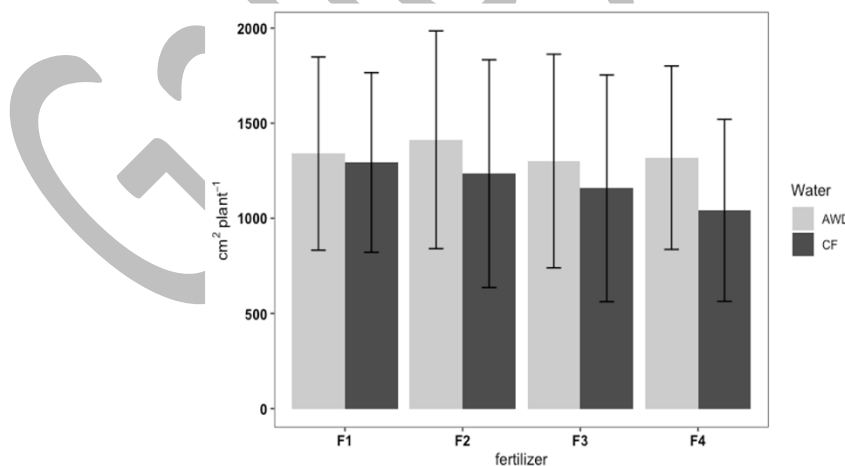
The highest above-ground biomass weight was recorded in F1 treatment under both CF (236 g plant<sup>-1</sup>) and AWD practices (266 g plant<sup>-1</sup>) (Table 2). The highest below-ground biomass was found

in F1 treatment under CF (197 g plant<sup>-1</sup>) and AWD practices (160 g plant<sup>-1</sup>). Root lengths (cm) were not affected by the different treatments, however root volume (cm<sup>3</sup>) 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<sup>3</sup>) compared to the AWD practice. In contrast, AWD resulted in significantly more tillers per plant compared to the CF practice (Table 2).



**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<sup>-1</sup>, Phosphorus (P) 30 kg ha<sup>-1</sup> and Potassium (K) 20 kg ha<sup>-1</sup>]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha<sup>-1</sup>, Phosphorus (P) 5 kg ha<sup>-1</sup> and Potassium (K) 6 kg ha<sup>-1</sup>]; F3 = Organic manure application (cow manure - 5 t ha<sup>-1</sup>); 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<sup>2</sup>) of rice**

CF = Continuous flooding; AWD = Alternate wetting and drying; F1= Inorganic fertilizer application [Recommended rate: Nitrogen (N) 50 kg ha<sup>-1</sup>, Phosphorus (P) 30 kg ha<sup>-1</sup> and Potassium (K) 20 kg ha<sup>-1</sup>]; F2 = Inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha<sup>-1</sup>, Phosphorus (P) 5 kg ha<sup>-1</sup> and Potassium (K) 6 kg ha<sup>-1</sup>]; F3 = Organic manure application (cow manure - 5 t ha<sup>-1</sup>); 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 <sup>-1</sup> )		Below-ground biomass (g plant <sup>-1</sup> )		Root length (cm)	Root volume (cm <sup>3</sup> )	No. of tillers plant <sup>-1</sup>
	Fresh	Dry	Fresh	Dry			
<b>Water management (W)</b>							
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
<i>Pr (&gt;F)</i>	0.131	0.062	0.388	0.258	0.965	0.003**	0.002**
<b>Fertilizer application (F)</b>							
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
<i>Pr (&gt;F)</i>	0.561	0.374	0.465	0.163	0.389	0.206	0.464
<b>Interaction (W x F)</b>							
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 a	42.5	106.7	44.7
<i>Pr (&gt;F)</i>	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<sup>-1</sup>, Phosphorus (P) 30 kg ha<sup>-1</sup> and Potassium (K) 20 kg ha<sup>-1</sup>]; F2 = inorganic fertilizer application [Farmer practices: Nitrogen (N) 21 kg ha<sup>-1</sup>, Phosphorus (P) 5 kg ha<sup>-1</sup> and Potassium (K) 6 kg ha<sup>-1</sup>]; F3 = Organic manure application (cow manure - 5 t ha<sup>-1</sup>); F4 = Control. \*\*Significant at 1% level of significance

## 4. DISCUSSION

### 4.1 Effects of Water Management Practices and Fertilizer Applications on the CO<sub>2</sub> Fluxes

Studies investigating CO<sub>2</sub> 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<sub>2</sub> sink during the day but act as the CO<sub>2</sub> source during the night [6]. Similar tendencies were found also in our present study. Declining CO<sub>2</sub> concentrations, hence negative CO<sub>2</sub> 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<sub>2</sub> concentration and positive fluxes might indicate prevalent respiration of the rice plant together with soil and water biota [49]. Lower CO<sub>2</sub> uptakes with lower photosynthetic activities can be attributed to relatively higher net CO<sub>2</sub> fluxes during an early period of plants (Fig. 5). In this study, we focused mainly on CO<sub>2</sub>

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<sub>2</sub> 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<sub>2</sub> fluxes did not differ significantly between different water management practices in our experiment which seems contradictory to previously published data, which suggested functioning as net sinks of atmospheric C. Weekly mean for total CO<sub>2</sub> fluxes under CF treatment was -30.82 g CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> (-8.3 g C m<sup>-2</sup> d<sup>-1</sup>) during daytime and 29.64 g CO<sub>2</sub>

$\text{m}^{-2} \text{d}^{-1}$  ( $8 \text{ g C m}^{-2} \text{d}^{-1}$ ) 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  $\text{CO}_2$  fluxes in a range between 5 and  $-39 \text{ g C m}^{-2} \text{d}^{-1}$  [51-55]. While in AWD treatment, weekly mean for total  $\text{CO}_2$  fluxes were similar to CF  $-31.06 \text{ g CO}_2 \text{ m}^{-2} \text{d}^{-1}$  ( $8.3 \text{ g C m}^{-2} \text{d}^{-1}$ ) during daytime and  $29.42 \text{ g CO}_2 \text{ m}^{-2} \text{d}^{-1}$  ( $7.94 \text{ g C m}^{-2} \text{d}^{-1}$ ) during nighttime (Table 1). However, reported data from intermittently flooded systems showed more positive values of the  $\text{CO}_2$  fluxes [7,47,56]. These finding suggest that slightly effects on total  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  emission flux was by CF 35% higher than under AWD. Positive  $\text{CO}_2$  fluxes (emissions) found during both day and nighttime indicate that anoxic respiration and/or fermentation performed by anaerobic microorganisms release  $\text{CO}_2$ , which quantity is comparable with the  $\text{CO}_2$  fluxes produced under aerobic conditions. Thus, soil moisture could greatly contribute to the decomposition process of organic residues and  $\text{CO}_2$  flux [60]. It remains therefore arguable whether AWD practice is really appropriate method to mitigate GHGs. Nevertheless, contribution of soil respiration to overall  $\text{CO}_2$  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  $\text{CO}_2$  flux [62]. The major  $\text{CO}_2$  emission sources in rice fields are plant and soil

respiration [63], with the total  $\text{CO}_2$  flux varying depending on the applied fertilizing regime. Indeed, the highest net  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  flux during the night [62,63]. Organic manure treated pots (F3) showed negative net  $\text{CO}_2$  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  $\text{CO}_2$  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 ( $\text{cm}^2$ ) 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<sup>3</sup>), although, there was found no significant difference among fertilizer treatments.

## 5. CONCLUSION

Surprisingly, net CO<sub>2</sub> fluxes were negative under both CF and AWD water management practices, indicating that CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> fixation.

Net CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub> in rice paddy fields.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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## 7.2 Comparison of Carbon Dioxide (CO<sub>2</sub>) Fluxes between Conventional and Conserved Irrigated Rice Paddy Fields in Myanmar

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Article

# Comparison of Carbon Dioxide (CO<sub>2</sub>) Fluxes between Conventional and Conserved Irrigated Rice Paddy Fields in Myanmar

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**Abstract:** Rice (*Oryza sativa*. L.), a major food crop widely grown in Myanmar, is the most prominent cause of greenhouse gas (GHG) emissions in agriculture. Moreover, as a result of modification in agricultural management practices (such as soil tillage), the soil organic matter is exposed to more oxidizing conditions, releasing CO<sub>2</sub> into the environment, contributing to global warming. Therefore, we studied the effects of both conventional and conservation soil tillage management practices on CO<sub>2</sub> fluxes on an experimental rice paddy field in Myanmar. Total CO<sub>2</sub> emissions during the night from paddies farmed under conventional practices were significantly higher than those from paddies farmed under conservation practices; however, no net CO<sub>2</sub> flux differences were found between practices. Total net CO<sub>2</sub> fluxes ranged from −59 to 1614 mg CO<sub>2</sub> m<sup>−2</sup> h<sup>−1</sup> in conventional practices and from −282 to 1082 mg CO<sub>2</sub> m<sup>−2</sup> h<sup>−1</sup> in conservation practices, respectively. Significantly higher rice biomass and grain yields were observed in conventional practices when compared to those in conservation practices, causing a significant rise in both CO<sub>2</sub> uptake and emissions during the day and night, respectively. In addition, the results of this study revealed that CO<sub>2</sub> emissions in rice fields could be much higher than expected, requiring further study to elucidate key factors driving the dynamics of CO<sub>2</sub> in rice paddy systems.

**Keywords:** CO<sub>2</sub> emission; conventional; conservation; grain yields; rice paddy

## 1. Introduction

Global warming and increased emissions of anthropogenic greenhouse gases (GHGs) has become an international issue of great concern. Comprehending the dynamics of global climate change requires an understanding of the exchange of greenhouse gases between terrestrial ecosystems and the atmosphere [1]. Carbon dioxide (CO<sub>2</sub>) is considered to be the major contributor to anthropogenic GHGs, accounting for 76% of total emissions in 2010 [2]. Based on various population growth and energy use scenarios, the current CO<sub>2</sub> concentration of 379 ppm is expected to rise to a concentration between 485 and 1000 ppm by 2100 [3]. The carbon cycle in cropland ecosystems is strongly affected by human activities. Emissions of CO<sub>2</sub> from agricultural systems can occur via plant respiration, the oxidation of organic carbon in soil (soil respiration) and crop residues, the use of fossil fuels in agricultural machinery, and the use of fossil fuels in production of agricultural production inputs [4,5]. Organic carbon in soil is the largest of the terrestrial carbon pools [6]; therefore, despite relatively small changes in soil CO<sub>2</sub>, it can significantly affect both the atmospheric CO<sub>2</sub> concentration and soil carbon sequestration processes [7]. Despite significant changes of CO<sub>2</sub> between the atmosphere and agricultural land, net flux is considered to be approximately balanced, although there is limited evidence for this presumption [8]. Many studies investigated CO<sub>2</sub> emissions from various terrestrial

ecosystems [9,10], including natural grasslands [11,12] and croplands with various types of crop cultivation [13,14]. However, CO<sub>2</sub> fluxes from agricultural soils depend on complex interactions between the climate and the physical, chemical, and biological properties of the soil [15]. Different kinds of land use practices on agricultural soil may also affect the properties of soil and thereby influence the CO<sub>2</sub> dynamics [15,16].

Rice is a major food crop around the world, with a total harvested area of approximately 160 million hectares, producing more than 700 million tons annually [17]. The Food and Agriculture Organization (FAO) Rice Market Monitor (2018) reported that 681 million tons of rice were grown in Asia, representing 90% of the global rice production [18]. To keep pace with the increasing global population, rice production needs to increase approximately 40% by the end of 2030 [19]. The country of Myanmar, with 1.19 million hectares under summer rice paddies and an average yield of 4.19 Mt ha<sup>-1</sup> from 2015 to 2016, is listed among the top 10 major rice producers in the world [20]. The actual sown area of paddies from 2015 to 2016 was 7.21 million hectares, producing 28.21 million metric tons of grain yield [21]. Summer rice grown under irrigated conditions can be very productive due to increased summertime day length [22]. Paddy fields are one of the major agricultural land uses, having substantial effects on the carbon cycle and contributing to global climate change [23]. According to the FAO database, rice cultivation contributed 10.1% of total agricultural GHG emissions worldwide [24]. Paddy ecosystems are considered to be a carbon (C) sink of atmospheric CO<sub>2</sub> and sensitive to changes in the C pool [25]. The carbon cycle in these systems is easily affected by different management practices of tillage and application of nitrogen fertilizer [26]. Several studies have demonstrated that no or minimum tillage increases the carbon sequestration in soil [27]. No-till management is a practice that leaves the soil surface without disturbance from harvesting to planting, leaving 30% or more of the ground cover with crop residues.

Conservation agriculture (CA) involves minimum soil disturbance, providing soil cover with crop residues or cover crops and crop rotation to achieve higher yields [28]. Conservation tillage is the most sustainable form of agriculture and requires minimum input (fertilizer, pesticides, herbicides, etc.) [29]. Conventional tillage is the management of the soil surface with crop residues not exceeding 15% subsequent to planting [4]. Application of commercial fertilizer and chemicals used for crop protection in the conventional practice on paddy fields can undoubtedly affect soil CO<sub>2</sub> flux by increasing the C input from increased crop biomass and crop residues returned to the soil [30]. The soil carbon balance can be altered by tilling the soil and exposing the soil organic matter to microbial decomposition, resulting in increased CO<sub>2</sub> emissions [31].

Tillage can change physical, chemical, and biological soil properties, affecting the release of CO<sub>2</sub> from soils [15]. Thus, shifting from conventional to conservation management practices could reduce CO<sub>2</sub> emissions and increase the accumulation of soil organic carbon (SOC) [32,33]. However, some researchers reported similar soil CO<sub>2</sub> flux from different tillage management practices [34,35]. Different agricultural practices also affect the resulting plant biomass and yield of rice. For instance, plant height is directly associated with plant biomass production, an essential morphological trait that affects the harvestable yield of rice [36]. Levels of night-time CO<sub>2</sub> production by rice plants and soil respiration are expected to be higher than in the day, when photosynthesis of the rice plants and aquatic algae covering the paddy soil prevails. However the mechanism of CO<sub>2</sub> exchange between rice paddies and the atmosphere has been explored in only a few studies and is not yet fully understood [37–42]. Hence, further evaluations of CO<sub>2</sub> flux from rice crops are necessary for understanding the dynamics of agro-ecosystems and predicting future climate change [43].

The aims of our field experiment were to (1) investigate the effects of conventional and conservation management practices on CO<sub>2</sub> emission fluxes during the summer rice growing season including the fallow period, (2) elucidate the effects of plant biomass on total CO<sub>2</sub> fluxes, and (3) compare the effects of different soil tillage practices on grain yield and quality in a common Myanmar rice cultivar. Whereas previous studies focused on the measurement of CO<sub>2</sub> fluxes only during a short portion of the paddy crop season, in this study, diurnal CO<sub>2</sub> flux was measured during the entire summer rice

paddy growing season and the fallow period. During the day, the quantity of CO<sub>2</sub> produced by rice plants was expected to be very low due to photosynthetic activities by the plants, while respiration during the night was expected to be high due to respiration from rice plants and paddy soil.

## 2. Material and Methods

Trials were conducted from mid-January through June 2018 at the experimental paddy fields of the Department of Agronomy, Yezin Agricultural University, Myanmar. These fields are situated in the Yezin area, Pyinmana Township, Naypyitaw district (19°50′03″ N, 96°16′03″ E), and have been used for rice production for several years. The area is characterized by annual average temperature and rainfall of 21–33 °C and 1000 mm, respectively. The fields in our study were located in an area vulnerable to drought and, therefore, the vast majority of rice paddy fields in Myanmar are comparable to current experimental field conditions. In this region, summer rice cultivation uses irrigation water, which is normally initiated from January to May, depending on water availability and type of cultivars. Rice seeds of *Oryza sativa* subsp. *indica* (Manawthukha, Masuri-M) (135 days), a commonly grown cultivar in Myanmar, were sown in the nursery during the second week of January 2018 and transplanted manually in the first week of February. Rice seedlings were prepared in the nursery before land preparation and transplanted as 25-day-old seedlings.

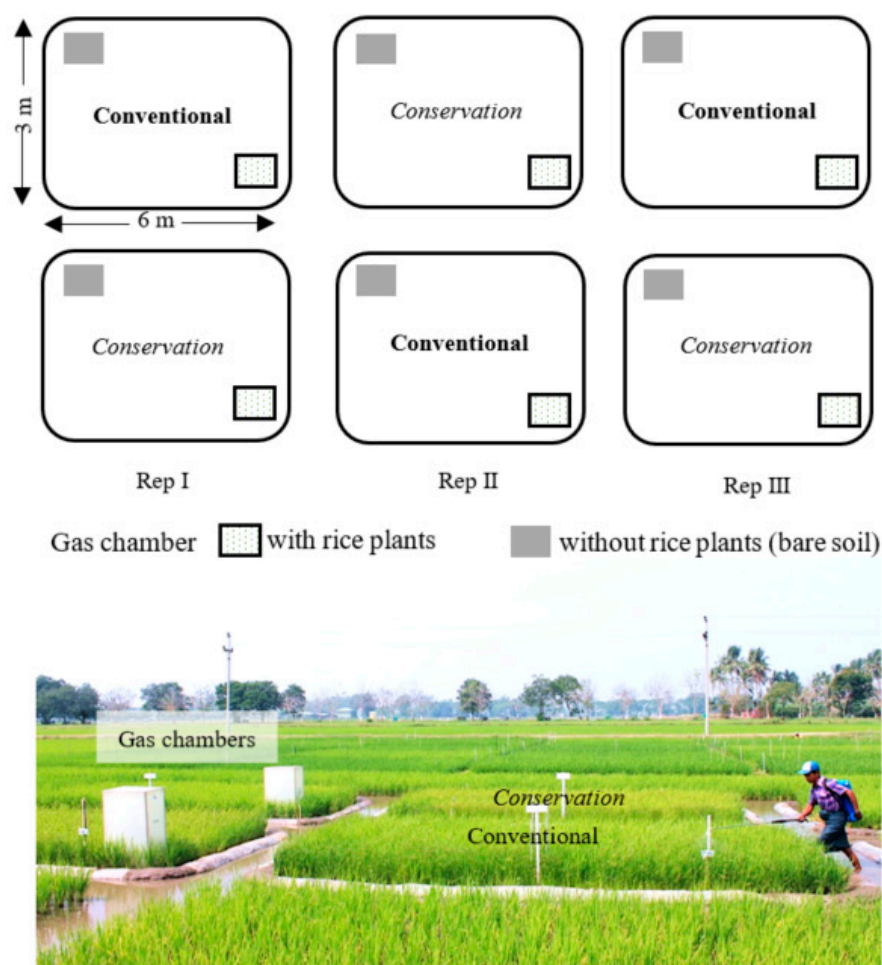
Six experimental plots, three under conventional and three under conservation practices, were constructed adjacent to one another. Plot size covered an area of 6 × 3 m, separated by bunds covered with plastic film serving as a barrier to prevent contamination of applied inorganic fertilizer from conventional plots to non-treated conservation plots. In a corner of every plot, a small area was left without rice plants for measuring bare soil respiration (Figure 1). The entire duration of the experiment lasted 155 days, including the fallow period. Carbon dioxide (CO<sub>2</sub>) gas sampling started one week after transplanting the rice plants and lasted throughout the fallow period.

Manual tillage was conducted using a spade, up to a maximum of 20 cm depth. The conventional practice (Conv) treatment was tilled three times and prior to transplanting, compound fertilizer (NPK 15:15:15) was added as a basal application at the rate of 61.75 kg ha<sup>-1</sup>. Urea fertilizer (46% N) was applied two times (Tillering and Panicle Initiation/Heading—see Table 1) as a split application at the rate of 50 kg N ha<sup>-1</sup> [44]. Other standard practices such as application of pesticides and weed control were conducted using conventional concentrations and rates. By contrast, in conservation practice (Cons) treatment, tillage was carried out only once and without application of chemical fertilizer, pesticides or herbicides. Water was drained two weeks before harvesting, and the fallow paddy field was flooded for four weeks before the next planting season started.

Two airtight gas chambers with frosted acrylic sheets were constructed, one chamber for each treatment (Conv vs Cons) for the sampling of CO<sub>2</sub> gas emitted from the paddy soil and rice plants. Acrylic sheet was used because it is non-reactive with the CO<sub>2</sub> trapped inside the chambers. Each chamber (1 × 0.4 × 0.6 m) was equipped with a fan for mixing air inside the chamber, and the CO<sub>2</sub> sensor was installed at the top of the chamber [29]. The fan was operated by a power bank device (2600 mAh), while carbon dioxide gas was measured immediately, using a SenseAir® CO<sub>2</sub> sensor module K33 ELG, designed to measure and record data for environmental parameters such as temperature (T/°C), relative humidity (%) and carbon dioxide (CO<sub>2</sub>) concentration (up to 5000 ppm) [45].

CO<sub>2</sub> flux was measured once a week during the entire summer paddy growing season (from transplanting to throughout the fallow period). Due to the high variability of CO<sub>2</sub> fluxes by photosynthetic activities during the day [46], CO<sub>2</sub> fluxes were consistently measured at a fixed time for all treated plots: between 9:00 and 12:00 for the day, and 21:00 and 24:00 for the night. CO<sub>2</sub> values were recorded simultaneously for each replication under Conv and Cons management practices, with 2 min logging time for 30 min. To distinguish between respiration from the rice plants and paddy soils alone for the calculation of the CO<sub>2</sub> gas balance, CO<sub>2</sub> flux was measured separately with and without rice plants for each treatment. There were five rice growth periods (T-Tillering,

PI/H-Panicle Initiation/Heading, FL-Flowering, GF-Grain Filling, and M-Maturity) analyzed for net CO<sub>2</sub> emissions between Conv and Cons practices.

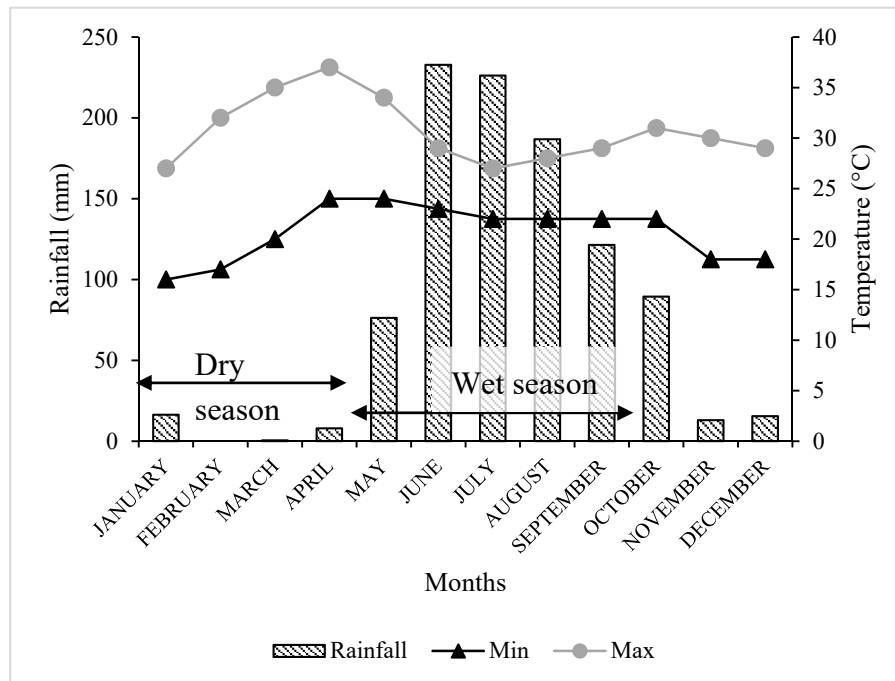


**Figure 1.** Experimental field layout at Yezin Agricultural University, Myanmar.

**Table 1.** Description of different rice growth stages according to duration of each period as observed in the present study.

Growth Stages	Abbreviation	Description	Duration	No. of Days
Seedling	-	Includes seedlings in the nursery and transplanting.	January 10–February 14	35
Tillering Stage	T	Two weeks after transplanting, plants grow quickly, increasing number of tillers as well as plant height.	February 15–March 26	40
Panicle Initiation/Heading	PI/H	In addition, also called the booting stage. Tip of developing panicle emerges from stem and continues to grow until panicle fully visible.	March 31–April 19	20
Flowering	FL	Flowering can occur one day after heading, followed by pollination.	April 20–April 31	10
Grain Filling	GF	Begins within 1–5 days after heading and grain filling is complete within 3 weeks.	May 1–May 15	15
Mature	M	Starts after flowering and ends at harvesting, usually lasting 30–65 days depending on the variety.	May 16–June 1	17

Daily mean ambient air temperature and relative humidity (%) were recorded by an automated weather station nearby the experimental fields. The meteorological data for this study year (2018) is presented in Figure 2 and daily data were downloaded from the server of the Agro-Meteorological Department of Yezin Agriculture University [47]. Soil temperature (°C) was recorded at three different depths of 0–5 cm, 0–10 cm, and 0–20 cm using a T&D TR-7wf/nw series soil temperature data logger. Soil samples for soil organic carbon (SOC%) analysis were taken before soil preparation and at the time of harvest. The sub-samples were analyzed at Palacký University Olomouc, Czech Republic. Redox potential (Eh) and soil water pH were measured using a Hanna Instruments HI83141 portable meter before and after rice harvest on the same date as CO<sub>2</sub> flux measurements.



**Figure 2.** Mean monthly rainfall and temperature in Yezin, Naypyitaw, 2018 (Source: Agrometeorology station, Department of Agronomy, Yezin Agricultural University).

For vegetation analysis, plant characteristics such as plant height (cm) and leaf area (cm<sup>2</sup>) were recorded throughout the experiment, whereas characteristics such as fresh and dry biomass weight, yield and yield component characteristics were measured at harvest. Three randomly selected hills (plants) were used for plant height and five hills for leaf area measurements for each treatment. For leaf area measurements, a CI-203 handheld portable Laser Leaf Area Meter (CID Bioscience, Inc., USA) was used. Rice quality was evaluated based on measurements of the head rice, chalky rice, amylase content, gel consistency and protein content. After the rice harvest, 1000 grains were evaluated for each treatment. Grain quality analysis was conducted at the Department of Agricultural Research (DAR, Yezin) by using UV-Vis Spectrophotometer, Jenway-6305 with the KI solution method for Amylose (%), by the Kjeldahl digestion and distillation method for protein %, and by the Gel Flow rate Method for Gel Consistency (mm) [48].

### 3. Flux Calculation

CO<sub>2</sub> emission flux, namely the change of the gas quantity over the soil covered by a chamber per hour per unit area, was calculated using the following equation [49]:

$$F = \Delta C / \Delta t \frac{V}{A} \quad (1)$$



where

$F$  is the total flux density of gas ( $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ );

$\frac{\Delta C}{\Delta t}$  is the slope of regression obtained by plotting concentration of  $\text{CO}_2$  vs time recorded during sampling;

$V$  is the total volume of the chamber ( $\text{m}^3$ );

$A$  is the area of the chamber's base ( $\text{m}^2$ ).

The  $\text{CO}_2$  flux produced by the rice plants only was calculated by using the equation:

$$F_{\text{Rice}} = F_{\text{Total}} - F_{\text{Soil}} \quad (2)$$

where  $F_{\text{Total}}$  is total  $\text{CO}_2$  flux (which is the sum of  $\text{CO}_2$  respired by the rice plants and  $\text{CO}_2$  respired by the soil),  $F_{\text{soil}}$  is  $\text{CO}_2$  respired by the bare soil only, and  $F_{\text{Rice}}$  is the  $\text{CO}_2$  emission flux from the rice plants only.

The *net  $\text{CO}_2$  flux* refers to a difference in total  $\text{CO}_2$  fluxes measured during the night and day, and annual flux was estimated by extrapolating each measurement based on this flux for rice paddy fields in Myanmar.

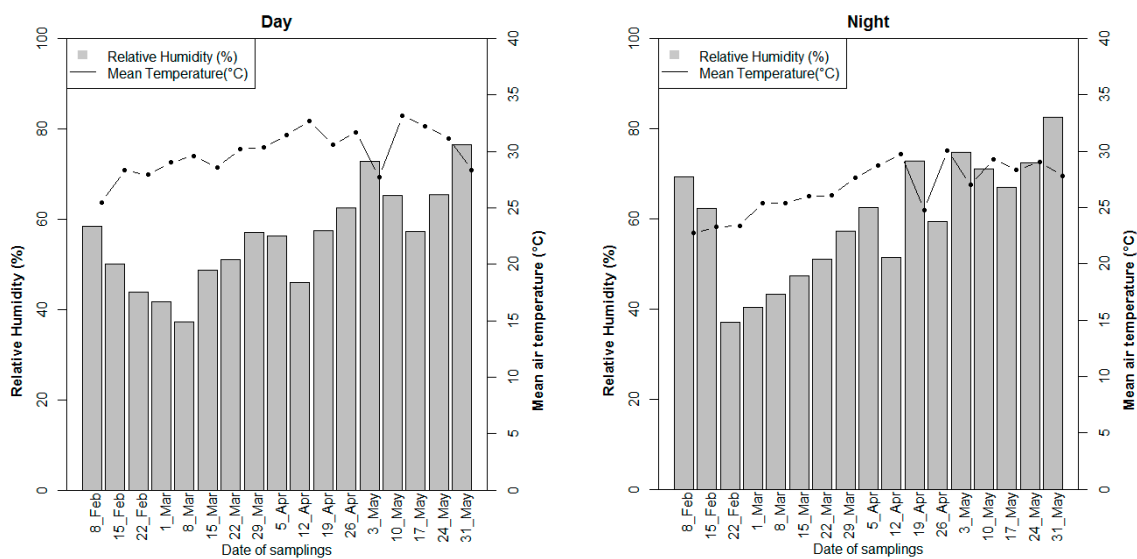
#### 4. Statistical Analysis

Analysis of variance (ANOVA) was performed for total  $\text{CO}_2$  fluxes and soil respiration from bare paddy soil in each of the sampling dates separately, to evaluate the differences between land management practices and time on  $\text{CO}_2$  fluxes during day and night. Significance was tested at the  $p < 0.05$  level. In addition, differences in mean values were calculated by using Tukey's Honest Significance Test pair-wise comparisons at a significance level of  $p \leq 0.05$ . Statistical software (R version 3.6.3) was used to perform ANOVA and plot graphs. Linear regression analysis was performed in Microsoft Excel to determine the relationship between plant height and  $\text{CO}_2$  fluxes influenced by the rice plants and paddy soil.

#### 5. Results

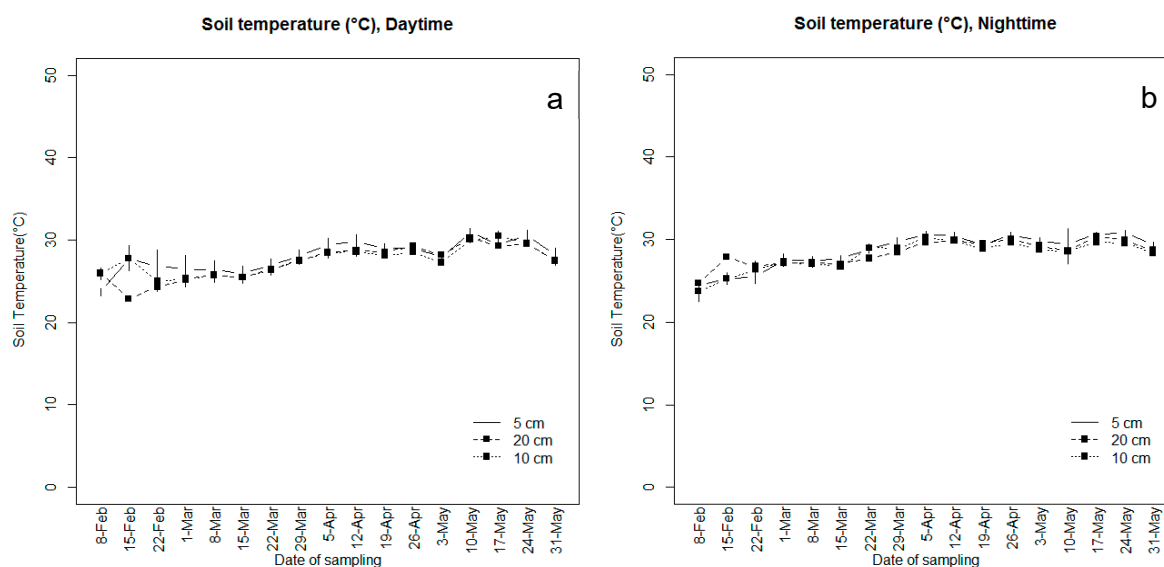
##### 5.1. Ambient Air Temperature, Relative Humidity and Soil Temperature

Mean ambient air temperature ( $^{\circ}\text{C}$ ) and relative humidity (%) showed only slight fluctuations during the day and night. The highest air temperature during the day of  $33.13^{\circ}\text{C}$  was recorded on May 10, 2018 and the lowest temperature at night of  $22.75^{\circ}\text{C}$  on February 8, 2018 (Figure 3). Air temperature ( $^{\circ}\text{C}$ ) and relative humidity (%) recorded inside the gas chambers containing rice plants and bare paddy soil closely matched the same patterns during day and night-time periods. Air temperature ranged between  $37.26$  and  $65^{\circ}\text{C}$  during the day in the chambers with rice plants, the maximal and minimal temperatures at night measurement showed  $26.83^{\circ}\text{C}$  and  $21.53^{\circ}\text{C}$ , respectively. Mean air temperature from bare paddy soil during the day ranged between  $35.95$  and  $63.97^{\circ}\text{C}$  and  $17.46$  and  $30.48^{\circ}\text{C}$  during the night. The relative humidity (%) with rice plants ranged between  $22.14\%$  and  $47\%$  during the day and  $76.08\%$  and  $96.06\%$  during the night. By contrast, relative humidity in the bare soil treatment ranged between  $20.4\%$  and  $44.2\%$  during the day and  $79.3\%$  and  $97.6\%$  during the night (Figure S1a–d; where "S1" denotes supplementary material).



**Figure 3.** Average weekly relative humidity (%) and ambient air temperature (°C) recorded day and night during the experimental period (February–May 2018).

There were no significant differences among mean soil temperatures measured at three different depths (5, 10, and 20 cm) during the day and night. As expected, the highest mean soil temperature during the day and night was observed at the 5 cm depth (30.97 °C and 31.17 °C, respectively), whereas the lowest temperature during the day and night was recorded at a depth of 20 cm (13.9 °C and 12.53 °C, respectively) (Figure 4a,b).

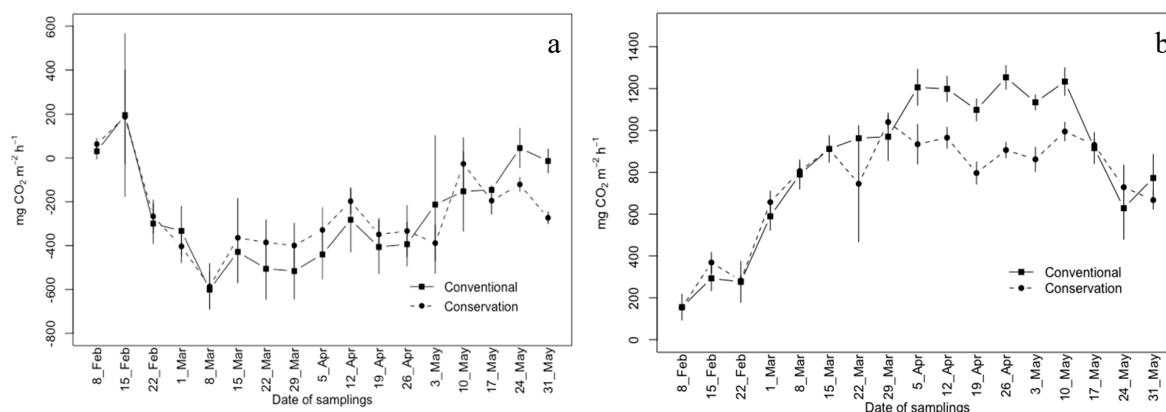


**Figure 4.** Mean soil temperature (°C) recorded at three different soil depths (0–5 cm, 0–10 cm, and 0–20 cm) during (a) Day and (b) Night from irrigated summer rice paddy fields.

## 5.2. Patterns of CO<sub>2</sub> Fluxes under Different Soil Management Practices

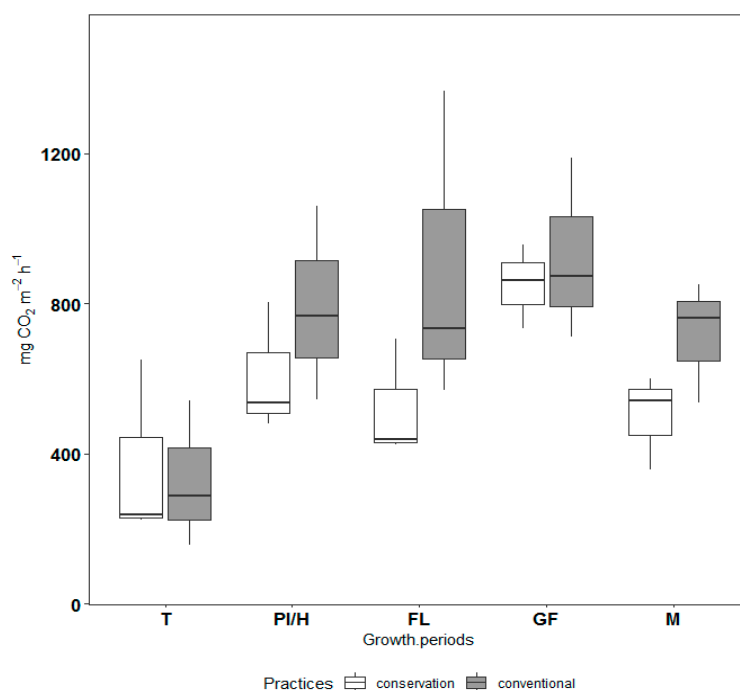
CO<sub>2</sub> emission fluxes measured during the day were always negative for both soil management practices, except during the first 2 weeks after transplanting the rice seedlings and the period after harvesting (Figure 5a). As expected, CO<sub>2</sub> emission flux at night was highly positive for both soil management practices during the entire rice growing season (Figure 5b). The highest fluxes were observed in Conv practice on April 26 and May 10, peaking at 1254 mg CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>.





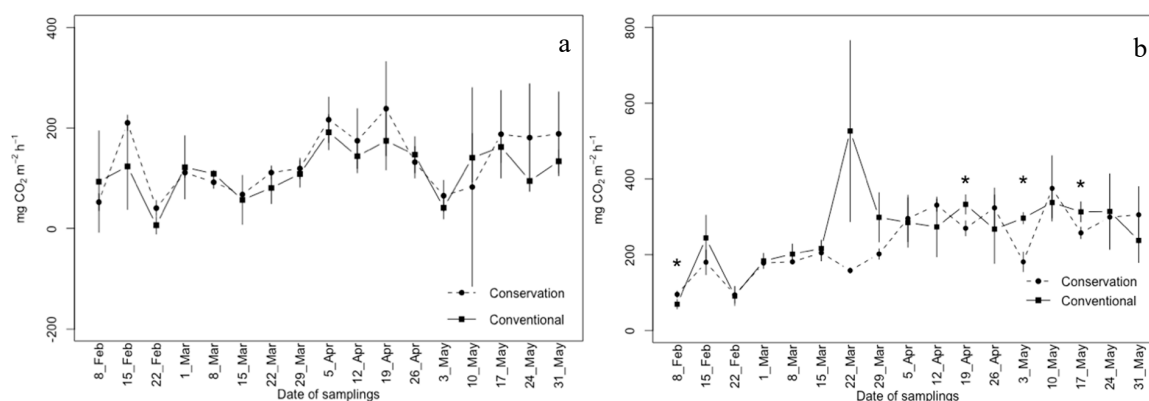
**Figure 5.** Effects of different soil management practices on total CO<sub>2</sub> fluxes from irrigated rice paddy fields during: (a) day and (b) night  $\pm$  SE.

Net CO<sub>2</sub> fluxes from different crop growth periods are presented in Figure 6. Significant differences of net CO<sub>2</sub> flux emissions were observed among the different growth periods; however, no significant difference was found between Conv and Cons tillage management practices. The highest net CO<sub>2</sub> emission was observed in the Conv practice during the grain filling (GF) period, upwards of 925.40 mg CO<sub>2</sub> m<sup>2</sup> h<sup>-1</sup> and followed by emissions during the flowering (FL) period at 890.72 mg CO<sub>2</sub> m<sup>2</sup> h<sup>-1</sup>. The lowest observed emission of net CO<sub>2</sub> fluxes was found during the tillering (T) period under Conv and Cons practices as 329.08 and 370.04 mg CO<sub>2</sub> m<sup>2</sup> h<sup>-1</sup>, respectively.



**Figure 6.** Effects of different soil management practices on net CO<sub>2</sub> fluxes ( $\pm$ SE) from various rice growth stages (see Table 1 for abbreviations of different growth stages).

Due to the absence of photosynthesis, CO<sub>2</sub> fluxes from bare soil respiration under both Conv and Cons practices were always positive during the day and night (Figure 7a,b). Soil CO<sub>2</sub> emission fluxes showed no significant differences between management practices except for the night measurements on February 8, April 19, May 3, and May 17 (Figure 7b).



**Figure 7.** Effects of different soil management practices on soil CO<sub>2</sub> fluxes from irrigated rice paddy fields during: (a) day and (b) night  $\pm$  SE.

Total CO<sub>2</sub> emission fluxes from Conv practices were significantly higher than for Cons during night (Table 2). However, a non-significant difference between practices was observed during the day. In contrast, total CO<sub>2</sub> flux from bare soil respiration showed significant differences between the two management practices for both the day and night measurements. The average contribution of soil respiration to the total CO<sub>2</sub> emission fluxes during the night was 31.4% (ranging from 27% to 36%) in Conv, and 29.3% (range 28% to 33%) in Cons practice. Higher uptake of CO<sub>2</sub> by rice plants during the day was observed in Conv practices compared to Cons practices, as well as higher net emissions of CO<sub>2</sub> observed in Conv practices. During the fallow period, significantly higher CO<sub>2</sub> flux was observed in the Conv versus Cons practices during the day, but non-significant differences of the CO<sub>2</sub> fluxes were observed during the night between the practices (Table 2).

**Table 2.** Effects of soil management practices on the carbon dioxide (CO<sub>2</sub>) flux from irrigated rice paddy fields in Myanmar. Mean value  $\pm$  standard deviation (SD).

Periods (Day/Night)	Treatments (Practices)	Total CO <sub>2</sub> Fluxes (mgCO <sub>2</sub> m <sup>-2</sup> h <sup>-1</sup> )	Soil Respiration (mgCO <sub>2</sub> m <sup>-2</sup> h <sup>-1</sup> )	Fallow (mgCO <sub>2</sub> m <sup>-2</sup> h <sup>-1</sup> )
Day	Conventional	-262.5 $\pm$ 98.5	151.6 $\pm$ 15.4	318.8 $\pm$ 71.6
	Conservation	-257.2 $\pm$ 73.2	95.7 $\pm$ 11.7	170 $\pm$ 38.0
		ns	**	ns
Night	Conventional	846.5 $\pm$ 31.3	266.2 $\pm$ 16.1	592.1 $\pm$ 39.8
	Conservation	749.9 $\pm$ 34.2	219.5 $\pm$ 19.2	450.8 $\pm$ 32.3
		**	*	*

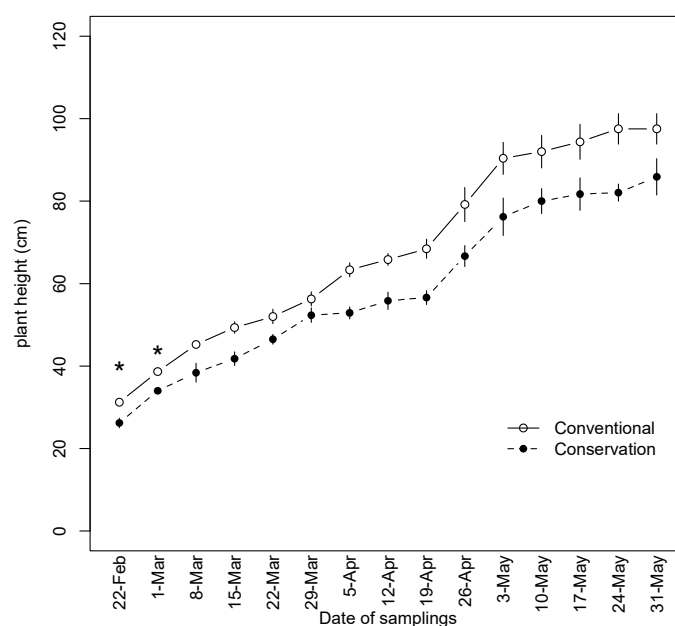
\*, significant at the 0.05 probability level; \*\*, significant at the 0.01 probability level; ns, not significant.

### 5.3. Effects of Different Agricultural Tillage Practices on Plant and Soil Characteristics

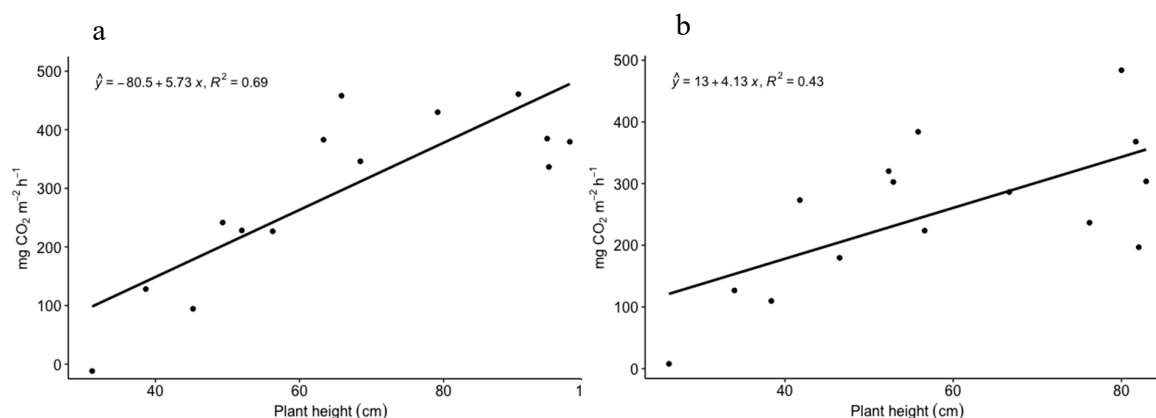
Plant height increased over time as the rice growing stage extended and peaked at maturity. A statistically significant difference was observed in plant height between Conv (range 31.2–97.5 cm) and Cons practices (range 26.2–82.9 cm at the early growth stages, Feb 22 and March 1) (Figure 8); however, no apparent difference was found during late growth stages. Maximum plant heights of 97.5 and 82.9 cm were observed in Conv and Cons practices, respectively.

Despite different management practices, we found no significant differences between soil organic carbon (SOC%), phosphorus, and nitrogen contents in the soil. Nevertheless, a slight decrease in SOC was observed during the harvest, as compared to before planting. Soil water pH and redox potential (mV) before and after harvesting of the paddy field showed no significant differences among Conv and Cons practices. However, soil water pH showed a slight increase after harvest (Table S1; S1 denotes supplementary appendix). Total CO<sub>2</sub> fluxes from the Conv and Cons practices were positively correlated with plant height, while the coefficient of determination ( $R^2$ ) in Conv practices

accounts for 69% which had more fitted values than Cons practices (43%) (Figure 9a,b). However, no significant difference was found when comparing the two different slopes.



**Figure 8.** Average plant height in cm ( $\pm$  SE) measured under different soil management practices from irrigated rice paddy fields.



**Figure 9.** Relationship between plant height and total CO<sub>2</sub> fluxes from irrigated rice paddy under (a) Conventional and (b) Conservation practices.

Significant differences for the harvestable yield were observed between Conv and Cons practices, with 5.52 metric tons (Mt) ha<sup>-1</sup> and 2.63 Mt ha<sup>-1</sup>, respectively (Table 3). However, the nutritional, edible and visual quality of rice such as amylose (%), protein (%), gel-consistency (mm), head rice rate (%), and chalky rice rate (%) was not affected by the different soil management practices as presented in Table 4. Plant height (cm) and other yield component characteristics of rice such as number of spikelets per panicle, filled grain (%), unfilled grain (%), and 1000 grain weight also showed no significant differences between the practices. However, we found the number of effective tillers per hill to be significantly higher than in Cons practices (Table 3). At harvest time, fresh and dry biomass weight (g hill<sup>-1</sup>) was significantly different between the different practices. According to the crop growth stages, leaf area during the tillering stage (43 DAT) showed significant differences between Conv (36.22 cm<sup>2</sup> per hill) and Cons practices (28.82 cm<sup>2</sup> per hill). Leaf area was significantly different between different practices during the flowering stage (88 DAT) (Table 3).

**Table 3.** Effects of different soil management practices on yield and yield components, biomass, and leaf area from irrigated summer rice paddy.

Practices	Yield (t ha <sup>-1</sup> )	Plant Height at Harvest (cm)	Number of Tillers hill <sup>-1</sup>	Effective Tillers/Hill (g hill <sup>-1</sup> )	Number of Spikelet/ Panicle	Filled Grain (%)	1000 Grain Weight (g)	Fresh Weight (g hill <sup>-1</sup> ) (at Harvest)	Dry Weight (g hill <sup>-1</sup> ) (at Harvest)	Leaf Area (cm <sup>2</sup> hill <sup>-1</sup> ) (43 DAT)	Leaf Area (cm <sup>2</sup> hill <sup>-1</sup> ) (88 DAT)
Conventional	5.52	95.7	11.40	10.47	114.9	92.17	20.10	103.7	68.9	672	1402
Conservation	2.63	82.1	7.67	6.73	82.9	93.57	18.70	68.8	35.0	368	539
Pr value	0.018	0.086	0.013	0.019	0.181	0.472	0.205	0.052	0.046	0.01	0.006
CV%	40.3	9.4	23.5	24.9	24.1	2.8	7.3	23.7	37.6	5.7	13.3
LSD <sub>0.05</sub>	1.693	18.44	1.881	2.240	68.34	6.823	3.248	36.72	32.44	139.8	281.7

DAT = Days after transplanting.

**Table 4.** Effects of different soil management practices on rice grain quality analysis.

Practices	Amylose (%)	Protein (%)	Gel Consistency (mm)	Head Rice Rate (%)	Chalky Rice Rate (%)
Conventional	22.33	7.10	32.50	33.5	6.33
Conservation	23.26	7.77	28.67	49.4	5.33
Pr value	0.475	0.149	0.063	0.112	0.580
CV%	4.5	9.9	8.5	28.8	27.5
LSD <sub>0.05</sub>	4.616	1.250	4.362	25.04	6.572

## 6. Discussion

Previous measurements of CO<sub>2</sub> emission fluxes from paddy fields were reported in other studies in various Asian countries such as China, Japan, and Thailand. Rice fields in Myanmar occupied 34% of the total sown area [21]; however, relatively few studies have investigated the effects of land-use practices in rice paddy cultivation on CO<sub>2</sub> fluxes. In the present study, we focused mainly on the effects of Conv and Cons agricultural tillage practices on CO<sub>2</sub> emission fluxes from the paddy fields during the summer season in Myanmar. Whereas a broad range of paddy fields in Myanmar are farmed under similar conditions as our experimental field, the current CO<sub>2</sub> emissions from Myanmar may be highly underestimated.

The CO<sub>2</sub> concentration inside of the acrylic chamber was negative during the day, indicating prevalent uptake of CO<sub>2</sub> via plant photosynthesis [5,50–53]. Variation of the CO<sub>2</sub> gas exchange pattern can be influenced by leaf photosynthesis, which is strongly affected by high temperatures or light conditions [54]. Nevertheless, there is little temperature effect on leaf photosynthesis in rice from 20 to 40 °C [55]. High temperatures can reduce photosynthetic rate by 40%–60% at different growth stages. The photosynthetic rate of leaves under light dependence conditions is highly correlated with atmospheric CO<sub>2</sub>, and also varies with the growing temperature [56].

During the day, CO<sub>2</sub> is consumed from the ambient atmosphere and CO<sub>2</sub> is emitted by flooded soil. However, CO<sub>2</sub> fluxes from respiration in bare paddy soil were positive during both the day and night, and lower during the day. This was likely due to CO<sub>2</sub> uptake/release by aquatic weeds and algae present in the overlying paddy water [6]. Net soil CO<sub>2</sub> flux throughout the growing season was generally positive, indicating the dominance of respiratory CO<sub>2</sub> release by the soil microorganisms as well as by aquatic weeds and algae in paddy water. Generally, flooded bare paddy soil acted as a CO<sub>2</sub> source throughout the day (Table 2). Similar results were reported by Nishimura et al., (2015) [5], who found the net soil CO<sub>2</sub> flux was generally near zero during the submerged period, with paddy rice cultivation having a slight CO<sub>2</sub> influx in the daytime and efflux at night-time.

Plant respiration in the absence of photosynthesis at night always resulted in a positive flux (efflux), suggesting that the field overgrown with rice plants was acting as a CO<sub>2</sub> source during the night (Figure 5b). In this study, a peak of net CO<sub>2</sub> emission fluxes from rice plants was observed in both the Conv and Cons practices during the grain filling period (Figure 6). On the contrary, Dutta and Gokhale (2017) [29] found the peak of net CO<sub>2</sub> emission fluxes earlier, during the flowering period, probably due to higher ambient air temperature and development of root growth. On the other hand, a decrease in net CO<sub>2</sub> emissions occurred during the maturity or ripening period. Due to the maturation of leaves such as leaf rolling, senescence, and yellowing, CO<sub>2</sub> uptake rate gradually declined during the late growth stage or ripening/mature stage [57]. In addition, respiration of plants also decreased at night-time during this period (Figure 5b).

In the fallow period after removal of all aboveground biomass, CO<sub>2</sub> flux was mediated only by the soil itself. Nevertheless, average values of CO<sub>2</sub> emissions were higher compared to those from bare soil respiration (see Table 2). This could be associated with higher root residues and the decomposition of organic litter after removal of the aboveground biomass [49] and to intensive aerobic respiration initiated just after the drainage of water. Typically, higher CO<sub>2</sub> emissions from Conv practices during

the fallow period were observed when compared to the Cons practices. Similarly, lower CO<sub>2</sub> emission in the non-tillage versus tillage treatment during the fallow period was noted by Wei et al. (2007) [58].

Greenhouse gas emissions, including CO<sub>2</sub> from Conv tillage are usually reported as higher than emissions from Cons agricultural practices with minimal tillage [59–65]. Data from the present study were consistent with the observations from the previous findings mentioned above. Total CO<sub>2</sub> emission fluxes from the Cons practice were significantly lower than those from the Conv practice at night (Table 2), but there was no significant difference during the day. Practitioners of Conv practices often apply nitrogen fertilizer (urea), resulting in increased plant biomass and subsequent stimulation of biological activity and increased CO<sub>2</sub> emissions [66].

In the present study, higher biomass yields (Table 3) and net CO<sub>2</sub> emissions (Table 2) were observed in Conv versus Cons practices, indicating that aboveground biomass was an important factor influencing CO<sub>2</sub> fluxes in this field experiment. Similar findings were noted by Maraseni et al., (2009) [64] who found that higher GHG emissions were directly linked to increasing rice productivity by using higher farm inputs. The aboveground biomass also contributes organic matter to the soil [67,68]. A quantity of soil CO<sub>2</sub> emission is often linked to the amount of aboveground biomass produced [69] because plant biomass is a primary source of the soil C pool. Moreover, Conv tillage practices also increase CO<sub>2</sub> emissions by exposing organic matter to increased aerobic conditions, thus enhancing the soil organic matter decomposition process [70,71]. The results of our experiment revealed that fresh biomass from the Conv practice was higher than that from Cons practice, and the soil CO<sub>2</sub> fluxes released by Conv practice were also always higher than those produced by Cons practice for both day and night measurements. The peak CO<sub>2</sub> fluxes in soil from the Conv practice observed during flowering (FL) and grain filling (GF) stages (Figure 6) were likely due to increased substrates derived from root exudation and microbial decomposition of the remaining residues from previous crops [72] and photosynthates translocated from the aboveground biomass [73].

Plant height usually exhibits a highly significant relationship with leaf area, aboveground biomass, and yield [74]. In our study, the mean plant height from the Conv practice was greater in the Conv versus Cons practice. Consequently, higher biomass weight (g hill<sup>-1</sup>) and leaf area (cm<sup>2</sup>) was observed in the Conv versus Cons practice. These findings are similar to the results of Tilly et al., (2013) [75], indicating that increased plant height is followed by higher plant biomass. As expected, rice grain yield was also affected by the different soil management practices, with the Conv practice yielding twice as much as the Cons practice. Additionally, a significantly higher number of effective tillers per hill was observed in the Conv practice compared to the Cons practice. Wu et al., (2013) [76] pointed out that the Cons practice using minimum tillage significantly reduces the ratio of effective tillers to the total number of tillers. Lower grain yield might be also be affected by the decreased uptake of nitrogen by the rice plants due to weed infestation and high loss of nitrogen fertilizer [77,78]. On the other hand, some researchers suggested that no tillage practice improves both physical and chemical soil properties [79]; hence, the soil condition would favor germination and plant growth, consequently increasing rice grain yields [80].

#### *Annual CO<sub>2</sub> Flux from Various Rice Paddy Fields in Asian Countries*

We estimated average emissions of CO<sub>2</sub> from summer rice fields at 2347 g CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>. Compared to CO<sub>2</sub> fluxes from other rice fields under different water management, our findings are within the range of the previously found values presented in Table 5, despite the fact that most of the studies were focused mostly on CO<sub>2</sub> emission from the soil only. It is also important to note that our findings are consistent with these measurements although they were carried out using different methodologies under flooded/irrigated conditions. Annual CO<sub>2</sub> flux was extrapolated from the summer rice area based on Department of Agriculture (MoALI, 2016) [21] data, and resulted in 28.2 ± 3.97 Tg CO<sub>2</sub> yr<sup>-1</sup> (7.6 Tg C yr<sup>-1</sup>). This value is 21.3% higher than estimated CO<sub>2</sub> emissions from Myanmar rice cultivation from both dry and wet seasons, as assessed from global emissions in the FAO Statistical Database [81].

**Table 5.** Annual CO<sub>2</sub> (C) emissions from various rice fields under different management practices in Asia.

Type of Rice Field	Management Practices	CO <sub>2</sub> Emissions (g CO <sub>2</sub> m <sup>-2</sup> yr <sup>-1</sup> )		Authors
		Soil Respiration	With Rice Plants	
Flooded rice field	Conventional tillage	381–572	1362–3816	[82] (Taiwan)
Irrigated rice field	Non-tillage Conventional tillage	770–831 (2008) 466–519 (2009) 772–793 (2008) 371–383 (2009)		[72] (China)
Irrigated rice field	Ridge non-tillage Flat non-tillage	1042–1489 724–1016		[73] (China)
Flooded rice field	Conventional tillage	1563–1922		[83] (Japan)
Flooded rice field	Conventional tillage	1731		[84] (DNDC model, Malaysia)
Tropical lowland rice field	Conventional tillage	1693		[85] (DNDC model, India)
Summer Irrigated rice fields	Conservation tillage	1383	2137	This study (2018) (Myanmar)
	Conventional tillage	1830	2558	

## 7. Conclusions

Our field experiment demonstrated that the Conv practice of rice cultivation produced higher aboveground biomass and, also, grain yield, compared to the Cons practice (Table 3). However, this Conv management also emitted significantly higher CO<sub>2</sub> fluxes than Cons management using minimum tillage and inputs of agrochemicals. Although a positive relationship between plant height and total CO<sub>2</sub> emission was observed (Figure 9), fluxes indicated that the rice plant biomass is associated with CO<sub>2</sub> production; we also found significantly higher production of CO<sub>2</sub> from bare paddy soils managed under the Conv practice.

These findings suggest that management of soil is a primary factor with influence on resulting rice biomass as well as final CO<sub>2</sub> flux. In comparison with methane, another greenhouse gas emission very often studied from rice paddies, the atmospheric CO<sub>2</sub> concentration above a rice field shows a conspicuous diurnal pattern, with the lowest values during the day and highest during the night. Unsurprisingly, both plant photosynthesis and respiration are responsible for these diurnal changes in CO<sub>2</sub> concentrations as contributions from soil to total CO<sub>2</sub> emissions were generally less than 40%. Hence, CO<sub>2</sub> flux was affected by the metabolic activity of rice plants rather than aerobic/anaerobic conditions of the soils, as is typically the case for methane.

In the context of global climate change and ongoing mitigating approaches aiming to reduce emissions of GHGs from rice fields focusing mostly on methane [86–88], it is worth noting that modification of current cultivation systems toward Cons practices that emit less CO<sub>2</sub> requires farmers to be motivated, as this practice results in lower plant biomass as well as lower grain yields. Another noteworthy finding from our study was that the emissions of CO<sub>2</sub> by rice fields may be much higher than previously expected [87], requiring verification from further studies. Therefore, additional studies are also needed to incorporate a range of multi-year/season assessments to determine seasonal variation of CO<sub>2</sub> fluxes exchange from rice production in Myanmar.



**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2071-1050/12/14/5798/s1>, Table S1. Effects of different agricultural tillage practices on soil characteristics of the experimental field, Figure S1. Relative humidity (%) and mean temperature (°C) inside the chamber with rice plants (a,b) and without rice plants (c,d) recorded day and night during the experimental period (February–May 2018).

**Author Contributions:** S.M. and M.R. conceived the study design, S.M. implemented the field research collected and analyzed the field data, S.M. wrote the paper with the help of M.R., M.R. commented on and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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## 8. CURRICULUM VITAE

**Name:** Saw Min

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### Education

- |                 |   |
|-----------------|---|
| 2016 to Present | PhD study-Ecology<br>Faculty of Science, Palacky University, Olomouc<br>Czech Republic  |
| 2012-2014       | Master of Science – Agriculture Sciences and Resource<br>Management in the Tropic and Sub-Tropics (ARTS)<br>University of Bonn, Bonn, Germany |
| 2007-2008       | Diploma in Agri-Business, ARAVA International Centre<br>for Agriculture Training Centre (AICAT), Israel                                       |
| 2001-2005       | Bachelor of Agricultural Science<br>Yezin Agricultural University, Naypyitaw, Myanmar   |

### Work experience

- |           |   |
|-----------|---|
| 2015-2016 | Bamboo Sustainable Management Manager, UKAid<br>Yangon, Myanmar                                 |
| 2005-2012 | Assistant Lecturer, Department of Agronomy<br>Yezin Agricultural University, Naypyitaw, Myanmar |

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| 01/2018-06/2018 | Research Stay, Department of Agronomy, Yezin<br>Agricultural University, Naypyitaw, Myanmar       |
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### **Publications in peer-reviewed journals**

**Min, S.;** Rulík, M. Effects of Different Water Management and Fertilizer Applications on CO<sub>2</sub> Fluxes from a Selected Myanmar Rice (*Oryza sativa* L.) Cultivar. International Journal of Plant and Soil Science (Accepted in January 2021).

**Min, S.;** Rulík, M (2020). Comparison of Carbon Dioxide (CO<sub>2</sub>) Fluxes between Conventional and Conserved Irrigated Rice Paddy Fields Myanmar. Sustainability 2020, 12,5798. [http:// https://doi.org/10.3390/su12145798](https://doi.org/10.3390/su12145798)

### **Presentations on International Conferences**

**Min S,** Rulík M (2017): Carbon dioxide (CO<sub>2</sub>) emissions from paddy rice ecosystems – Investigations for the potential mitigation options, Fresh Blood for Fresh Water (FBFW 2017) (Oral Presentation)

<https://www.hbu.cas.cz/data/files/HBU/AME/5th%20meeting%20of%20FBFW%202017.pdf>

**Min S,** Rulík M (2019): Do conventional and conservation agricultural management practices play an important role for CO<sub>2</sub> flux in summer paddy production of Myanmar? Symposium for European Freshwater Sciences 2019 (SEFS 11).

(Oral presentation)

<http://www.sefs11.biol.pmf.hr/wp-content/uploads/2019/07/Book-of-abstract.pdf>