Varietal Effects of Five Contrasting Rice Varieties on Diurnal Methane Emissions at Different Development Stages

UNIVERSITY OF
HOHENHEIM

MSc. CANDIDATE: Theresa Detering
MATRICEL # 710501

Institute of Agricultural Science in the Tropics (490)

Management of Crop Water Stress in the Tropics and Subtropics (490g)

Advisor: Prof. Dr. Folkard Asch

Second Reader: Dr. Reiner Wassmann

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Abstract

Currently, concentrations of the greenhouse gas methane (CH_4) are rising faster than at any time in the last two decades. In order to combat climate change, it is essential to quantify and mitigate the primary anthropogenic sources of CH_4, including rice production. Methane emissions from rice paddies exhibit significant diurnal variations. These are often neglected when field measurements are scheduled at a fixed time of a given measuring day, leading to over or underestimation of total daily or seasonal CH_4 fluxes. Although several other crop management practices have already been researched, there is still a lack of information concerning the influence of different rice varieties on greenhouse gas emissions.

A field experiment was conducted during the dry season 2019/20 in An Giang province of the Vietnam Mekong Delta to assess the magnitude of diurnal CH_4 flux variations by considering varietal as well as development stage dependent differences. Methane samples were collected at 9:00, 12:00, 15:00, 18:00 and 6:00 at the consecutive day from five rice varieties (Dai Thom 8, ML202, OM18, OM5451, OM576) at tillering, panicle initiation, and flowering stage, using a manually operated, closed chamber method. Analyses were performed with an SRI 8610C gas chromatograph at the IRRI laboratory in Los Baños, Philippines.

All varieties showed a distinct, diurnal emission pattern, with a single peak in the afternoon around 12:00 to 15:00 at tillering and flowering stage. Ratios of maximum to minimum CH_4 emission rates varied from 3.1 to 5.3 at tillering stage, 1.5 to 2.4 at panicle initiation, and 4.2 to 19.8 at flowering stage. Total daily CH_4 emissions (E_{day}) were also affected by the variety and development stage. OM18 exhibited consistently high emissions with minor variations between developmental stages. An average daily CH_4 emission (Mean E_{day}) of 4.05 kg CH_4 ha^{-1} d^{-1} was calculated, which exceeded the least emitting variety Dai Thom 8 by about 17.8% or 0.72 kg CH_4 ha^{-1} d^{-1}.

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List of Abbreviations

AWD	Alternate Wetting and Drying
BC	Before Christ
CF	Continuous flooding
E_{day}	Total daily methane emission
DAT	Days after transplanting
GC	Gas Chromatograph
GHG	Greenhouse gas
GWP	Global warming potential
IPCC	Intergovernmental Panel on Climate Change
IRRI	International Rice Research Institute
LAI	Leaf Area Index
LTG	Lộc Trời Group
MTC	Methane transport capacity
Mean E_{day}	Average daily methane emission
OM	Organic matter
ppb	Parts per billion
ppm	Parts per million
RCP	Representative Concentration Pathway
RF	Radiative forcing
RFES	Rice field ecosystems
SSC	Soil saturated culture
Tg	Teragram

1. Introduction

1.1 Status Quo

The evidence of impending climate change can no longer be ignored. The global mean temperature has risen by 0.8 °C since the 1850s (Wheeler & Braun, 2013). Each of the three last centuries has continuously been hotter at the earth´s surface than any prior decade since 1850 (IPCC, 2013). It is more than likely that at least half of the observed increase in global average surface temperature is caused by the anthropogenic emission of greenhouse gases (GHG) and aerosols. In 2011, the concentrations of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) exceeded pre-industrial levels from 1750 by about 40 %, 150 % and 20 % with concentrations of 391 ppm, 1803 ppb and 324ppb, respectively (Ibid).

As the second most important GHG with a global warming potential (GWP) 28 times more potent on a 100-year time scale than CO_2, methane plays a crucial role in global climate global change (Olivier Boucher *et al.*, 2009). Increasing CH_4 concentrations have both, direct (radiative forcing of about 0.48 W m^{-2}) (Etminan *et al.*, 2016) and indirect (production of tropospheric ozone and stratospheric water vapour) impacts on the climate (Shindell *et al.*, 2005). Methane concentrations in the atmosphere have more than doubled since pre-industrial times (Saunois *et al.*, 2019). At this point, the Intergovernmental Panel on Climate Change (IPCC) projects a rise of CH_4 concentrations to as much as 3500 ppm in 2100 - a value almost twice as high as today's levels (van Vuuren *et al.*, 2011).

Methane emissions originate from natural (mostly wetlands) and anthropogenic sources such as wastewater treatment, combustion of biomass, rice paddy agriculture, enteric fermentation and land management activities (Denman *et al.*, 2007). Particularly the agricultural sector plays an essential role as the largest man-made source of CH_4 (Reddy *et al.*, 2013; Crippa *et al.*, 2018). Rice cultivation emits 31 - 112 Tg CH_4 yr^{-1} of methane, accounting for about 10 % of total anthropogenic emissions (Dlugokencky *et al.*, 2011).

Since its domestication about 8000 years BC (Callaway, 2014), Rice (*Oryza sativa*), has fed a greater number of people for more years than any other cereal of the world (Anderson, 1998). Nowadays, rice is grown on about 167.1 million hectares, representing the most important staple food for more than 3 billion people (FAOSTAT, 2020a). Based on an overall growing world population, total rice consumption is projected to increase by 13 % by 2027, with Asian countries accounting for over 70 % of the expected rise in global consumption (FAO and OECD, 2018).

To meet the global demand, farmers need to produce at least 8 – 10 million tons more paddy rice each year which is equivalent to an annual increase of 1.2 – 1.5 % over the coming decade (Maclean *et al.*, 2013). Irrigated lowland rice systems encompass different cropping cycles ranging from single to triple crops per year. These are much more productive than upland, deepwater or rainfed rice ecosystems on an annual basis (Fairhurst & Dobermann, 2002). Most upland rice varieties yield one to four t/ha, whereas the average rice yield from irrigated rice fields is up to seven t/ha in some countries (Datta, 1981). From a farmer perspective, the practice of flooding rice fields has several benefits, such as maintaining soil moisture and temperature, increasing soil carbon and suppressing soil-borne diseases and weeds (Reeves *et al.*, 2016).

Approximately 75 % of rice is already produced under irrigated, flooded or submerged conditions, making rice to one of the largest consumers of water in the world (van der Hoek *et al.*, 2001). Bouman (2007) estimated that 34 to 43 % of the world´s irrigation water or 24 to 30 % of the total world’s freshwater is used for rice production. The production of one kg of rice requires 2672 L water, which is 2.5 times higher compared to wheat or maize production. (Chapagain, 2009). Furthermore, flooding results in anaerobic conditions and therefore promotes methanogenesis and CH_4 emissions (Smith *et al.*, 2014), making rice production a larger source of GHG in comparison to other crops (Scheehle *et al.*, 2006).

Methane emission rates from rice paddies are a function of production, transport, and oxidation processes and are influenced by complex interactions between plant, soil and the atmosphere (Gogoi *et al.*, 2005). Several factors such as atmospheric, soil and water temperature (Yun *et al.*, 2013), solar radiation, plant physio-morphological properties and development stages (Satpathy *et al.*, 1997) can have a direct or indirect effect on these processes. Since all of these factors can vary daily, weekly or seasonally, CH_4 emissions show pronounced temporal fluctuations (Yun *et al.*, 2013). However, most of the field measurements are scheduled at a given time of day, thereby neglecting diurnal variations. For a more accurate estimation of seasonal CH_4 emissions, it is necessary to obtain representative daily flux values (Weller *et al.*, 2015).

In Vietnam, rice is grown on about 7.6 million hectares (FAOSTAT, 2020b) whereby lowland rice represents the predominant production system. Especially the Mekong River Delta, popularly known as “Rice Bowl” of Vietnam, accounts for more than half of the total rice production in Vietnam (Vo *et al.*, 2018). Although the delta comprises only about 12 % of the country's total land area, its rice production is of considerable importance for the national economy (Schneider & Asch, 2020). It represents the primary source of livelihood for about 60 % of the 17 million inhabitants (Vo *et al.*, 2018). However, rice production also accounts on a national scale for 50.5 % of emissions from agriculture and 18.1 % of all GHG emissions in Vietnam (Ibid). Therefore, a promising GHG mitigation strategy must be developed that combines CH_4 emission reductions with economic benefits such as stable or increased rice yields and water savings. Several publications already exist which have examined and confirmed the positive effects (water saving, reduction of the greenhouse potential) of water-saving management techniques such as Alternate Wetting and Drying (AWD) or similar methods (Sibayan *et al.*, 2018). Although a number of other crop management practices have also been researched, there is still a lack of information concerning the influence of different rice varieties on trace gas emissions.

1.2 Research Objectives and Hypotheses

The field experiment for this study was performed in the Mekong River Delta, South Vietnam, from the beginning of November 2019 until the end of March 2020. The study on diurnal cycles presented in this thesis was an integral part of a larger assessment of the combined impact of rice varieties and water management on CH_4 emissions (Vo, personal communication, 2020). This field trial will be repeated in 2020/21 and the data evaluation of the comparative measurement stretching over the entire season is not yet available at this point. The focus of this thesis was to investigate the effects of different rice varieties and the stage of development on diurnal CH_4 emissions. In total, five rice varieties including Dai Thom 8, ML202, OM18, OM5451 and OM576 were tested under saturated soil culture. The field layout was arranged as a randomized split block design with three replications consisting in total of 180 plots, and samples were manually taken using a closed chamber method.

The objectives of this study were to:

- identify typical diurnal variation in CH_4 emission
- elucidate the underlying mechanisms for this variation (correlation with plant, soil or atmospheric variables)
- estimate the best daytime for manual measurements in view of optimum extrapolation of daily CH_4 emission
- quantify the CH_4 emissions as a function of different rice varieties
- assess CH_4 emissions at different rice development stages

The study was based on the following hypotheses:

1. Methane emissions from rice paddies under tropical conditions show distinct diurnal patterns with maximum emissions at the early afternoon.
2. Atmospheric and soil temperature influence the formation of diurnal variations.
3. Rice varieties affect both the quantity of CH_4 released and the diurnal emission pattern.
4. The development stage influences the characteristics (intensity and pattern) of diurnal emissions.

2 Literature Review

2.1 Methane – a Global Greenhouse Gas

2.1.1 Sources and Sinks

The compound methane with the chemical formula CH_4 is the simplest hydride of carbon and a principal constituent of natural gas (> 90 %) (Crabtree, 1995). It is released into the atmosphere from a wide variety of sources of both anthropogenic and natural origin. Anthropogenic emissions, which account for about 50 to 65 % of total emissions (IPCC, 2013) arise from wastewater treatment, landfills, combustion of biomass, rice paddy agriculture, enteric fermentation and land management activities (Denman *et al.*, 2007). Fossil-fuel related emissions come from the exploitation, transport and usage of coal, oil and natural gas. Methane gas that was formed in the process of coalification and trapped between coal seams and the surrounding rock is released by natural erosion, faults or mining processes (United States Environmental Protection Agency, 2019).

With a share of about half of the total anthropogenic CH_4 emissions (Figure 1), agriculture plays an essential role as the largest man-made source of CH_4 (Reddy *et al.*, 2013; Crippa *et al.*, 2018). Enteric fermentation contributes thereby with 28 % of CH_4 emissions, due to release from domestic ruminants such as cattle, goats, camels or sheep as a by-product of the anaerobic microbial activity in their digestive systems (Ji & Park, 2012). Rice cultivation which is mostly practised under flooded conditions is amounting to 31 - 112 Tg CH_4 yr^{-1} of methane accounting for about 10 % of total anthropogenic emissions. Methane is also emitted during manure management and applications. (Dlugokencky *et al.*, 2011).

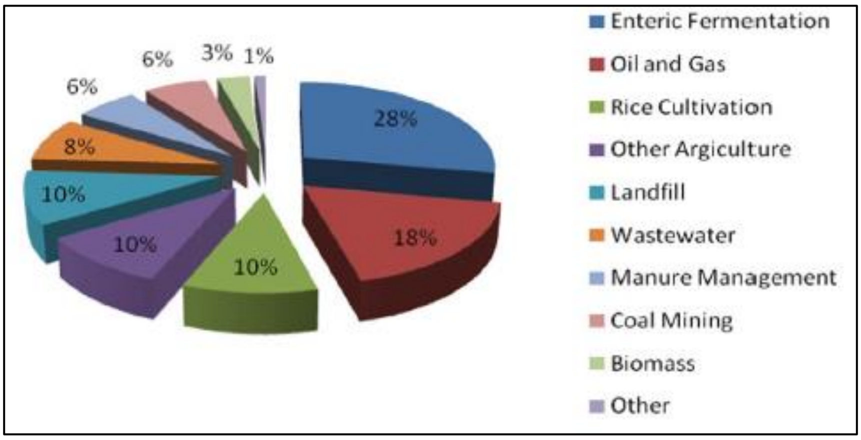

Figure 1: Anthropogenic methane emissions from different source sectors (Zakaria & Kamarudin, 2016).

Wetlands are the largest natural source of CH_4 gas globally. Annual emissions up to 231 Tg originating from swamps, bogs and the tundra are possible, depending on precipitation and temperature (Dlugokencky *et al.*, 2011). Additional natural sources include termites, wild ruminants, terrestrial permafrost, forest fires, oceans, hydrates and other geological sources (Wuebbles & Hayhoe, 2002; Denman *et al.*, 2007; IPCC, 2013). Annual CH_4 emissions from sources and sinks are summarized in Table 1.

In contrast to the numerous sources, there are only a few sinks for tropospheric CH_4, including biological CH_4 oxidation by methanotrophic bacteria and the upwards loss into the stratosphere. Both are contributing only to a minor CH_4 removal of about 30 Tg CH_4 yr^{-1} (Dlugokencky *et al.*, 2011). The primary loss mechanism of CH_4 is the reaction with the hydroxyl radical (OH), which can be described by the following equation:

$$CH_4 + OH \rightarrow H_2O + CH_3$$

The hydroxyl radical, which is formed through the photodissociation of tropospheric ozone and water vapour, leads to oxidation of CH_4, especially in the strong sunlight in the tropics (Owens *et al.*, 1982). This process is responsible for the removal of approximately 500 Tg CH_4 yr^{-1} corresponding to 90 % of the total sink (Wuebbles & Hayhoe, 2002). Increasing CH_4 concentrations can therefore, be attributed to higher CH_4 emissions (source) or a decrease in OH concentrations (sink).

Table 1: Edited annual CH_4 emissions by source type for 1984 to 2003 from the electronic supplementary material in Bousquet *et al.* (2006) in the middle column and a range of years from 1997 to 2006 from Solomon (2007) (IPCC). Units are Tg CH_4 yr^{-1}, where 1Tg = 10^{12} g.

Source	**Bousquet et al.**	**IPCC**
anthropogenic		
energy	110 ± 13	74–106
enteric fermentation	90 ± 14	76–92
rice agriculture	31 ± 5	31–112
waste	55 ± 11	35–69
natural		
wetlands	147 ± 15	100–231
termites	23 ± 4	20–29
total	525 ± 8	503–610

Sinks	**Bousquet et al.**	**IPCC**
troposphere	448 ± 1	428–511
stratosphere	37 ± 1	30–45
soil	21 ± 3	26–34
total	506	492–581

2.1.2 Climate Effects and Atmospheric Chemistry

With a GWP 28 times higher than CO_2 at warming the earth on a 100-year time scale and 80 times more potent over 20 years, CH_4 is a severe GHG and plays a crucial role as the second strongest anthropogenic GHG in climate change (IPCC, 2013). Changes in CH_4 concentrations have both direct radiative impacts on the climate and indirect effects on ozone (O_3), water vapour (H_2O), OH and the abundance as well as the lifetime of many other pollutants as they are intricately coupled via atmospheric chemistry (Shindell *et al.*, 2005). Like other GHG, methane absorbs and re-emits infrared radiation, trapping the longwave terrestrial radiation from the planetary surface in the atmosphere, a process commonly known as the greenhouse effect (Lelieveld *et al.*, 1993).

Methane belongs to the well-mixed gases in the atmosphere with a tropospheric chemical mean lifetime of 9.8 ± 1.6 yr. As a precursor of O_3 as well as H_2O and an essential source of carbon monoxide (CO), methane has a severe impact on tropospheric and stratospheric chemistry and their climatic feedback (Lelieveld *et al.*, 1993). Roughly a quarter of CO in the troposphere is derived from the CH_4 oxidation-circle (Wuebbles & Hayhoe, 2002). Additionally, the distribution of O_3 in the atmosphere is affected. Increasing CH_4 concentrations, promote the net O_3 production in the upper troposphere and lower stratosphere as well as net O_3 destruction in the upper stratosphere (Albritton *et al.*, 1992). Long term exposure to rising O_3 levels can cause health impacts, including mortality from respiratory diseases. Also, there is strong evidence that it negatively affects vegetation physiology, causing yield losses (IPCC, 2013; Crippa *et al.*, 2018).

Moreover, CH_4 oxidation produces water vapour, a particularly important GHG which itself is an influential driver of climate change, causing about two-thirds of the total global greenhouse effect (Lelieveld *et al.*, 1993). Rising stratospheric water vapour concentrations contribute to a warming of the troposphere (Solomon *et al.*, 2010). It is estimated that the destruction of one mole of CH_4 by the oxidation with the hydroxyl radical produces about two moles of water vapour. Consequently, not only does the increase in CH_4 levels itself contribute to global warming, but also the increase in water vapour has an indirect effect (Wuebbles & Hayhoe, 2002).

2.1.3 Trends and Future Projections

Methane concentrations have more than doubled since pre-industrial times. They increased by a factor of 2.5 from 722 ppb in 1750 to 1803 ppb in 2011 (IPCC, 2013) corresponding to an average increase of 4.3 ppb per year. Since 2007, CH_4 concentrations increased rapidly with a growth rate of 12.5 ppb yr^{-1} in 2014 and 9.9 ppb yr^{-1} in 2015, reaching an annual average of 1834 ppb in 2015. (Dlugokencky *et al.*, 2009; Saunois *et al.*, 2016; Crippa *et al.*, 2018). Now the development of atmospheric CH_4 emission most closely aligns with the Representative Concentration Pathway (RCP) 8.5 W m^{-2} due to an acceleration of CH_4 concentrations in the last few years (Saunois *et al.*, 2016). The RCPs are four new emission scenarios which have been defined by the scientific community for the fifth Assessment Report of IPCC. The scenarios are named according to their radiative forcing target in 2100 (RCP2.6, 4.5, 6 and 8.5) (IPCC, 2013). Emission scenarios are a relevant tool in climate research to describe possible future outcomes concerning a range of variables, including physical and ecological aspects, socio-economic and technological change, energy and land use as well as the emission of GHG and air pollutants (van Vuuren *et al.*, 2011). The last and highest RCP predicts that radiative forcing levels will reach, 8.5 W m^{-2}, followed by a further increase up to 12 W m^{-2} by 2250, and finally stabilize. When the current development of CH_4 concentrations is compared with the four different scenarios, it is evident that the development is most consistent with the RCP8.5 (Figure 2). This represents in terms of CH_4 concentrations an increase up to 3500 ppm in 2100, which is almost more than twice as high as today's levels (1834 ppb) (Saunois *et al.*, 2019).

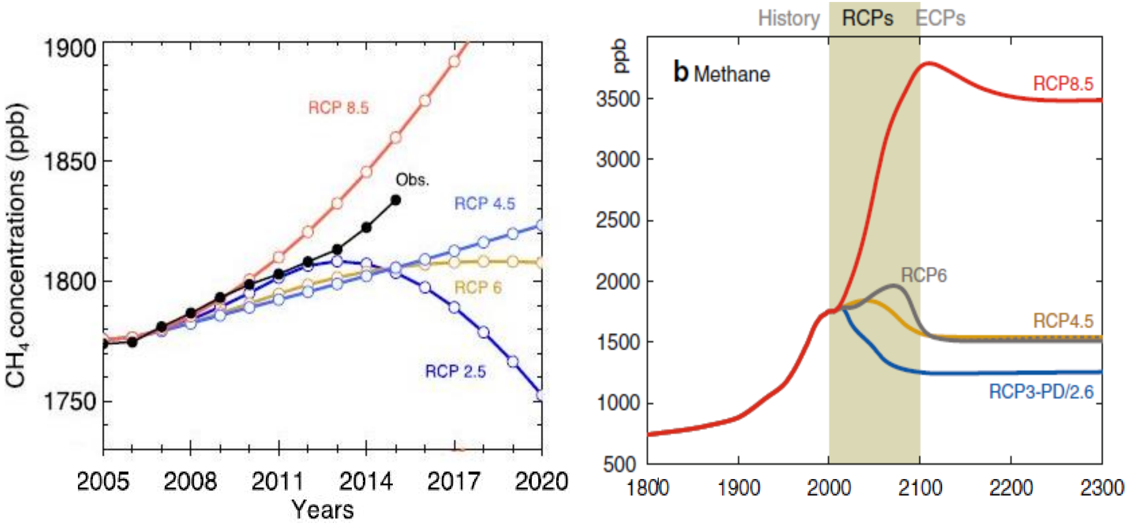

Figure 2: GHG concentrations of atmospheric methane in ppb Left: Four RCPs and observation of current atmospheric methane concentrations (black line) (Saunois *et al.*, 2016). Right: RCPs and ECPS (Meinshausen *et al.*, 2011). RCP – Representative Concentration Pathway (from year 1850 to 2100). ECP - Extended Concentration Pathways (beyond 2100).

2.2 Methane Emission from Rice Paddies

Methane emission rates from rice paddies are a function of production, transport, and oxidation of CH_4 and are influenced by complex interactions between plant, soil and the atmosphere (Gogoi *et al.*, 2005). These principal processes are affected by various variables which include: soil characteristics and redox potential, rice variety, climate, temperature, fertilization with organic carbon, water management and other agricultural practices (Sethunathan *et al.*, 2000; Conrad, 2002). The following section reviews in detail the main processes which are prerequisite for any CH_4 flux and the most important factors influencing them.

2.2.1 Production

Biogenic methane is produced by a process called methanogenesis, as the end product of the organic carbon decomposition cascade in anoxic, reduced soil conditions in flooded rice fields (Schütz *et al.*, 1989b; Minamikawa *et al.*, 2015). This is caused exclusively by anaerobic, methanogenic microorganisms that are phylogenetically associated with the archaea domain (Conrad, 2002). Only a limited number of substrates can be utilized by these methanogens to produce CH_4, including H_2/CO_2, acetate, formate, CO_2 and H_2 (Seiler *et al.*, 1983). These substrates are products of the microbiological degradation process of organic matter (OM), where complex compounds are broken down into substrates that are readily available to methanogenic archaea (Conrad, 2002). This process is divided into the following reactions, each catalysed by a specific group of microorganism: (1) Hydrolysis of macromolecules (e.g., polysaccharides), (2) Primary fermentation of the hydrolyzed products to form organic acids, alcohols, CO_2 and H_2 (3) Secondary fermentation and anaerobically degradation into methanogenic substrates such as H_2/CO_2 and acetate and the final reaction (4) Methanogenic conversion to CH_4 and CO_2 (Conrad, 2002; Kato & Watanabe, 2010). Methanogens only achieve the formation of CH_4 in the strict absence of oxygen and at redox potentials of less than -200 mV. Generally, the production of CH_4 is negatively correlated with Eh (-150 and -230 mV) and electric conductivity but positively correlated with soil carbon and pH (between 5.5 and 7.0) while the optimum pH is near neutral (Wang *et al.*, 1993). The amount of CH_4 that is produced through methanogenesis depends highly on primary production and the input of organic matter into the soil (Minamikawa *et al.*, 2015). Primary sources of OM include the soil, organic fertilizers, plant residues from rice, weeds and algae as well as rhizodeposition. The supply and decomposition of these materials are highly diverse and are closely related to field management (Kimura *et al.*, 2004).

2.2.2 Transport Pathways of Methane to the Atmosphere

The transfer of CH_4 from the soil to the atmosphere in rice paddies takes place over three main transport pathways: diffusion, ebullition, and transport through the rice plant (Figure 3).

The transport via molecular diffusion across the flooded soil and overlying water to the atmosphere is dependent on the concentration of the surface-water, wind speed as well as the CH_4 supply to the surface water and strongly limited due to low solubility of CH_4 in aqueous media (Sebacher *et al.*, 1983; Wassmann *et al.*, 1993). The diffusion rate in the liquid phase is about 10^4 times slower than diffusion in the gas phase (Aulakh *et al.*, 2001c). Only an exceedingly small proportion of dissolved CH_4 reaches the atmosphere since 60 to 90 % of CH_4, produced during a cropping season, is consumed by methanotrophs and oxidized in the aerobic soil layer, floodwater or in the rice rhizosphere (Wassmann *et al.*, 1993; Minami & Neue, 1994).

Ebullition is the ascent of CH_4 gas bubbles from the soil to the atmosphere. This physical process contributes about 4 - 9 % to CH_4 emissions (Denier van der Gon, 1996) and is strongly dependent on the partial pressure of CH_4, soil bulk density, soil texture and aggregation as well as bioturbation (Minami & Neue, 1994). Aquatic earthworm, for example, provide vents for the gas release but also increase the oxic soil surface (Ibid). Ebullition occurs predominantly in the early growth stages of rice plants when the root system is not yet well developed and can additionally be triggered by mechanically soil disturbance mediated by the soil fauna or crop management (Wassmann *et al.*, 1993). The escape of entrapped CH_4 happens very rapidly and forceful so that emerging bubbles are not oxidized when passing through oxic layers (Denier van der Gon, 1996).

However, 80 to 90 % of CH_4 is released by plant-mediated transport via the aerenchyma. A well-developed system of spongy, air-filled tissues that has evolved as a morphological adaptation to flooding in hydrophilic and wetland plants (Denier van der Gon, H.A & van Breemen, 1993; Wassmann *et al.*, 1993). The aerenchyma and additional parts of this transport system including leaf blades, leaf shades and culms, are required to supply atmospheric O_2 to the roots for respiration in anaerobic paddy soils (Minami & Neue, 1994). The oxygen is transported by diffusion or mass flow over the ventilation system from the leaf parts above water to the submerged plant organs (Nouchi *et al.*, 1990). Additionally, to the downward transport of O_2 to submerged roots, several gases, including CH_4, are transported upwards from the roots to plant parts above the soil surface and are released into the atmosphere (Aulakh *et al.*, 2001c). The potential of a rice plant and the amount of CH_4 that is emitted depends upon several, variety dependent factors which will be discussed in the next section below. Although stomata are generally considered to play an important role in gas absorption and release, Nouchi *et al.* (1990) have shown that the aerenchyma is not associated with stomatal gas exchange. They suggest the existence of another transport pathway. Methane is most probably transported over diffusion, driven by a concentration gradient between the soil (source) and the atmosphere which is acting as an unlimited sink due to low CH_4 concentrations of the ambient air (Denier van der Gon, H.A & van Breemen, 1993).

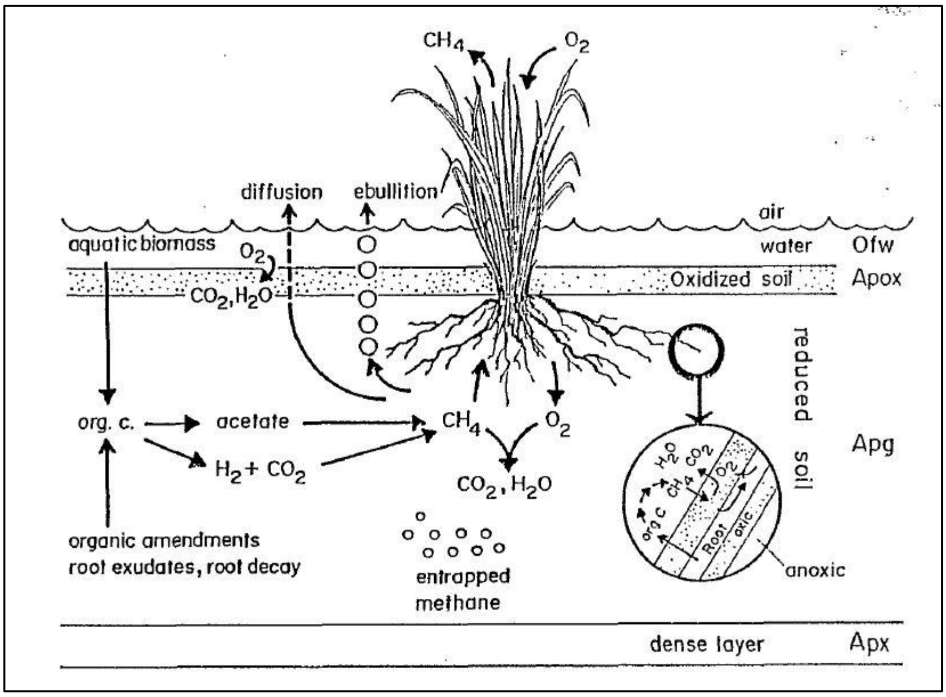

Figure 3: Schematic of production, oxidation, and emission of methane in rice paddies (Neue & Roger, 1993).

2.2.3 Oxidation

The volume of CH_4 emitted by rice paddy fields is considerably lower (< 20 %) than the initially produced amount in anoxic, reduced soil layers. Methane oxidation by methanotrophic bacteria in aerobic soil surface layers and the roots rhizosphere is generally reflected by the difference between CH_4 production rates and the released amount into the atmosphere (Conrad & Rothfuss, 1991). Methanotrophy thus poses great potential for moderating emissions from rice soils. The largest part of a flooded soil is reduced within a few days after submergence. Only the top millimetres of the soil surface is in the oxidized state, where methanotrophs can operate (Kumaraswamy *et al.,* 1997). The CH_4 oxidising activity decrease with increasing depth (Kimura *et al.*, 2004). Undisturbed soils have higher CH_4 uptake capacities than agricultural soils. Ones the soil is disturbed, the oxidation can be inhibited by months or even years

(Sethunathan *et al.*, 2000). The degree of CH_4 oxidation in the aerobic soil surface has been studied by several scientists (Conrad & Rothfuss, 1991; Banker *et al.*, 1995). Their results indicate that absolute rates of CH_4 oxidation at the soil surface are significantly lower compared to those in the rhizosphere (Gilbert & Frenzel, 1998).

Oxygen which diffuses through the aerenchyma to the roots, to meet the metabolic O_2 demand, leaks into the surrounding soil, creating another oxic-anoxic boundary layer in the rice ecosystem. This root-soil interfacial region is supplied with CH_4, which diffuses from the anaerobic parts of the soil towards the rhizosphere (Gilbert & Frenzel, 1998) and oxygen, partially leaking from the roots into the soil, allowing methane-oxidizing bacteria to be active (Gilbert & Frenzel, 1998; Kimura *et al.*, 2004). Thereby does O_2 supply, which is highly variable, most probably limit microbial activity and thus CH_4 oxidation (Armstrong, 1969).

2.2.4 Effect of Rice Cultivars on Methane Emission

Rice plants play a crucial role in the regulation of CH_4 fluxes from the soil to the atmosphere (Aulakh *et al.*, 2000a). They act as: (1) a source of methanogenic substrate (above-ground biomass or rhizodeposition) (Aulakh *et al.*, 2001b), (2) a conduit for CH_4 through a system of air-filled intercellular spaces (aerenchyma) (Nouchi *et al.*, 1990) and (3) a CH_4 oxidation site and habitat for methanotrophs in the rhizosphere (Gilbert & Frenzel, 1998). These three functions are additionally influenced by morpho-physiological parameters like growth characteristics, photosynthetic efficiency (Gogoi *et al.*, 2005) and the stage of development (Aulakh *et al.*, 2000a).

Rhizodeposition in the form of root exudates (e.g. organic acids, phenols and amino acids), sloughed-off cells and decayed roots from rice are an essential source of OM for CH_4 production (Conrad, 2002). On average, 30 - 60 % of photosynthetically synthesized organic C is allocated to the roots, and a considerable part of this is released into the rhizosphere (Clarkson, 1996). Aulakh *et al.* (2001b) pointed out, that root exudation rates differ among varieties and developmental stages of rice plants depending on their root system size and the above-ground biomass. GHG experiments with different rice cultivars showed a positive correlation between root biomass yield and CH_4 flux rates (Wang & Adachi, 2000). Higher root biomass could indicate the potential to provide larger amounts of organic materials (root exudation and degradation of dead roots) as substrates for CH_4 production (Kerdchoechuen, 2005)

After the production, by methanogenic bacteria in the anaerobic sites of the paddy soil, CH_4 is dissolved and diffuses to the soil-root interface (Gilbert & Frenzel, 1998), where it is immediately oxidized by oxidising bacteria or absorbed by roots, gasified in the root cortex and transported through the aerenchyma system (Nouchi *et al.*, 1990). Several studies documented a close relationship between varietal, anatomical and morphophysiological properties and CH_4 release via plant-mediated transport (Gogoi *et al.*, 2005; Das & Baruah, 2008a). Aulakh *et al.* (2000b) observed significant differences in methane transport capacities (MTC) among 12 cultivars as a function of plant biomass, tiller number and the amount/ density of aerenchyma. For example, the number of developed tillers at three growth stages (panicle initiation, flowering, and maturity) showed a significant positive relationship with the MTC.

O_2 is transported from the atmosphere to the roots through the same system of air-filled intercellular spaces which are acting as a vent for CH_4 diffusion (Denier van der Gon, 1996). The oxidizing activity of rice roots varies with the growth stage of the plant, leading to a development dependent CH_4 oxidation of the rhizosphere (Denier van der Gon & Neue, 1996). Wang & Adachi (2000) observed varying population levels of methanotrophs among different rice cultivars at booting and flowering stage, which could be explained by higher oxygen transport capacities in these growth stages.

2.2.5 Diurnal Patterns and Temperature Dependencies

Methane fluxes from flooded paddy soils to the atmosphere are directly or indirectly influenced by a number of biotic or environmental factors such as temperature (air, soil, water) (Yun *et al.*, 2013), photosynthesis, photosynthetically active radiation (Hatala *et al.*, 2012) and rice plant development stage (Satpathy *et al.*, 1997). However, all these factors vary hourly, weekly or seasonally and can, therefore lead to temporal variations (Yun *et al.*, 2013).

Several studies have shown significant positive correlations between air, soil, or water temperature and CH_4 emissions. Temperature strongly influences the activity and growth rate of microorganism. Activity and growth rate generally increase with rising temperatures until an optimal level is reached and then decline due to inactivation and death of the bacteria (Schütz *et al.*, 1990). In a laboratory experiment, pure cultures of hydrogenotrophic methanogenic bacteria were incubated at different temperatures. Depending on the methanogen species, minimum temperatures with detectable CH_4 formation were about 15 °C, the optimum temperatures were generally around 35 °C, and the maximum temperatures were higher than 40 °C (Schütz *et al.*, 1990). However, temperature effects in anaerobic rice soils are overly complex, not only affecting microbiological growth and activity but also the structure and function of microbial communities (Chin *et al.*, 1999) as well as the pathway of carbon flow (Chin & Conrad, 1995) since some microbial processes are more sensitive to temperature than others.

Abiotic factors such as decreased CH_4 solubility or increased CH_4 diffusion velocity caused by higher temperatures additionally contribute to temporal variations in CH_4 fluxes (Hosono & Nouchi, 1997).

Diurnal CH_4 emission rates have been studied in various rice ecosystems of different climate zone. It has been observed that diurnal patterns of CH_4 fluxes can show considerable variations (Weller *et al.*, 2015), but mostly exhibit maximum emission rates at the early afternoon (12:00 – 15:00) followed by a decline to a minimum around midnight (Satpathy *et al.*, 1997).

2.3 Rice Production and its Importance as Staple Food

Rice, belongs to the family Poaceae (Gramineae), subfamily Bambusoideae, genus Oryza (Khush, 2005) and has 24 species of which 22 are wild species (Jena, 2010) and two are distinct types of domesticated, cereals: *Oryza sativa* L. and *Oryza glabberrima* Steud (Sweeney & McCouch, 2007). *O. sativa* L., often called Asian rice, was domesticated about 8000 years BC (Ibid). It has two major subspecies namely *japonica* and *indica* and is distributed worldwide but mostly cultivated and consumed as a staple food in Asia (Callaway, 2014). The subspecies are more closely related to wild varieties than to each other. They became domesticated in different regions: *japonica* in China and *indica* in India (Gross & Zhao, 2014). The cultivated African Rice, *O. glaberrima* Steud., is grown on a limited cultivation area in a few African countries and has been domesticated about 3500 years ago (Jena, 2010).

Rice is cultivated under diverse agro-climatic conditions, in a wide range of environments in Asia, Africa, Australia, North America, South America and to a limited extent in Europe (Prasad *et al.*, 2017). The International Rice Research Institute (IRRI) has categorized four types of rice field ecosystems (RFES) depending upon hydrological characteristics: upland, irrigated, rainfed lowland and flood-prone, respectively deepwater rice ecosystems (Maclean *et al.*, 2013). The upland RFES covers less than 13 % of the world´s rice land, varies from low-lying valleys to steep sloping land with high runoff and lateral water movement and is characterized by rarely flooded aerobic soils (Chauhan *et al.*, 2017).

Irrigated rice accounts for 55 % of the global harvested area and contributes 75 % of global rice production, which is about 586 million tonnes of rice per year (Bouman *et al.*, 2007). Of this, 56 % of irrigated land is located in Asia, where rice is mostly grown as a monoculture with two crops per year receiving on a field level, 2 - 3 times more water per hectare than any other irrigated crop (Maclean *et al.*, 2013). In the wet season irrigated rice is grown with supplementary water, while it is reliant on irrigation in the dry season (Ibid).

Rainfed environments cover about one-quarter of world´s rice fields (Chauhan *et al.*, 2017), but they are subjected to several abiotic stresses due to high uncertainty of the duration, timing and intensity of rainfall (Maclean *et al.*, 2013). Here, rice is usually transplanted and cultivated in levelled, bunded fields which are shallowly flooded with rainwater (Mackill *et al.*, 1996).

Flood-prone environments are exposed to uncontrolled flood events (Chauhan *et al.*, 2017), in which rice fields are submerged from 10 days to a few months under 50 to more than 300 cm of water (Maclean *et al.*, 2013). Rice grown in deepwater environments distinguishes itself from most modern rice varieties by its ability to grow at rates of 20 to 25 cm/d when partially submerged. Deepwater rice can reach a length of up to 7 m to survive in water for at least one month (Catling, 1992). Flood-prone rice environments cover about 8 % of the world´s rice area (Chauhan *et al.*, 2017).

In 2018, the total global rice harvest area of paddy rice amounted an area of 167.1 million hectares. The production quantity was 782 million tons, led by China with 212.1 million tons followed by India and Indonesia with 172.5 and 83 million tons, respectively (FAOSTAT, 2020b). Together with Bangladesh, the Philippines, Vietnam, Thailand and Myanmar, these major producers account for 80 % of world´s total production. However, they also hold 46.6 % of the global population (Chauhan *et al.*, 2017). At present, rice is the most relevant staple food for the dietary energy requirements of almost half the world’s population, and virtually all of East and Southeast Asia are entirely dependent upon rice (Fairhurst & Dobermann, 2002). Rice is the largest single source of calories for a significant majority of Asian consumers. For example, on average Vietnam consumed 1629 calories of rice per capita per day in 2007, accounting for 57.8 % of total caloric intake (C. Peter Timmer, 2010). Especially in the poorest countries such as Myanmar or Bangladesh with an annual per capita consumption in 2003 of 197 and 169 kg respectively, rice plays a vital role. For comparison, in the same year, the per capita consumption in the United Kingdom was 5 kg, with a world average of 57 kg (Kole, 2006). Moreover, it is projected that the total consumption of rice will increase by 13 % by 2027 due to population growth rather than per capita gains (FAO and OECD, 2018).

2.4 Mitigation and Water Saving Strategies in Rice Production

From the previously described topics on CH_4 emission from rice paddies, it can be concluded that possible mitigation strategies need to consider either production, oxidation or transport processes to control CH_4 emissions. Several options can be contemplated to achieve this goal including water management, soil amendments and fertilizers, organic matter management, cultivar selection and altered agricultural field practices such as tillage (Yagi *et al.*, 1997; Aulakh *et al.*, 2001c). Also, water-saving strategies need to be considered since 24 – 30 % of the total world's freshwater is used for rice production (Chapagain, 2009). In the following, the mitigation strategies of water management and variety selection are illuminated in more detail. Besides, saturated soil culture as a water-saving strategy in rice production will be described briefly.

2.4.1 Alternated Wetting and Drying

Altered water management represents a way to decrease water usage and increases rice water productivity, making a large volume of water available for alternative applications (Lampayan *et al.*, 2015) and at the same time decreasing CH_4 production rates in soils (Yagi *et al.*, 1997). Since the activity of methanogens is constrained to reduced soil conditions (Zeikus, 1977), a substantial reduction of CH_4 production can be achieved by aeration of the soil during flooding (Yagi *et al.*, 1997). IRRI and its national agricultural research and extension system partners have worked together to develop and promote an alternative water management system, namely "Safe" Alternate Wetting and Drying (AWD). This water management technique consists of three key elements (Bouman *et al.*, 2007; Tran *et al.*, 2018): (1) shallow flooding of rice fields for the first two weeks after transplanting, to help seedlings to recover from transplanting shock and suppress weeds; (2) shallow ponding in the crop growth stages from heading to flowering which are very sensitive to water deficit stress and at times of high crop growth rates and water requirements (Bouman & Tuong, 2001); (3) application of AWD during all other periods. In these periods, flooded conditions are rotated with multiple aerations where the surface water level declines up to 15 cm below the soil surface before irrigation water is resupplied (Tran *et al.*, 2018). Several studies report on the reduction of CH_4 emissions and water consumption through the application of AWD combined with maintained or even increased grain yields (LaHue *et al.*, 2016). The reduction of CH_4 emissions due to soil aeration varies in a wide range between 7 % and 80 % and also implies some limitations because of a reverse effect on N_2O emissions (Wassmann *et al.*, 2004). However, AWD treatments resulted also in yield reductions varying from negligible to 70 % when compared with the yield of the flooded checks. (Lagomarsino *et al.*, 2016).

2.4.2 Soil Saturated Culture

Soil saturated culture (SSC) has the potential to achieve large reductions in water inputs at the field level by reducing the seepage and percolation flows as well as evaporation losses (Bouman *et al.*, 1994). Tabbal *et al.* (2002) reported on a reduction of irrigation water by 31 – 58 % in transplanted, 32 – 49 % in direct wet-seeded rice and 6 – 34 % in direct dry-seeded rice. In contrast to continuously flooded soils, were a ponded water layer of 5 - 10 cm is maintained, in SSC the floodwater depth is decreased. Therefore, shallow irrigation is given to about 1 - 2 cm flood water depth after the disappearance of standing water to keep the soil saturated. Implementation of this water-saving irrigation technique requires good water control at the field level and frequent shallow irrigations which can be labour intensive (Amudha *et al.*, 2009).

2.4.3 Variety Selection

The breeding and selection of low emitting cultivars represent another desirable mitigation strategy, as this can be easily adopted and implemented by farmers (Yagi *et al.*, 1997). There are three variety dependent factors for the selection, which should be considered:

1) selecting varieties with high CH_4 oxidation rates in the rhizosphere but low CH_4 transport efficiency since 60 % to 90 % of methane is emitted from the soil to the atmosphere via transportation through the aerenchyma of rice plants (Wassmann & Aulakh, 2000)
2) selecting varieties with low exudation of carbon from roots since carbon is the primary source for the production of methanogenic substrate
3) selecting varieties which have a high harvest index with a smaller number of unproductive tillers in order to minimize organic matter input into the soil after harvest (Aulakh *et al.*, 2001b)

3 Material and Methods

3.1 Site Description and Experimental Design

The field experiment was conducted during the dry season of 2019/2020 (December to March) at the experimental site of the Dinh Thanh Agricultural Research Center owned by the Lộc Trời Group (LGT) located in Định Thành – Thoại Sơn District – An Giang Province, South Vietnam (10°18’44.9 N 105°19’08.3 E). This location is characterized by a tropical climate with an annual mean rainfall of about 1415 mm and an average temperature of 27.4 °C. The air temperature at the experimental location varied during the test period from a minimum of 19.3°C in December to a maximum of 34.6°C in March, while the average air temperature was 26.4°C. No precipitation was recorded in December and January. However, a total of 5.33 mm was measured in February and 5.83 mm in March. As part of a doctoral thesis, the field layout was arranged as a randomized split block design with three replications consisting in total of 180 plots, each with a dimension of 4 x 5 m. The main experimental factor was the three water management treatments: (i) continuous flooding (CF), (ii) alternate wetting and drying (AWD) and (iii) soil saturated culture (SSC). The sub-factor was comprised of 20 different rice varieties. The field layout is shown in Figure 4.

This master thesis was integrated into a field trial, with its various parameters, randomization as well as measurement schedules, already predefined by the experimental set-up established for the PhD thesis. Complementing to these broader research questions, this MSC thesis focuses on the varietal effects of five rice varieties under one water treatment (SSC) at three different development stages, on diurnal emissions of CH_4. Therefore, three diurnal measurements, each consisting of five GHG samplings were conducted. Furthermore, CH_4 fluxes of the selected five rice varieties were determined over the entire growing period at a 7-day interval. Auxiliary measurements included weekly soil moisture recordings, the determination of Leaf Area Index (LAI), soil temperature measurements, and weather data collection. Information about land management and measurement schedule can be seen in Table 2.

3.2 Water, Land and Crop Management

Field preparations started with ploughing, on 04.12.2019 followed by the establishment of the irrigation, and drainage canals five days later (see Appendix 1). The main water canals along the repetitions and between blocks were 0.5 m in width, whereas the canals supplying individual plots with irrigation water were 0.3 m. Bunds were then constructed for separating 180 plots, each with a dimension of 4 x 5 m. The different water management practices were initiated 17 days after transplanting (DAT). Water was pumped from the basin irrigation system through a pipe into main field canals and fed directly into the plots by manually opening ‘bundbreaks’. The SSC plots were flooded twice a week (every Monday and Thursday) to maintain soil saturation, with irrigation water to a depth of about four centimetres and subsequently drained to a saturated level. The water level was controlled before every irrigation event (see Appendix 1) and soil moisture recorded with a PR2/6 capacity sensor. Fertilization was carried out according to LTG´s common practice of 90-40-40: a total amount of 90 kg/ha nitrogen, 40 kg/ha phosphorus (P_2O_5) and 40 kg/ha potassium (K_2O), which was divided between three fertilizer treatments and applied as listed in Table 2. The fertilizer for each application was equally divided into 180 small portions for 180 plots (see Appendix 2).

Table 2: Schedule of important field management and measurement activities. DAT – Days after transplanting

Management / Measurement Activity	**Date**	**DAT**
Sowing, Plot preparation	05.12.2019	-13
Transplanting	**18. - 20.12.2019**	**0**
1*st* Fertilizer application (27 kg N + 16 kg P_2O_5 + 8 kg K_2O)	23.12.2019	3
First regular Gas sampling	24.12.2019	4
Soil moisture access tubes insertion	30.12.2019	10
2*nd* Fertilizer application (36 kg N + 20 kg P_2O_5 + 12 kg K_2O)	03.01.2020	14
Start of water management practices	06.01.2020	17
1st Diurnal measurement- Tillering	**07. - 08.01.2020**	**18 - 19**
1st LAI measurement	09./10. /13.01.2020	20,21,24
2nd Diurnal, LAI - **Panicle initiation**	**20. - 21.01.2020**	**31 - 32**
3*rd* Fertilizer application (27 kg N + 4 kg P_2O_5 + 20 kg K_2O)	21.01.2020	32
3rd Diurnal, LAI - **Flowering**	**19. - 20.02.2020**	**61 - 62**
Flowering period, all plots are flooded	13.02.-26.02.2020	55 - 68
4th LAI	04.-05.03.2020	75 - 76
5th LAI, Harvest: OM5451, OM18/	16.03.2020	87
Harvest: DT8, ML202	19.03.2020	90
Harvest: OM576	23.03.2020	94

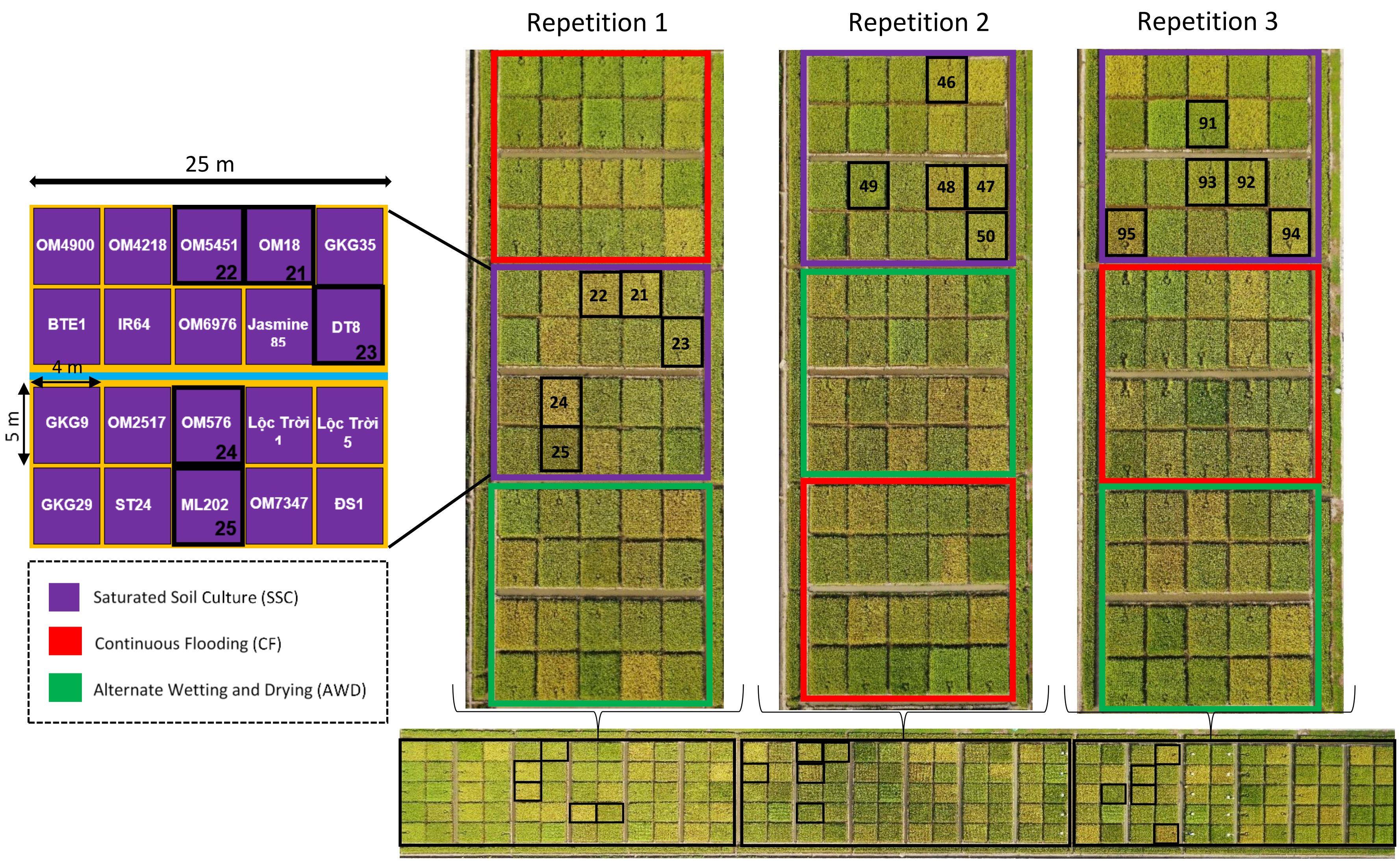

Figure 4: Experimental setup arranged as a randomized split block design with three replications consisting in total of 180 plots seen from above. Different colours indicate water treatments. Black frames and numbers show the position of experimental plots (pictures provided by LTG, 2020)

3.3 Rice Cultivation

While the overall experiment comprised 20 different rice varieties, this thesis focuses on five rice cultivars shown in Figure 5. On the 05.12.2019, rice varieties were sown separately into a soil substrate (see Appendix 2). The seedbeds were covered with coconut mulch and a layer of synthetic netting to protect seeds and seedlings from direct sunlight, birds, and rodents. One day before transplanting seedlings were translocated into their designated plots for acclimatization. Thirteen-day-old seedlings were manually transplanted (see Appendix 3) at a density of two seedlings per hill at 20 cm × 20 cm spacing on the 18.12.2019. Phenology development was monitored and recorded by the LTG staff. Information about rice variety characteristics are given in Table 3.

Figure 5: Morphology of rice varieties. From left to right: Dai Thom 8, ML202, OM18, OM5451, OM576.

Table 3: Properties of rice varieties. Blast – Rice Blast (*Magnaporthe grisea)*; BPH – Brown Planthopper (*Nilaparvata lugens)*; S – susceptible; R – resistant; Mod. - moderately; DX – Winter-Spring season; HT – Summer-Fall season. Information provided by LTG (2020)

Name	Origin	Released year	Duration (day)	Yield (tons/ha)	Amylose content	Quality	Respond to pest	Adaptability	Additional Information
Dai Thom 8	BVN/OM4900 Hybrid Semi-Dwarf	2017	90-95	DX: 7-8 HT: 5-6	16-17%	slender, less chalkiness, scent	Blast: S BPH: S	Adapt to moderate acid sulfate soil and Salinity	elongated, limpid grain, sticky cooked rice and gentle scent
ML202	Hybrid Semi-Dwarf	2013	90-100	DX: 7-10 HT: 5-6	28%	medium grain for starch production	Blast: S BPH: R	Adapt to moderate acid sulfate soil and Salinity	High-yielding, but very low rice quality, mainly used for rice flour, rice noodle and feeding animals High-tillering capacity
OM18	OM8017/OM5166	2019	95-100	DX: 7-8 HT: 5-6	17-18%	slender, less chalkiness, scent	Blast: Mod. R BPH: Mod. S	Acid sulfate soil Salinity: tolerant at 3-4‰	High-quality aromatic rice with a high tillering capacity
OM5451	Jasmine 85/OM2490 Hybrid Semi-Dwarf	2011	90-95	DX: 7-8 HT: 5-6	16-17%	slender, less chalkiness	Blast: Mod. R BPH: Mod. R	Wide adaptability	Long-grain white rice
OM576	IR48/Hungary Hybrid Semi-Dwarf	1991	100-105	DX: 7-9 HT: 5-6	27%	medium grain for starch production	Blast: Mod. R BPH: Mod. R	Adapt to moderate acid sulfate soil and Salinity	High-tillering capacity

3.4 Gas Sampling Preparations

Methane fluxes were determined using a manually operated, closed chamber method as described by Minamikawa *et al.* (2015). However, to implement this method, several preparations had to be made in the plots. Scaffolding had to be set up around each chamber location to minimize physical soil disturbance and artificial CH_4 ebullition during chamber deployment and sampling (see Appendix 3). Portable, one-meter long boardwalks with a metal frame at each end for greater stability were built, and four wooden poles installed in each plot. Each gas sampling unit consisted of a base permanently installed into the soil with an open bottom and a removable top compartment. A total of 15 rectangular gas sampling chambers, with a height of 96 cm and a length and width of 46 cm made of PVC, were built. As a lightweight, break-resistant, inert, and transparent material, PVC combines multiple useful properties, which makes it ideal for these kinds of measurements (Minamikawa *et al.*, 2015). Two computer fans were installed inside each chamber, ensuring a constant mixing and circulation of the air. Moreover, chambers were equipped with a gas sampling port and a thermometer, which could be inserted through a circular opening in the upper part of the chamber. The base consisted of an aluminium frame with a water-filled groove (4 cm in depth) which provided a gas-tight chamber closure and kept soil disturbance during the sampling procedure to a minimum. Chamber and base designs are illustrated in Figure 6.

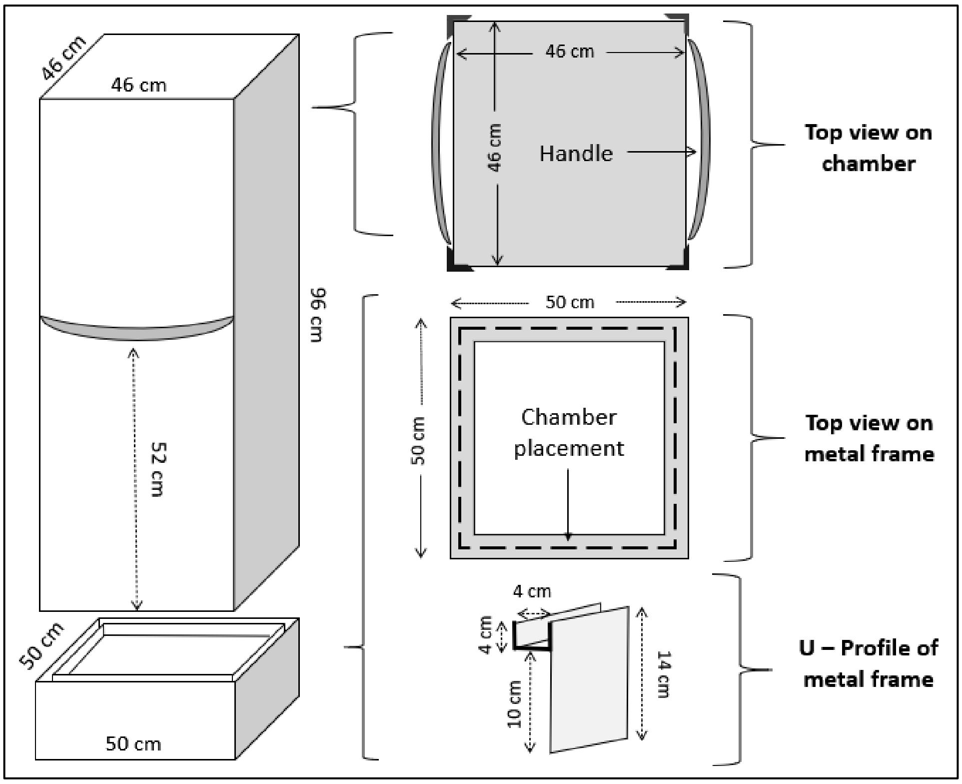

Figure 6: Chamber and Base design

One metal base per plot, covering a total area of four rice hills, was inserted 10 cm into the soil two days after transplanting (Figure 7). Furthermore, a total of 45 access tubes for soil moisture measurements were individually inserted into each plot that contained one of the five selected varieties. An auguring kit provided by the University of Hohenheim was used.

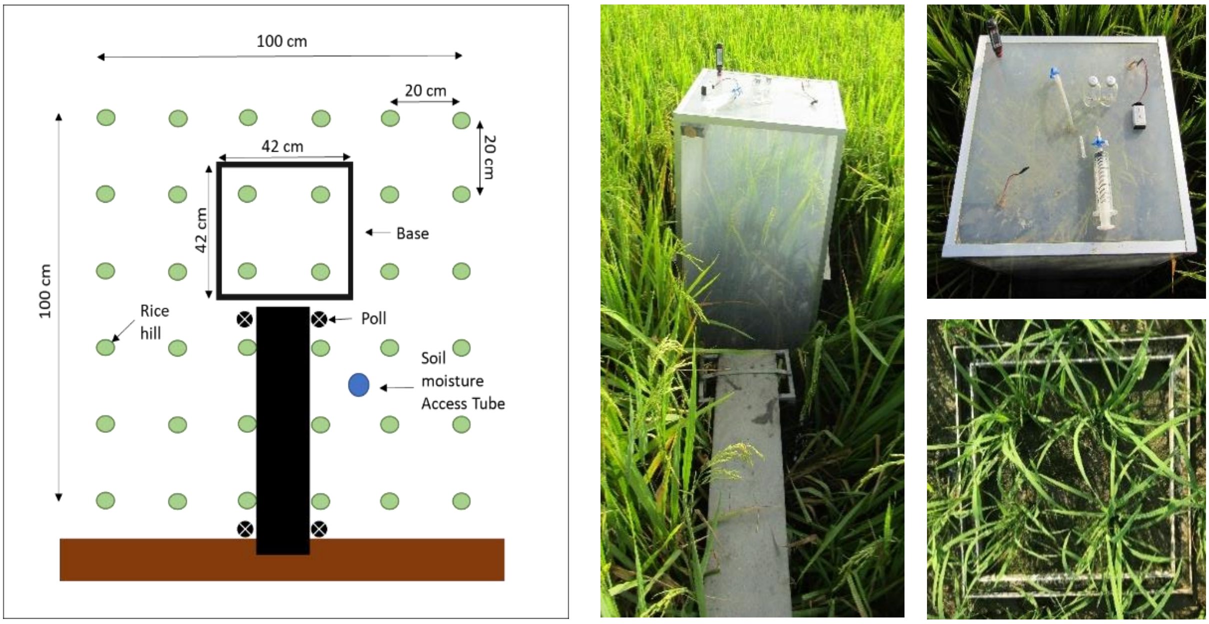

Figure 7: Greenhouse gas sampling using the manual operated, closed chamber method described by Minamikawa *et al.* (2015). Left: Gas sampling set up. Middle: Wooden scaffolding to reach chamber. Right(top): Top view of chamber with thermometer,syringe, battery and vials. Right (down): Aluminium base with four rice hills.

3.5 Measurements

3.5.1 Collection and Analyses of Gas Samples

The regular CH_4 fluxes were measured starting from the 24.12.2019 until the 26.03.2020. While the regular gas sampling was implemented weekly over the whole growing period and samples collected during mid-morning from 8:00 till 12:00, diurnal measurements were carried out at tillering stage (19 DAT), panicle initiation (32 DAT) and flowering (62 DAT). Diurnal samplings consisted of a set of five measurements taken at 9:00, 12:00, 15:00, 18:00 and 6:00 (on the following day), respectively. Due to a limited number of available field helpers, each measurement had to be divided into two rounds. The first round started at the full hour (9:00, 12:00, 15:00 etc.) and the second followed 30 min. after (9:30, 12:30, 15:30 etc.). For each measurement, six to nine chambers were used, handled by three persons in charge of two to three chambers each. Before chamber deployment, a thermometer for temperature monitoring and a 9-volt, rechargeable battery was installed to power the fan inside the chamber. For each gas collection, chambers were placed completely on top of the metal bases and sealed gas-tight by the water-filled groove. Samples were taken at 15-min intervals at 0, 15 and 30 minutes after chamber closure. For gas collection, a 60 ml syringe fitted with a stopcock attached via a valve to the gas sampling port at the chamber headspace was used. After flushing the plastic tube connected to the port five times with air out of the chamber, gas samples were extracted and immediately inserted with a needle into pre-evacuated vacuum glass vials provided by IRRI. The experimental setup and measurement procedures are illustrated in Figure 7 and 8. The vials were intermediately stored at an LTG facility and shipped in batches (see Appendix 4) for analysis to the IRRI laboratory in Las Baños, Philippines. The samples were analysed with an SRI 8610C gas chromatograph (GC) with flame ionization detector (Artisan Technology Group ®, Champaign, IL, USA). For analysis, the sample was injected into the GC, and the components of the gas mixture separated due to differences in their partition between a stationary phase, with a large active surface area, and a mobile phase usually an inert gas such as helium or nitrogen that flows over the stationary phase (Smith & Cresser, 2004).

Figure 8: Left: Measurement procedure in the field with a total of 15 chambers. Right: Top view of the field site.

CH_4 emissions were determined by the increase of gas concentration in the gas-sampling chamber over time. The hourly CH_4 fluxes (mg CH_4 m^{-2} h^{-1}) were calculated using Equation 1 given by Minamikawa *et al.* (2015) as follows:

$$Flux_{CH4} = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \rho \times \frac{273}{273 + T} \qquad (1)$$

where $\Delta C / \Delta t$ is the concentration change over time (ppm-CH_4 h^{-1}); V is chamber volume (m^3); A is chamber area (footprint; m^2); ρ is gas density (0.717 kg m^{-3} for CH_4), and T is the mean temperature in the chamber (°C). Total daily CH_4 emission (E_{day}) for each variety and development stage was calculated using Equation 2:

$$E_{day} = \bar{X}_9 * 3 + \bar{X}_{12} * 3 + \bar{X}_{15} * 3 + \bar{X}_{18} * 12 + \bar{X}_6 * 3 \qquad (2)$$

where E_{day} is the total daily CH_4 emission (kg CH_4 ha^{-1} d^{-1}) for the respective variety and development stage, $\bar{X}$ is the average of two subsequent measured CH_4 fluxes (mg CH_4 m^{-2} h^{-1}) for the respective time (e.g. $\bar{X}_9$ is the average from the flux measurements at 9:00 and 12:00) multiplied by the number of hours of the interval. For the calculation of $\bar{X}_6$ the average of the flux measurement at 9:00 and 6:00 on the following day was formed. Figure 9 illustrates the described calculation more precisely.

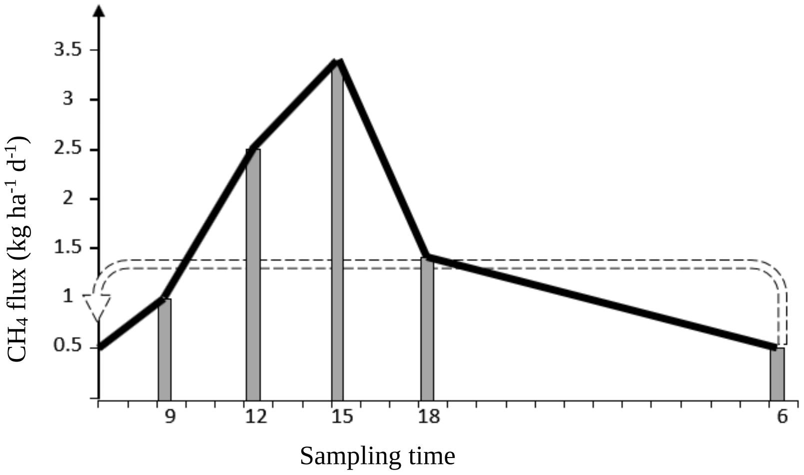

Figure 9: Schematic presentation of sampling points within one 24-h cycle. The dotted arrow represents the conceptual shift of this data point to calculate E_{day} values per calendar day

3.5.2 Leaf Area Index

Leaf area index (LAI) was firstly defined by Watson (1947) as the total, one-sided area of leaf surface per unit ground area. For this study, an indirect method for Leaf Area Index determination was chosen. LAI estimates were performed with the LAI-2000 Plant Canopy Analyzer (Li-Cor, Lincoln, Nebraska, USA); an optical sensor that projects its hemispherical view onto five detectors. Using the diffuse radiation (gap fraction), respectively light transmission at zenith angles of 7, 23, 38, 53, and 68°, the LAI-2000 calculates foliage amount and orientation (mean foliage tilt angle) (Stroppiana *et al.*, 2006). While direct methods are extremely time-consuming, labour intensive, and not compatible with long term monitoring regarding their destructive character, indirect methods can be used *in-situ* without withdrawal of biomass (Jonckheere *et al.*, 2004). LAI-2000 measurements were acquired in the mornings from 8:30 – 11:30 and in the afternoons from 15:00 – 17:30. Due to weather conditions, direct sunlight could not be avoided. Consequently, all A and B readings were obtained with the sensor shaded, facing the opposite direction of the sun. Each measurement-sequence consisted of one A reading above the canopy and eight B readings below, using a 45° view-cap to reduce the influence of the adjusted plots and the operator. A total of five LAI measurements were conducted over the entire growing season. The first three were carried out almost simultaneously with the diurnal sampling. The fourth was performed 77 - 78 DAT and the last one 89 DAT when the first varieties got harvested. The LAI 2000 outputs were processed, using the FV2200 ver.2 software from LI-COR. The data was reprocessed discarding the wide viewing angle readings since Wilhelm *et al.* (2000) showed that this would improve LAI estimates.

3.5.3 Auxiliary Measurements

Weather parameters (air temperature, humidity, wind speed etc.) and phenology of rice plants (onset of tillering, panicle initiation, booting etc.) were recorded and provided by LTG. Biomass and yield components were also acquired and provided by LGT staff (see Appendix 4 and 5). Soil temperature was recorded at about 10 - 15 cm soil depth with a TGP-4017 Temperatur-Datenlogger Tinytag Plus2 (BMC Solutions GmbH, Puchheim, Bayer, Germany) from the 08.01. until 09.01.2020 and from 19.02. until 20.02.2020, at the same days as the first and the third diurnal measurement was implemented.

3.6 Statistical Analysis

For statistical analysis, measured values were first sorted in Excel (Microsoft Ireland Operations Limited, Dublin, Ireland) followed by descriptive statistics for the sample replicates. Methane flux rates were plotted against time according to growth stages for evaluation of emission pattern. Emission rates are presented as the mean value ± standard error unless otherwise indicated. Correlation between CH_4 emissions and climate as well as plant parameters were tested with a bivariate Pearson correlation using SPSS (SPSS Statistics, IBM Deutschland GmbH, Ehningen, Germany). A T-test for independent samples was carried out to test for significant differences between varieties in tiller number and height. The significance level was set at $p < 0.05$

4 Results

4.1 Diurnal Emission Pattern

Three diurnal samplings consisting of a set of five measurements taken at 9:00, 12:00, 15:00, 18:00 and 6:00 on the consecutive day were carried out at tillering stage (19 DAT), panicle initiation (32 DAT) and flowering (62 DAT). Methane emission rates differed greatly during the 24-h cycle among varieties and development stages (Figure 10). Ratios of maximum to minimum CH_4 emission rates during a 24 h period varied from 3.1 to 5.3 at tillering stage, 1.5 to 2.4 at panicle initiation, and 4.2 to 19.8 at flowering stage among the five cultivars.

At tillering stage, all varieties showed a distinct, diurnal emission pattern, with a single peak in the afternoon around 12:00. After sunrise, emission rates increased rapidly, peaked at noon, and then declined gradually until they levelled off at night. Dai Thom 8 and OM18 exhibited the highest CH_4 emission rates with peak values of 49.86 ± 8.27 and 45.84 ± 2.97mg CH_4 m^{-2} h^{-1}, respectively (see Appendix 6). While ML202, OM5451 and OM576 showed lower but nearly identical maximum emission rates of around 35 mg CH_4 m^{-2} h^{-1}.

At panicle initiation, CH_4 emission patterns varied strongly between varieties. Fluctuations in CH_4 emissions were less pronounced, and peak emission values significantly lower. Dai Thom 8 exhibited two peaks of emission with rates of 16.49 ± 3.13 mg CH_4 m^{-2} h^{-1} at 12:00 and 19.53 ± 4.30 mg CH_4 m^{-2} h^{-1} at 18:00 (see Appendix 7). Lowest fluctuations in CH_4 emissions among the five varieties were observed in OM5451 with a ratio of 1.5 of maximum to minimum CH_4 emission rates. For ML202 and OM576, highest emission rates were recorded at noon, followed by a decrease between 12:00 and 15:00, and a further increased until 06:00 the following day. The diurnal CH_4 emission patter of OM18 at panicle initiation was similar to its pattern at tillering stage with the difference that peak emissions occurred at 15:00.

At the flowering stage, emission pattern differed only slightly among varieties. However, diurnal variations in CH_4 emission were more pronounced as compared to tillering and panicle initiation stage. It was found that peak emissions shifted from 12:00 to 15:00 for Dai Thom 8, OM18 and OM5451. Exceptional high, peak values with also high standard errors of 70.46 ± 12.96 mg CH_4 m^{-2} h^{-1} at 12:00 and 59.09 ± 24.75 mg CH_4 m^{-2} h^{-1} at 15:00, followed by a sharp drop in CH_4 emission rates were identified for ML202 and OM18, respectively (see Appendix 8). Mean values for CH_4 emission rates were obtained from three repetitions. High variations in CH_4 emission rates among repetitions within one variety were found, which might have been caused by differences in soil properties, sampling procedure or plant characteristics. These could explain high standard errors observed for all varieties and unusual high emission peaks observed especially for ML202 and OM18 at flowering stage.

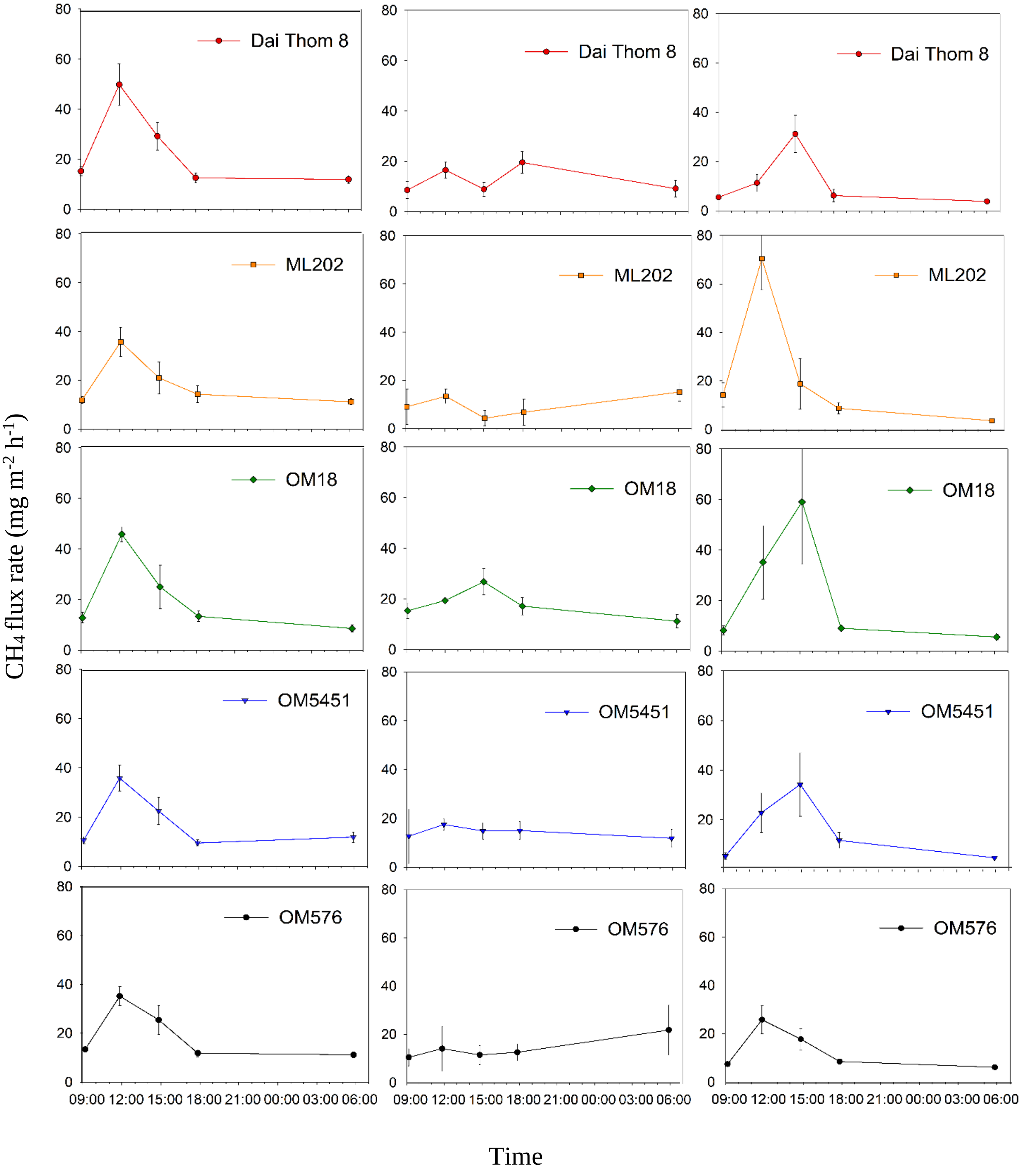

Figure 10: Diurnal methane emission pattern of five rice varieties measured at tillering stage, panicle initiation stage and flowering stage. Error bars indicate standard error.

4.2 Correlation between Diurnal Methane Emissions and Climate Parameters

Atmospheric temperature and solar radiation were recorded with a 30-minute resolution by the LTG weather station located in direct proximity of the experimental site. Soil temperature was measured at 10 to 15 cm depth with a Tinytag Plus2 Datalogger. No soil temperature data could be recovered for the panicle initiation stage due to a malfunction of the logger. The diurnal change of atmospheric temperature, soil temperature at 10 to 15 cm depth and solar radiation, are illustrated in Figure 11. For illustrative purposes, CH_4 emission patterns which were already shown in Figure 10, are shown again in a compact format.

The solar radiation (Sr) showed strong fluctuations, characterised by several sharp drops at tillering and panicle initiation. However, at the flowering stage, maximum values were reached around noon, followed by a decline. Atmospheric temperature (T_a) began to rise with increasing Sr until it reached its maximum of 31.9 °C around 14:00 at tillering. Whereas at panicle initiation and flowering peak values of 30.9 °C and 31.7 °C, respectively, were recorded about two hours later at 16:00. The soil temperature (T_s) followed a similar trend, though, maximum values were not reached until 19:00 in the evening. The amplitude between maximal and minimal T_s was higher at tillering than at flowering stage; values ranged from 27.7 °C to 30.5 °C. Statistical analysis showed, that correlation of CH_4 emissions with respect to Sr and T_a was highest at tillering and flowering stage, while the values were low or even negatively correlated at panicle initiation (Table 4). All varieties showed high correlation coefficients ranging from 0.67 to 0.72 for T_a and 0.81 to 0.85 for Sr at tillering stage. At panicle initiation, positive but also negative correlation between CH_4 emission rates and T_a as well as Sr were found. Furthermore, the data from Table 4 indicates, that T_s and CH_4 fluxes correlated very poorly or even negatively, although the general trends of both were similar.

Table 4: Correlation coefficients between methane emission rate and atmospheric temperature (T_a), soil temperature (T_s) and solar radiation (Sr)

	Tillering			**Panicle initiation**			**Flowering**		
Variety	T_a	T_s	Sr	T_a	T_s	Sr	T_a	T_s	Sr
Dai Thom 8	0.671	-0.331	0.862	0.358	-	-0.413	0.729	0.078	0.497
ML202	0.699	-0.216	0.820	-0.764	-	-0.334	0.332	-0.455	0.836
OM18	0.712	-0.256	0.856	0.912	-	0.529	0.745	-0.077	0.687
OM5451	0.629	-0.337	0.815	0.739	-	0.224	0.839	0.066	0.641
OM576	0.725	-0.285	0.820	-0.777	-	-0.701	0.596	-0.306	0.868

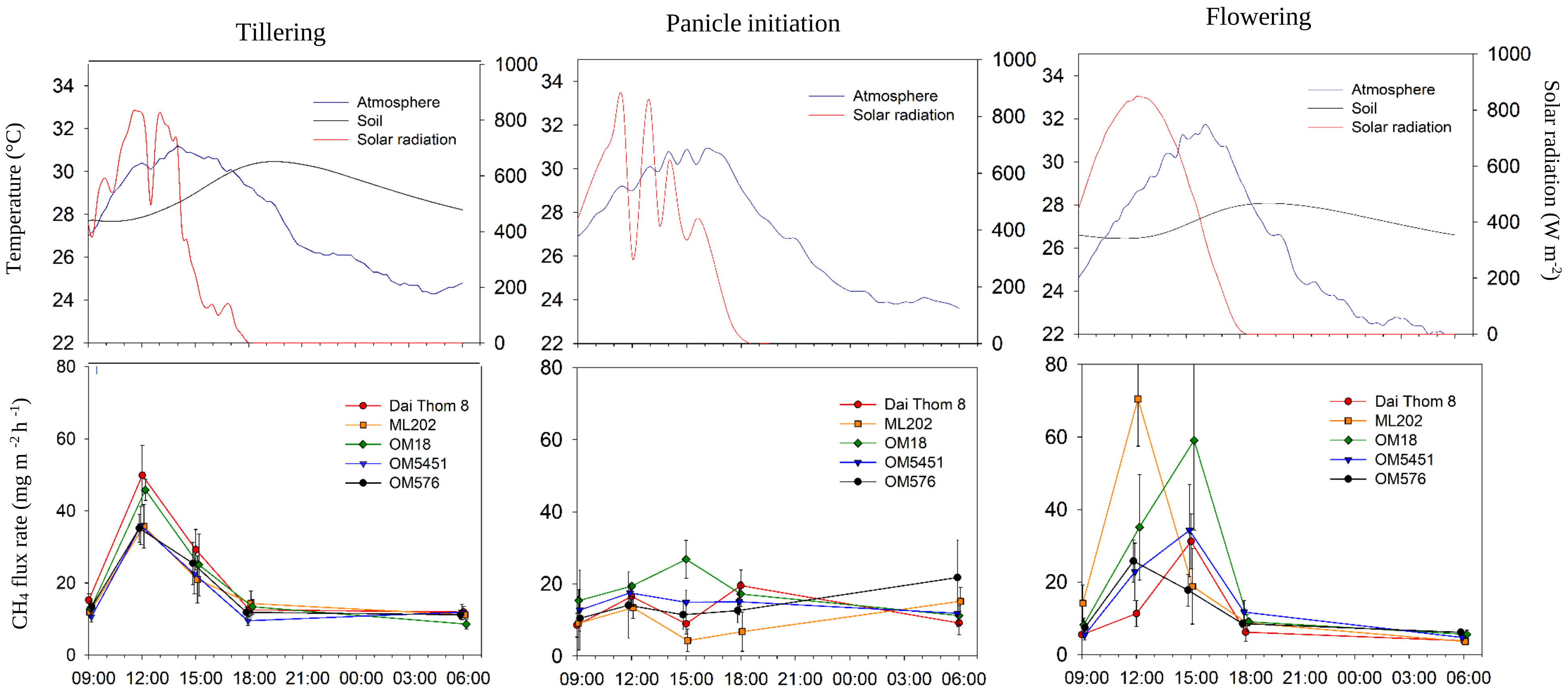

Figure 11: Diurnal change of atmospheric temperature, soil temperature at 10 to 15 cm depth, solar radiation, and the rates of CH_4 emissions for five rice varieties at tillering, panicle initiation and flowering stage. Error bars indicate standard error.

4.3 Total Daily Methane Emission

The CH_4 flux rates from single measurements were summed to daily fluxes. E_{day} (kg CH_4 ha^{-1} d^{-1}) for each variety and development stage was calculated by multiplying the average CH_4 emission rate of two sequential measurements by the number of hours of the interval. The related equation can be found above, in chapter 3.5.1. Results for E_{day} can be found in Table 5 and are illustrated in Figure 12. Mean E_{day} was calculated by averaging E_{day} values for each variety of the three developments stages and can be seen in Figure 13. E_{day} were highest for the rice cultivars Dai Thom 8, OM5451 and OM576 at tillering stage and declined with advancing plant development, showing lowest values at flowering. However, ML202 and OM18 showed a different pattern, with lowest values at panicle initiation followed by an increase in E_{day} at flowering stage. The decrease of CH_4 emission in later stages of development was particularly strong in the Dai Thom 8 rice variety. A maximum CH_4 emission of 4.66 kg CH_4 ha^{-1} d^{-1} was found at tillering stage, which then dropped to a value of 2.18 kg CH_4 ha^{-1} d^{-1} at flowering, corresponding to a reduction of about 53.22 %. The same evolution of CH_4 emission from tillering stage to flowering stage could be observed for OM5451 and OM576. Nevertheless, the decline was less pronounced with a reduction of 15.53 % and 34.43 % for OM5451 and OM576, respectively. OM18 exhibited consistently high emissions with minor variations between developmental stages, ranging from 3.82 to 4.16 kg CH_4 ha^{-1} d^{-1}. For ML202, E_{day} values were also relatively high at tillering and flowering stage though at panicle initiation CH_4 emission

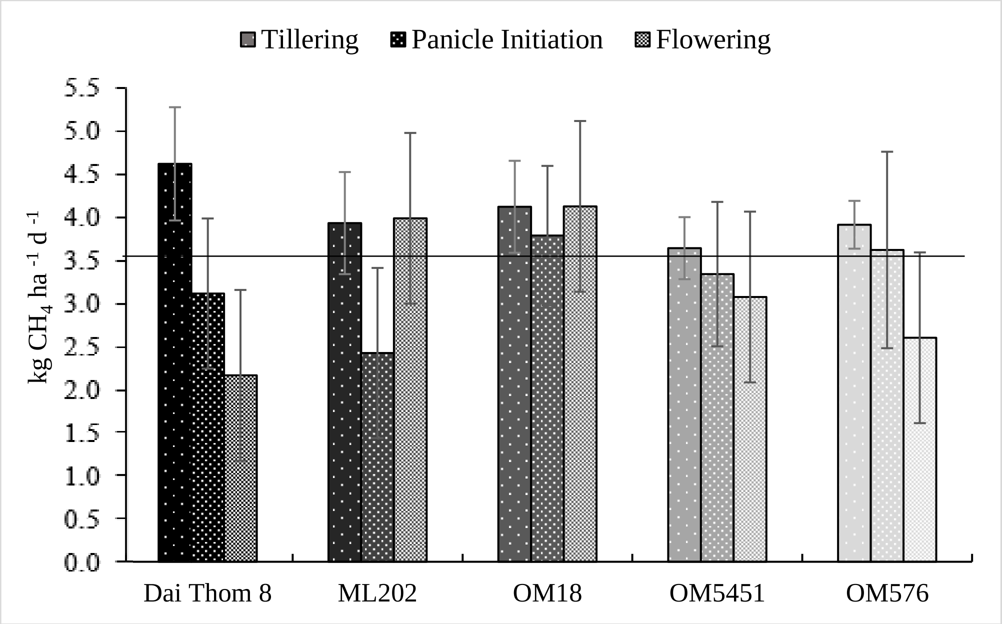

Figure 12: Total daily methane emissions (E_{day}) illustrated for five rice varieties at three development stages. Horizontal line indicates overall average (3.53 kg CH_4 h^{-1} d^{-1}) for all varieties and development stages. Error bars indicate standard error.

dropped sharply to a minimal value of 2.44 kg CH_4 ha^{-1} d^{-1}. The average E_{day} values for five varieties at tillering, panicle initiation and flowering were 4.08, 3.29 and 3.22 kg CH_4 ha^{-1} d^{-1} respectively (Table 5). It was observed that E_{day} values calculated for OM18 were constantly higher than the average CH_4 emission from the individual development stages.

Table 5: Total daily methane emission (E_{day}) for five rice varieties at tillering, panicle initiation and flowering. Average daily emissions from different development stages, Mean E_{day} and the overall average calculated from mean values of single varieties.

Variety	**Tillering**	**Panicle initiation**	**Flowering**	**Mean E_{day}**
Dai Thom 8	4.66 ± 0.66	3.14 ± 0.88	2.18 ± 0.72	3.33 ± 0.72
ML202	3.97 ± 0.60	2.44 ± 1.00	4.02 ± 0.52	3.48 ± 0.52
OM18	4.16 ± 0.54	3.82 ± 0.82	4.16 ± 0.11	4.05 ± 0.11
OM5451	3.67 ± 0.36	3.37 ± 0.85	3.10 ± 0.17	3.38 ± 0.17
OM576	3.95 ± 0.28	3.65 ± 1.15	2.62 ± 0.40	3.41 ± 0.40
Average	**4.08** ± 0.15	**3.29** ± 0.21	**3.22** ± 0.35	**3.53 ± 0.13**

No significant differences between the varieties in Mean E_{day} were found, but trends could be identified. The overall average daily emission calculated from five varieties and three different development stages was 3.53 kg CH_4 ha^{-1} d^{-1}. OM18 had the highest Mean E_{day} emission compared to the other varieties. A Mean E_{day} of 4.05 kg CH_4 ha^{-1} d^{-1} was calculated, which exceeds the least emitting variety Dai Thom 8 by about 17.8 % or 0.72 kg CH_4 ha^{-1} d^{-1} and the overall average by 12.87 % or 0.52 kg CH_4 ha^{-1} d^{-1}. OM5451 and OM576 showed remarkably similar Mean E_{day} values which differed only by 0.03 kg CH_4 ha^{-1} d^{-1}.

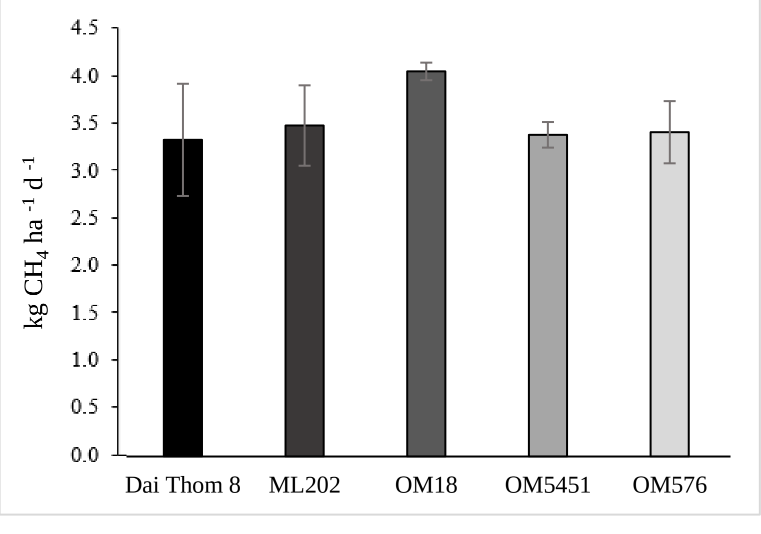

Figure 13: Mean daily methane emission (Mean $E_{day)}$ of five varieties. Error bars indicate standard error.

4.4 Physio-morphological Characteristics

4.4.1 Phenology

Phenological parameters such as the development of rice plants (onset of tillering, panicle initiation, and flowering), biomass and yield components were monitored, recorded, and provided by LTG staff. Observed crop phenology stages with corresponding dates can be seen in Table 6. No differences could be found among the varieties Dai Thom 8, ML202, OM18 and OM5451 in plant development. They reached the crop growth stages of panicle initiation, flowering, and physiological maturity at a similar date. However, the cultivar OM576 showed a slower phenological development reaching the same crop growth stages with an increasing delay of four, eight and twelve days, respectively than the other cultivars. Due to this delay, differed the development stage of OM576 from the other varieties at the time of the second and third diurnal sampling.

Table 6: Rice development stages with corresponding dates recorded by LTG staff. DAS - days after sowing. DAT - Days after transplanting.

	Panicle initiation			**Flowering**			**Physiological maturity**		
Variety	Date	DAS	DAT	Date	DAS	DAT	Date	DAS	DAT
Dai Thom 8	20-Jan-20	46	33	19-Feb-20	76	63	29-Feb-20	86	73
ML202	20-Jan-20	46	33	19-Feb-20	76	63	29-Feb-20	86	73
OM18	20-Jan-20	46	33	19-Feb-20	76	63	29-Feb-20	86	73
OM5451	20-Jan-20	46	33	18-Feb-20	75	62	28-Feb-20	85	72
OM576	24-Jan-20	50	37	27-Feb-20	84	71	12-Mar-20	98	85

Five rice hills per repetition were counted to determine the number of tillers per hill and ten plants were selected to assess tiller height in three sequential measurements, which were conducted 20, 32 and 73 DAT. The average number of tillers per hill is displayed in Table 7, whereas the average height of tillers is reflected in Table 8.

ML202 showed the highest tillering capacity in all three measurements with a maximum of about 19 tillers per hill, 32 DAT. The cultivar produced significant more tillers than Dai Thom 8, OM18 and OM5451 about 20 days after transplanting. The rice variety OM576 showed similar results, producing the second highest number of tillers per hill in all samplings. The least number of tillers were produced by the cultivar Dai Thom 8 at the first and third sampling with an average of 11.33 tillers per hill. OM18 produced, on average a lower number of tillers than most of the other varieties. However, tillers grew significant higher, reaching a peak value of 100.53 cm. Differences in size among rice varieties amplified with time and development.

In the first measurement Dai Thom 8, ML202, OM5451 and OM576 showed little difference in tiller height, whereas in the third measurement the values ranged from a minimum height of 88.73 cm (ML202) to a maximum height of 92.07 cm (OM5451) for the varieties listed.

Table 7: Average number of tillers per hill. DAT – Days after transplanting. S.E. – Standard Error.

Date	**09-Jan-2020 20 DAT**		**21-Jan-2020 32 DAT**		**02-Mar-2020 73 DAT**	
Variety	Mean	S.E.	Mean	S.E.	Mean	S.E.
Dai Thom 8	11.33^{a}	0.72	16.00^{ac}	0.73	11.27^{a}	0.21
ML202	15.13^{b}	1.01	18.87^{bd}	0.82	13.60^{bd}	0.27
OM18	12.07^{a}	0.73	14.73^{a}	0.71	12.27^{a}	0.38
OM5451	12.13^{a}	0.99	15.27^{a}	0.56	12.87^{abcd}	0.34
OM576	14.60^{b}	0.83	18.20^{bcd}	1.05	13.00^{ad}	0.45

Different letters ($^{a-d}$) indicate significant ($p < 0.05$) differences between cultivars.

Table 8: Average tiller height of five rice varieties in centimetres. S.E. – Standard Error

Date	**09-Jan-2020 20 DAT**		**21-Jan-2020 32 DAT**		**02-Mar-2020 73 DAT**	
Variety	Mean	S.E.	Mean	S.E.	Mean	S.E.
Dai Thom 8	40.60^{a}	0.45	59.02^{a}	0.53	91.83^{a}	0.63
ML202	39.33^{a}	0.45	57.40^{a}	0.87	88.73^{b}	0.61
OM18	43.69^{b}	0.49	63.87^{b}	0.43	100.53^{c}	0.50
OM5451	39.82^{a}	0.39	56.93^{a}	0.43	92.07^{a}	0.62
OM576	39.73^{a}	0.50	63.68^{b}	0.44	91.18^{a}	0.67

Different letters ($^{a-d}$) indicate significant ($p < 0.05$) differences between cultivars.

4.4.2 Leaf Area Index

Leaf area index was acquired with an LAI 2000 Plant canopy analyser. The first three measurements were carried out at the same days as diurnal GHG samplings were conducted. Two additional LAI measurements were performed 78 DAT and 89 DAT. Figure 14 shows the temporal trend of the average LAI for five rice varieties. Corresponding LAI values are displayed in Table 9.

LAI values ranged between 1.07 m^{2} m^{-2} (Dai Thom 8) and 1.36 m^{2} m^{-2} (OM18) at tillering stage (19 DAT) and 2.13 m^{2} m^{-2} (Dai Thom 8) and 2.48 m^{2} m^{-2} (OM576) at panicle initiation (32 DAT); showing only small variations between varieties. Differences within the leaf area index of rice varieties became more distinct at flowering stage about 62 days after transplanting. The leaf area index of Dai Thom 8 and OM18 increased steeply, reaching maximum values of 4.25 m^{2} m^{-2} and 4.12 m^{2} m^{-2} at flowering, whereby ML202 and OM5451 exhibited peak values of about 3.40 m^2 m^{-2}, which is about 18 % less. A decrease in LAI was observed in four varieties at grain filling and physiological maturity. However, the LAI of OM576 continued to increase, reaching a maximum value of 4.14 m^2 m^{-2} at the time of grain filling about 78 days after transplanting. The plant development stage of OM576 did not correspond to the development stages of the other varieties at the time of measurement, which could explain the delayed peak in leaf area index.

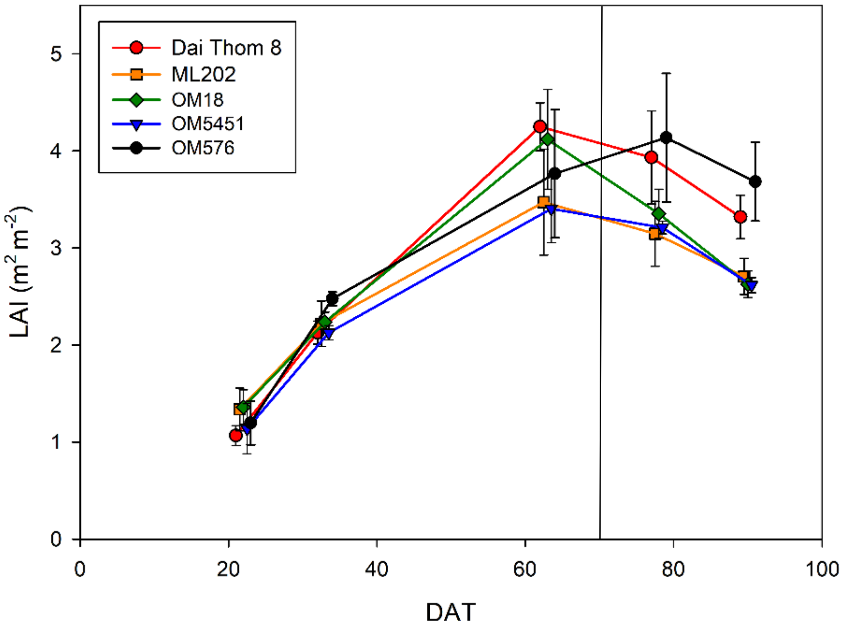

Figure 14: Leaf area index of five different rice varieties. Vertical line separates additional measurements, which were not associated with diurnal samplings Bars indicate standard error. DAT – Days after transplanting

Table 9: Leaf area index (m^2 m^{-2}). DAT - Day after transplanting

Variety	**Tillering** (19 DAT)	**Panicle initiation** (32 DAT)	**Flowering** (62 DAT)	**Grain filling** (78 DAT)	**Maturity** (89 DAT)
Dai Thom 8	1.07 ± 0.10	2.13 ± 0.12	4.25 ± 0.43	3.93 ± 0.48	3.32 ± 0.23
ML202	1.34 ± 0.22	2.22 ± 0.24	3.47 ± 0.54	3.15 ± 0.33	2.71 ± 0.19
OM18	1.36 ± 0.18	2.24 ± 0.10	4.12 ± 0.51	3.35 ± 0.25	2.63 ± 0.14
OM5451	1.14 ± 0.26	2.13 ± 0.07	3.40 ± 0.35	3.21 ± 0.06	2.62 ± 0.08
OM576	1.20 ± 0.13	2.48 ±0.04	3.77 ± 0.38	4.14 ± 0.33	3.68 ± 0.23

Number and height of rice tillers, and LAI were correlated with E_{day} at three development stages. Correlation coefficients are displayed in Table 10. LAI and tiller height of Dai Thom 8, OM5451 and OM576 as well as tiller number of ML202 and OM18 were negatively correlated with CH_4 emissions. Only a poor, positive correlation between plant height and LAI and CH_4 fluxes for ML202 and OM18 were found.

Table 10: Correlation between selected plant parameters and methane emission of cultivars.

Variety	No. of tillers	Plant height	Leaf area index
Dai Thom 8	-0.118	-0.958	-0.949
ML202	-0.967	0.181	0.127
OM18	-0.998	0.165	0.205
OM5451	-0.255	-0.974	-0.995
OM576	0.574	-0.965	-0.954

5 Discussion

5.1 Climatic Effects on Diurnal Emission Pattern

Methane emissions of five contrasting rice cultivars under SSC measured over a 24-hr period showed a distinct diurnal emission pattern with a single peak at the early afternoon at tillering and flowering stage. The rates of CH_4 fluxes increased from about 6:00 in the morning, exhibiting a maximum at 12:00 – 15:00, followed by a decline until sunset. This type of diurnal pattern closely resembled the trend of atmospheric temperature and solar radiation, which were also monitored. Statistical correlations between diurnal CH_4 fluxes and Ta as well as Sr differed with respect to the development stage and rice variety. The correlation coefficients between CH_4 emission rate and T_a and Sr was high for all varieties at tillering stage, whereby at flowering ML202 and OM576 showed a higher correlation with Sr than T_a and at panicle initiation, the correlation coefficients were low or negative for most varieties. Generally, the rates of CH_4 emissions followed the trend of solar radiation and atmospheric temperature. A possible explanation for this coherence might be an increase in photosynthetic activity during the day.

Sass *et al.* (1991) postulated that plant photosynthesis, which is directly associated with solar radiation, has the potential to affect CH_4 production. During plant photosynthesis, light energy transfers electrons from water to carbon dioxide, to produce carbohydrates and O_2. On average, 30 to 60 % of this photosynthetically synthesized organic C is allocated to the roots, and a considerable part of this is released into the rhizosphere (Clarkson, 1996). This rhizodeposition constitutes a major source of OM input into the soil (Kimura *et al.*, 2004). Since methanogens drive only on a limited number of substrates such as acetate or H_2 which are products of the microbial degradation of organic matter (Conrad, 2002), the input of OM into the soil via rhizodeposition represents a key element of CH_4 production (Minamikawa *et al.*, 2015b). Parts of this photosynthesized carbon may serve immediately (e.g., 2-3h after C incorporation into the plant) as root exudates for methanogens (Kimura, 1997; Aulakh *et al.*, 2001b) leading to an increase in CH_4 production during the day. Solar radiation could also have a substantial indirect effect on diurnal variation in CH_4 emission via controlling T_a and $T_{s,}$ which also demonstrated a strong diurnal pattern. Temperature affects microbial activity and the growth of microbial populations.

Consequently, an increase in temperature may result in a response of CH_4 production (Conrad, 2002). Holzapfel-Pschorn & Seiler (1986) reported that CH_4 release rates in Italian rice paddies showed diurnal variations, with highest values at the late afternoon which coincided with the temperature variation in the upper soil layer. Methane flux doubled as temperature increased from 20 to 25 °C in 5 cm soil depth. The temperature dependency of CH_4 production rates is often described by the Arrhenius equation, (Schütz *et al.*, 1990) which is a remarkably accurate, formula specifying the relation between the rate of reaction and temperature (Ashter, 2014). However, changes in T_a and T_s not only influence biological processes. They can also physically affect the rate of diffusive CH_4 transport. Diffusion is a process of passive transport in which molecules move from an area of higher concentration to one of lower concentration. Higher temperatures increase the energy and therefore, the movement of the molecules. (Jacobs, 1967). This leads to the assumption that an increase of T_a and T_s during the day, also increases the diffusion-controlled transport of CH_4 through the ventilation system of rice plants (Denier van der Gon, & van Breemen, 1993).

Measurements showed that the pattern of diurnal CH_4 emission was more closely correlated with the pattern of T_a rather than of $T_{s.}$ The fluctuation of T_s was lower than T_a, reaching maximum values about 5 to 7 hours after CH_4 emission peaks. These results are in agreement with findings from Holzapfel-Pschorn & Seiler (1986). They found that daily CH_4 release rates from rice paddies in Vercelli, Italy correlated well with the soil temperature at 1 to 10 cm depth but did not show any correlation with soil temperature at 15-cm depth. Besides, Schütz *et al.* (1990) reported on decreasing diurnal amplitudes and increasing delays of maximum temperatures with increasing soil depth. In this study, soil temperature measurements were conducted in a depth of about 10 to15 cm, which could be the reason for low correlations between T_s and CH_4 emissions.

With the findings presented, the first working hypothesis can be partially confirmed. Methane emissions from rice paddies under tropical conditions showed distinct diurnal patterns with maximum emissions at the early afternoon at tillering and flowering stage. It has also been shown that the emission pattern was influenced by atmospheric temperature and solar radiation rather than by soil temperature. However, the emission pattern at panicle initiation differed from the pattern of the other development stages. Lower emission ratios and a variable distribution and number of peak emissions were observed. In addition, climate parameters correlated only poorly with CH_4 emission rates. Even though a “typical” pattern, has been described by the majority of the scientist (Satpathy *et al.*, 1997; Yang, 1999), a few studies report on night and early morning peaks (Wang *et al.*, 1990), afternoon – night, double peaks or random peaks (Wang & Li, 2002). These divergent results indicate that diurnal fluctuations in CH_4 emissions are not only linked to climatic parameters but are instead controlled by complex mechanism involving interactions between abiotic and biotic factors which are in turn also influenced by plant physio-morphological characteristics. The level of fluctuation in diurnal CH_4 emission rates at different growth stages is an indication of the needed frequency of flux measurements to accurately determine seasonal CH_4 emissions. Therefore, diurnal samplings are an important tool to identify the optimum time of a day to avoid CH_4 emission extremes, which would result in the under- or overestimation in extrapolated daily CH_4 emission.

5.2 Effect of Rice Varieties on Methane Emissions

Five rice varieties were analysed, which differed in their physio-morphological characteristics (No. of tiller, tiller height, yield), duration, quality, and other aspects. As displayed in Figure 13, the OM18 rice variety tended to emit larger quantities of CH_4 per day than the other varieties. An average emission (Mean E_{day}) of 4.05 ± 0.11kg CH_4 ha^{-1} d^{-1} was calculated, which exceeded the least emitting variety Dai Thom 8 by about 17.8 % or 0.72 kg CH_4 ha^{-1} d^{-1} and the overall average by 12.87 % or 0.52 kg CH_4 ha^{-1} d^{-1}.

Plant characteristics play a crucial role in the production, oxidation, and transport of CH_4. Up to 90 % of CH_4 is released by diffusive transport through the plant's aerenchyma from rice fields to the atmosphere (Schütz *et al.*, 1989a). The potential or capacity of rice plants to transport CH_4 from the root to the atmosphere depends on several physio-morphological properties. These include plant size and its growth stage, amount/density and characteristics of the aerenchyma and plant biomass (Aulakh *et al.*, 2001c). To interpret varietal variations in diurnal CH_4 emissions, rice physio-morphological characteristics were recorded and with CH_4 emissions rates correlated. In all varieties, it was observed that plant height increased with every measurement whereby plant tiller number increased up to panicle initiation and declined thereafter.

Various, often contradictory results can be found in the literature regarding the relationship between biomass and CH_4 emissions. Several scientists report on a positive correlation between above-ground biomass and CH_4 emission rates (Singh *et al.*, 1998; Aulakh *et al.*, 2000a; Setyanto *et al.*, 2016). For example, Gogoi *et al.* (2005) related the increase in CH_4 emission with advancing crop growth stage to the increase in leaf number and LAI. Singh *et al.* (1998) indicated that there was a significant positive correlation of CH_4 emission with shoot height and weight; the number of tillers, and root weight and volume (r^2 ranged from 0.582 to 0.947, $P < 0.001$). A positive relationship between the number of tillers and CH_4 emission has also been shown by Neue & Sass (1994). This might demonstrate the importance of the availability of aerenchyma channels through which CH_4 emission is affected. However, while studying the effect of rice cultivars on CH_4 emission, Mitra *et al.* (1999) certainly found a significant variation in the number of tillers, but no relationship with CH_4 emission could be established. Also, Watanabe *et al.* (1995) concluded, that neither the number of tillers nor the shoot length, shoot weight and root weight were correlated with CH_4 emission rates at tillering or reproductive growth stages.

In the present study, there was a significant difference in the number of plant tillers and plant size among varieties, but no statistically positive correlations between plant growth parameters and CH_4 emissions were found. However, a certain relationship can be recognised. On average, Dai Thom 8 emitted the least amount of CH_4 per day (3.33 ± 0.72 kg ha^{-1} d^{-1}) and also exhibited the lowest No. of tillers at tillering and flowering stage and the lowest LAI at tillering and panicle initiation. OM18 was found to emit on average, the highest quantity of CH_4 per day (4.05 ± 0.11 kg ha^{-1} d^{-1}). It also produced significantly larger tillers and exhibited a comparable high LAI at all three development stages. A similar relation between plant height and CH_4 emissions is described by Lindau *et al.* (1995) and Ding *et al.* (1999). Plant height may influence the ability to transport CH_4 through the aerenchyma of rice plants (Setyanto *et al.*, 2016). As described above, plant-mediated transport of CH_4 through the aerenchyma system of rice plants is known to be the primary route of CH_4 from the soil to the atmosphere. Therefore, differences in anatomical characteristics of the aerenchyma could affect the amount of CH_4 emitted and explain differences among *Oryza sativa* varieties. Das & Baruah (2008b) for example, recorded significant positive correlations between the size of the medullary cavities and the CH_4 emission. They compared ten different cultivars, from which five were traditional rice genotypes and five were improved high-yielding varieties. The larger sized medullary cavities in high- and medium-CH_4-emitting cultivars may increase the cross-sectional area of the CH_4 diffusion pathway, which increased CH_4 emissions. Additionally, the relationship is based on the assumption, that plant height represents an indicator of the level of biological activity of plants and as a consequence also of the level of the substrate (root exudates, size of the root system, above-ground biomass) which is available for CH_4 production.

The quantity and quality (composition) of substrate in the form of organic C-compounds released into the rhizosphere, varies greatly among varieties and is also influenced by the developmental stage of the rice plant. Organic acids in root exudates provide energy to microbial communities, including methanogens for the production of CH_4. Aulakh *et al.*

(2001b) investigated the impact of root exudates from five different rice varieties collected at different development stages on CH_4 production. They found that a high above-ground biomass was associated with a large root system which increased the methanogenic source strength of the soil. Aulakh *et al.* (2001a) characterized root exudates at different growth stages of ten rice varieties. They also reported on a positive correlation between the root and shoot biomass with carbon exudation, suggesting that it is driven by plant biomass. Ladha *et al.* (1986) demonstrated a wide variation among rice cultivars in the amount of carbohydrate material lost from rice roots. These observations implicate that variations among cultivars in their ability to emit CH_4 cannot solely be attributed to CH_4 transport properties. Also, C-exudation rates and oxidation processes must be taken into account. Based on varietal differences in the amount of daily methane emissions and emissions pattern which were demonstrated in this study, the third working hypothesis can be confirmed.

The selection of existing or breeding of new, low emitting rice cultivars, could offer an important CH_4 mitigation option which also represents an easily implementable strategy for local farmers as long as yields are not compromised. The combination of different agricultural practices such as water management and variety selection could even increase the mitigation potential while decreasing the amount of freshwater use.

5.3 Effect of the Plant Development Stage on Methane Emissions

Diurnal measurements were conducted at three development stages. The first sampling was carried out at the tillering stage in the vegetative phase of rice development, 18 to 19 DAT. The second, at panicle initiation, which represents the first stage in the reproductive phase of growth and the third diurnal sampling was carried out at the flowering stage about 62 DAT. It was observed that E_{day} values differed not only among varieties but also between development stages. Methane emission decreased in three rice varieties (Dai Thom 8, OM5451 and OM576), with advancing development stage. This decline could be attributed to an age-related change in plant morphological characteristics. For example, it has been suggested that the permeability of the root epidermal layer is reduced by ageing. This could negatively affect the diffusion of CH_4 from the anaerobic parts of the soil through the roots into the plant (Armstrong, 1971). Additionally, the aerenchyma system which determines the methane transport capacity of rice plants, changes with advancing development stage. Aulakh *et al.* (2000c) demonstrated that the amount and density of large aerenchyma spaces, which exhibit highly significant correlations with MTC, differed between rice varieties and was altered by ageing. In their study, three rice varieties were analysed. The tall rice cultivar Dular showed the largest amount of aerenchyma lacunae and consequently the highest methane transport capacity compared to the other varieties. Furthermore, they found that the amount and density of aerenchyma lacunae decreased with increasing development stage, which resulted in a decline in CH_4 emissions. With increasing age, aerenchyma lacunae appeared to collapse, resulting in a blockage of aerenchyma channels. These results suggest that the age or growth stage of rice affects CH_4 transport through the rice plant and consequently, the quantity of CH_4 emission.

Also, development stage related differences in the amount of excreted methanogenic substrate, which induced CH_4 production were described. Aulakh *et al.* (2001b) reported that plant-derived organic C produced 3 – 4-fold greater amounts of CH_4 during panicle initiation to flowering as compared to the seedling stage (early tillering stage). Aulakh *et al.* (2001a) also observed a general, increase in exudation rates, with plant development from seedling to panicle initiation or flowering but decrease at maturity. These findings do not precisely agree with results from the presented study, since in this study CH_4 emissions were highest at tillering and decreased with plant development in three varieties or showed a decline at panicle initiation followed by a rise at flowering. A large amount of CH_4 recorded at active tillering stage may be attributed to ebullition because the root system was not yet well established (Schütz *et al.*, 1989b). Ebullition is a more rapid process than the other gas-escape processes; CH_4 escaping in bubbles is not being oxidized at the soil floodwater interface or soil-root interface (van der Gon & Neue, 1995). The contribution of ebullition decreased from 90 % to 10 % with the progress of the season. The importance of plant-mediated transport shows a reverse pattern, i.e., from none at transplanting to almost 100 % with increasing plant growth (Wassmann *et al.*, 1996). If CH_4 production in a rice field is large during the early season, the contribution of ebullition to the total emission could be very high. It was also observed that the soil temperature amplitude and maximal temperatures decreased during the season. The temperature ranged at the tillering stage from 27.7 °C to 30.5 °C and at flowering from 26.4 °C to 28.1 °C. A possible reason could be the increased shading effect of the fully developed rice plant canopy. This decrease in soil temperature might also affect the CH_4 production leading to a decrease in CH_4 emission at flowering stage.

Finally, the comparison of CH_4 emissions at different stages of development showed that emissions decreased from tillering to panicle initiation in all varieties, with E_{day} being lowest for ML202 and OM18 at panicle initiation. Similar results have been found by van der Gon & Neue (1995). They calculated the percentage of the cumulative potential CH_4 production that is emitted. Calculations revealed that only a small fraction of CH_4 produced was emitted at panicle initiation. Differences between CH_4 production rates and the amount released into the atmosphere generally reflect the CH_4 oxidation by methanotrophic bacteria, since oxidation is the main sink for CH_4 in a rice field (Conrad & Rothfuss, 1991). Therefore, they suggested a high CH_4 oxidation rate at this growth stage. Seasonal variation in CH_4 oxidation rates may be an additional clue to explain patterns in CH_4 emission. However, additional experiments with direct measurements of CH_4 oxidation are necessary to confirm this hypothesis.

6 Conclusion

In the present study, distinct diurnal patterns could be identified for all varieties at tillering and flowering stage. Methane emission rates increased with accelerating rates in the morning, reached a maximum between 12:00 and 15:00 then decreased rapidly and levelled off during the night. Methane emission rates were related to climate parameters to elucidate the underlying mechanism. Diurnal CH_4 emission patterns correlated well with Sr and T_a at tillering and flowering stage. This was attributed to the increase in photosynthetic activity that might have increased excreted organic C compounds and the temperature response of the CH_4 producing microbial communities. T_s correlated very poorly or negatively with diurnal CH_4 emissions, which might be explained by the effect of measured soil depth. However, at panicle initiation, untypical CH_4 emission patterns were found which varied strongly between varieties, exhibiting double emission peaks, or increased CH_4 emission rates at the early morning. It can be assumed that not only environmental variables but also the combination of many other abiotic and biotic factors cause considerable variation in the diurnal pattern of CH_4 emission.

It was shown that rice variety and development stage also determine the amount and pattern of diurnal CH_4 emission. The quantity of daily CH_4 emission decreased from tillering to panicle initiation in all five rice varieties, followed by a further decline (Dai Thom 8, OM5451, OM576) or increase (ML202, OM18) at flowering stage. The underlying mechanism could not exactly be specified. However, it is suggested that age-related changes of aerenchyma lacunae or a decrease in root conductance influenced the methane transport capacity of rice plants. Moreover, differences in ebullition rates and soil temperature variations might also affected CH_4 emission. Among the five, analysed rice varieties, OM18 was found to emit the highest amount of CH_4 which exceeded the least emitting variety Dai Thom 8 by about 17.8 % or 0.72 kg CH_4 ha^{-1} d^{-1}. Morphophysiological characteristics of a genotype seem to affect the CH_4 emission rate from the rice plant. The identification and breeding of high yielding but low emitting rice varieties in combination with appropriate water management is a promising strategy to meet rising global food demands and combat climate change.

To evaluate the results presented in this study, it would be advisable to repeat the experiment with a higher number of repetitions to increase experimental and statistical precision. Morphophysiological properties such as aerenchyma characteristics or root exudation rates could also be investigated to gain more profound insights into the relationship with CH_4 emissions. Additional diurnal measurements for example at rice maturity would increase comparability with existing field studies. Data obtained in this study, which was an integral part of a larger assessment, might be helpful to correct regional emission factors to reflect the effects of diurnal variations and improve the accuracy of CH_4 extrapolations.

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