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Diurnal Methane Fluxes as Affected by Cultivar from Direct-Seeded, Delayed-Flood Rice Production

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Abstract

Methane (CH_4) emissions are known to differ between rice (*Oryza sativa* L.) cultivars, where CH_4 emissions from pure-line cultivars are often greater than from hybrids. Numerous field studies have shown that CH_4 emissions follow a diurnal pattern, typically reaching their maximum during afternoon hours. However, it is unknown whether cultivar affects CH_4 fluxes/emissions at various measurement times of day or how those cultivar effects may differ spatially across soil textures and temporally throughout the rice growing season. The objective of this field study was to evaluate the effects of time of day (300, 800, 1200, 1800, and 2300 hours) and cultivar (one hybrid and one pure-line) on CH_4 fluxes before and after heading from a silt-loam and clay soil in a direct-seeded, delayed-flood rice production system. Enclosed headspace chambers, 30 cm in diameter, were used for CH_4 gas sampling on 22 July and 19 August at a silt-loam site and on 29 July and 26 August, 2014 at a clay-soil site in the Lower Mississippi River delta region of eastern Arkansas. Methane fluxes measured pre- and post-heading ranged from 0.7 to 2.2 mg CH_4-C $m^{-2}\cdot hr^{-1}$ from the clay soil and from 2 to 7 mg CH_4-C $m^{-2}\cdot hr^{-1}$ from the silt-loam soil. Hourly CH_4 fluxes and estimated daily emissions differed among measurement times of day ($P < 0.05$) for a given cultivar or averaged across cultivars and differed between cultivars ($P < 0.05$) from the silt-loam soil, but not the clay soil. Results suggested that the optimum measurement time of day to capture either minimum, maximum, or average hourly CH_4 flux or daily emissions for a given day differs by soil texture and rice growth stage, but conducting CH_4 flux measurements around late morning to mid-day appear to be optimum to best capture the mean CH_4 emissions for the day.

Keywords

Methane Flux, Methane Emissions, Rice, Arkansas, Hybrid, Pure-Line, Silt Loam, Clay

1. Introduction

Measurement time of day is important for attaining the most accurate estimations of seasonal and/or annual methane (CH_4) emissions [1]. Consequently, temporally scaled CH_4 emissions may be under- or over-estimated depending on the time of day in-field CH_4 flux measurements are conducted. However, a reasonable balance must also be achieved between accuracy and practicality for conducting in-field CH_4 flux measurements for systematic research purposes.

As with many biologically mediated processes, CH_4 emissions from flooded soils under rice (*Oryza sativa* L.) production have been suggested to follow a diel pattern controlled by diurnal soil temperature fluctuations [2] [3] and/or gross ecosystem photosynthesis [4] [5]. However, there is some inconsistency for the time of day when daily peak or average CH_4 emissions occur. Most studies have reported peak emissions during the daytime, either in late morning to early afternoon [6] [7] [8] [9] or during mid- to late afternoon [2] [4] [7] [10] [11] [12] [13]. During the night and early morning have generally been reported as the times of day with the lowest CH_4 fluxes/emissions [6] [7] [8] [11] [13]. Previous reports indicate diurnal variations (*i.e.*, the amplitude and timing of flux minima and maxima) in CH_4 emissions may also differ over time within the growing season [8] [10] [12]. However, numerous studies have also reported no significant difference in CH_4 emissions between day and night [6] [14] [15].

Differences between cultivars have generally been consistent given the multitude of factors that have been shown to affect CH_4 fluxes and emissions throughout a growing season, such as soil texture [16] [17] [18] [19], fertilizer nutrient source [20], organic soil amendments [8] [12], residue management/previous crop [21] [22] [23] [24], water management scheme [8] [25] [26], and production system [27]. Typically, hybrid rice cultivars have lower season-long CH_4 emissions than do pure-line cultivars [23] [24] [28] [29].

Though cultivar effects on season-long CH_4 emissions are generally well-understood, it is unknown how cultivar may affect CH_4 fluxes/emissions at different measurement times of day. It is also unknown how potential cultivar effects on diurnal CH_4 emissions may interact with soil texture and/or rice growth stage. Furthermore, as many of the previous studies have been conducted decades ago, in-field methodological and analytical laboratory measurement advancements have subsequently been made, which makes revisiting the issue of diel CH_4 emissions warranted, particularly in the direct-seeded, delayed-flood rice production system for which no known previous diel emissions studies have been conducted. Therefore, the objective of this field study was to evaluate the effects of time of day and cultivar on CH_4 fluxes before and after heading on a silt-loam and clay soil in a direct-seeded, delayed-flood rice production system in Arkansas. It was hypothesized that, similar to other physiological plant-related processes, CH_4 fluxes would be greater during the day than during the night. Based on past reports of lower season-long CH_4 emissions from hybrid compared to pure-line cultivars, it was also hypothesized that CH_4 fluxes from a hybrid would generally be lower than from a pure-line cultivar regardless of measurement time of day over a 24-hr period.

2. Materials & Methods

2.1. Site Description

Research was conducted during the 2014 growing season on a silt-loam soil at the University of Arkansas System Division of Agriculture Rice Research and Extension Center (RREC) near Stuttgart in Arkansas County, Arkansas (34˚27'N, 91˚24'W) and on a clay soil at the Northeast Research and Extension Center (NEREC) at Keiser in Mississippi County, Arkansas (35˚40'N, 90˚05'W). At RREC, study plots were located on a Dewitt silt loam (fine, smectitic, thermic Typic Albaqualfs), while plots were located on a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) [30] at NEREC. The study site at RREC is located in the northern portion of major land resource area (MLRA) 131D, the Southern Mississippi River Terraces, while the study area at NEREC is located in MLRA 131A, the Southern Mississippi River Alluvium [31]. Mean annual precipitation is 124 and 126 cm and the mean annual air temperature is 16.7 and 15.5˚C at RREC and NEREC, respectively [32]. Both research locations reside in major rice-producing regions in Arkansas and the study areas at both locations have been in an annual soybean [*Glycine max* L. (Merr.)]-rice rotation for more than 20 years with crop residues incorporated between growing seasons into the top 15 cm of the soil.

2.2. Treatments and Experimental Design

Two common rice cultivars, one hybrid and one pure-line, were selected for field study. The hybrid cultivar CLXL745 (RiceTec, Inc., Houston, TX) is a short-season cultivar that heads at 77 days after emergence [33] and accounted for 22.0% of the total Arkansas rice production in 2014 [34]. The pure-line cultivar Roy J is a mid-season cultivar developed at the University of Arkansas [35] that heads at 85 days after emergence and accounted for 12.6% of the total Arkansas rice production in 2014 [34]. Both cultivars produce long-grain rice and are considered high-yielding cultivars with CLXL745 and Roy J yields averaging 10.0 and 9.9 $Mg \cdot ha^{-1}$, respectively, across numerous locations in the 2014 Arkansas Rice Performance Trials [33].

At both locations, a randomized complete block (RCB) design was established with four replicates of each cultivar. As described in more detail below, the five CH_4 flux measurements that were conducted over a 24-hr period on two different dates, representing different rice growth stages, were treated as a repeated measure.

2.3. Plot Management

Field plots, 1.6-m wide by 5-m long that encompassed nine rows of rice, were established in early March 2014 and managed throughout the rice growing season in accordance with University of Arkansas Cooperative Extension Service

(UACES) guidelines [36]. On 26 March 2014 at RREC (*i.e.*, the silt-loam-soil location), phosphorus (P) and potassium (K), 100 kg·ha^{-1} of each, and 11.2 kg·ha^{-1} of zinc (Zn) were manually surface-applied and tilled into the top 10 to 15 cm of soil throughout the study area as per soil-test recommendations. Soil-test results indicated that the study area at NEREC (*i.e.*, the clay-soil location) did not require any P, K, or Zn additions for optimal rice production. On 5 May at RREC and 7 May at NEREC, each plot was independently drill-seeded at an 18-cm row spacing with the pure-line cultivar Roy J at a seeding rate of 82 and 102 kg·ha^{-1} at RREC and NEREC, respectively, or the hybrid cultivar CLXL745 at a seeding rate of 34 kg·ha^{-1}. Clay soils require a greater seeding rate than silt loams and hybrids have lower seeding rates due to an increased capacity to tiller compared to pure-line cultivars [36]. After planting at both locations, levees were constructed around each study area to contain the permanent flood. On 16 June at RREC and on 18 June at NEREC, based on UACES guidelines at the time, N was applied as urea (46% N) in a split application, where Roy J received 100 and 135 kg N ha^{-1} and CLXL745 received 135 and 168 kg N ha^{-1} at RREC and NEREC, respectively, as the first application [37]. On 17 June at RREC and on 20 June at NEREC, at the 4- to 5-leaf stage, the permanent flood was established and maintained at an approximate depth of 10 cm throughout the rice growing season until draining. The split application of N occurred at the beginning of internode elongation on 10 and 17 July 2014 at RREC and NEREC, respectively, for Roy J (50 kg N ha^{-1}) and at the booting growth stage on 23 July and 4 August 2014 at RREC and NEREC, respectively, for the hybrid (33 kg N ha^{-1}). According to UACES guidelines [38] [39], insects and weeds were managed in all field plots throughout the season to remain below yield-affecting threshold levels of pests.

2.4. Soil Sampling

One set of five soil cores was collected prior to N fertilization and flooding from the top 10 cm in each plot using a 2-cm-diameter push probe and combined into one sample per plot. Samples were dried in a forced-draft oven at 70˚C for 48 hr, crushed, and sieved through a 2-mm mesh screen for soil chemical property determinations. Soil electrical conductivity (EC) and pH were determined potentiometrically on a 1:2 (mass:volume) soil-to-solution mixture. Soil organic matter (SOM) concentration was determined by weight-loss-on-ignition at 360˚C for 2 hr. Total C (TC) and N (TN) concentration were determined by high-temperature combustion (VarioMax CN analyzer, Elementar Americas Inc., Mt. Laurel, NJ). Total C and TN concentrations were used to calculate the soil C:N ratio. Mehlich-3 extractable nutrient (*i.e.*, P, K, Ca, Mg, Fe, Mn, Na, S, Cu, and Zn) concentrations [40] were measured by inductively coupled plasma atomic emission spectroscopy (Spectro Arcos ICP, Spectro Analytical Instruments, Kleve, Germany).

A second set of samples was collected from the top 10 cm using a slide hammer and 4.7-cm-diameter core chamber with a beveled core tip, dried at 70˚C for 48 hr, weighed for bulk density determinations, and ground to pass through a 2-mm mesh screen for particle-size analysis using a modified 12-hr hydrometer method [41]. Measured soil nutrient concentrations ($mg \cdot kg^{-1}$) and measured bulk densities from the top 10 cm were used to calculate and report nutrient contents ($kg \cdot ha^{-1}$ or $Mg\ ha^{-1}$).

2.5. Methane Gas Sampling

Methane fluxes were measured in all replications of both cultivars at 300, 800, 1200, 1800, and 2300 hours once before heading (*i.e.*, a vegetative growth stage; 22 and 29 July 2014 for RREC and NEREC, respectively) and once after heading (*i.e.*, a reproductive growth stage; 19 and 26 August 2014 for RREC and NEREC, respectively) using 30-cm-diameter enclosed headspace chambers [18] [19] [23] [24] [42] [43]. Elevated boardwalks were erected for minimally disturbing access into each plot prior to flooding. Polyvinyl chloride (PVC) base collars, 20 cm tall with 30-cm inside diameter (ID), were inserted approximately 10 cm deep in each plot immediately after flood establishment. To maintain atmospheric pressure during sampling, chamber caps, constructed from 30-cm-diameter PVC, were outfitted with 15-cm-long section of 4.5-mm ID copper tubing as a vent. Chamber caps were also outfitted with sealed, gray butyl-rubber septa (Voight Global, part number 73828A-RB, Lawrence, KS) for gas sampling and chamber temperature measurement ports. A 2.5-cm-diameter, 9V-battery-operated fan (Sunon Inc., MagLev, Brea, CA), mounted on the underside of each chamber cap, mixed the headspace air within the chamber throughout the duration of chamber closure.

At each diurnal measurement sampling time, chamber headspace gas samples were collected at 0, 20, 40, and 60 minutes after cap closure. Gas samples were extracted using 20-mL B-D syringes (Becton Dickinson and Co., Franklin Lakes, NJ) and injected into pre-evacuated 10-mL, crimp-top glass vials (part number 5182 - 0838, Agilent Technologies, Santa Clara, CA). All gas samples were analyzed within two days using a gas chromatograph (Agilent 6890-N, Agilent Technologies) with a flame ionization detector. As outlined by Parkin and Venterea [43] and used in several recent studies [18] [19] [23] [24], CH_4 fluxes were calculated based on the change in headspace CH_4 concentration over time on a chamber-by-chamber basis. Additional details of the gas sampling and analysis procedures have been described in previous studies [18] [19] [23] [24]. Daily CH_4 emissions were calculated independently for each of the CH_4 fluxes for statistical analyses. In addition, daily CH_4 emissions were calculated for statistical analyses from linear interpolation between all five CH_4 flux measurements, referred to hereafter on each measurement date as the all-times emissions.

2.6. Statistical Analyses

Based on the RCB design of the cultivar treatments at each location, an analysis of variance (ANOVA) was conducted, separately by location, in SAS (version

9.4, SAS Institute, Inc., Cary, NC) using the PROC Mixed procedure to assess potential cultivar effects on near-surface soil properties prior to beginning gas sampling. Based on visual inspection of normal probability plots of the studentized residuals, CH_4 flux data showed no indication of non-normal distribution. Consequently, separate ANOVAs were performed for each location-growth stage combination based on a RCB repeated-measures design, where measurement time of day was treated as the repeated measure, to evaluate the effects of time of day, cultivar, and their interaction on CH_4 fluxes and daily CH_4 emissions calculated from each different time-of-day flux measurement and for the all-times daily emissions. When appropriate, means were separated by least significant difference (LSD) at the 0.05 level. Due to planting and flooding date differences and differences in the timing of achieving various physiological growth stages between locations, location (*i.e.*, soil texture) was not formally assessed as an experimental variable in this statistical analysis. Similarly, due to differences in the timing of achieving various physiological growth stages between cultivars, growth stage was also not formally assessed as an experimental variable.

3. Results & Discussion

3.1. Initial Soil Properties

Initial, near-surface soil properties varied slightly to not at all between cultivars at each location. At both locations, cultivar did not affect ($P > 0.05$) soil physical properties, namely sand, silt, and clay concentration and bulk density, in the top 10 cm prior to flood establishment (Table 1). Similarly, at both locations, cultivar did not affect ($P > 0.05$) near-surface soil chemical properties, namely soil pH, extractable soil Fe, Cu, and Zn and TC and TN contents, or C:N ratio, prior to flood establishment. On the silt-loam soil at RREC, cultivar did not affect ($P > 0.05$) extractable soil Mg and S and SOM contents, while on the clay soil at NEREC, cultivar did not affect ($P > 0.05$) extractable soil P content. However, at both locations, extractable soil K, Ca, Mn, and Na contents in the top 10 cm were greater ($P < 0.05$) for the pure-line than the hybrid cultivar. Soil EC and extractable soil P in the top 10 cm were greater ($P < 0.05$) for the pure-line than the hybrid cultivar at RREC, but soil EC was greater ($P < 0.05$) for the hybrid than the pure-line cultivar at NEREC. At NEREC, extractable soil Mg and SOM contents were greater ($P < 0.05$) for the pure-line than the hybrid cultivar, while extractable soil S content was greater ($P < 0.05$) for the hybrid than the pure-line cultivar. Though some pre-flood differences in near-surface soil properties existed between cultivars at both locations, the soil property differences were small and not expected to affect rice growth or production at either location [36].

3.2. Hourly CH_4 Fluxes and Estimated Daily Emissions

Hourly CH_4 fluxes and estimated daily emissions differed by measurement time of day, rice cultivar, or both among location-growth stage combinations (Table 2). Hourly CH_4 fluxes and estimated daily emissions differed between cultivars

Table 1. Summary of the effects of cultivar on mean soil properties (n = 4 per cultivar per location/soil texture) in the top 10 cm prior to flood establishment in a silt-loam soil at the Rice Research and Extension Center (RREC) near Stuttgart, Arkansas and in a clay soil at the Northeast Research and Extension Center (NEREC) in Keiser, Arkansas during the 2014 growing season.

Soil Property	Silt-loam Soil		Clay Soil	
	Roy J	CLXL745	Roy J	CLXL745
Sand (%)	7 a†	7 a	16 a†	16 a
Silt (%)	76 a	76 a	32 a	32 a
Clay (%)	17 a	17 a	51 a	51 a
Bulk density ($g \cdot cm^{-3}$)	1.34 a	1.35 a	1.13 a	1.12 a
pH	6.4 a	6.5 a	7.4 a	7.4 a
Electrical conductivity ($dS \cdot m^{-1}$)	0.205 a	0.192 b	0.185 b	0.232 a
Extractable nutrients				
P ($kg \cdot ha^{-1}$)	70.3 a	67.8 b	74.0 a	74.0 a
K ($kg \cdot ha^{-1}$)	198 a	188 b	382 a	368 b
Ca ($kg \cdot ha^{-1}$)	2235 a	2229 b	5529 a	5388 b
Mg ($kg \cdot ha^{-1}$)	214 a	213 a	1126 a	1090 b
Fe ($kg \cdot ha^{-1}$)	493 a	494 a	495 a	497 a
Mn ($kg \cdot ha^{-1}$)	337 a	325 b	68.8 a	65.1 b
Na ($kg \cdot ha^{-1}$)	74.6 a	71.7 b	68.9 a	65.2 b
S ($kg \cdot ha^{-1}$)	18.1 a	15.3 a	16.3 b	18.8 a
Cu ($kg \cdot ha^{-1}$)	1.8 a	1.8 a	5.8 a	5.6 a
Zn ($kg \cdot ha^{-1}$)	7.0 a	6.8 a	4.0 a	3.9 a
Soil organic matter ($Mg\ ha^{-1}$)	27.0 a	27.0 a	39.1 a	38.2 b
Total N ($Mg\ ha^{-1}$)	1.21 a	1.22 a	1.45 a	1.47 a
Total C ($Mg\ ha^{-1}$)	11.6 a	11.9 a	15.6 a	15.7 a
C:N ratio	9.6 a	9.7 a	10.7 a	10.7 a

†Means within a row and location with different letters are significantly different at the $P < 0.05$ level.

among the various measurement times of day ($P < 0.023$) at the pre-heading growth stage from the silt-loam soil at RREC. Hourly CH_4 fluxes from the pure-line cultivar were greatest at 2300 hours, smallest at 800 hours, and did not differ among the 300, 1200, and 1800 hours measurement times of day (**Figure 1(a)**). Similar to the pure-line cultivar, hourly CH_4 fluxes from the hybrid were greatest at 2300 hours, but similar among the other four measurement times of day. Hourly CH_4 fluxes only differed between cultivars at the 2300 hours measurement time of day, where the hourly flux from the pure-line was 1.6 times greater than that for the hybrid.

Estimated daily CH_4 emissions from both cultivars followed similar patterns to hourly fluxes at the pre-heading growth stage from the silt-loam soil at RREC

Table 2. Summary of the effects of measurement time of day (TOD), cultivar, and their interaction on methane (CH_4) fluxes and estimated daily emissions from a silt-loam soil at the Rice Research and Extension Center (RREC) near Stuttgart, Arkansas and from a clay soil at the Northeast Research and Extension Center (NEREC) in Keiser, Arkansas for two growth stages (*i.e.*, pre-heading and post-heading) during the 2014 growing season.

Location-Growth Stage Combination/Variable	Source of Variation		
	TOD	Cultivar	TOD × Cultivar
		P	
RREC/Pre-heading			
CH_4 flux	<0.001	0.066	0.023
Estimated daily emissions	<0.001	0.066	0.014
RREC/Post-heading			
CH_4 flux	<0.001	0.003	0.128
Estimated daily emissions	<0.001	0.003	0.109
NEREC/Pre-heading			
CH_4 flux	<0.001	0.472	0.112
Estimated daily emissions	<0.001	0.472	0.093
NEREC/Post-heading			
CH_4 flux	<0.001	0.387	0.561
Estimated daily emissions	<0.001	0.388	0.585

(Table 2, Figure 1(b)). Numeric and/or statistically significant daily emissions minima were achieved at 800 hours, while maxima were achieved at 2300 hours for both cultivars (Figure 1(b)). Based on linear interpolation among the hourly CH_4 fluxes from five measurement times of day, the all-times estimated daily CH_4 emissions was achieved for both cultivars at the 300, 1200, and 1800 hours measurement times of day.

Hourly CH_4 fluxes and estimated daily emissions differed between cultivars ($P < 0.003$) and differed among the various measurement times of day ($P < 0.001$) at the post-heading growth stage from the silt-loam soil at RREC (Table 2). Similar to that hypothesized and previous Arkansas reports [23] [24] [28], averaged across measurement times of day, both hourly CH_4 fluxes and estimated daily emissions were more than 2.6 times greater from the pure-line (8.16 mg CH_4-C $m^{-2} \cdot hr^{-1}$ and 195.6 mg CH_4-C $m^{-2} \cdot day^{-1}$, respectively) compared to the hybrid cultivar (3.11 mg CH_4-C $m^{-2} \cdot hr^{-1}$ and 74.5 mg CH_4-C $m^{-2} \cdot day^{-1}$, respectively). Averaged across cultivar, hourly CH_4 fluxes were greatest at the 300 and 2300 hours, which did not differ, and lowest at the 800, 1200, and 1800 hours, which did not differ, measurement times of day (Table 3).

Estimated daily CH_4 emissions, averaged across cultivar, were greatest at 2300 hours and smallest at 800 and 1800 hours, which did not differ, at the post-heading growth stage from the silt-loam soil at RREC (Table 3). However, in

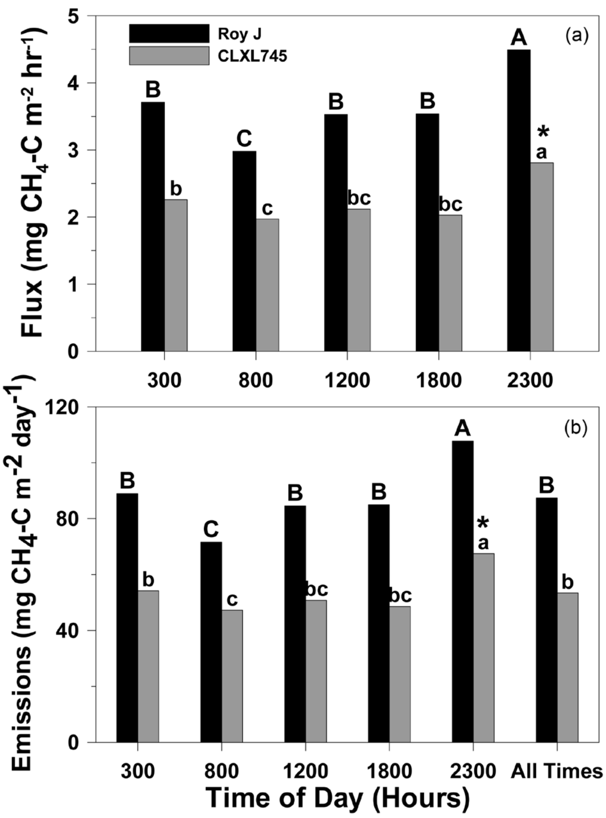

Figure 1. Hourly CH_4 fluxes (a) and estimated daily emissions (b) among measurement times of day at the pre-heading growth stage from the silt-loam soil at the Rice Research and Extension Center near Stuttgart, AR. Different letters above bars within a cultivar indicate a significant difference ($P < 0.05$) among measurement times of day. The asterisks (*) indicates a significant difference ($P < 0.05$) between cultivars within a given measurement time of day. The All Times label on panel B represents the estimated daily emissions from the linearly interpolated fluxes across all measurement times of day.

contrast to that at pre-heading, the all-times estimated daily CH_4 emissions was only achieved at the 1200 hours measurement time of day. Estimated daily emissions from the 300 and 2300 hours measurement times of day were greater, while that from the 800 and 1800 hours measurement times of day were smaller than the all-times estimated daily CH_4 emissions.

In contrast to that at the pre-heading growth stage from the silt-loam soil at RREC, hourly CH_4 fluxes and estimated daily emissions only differed among the various measurement times of day ($P < 0.001$), but were unaffected by cultivar ($P > 0.05$) at the pre-heading growth stage from the clay soil at NEREC (Table 2). Averaged across cultivar, hourly CH_4 fluxes and estimated daily CH_4 emissions were greatest at 300 hours and lower, but similar, among the other four measurement times of day (Table 3). The all-times estimated daily CH_4 emissions were achieved at the 800, 1800, and 2300 hour measurement times of day. Estimated daily emissions from the 300 hours measurement time of day were greater, while that from the 1200 hours measurement time of day were smaller than the all-times estimated daily CH_4 emissions.

Table 3. Summary of the effects of measurement time of day on methane (CH_4) fluxes and estimated daily emissions from a silt-loam soil at the Rice Research and Extension Center (RREC) near Stuttgart, Arkansas and from a clay soil at the Northeast Research and Extension Center (NEREC) in Keiser, Arkansas by growth stages (*i.e.*, pre-heading or post-heading) during the 2014 growing season.

Variable/Time of Day (Hours)	Location-Growth Stage Combination		
	RREC/ Post-heading	NEREC/ Pre-heading	NEREC/ Post-heading
CH_4 Flux (mg CH_4-C $m^{-2}\cdot hr^{-1}$)			
300	6.31a†	0.93 a	1.99 b
800	4.87 b	0.73 bc	1.66 d
1200	5.36 b	0.71 c	1.82 c
1800	4.65 b	0.74 bc	2.19 a
2300	6.99 a	0.78 b	1.82 c
Estimated Daily Emissions (mg CH_4-C $m^{-2}\cdot d^{-1}$)			
300	151.3 b	22.4 a	47.7 b
800	116.9 de	17.6 bc	39.8 d
1200	128.7 cd	17.1 c	43.6 c
1800	111.5 e	17.7 bc	52.5 a
2300	167.8 a	18.7 b	43.7 c
All-times mean	134.1 c	18.7 b	45.7 bc

†Means within a variable and location-growth stage combination with different letters are significantly different at the $P < 0.05$ level.

Contrary to that hypothesized, the maximum hourly CH_4 flux and estimated daily emissions occurred during the night (*i.e.*, at 2300 and/or 300 hours) rather than during the day at both the pre- and post-heading growth stages from the silt-loam soil at RREC and the pre-heading growth stage from the clay soil at NEREC. Though somewhat inconsistent with previous decades-old reports, peak emissions occurring during the night could be explained by a combination of improvements to in-field methodologies and cultivar genetics and differences in rice production systems used presently compared to those present at the time the previous studies were conducted. To our knowledge, no previous studies of diurnal CH_4 emissions have been conducted in the direct-seeded, delayed-flood production system in Arkansas, in which this production system differs somewhat from those used in Louisiana, Texas, and California. Enclosed-headspace, chamber-based, in-field measurement procedures have likely advanced to reduce in-field variability and advancements in analytical laboratory techniques (*i.e.*, gas chromatography) likely have enhanced sensitivity and accuracy compared to decades ago. In addition, cultivar breeding efforts, genetics, and trait manipulations have increased in complexity, such that the cultivars, both pure-lines and hybrids, which are being grown presently, are quite different than those that were grown decades ago. Even slight changes in plant physiological and metabolic processes could alter the semi-active, plant-mediated transport of CH_4 from the soil through the rice plant's aerenchyma tissues [4] [44] [45] [46] and potentially shift emissions minima and/or maxima to different times of the day.

Similar to that at the post-heading growth stage from the silt-loam soil at RREC and at the pre-heading growth stage from the clay soil at NEREC, hourly CH_4 fluxes and estimated daily emissions differed among the various measurement times of day ($P < 0.001$), but, similar to that at pre-heading from the clay soil, were also unaffected by cultivar ($P > 0.05$) at the post-heading growth stage from the clay soil at NEREC (**Table 2**). Averaged across cultivars, both hourly CH_4 fluxes and estimated daily emissions were greatest at 1800 hours and lowest at 800 hours at the post-heading growth stage from the clay soil at NEREC (**Table 3**). Though hourly CH_4 fluxes were greater at 1200 and 2300 hours, which did not differ, than at 300 hours, estimated daily emissions from 300, 1200, and 2300 hours were all similar to the all-times mean estimated daily CH_4 emissions.

Methane fluxes measured at the two growth stages in 2014 ranged from 0.7 to 2.2 mg CH_4-C $m^{-2} \cdot hr^{-1}$ from the clay soil and from 2 to 7 mg CH_4-C $m^{-2} \cdot hr^{-1}$ from the silt-loam soil (**Table 3**), which characterized fluxes from relatively low-emissions soils [47]. Though not formally assessed, the numerically lower emissions from the clay compared to the silt-loam soil are consistent with previous reports [16] [18] [19]. It is generally understood that lower gas diffusion rates associated with finer- (*i.e.*, clays) compared to coarser-textured soils (*i.e.*, silt loams) allow for greater CH_4 oxidation before being emitted to the atmosphere, thereby reducing CH_4 emissions [19]. Furthermore, clay soils tend to require a longer duration than do silt-loam soils to achieve the requisite oxidation-reduction potential for CH_4 production [18] [48], which further reduces CH_4 production and emissions from clay compared to silt-loam soils. The soil-texture effect on CH_4 emissions may also be responsible for the lack of a cultivar effect on CH_4 emissions from the clay (NEREC) compared to the silt-loam soil (RREC) in this study (**Table 3**).

In contrast to studies that reported no diurnal variation [6] [14] [15], CH_4 fluxes/estimated emissions differed among measurement times of day for each of the four location-growth stage combinations in the direct-seeded, delayed-flood production system measured in this study. These results were similar to the measured diurnal variations reported previously [2] [4] [6]-[13]. Though greater diurnal variations have been reported early compared to late in the growing season [8] [10], results of this study indicated the proportional range in diurnal CH_4 fluxes/estimated emissions was similar before and after heading from both soil textures (**Table 3**). The numeric peak CH_4 flux was 23% to 33% greater than the numeric low among the various measurement times of day across the four location-growth stage combinations, which was similar to the magnitude of variation reported by Yagi and Minami [13].

Based on results from four location/soil texture-growth stage combinations during the 2014 rice growing season in eastern Arkansas, several commonalities were observed in terms of a potential optimum measurement time of day to result in daily emissions estimates that are similar to the mean daily emissions. Actual CH_4 flux measurements made at 1200 hours at both the pre- and post-heading growth stages from the silt-loam soil at RREC, which were neither the daily minima nor maxima fluxes, resulted in daily estimated CH_4 emissions that were statistically similar to the all-times mean estimated daily CH_4 emissions (**Figure 1**, **Table 3**). In contrast, actual CH_4 flux measurements made at 2300 hours at both the pre- and post-heading growth stages from the clay soil at NEREC, which were also neither the daily minima nor maxima fluxes, resulted in daily estimated CH_4 emissions that were statistically similar to the all-times estimated daily CH_4 emissions. However, in three of the four location-growth stage combinations, with the exception of at pre-heading on the clay soil at NEREC, actual CH_4 flux measurements conducted at 1200 hours resulted in daily estimated CH_4 emissions that were statistically similar to the all-times estimated daily CH_4 emissions. Even less consistency occurred among location-growth stage combinations if the maximum daily emissions were to be the emissions target, where the maximum daily emissions occurred for flux measurements conducted at 2300 hours for both growth stages from the silt-loam soil at RREC compared to 300 and 1800 hours for pre- and post-heading, respectively, from the clay soil at NEREC (**Figure 1**, **Table 3**). The maximum daily emissions ranged from 14.8% to 26.4% and averaged 22% greater than the all-times daily emissions, whereas daily emissions calculated from measurements at all other times of day ranged from 18.1% lower to 12.8% greater and averaged 5.1% lower than the all-times daily emissions.

It is clear that CH_4 flux measurements that are conducted to represent the average daily emissions are likely somewhat conservative estimates of the actual daily emissions because the time of day the average daily emissions occurs differs from the time of day when the daily maximum occurs. However, conducting CH_4 flux measurements during the night to capture the maximum hourly flux or daily emissions is impractical; thus, targeting a measurement time of day to result in daily emissions that are similar to the average daily emissions appears to be a more practical goal. The 1200 hours measurement time of day that resulted in similar daily emissions to the all-times daily emissions in three of the four location-growth stage combinations in the direct-seeded, delayed-flood production system is only a few hours or less ahead of the measurement time range used in most recent field studies of 800 to 1000 [18] [19] [23] [24] [28] [49] [50] [51] and is similar to the timing suggested by Minamikawa *et al.* [1] from measurements in Japan and Weller *et al.* [52] from measurements in the Philippines.

4. Summary & Conclusions

Based on the results of this field study conducted among four location-growth stage combinations during the 2014 rice growing season from the direct-seeded, delayed-flood production system in eastern Arkansas, hourly CH_4 fluxes and estimated daily emissions differed among measurement times of day for a given cultivar or averaged across cultivars. Hourly CH_4 fluxes and estimated daily emissions also differed between cultivars from the silt-loam soil (RREC), but did not differ between cultivars from the clay soil (NEREC). In addition, it appears that the optimum measurement time of day to capture either minimum, maximum, or average hourly CH_4 flux or daily emissions for a given day differs by soil texture and rice growth stage. However, conducting CH_4 flux measurements around late morning to mid-day appears to be optimum to best capture the mean CH_4 emissions for the day. Considering measurement time of day when devising a field study will improve the accuracy of seasonal and/or annual estimates of CH_4 emissions. Though it is unknown exactly why peak measured CH_4 fluxes would occur at night, after photosynthesis for the day has ceased, the combination of recent advances in rice breeding, particularly with hybrid cultivars, and methodological improvements to in-field gas sampling and laboratory analytical techniques warrant revisiting conclusions drawn from studies conducted several decades ago.

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