Methane Emissions among Hybrid Rice Cultivars in the Mid-Southern United States

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Abstract. Rice (Oryza sativa L.) production systems have a greater global warming potential than upland row crops due to methane (CH4) emissions resulting from anaerobic conditions associated with flood-irrigated soils. Based on recent research indicating the potential for hybrid cultivars to mitigate CH4 emissions from rice, the objective of this study was to determine the influence of several commonly grown hybrid rice cultivars on CH4 fluxes and emissions from a silt-loam soil. Four cultivars were evaluated: the three hybrids CLXL729, CLXL745, and XL753 and the pure-line cultivar Roy J. Methane fluxes were determined by measuring changes in headspace CH4 concentrations over a period of 1 hour using 30-cm-inner-diameter polyvinyl chloride chambers. Only minor differences in CH4 fluxes occurred among the three hybrid cultivars, while the pure-line cultivar (Roy J) generally had greater (P < 0.05) fluxes. Peak CH4 fluxes occurred just after heading and were greater (P < 0.05) from Roy J (7.9 mg CH4-C m-2 h-1) than from the three hybrid cultivars, which did not differ and averaged 5.1 mg CH4-C m-2 h-1. Seasonal CH4 emissions were greater (P < 0.05) from Roy J (74.8 kg CH4-C ha-1 season-1) than from CLXL729, XL753, and CLXL745, which did not differ, and averaged 55.3, 53.0, and 48.9 kg CH4-C ha-1 season-1, respectively. Results of this study indicate the use of common hybrid cultivars may have potential for mitigation of CH4 emissions from rice production on silt-loam soils in the mid-southern United States.

Keywords: Methane emissions, rice cultivar, hybrid rice, methane mitigation

1 Introduction

Rice (Oryza sativa L.) is the world’s only major row crop that substantially contributes to global methane (CH4) emissions. While most crops are grown under aerated soil conditions and act as net sinks for atmospheric CH4, the majority of rice throughout the globe is produced in flooded fields [1] and acts as a net source of CH4 into the atmosphere. The anoxic conditions resulting from flooded soils lead to the production and release of CH4, a greenhouse gas with a global warming potential (GWP) 25 times stronger than carbon dioxide (CO2) [2]. Due to the production of CH4, rice cultivation has been estimated to have a GWP 2.7 and 5.7 times stronger than the production of maize (Zea mays L.) and wheat (Triticum aestivum L.), respectively, with 90% of the GWP of rice systems attributed to CH4 [3, 4]. It has been estimated, on a global scale, that approximately half of all anthropogenic CH4 emissions to the atmosphere are a direct result of agricultural activities [5, 6] and that 22% of those agricultural CH4 emissions occur due to rice cultivation [7]. Arkansas is the leading rice-producing state in the US, representing over 49% of harvested area in 2014 and resulting in an estimated 39% of total CH4 emissions from rice cultivation in the US in 2014 [8].

Methane emissions from a rice cultivation system are governed by the magnitude and balance between the two microbial processes of methanogenesis, the production of CH4, and methanotrophy, the oxidation of CH4, both of which are strongly influenced by various soil, plant, and environmental factors. Methanogenesis occurs in anaerobic soils as a specific group of Archaea utilize acetate or hydrogen gas and CO2, which are formed by the fermentation of organic matter by a large consortium of anaerobic bacteria, as substrates to form CH4 [9, 10]. A specific group of methanogens referred to as Rice Cluster I was identified by Grosskopf et al. [11] and was determined to represent up to 50% of methanogens in rice systems [12], occupying a niche surrounding rice roots by producing CH4 from root exudates released into the soil [13, 14]. Methane oxidation in flooded-rice systems occurs at oxic-anoxic interfaces where a group of aerobic Proteobacteria, known as methanotrophs, utilize CH4 or methanol as a source.
of energy and carbon [9]. Research has consistently indicated that up to 90% of CH$_4$ produced in rice cultivation systems is oxidized by methanotrophs [15, 16, 17, 18, 19] as CH$_4$ moves through the oxygenated zones of soil surrounding rice roots [20, 21] and near the soil surface [19, 22].

A CH$_4$ mitigation strategy that has been recently investigated is the selection of specific rice cultivars that could potentially reduce CH$_4$ emissions. While the implementation of certain mitigation options by producers may be restricted due to required changes in management practices and equipment or the possibility of yield reductions, the selection of low-emitting cultivars can be implemented without major changes in crop management while maintaining grain yields. Studies have indicated that about 90% of CH$_4$ emissions from rice fields occur by plant-mediated transport through the aerenchyma tissues of rice plants [16, 19, 23, 24]. Due to the strong influence of rice plants themselves on CH$_4$ emissions from the system, innate physiological differences between cultivars may lead to differences in CH$_4$ transport capacities [23], differences in biomass accumulation [25] and root exudation [26, 27, 28], or differences in microbial populations in the rhizosphere [29]. Many studies throughout rice-growing countries have reported differences in CH$_4$ emissions among rice cultivars; however, the complexity of the system and processes involved have led to differing results and, at this point, no single parameter has been identified to consistently explain differences in CH$_4$ emissions among cultivars. Due to the wide variety of environmental conditions, production practices, and rice cultivars that occur throughout the world, it seems that CH$_4$ emissions measurements need to be conducted to account for the specific production practices and cultivars that predominate in each rice-growing region in order to mitigate CH$_4$ emissions while maintaining high yields and profitability.

Research has recently been conducted to evaluate rice cultivar effects on CH$_4$ emissions in the US with studies occurring in California and Arkansas. The studies conducted in Arkansas observed reductions in CH$_4$ emissions ranging from 25 to 37% from a japonica/indica hybrid cultivar relative to pure-line conventional cultivars [30, 31, 32], while Simmonds et al. [31] measured no difference in CH$_4$ emissions from the same hybrid cultivar (CLXL745) and pure-line cultivars in California. Although limited data have been collected in the US, cultivar selection has been shown to significantly impact CH$_4$ emissions from rice. An examination of the mitigation potential of hybrid rice cultivars is important in Arkansas, where over 40% of rice area has been planted with hybrid cultivars since 2008 [33]. Previous studies have observed reduced emissions from the hybrid cultivar CLXL745 in Arkansas; however, no studies have been conducted to investigate if the reduced emissions are consistent with other hybrid rice cultivars. This study focused on comparing a single pure-line cultivar to several hybrid cultivars to investigate if other hybrids showed similar CH$_4$ mitigation potential as was observed from CLXL745. This study adds significant data from drill-seeded, delayed-flood rice production systems in the mid-southern US concerning cultivar effects on CH$_4$ emissions that will be instrumental in estimating current CH$_4$ emissions as well as mitigating potential future emissions. Thus, the objective of this study was to evaluate hybrid rice cultivar effects on CH$_4$ emissions from a silt-loam soil during the 2014 growing season. It was hypothesized that CH$_4$ fluxes and emissions would not differ among hybrid cultivars, but that all hybrid cultivars collectively would produce lower mean emissions compared to a pure-line cultivar.

2 Materials and Methods

2.1 Site Description

Research was conducted during the 2014 growing season, from May to September, at the University of Arkansas System Division of Agriculture Rice Research and Extension Center near Stuttgart in Arkansas County, Arkansas (34°27'58"N, 91°24'47"W). Study plots were located on a Dewitt silt loam (fine, smectitic, thermic Typic Albaqualfs) [34]. The study site is located in the northern portion of the Southern Mississippi River Terraces Major Land Resource Area (MLRA 131D) known as the Grand Prairie in east-central Arkansas. Cropland makes up approximately 42% of MLRA 131D, where precipitation, which mostly occurs in the spring and early summer, averages between 124.5 and 142 cm annually and the annual average air temperature ranges from 16 to 18 °C [35]. The crops at this study location have been rotated annually between soybean and rice for more than 20 years and previous crop residues are incorporated into the top 10 to 15 cm of soil between growing seasons.
2.2 Treatments and Experimental Design

The purpose of this study was to determine if other hybrid rice cultivars produced similar CH₄ emissions results to the hybrid cultivar CLXL745, which has been previously studied. Cultivars were selected in this study in an attempt to represent the most common hybrid cultivars as well as a comparison to one of the most predominant pure-line cultivars currently grown in Arkansas. The three hybrid rice cultivars selected for this study were “CLXL745”, “XL753”, and “CLXL729” (RiceTec, Inc., Houston, TX), which accounted for 22.0, 11.8, and 4.2%, respectively, of Arkansas rice production in 2014 and 21.3, 13.6, and 4.1%, respectively in 2016 [36]. The final selection in this study was the pure-line cultivar “Roy J”, developed at the University of Arkansas [37], which accounted for 12.6% of Arkansas production in 2014 and 19.6% in 2016 [36]. All four cultivars produce long-grain rice, and, while the pure-line cultivar, Roy J, is a mid-season cultivar, heading at 85 days after emergence, the hybrid cultivars CLXL745, XL753, and CLXL729 are all short-season cultivars, heading at 77, 78, and 80 days after emergence, respectively [38]. All four rice cultivars are high yielding with CLXL729, CLXL745, XL753, and Roy J averaging 9.9, 10.0, 12.7, and 9.9 Mg ha⁻¹, respectively, in Arkansas Rice Performance Trials in 2014 [38].

Research plots, which were arranged in a randomized complete block (RCB) design with four replicates of each cultivar, were 1.6 m wide by 5 m long and encompassed nine rows of rice. Methane sample date was treated as a repeated measure in analyzing CH₄ flux data.

2.3 Plot Management

Study plots were managed in accordance with University of Arkansas Cooperative Extension Service (UACES) guidelines [39]. As per soil test recommendations, 100 kg ha⁻¹ each of phosphorus (P) and potassium (K) and 11.2 kg ha⁻¹ of Zn were incorporated by tillage into the top 10 to 15 cm of soil on 26 March 2014 throughout the study area. Research plots were then independently drill-seeded, using 18-cm row spacing, on 5 May 2014. The pure-line cultivar, Roy J, was seeded at a rate of 82 kg ha⁻¹, while all three hybrid cultivars were seeded at a lower rate of 34 kg ha⁻¹ due to an increased tillering capacity in hybrid rice cultivars. Levees were constructed to surround the study area following seeding and plots were flush-irrigated with nearby reservoir water as necessary prior to the establishment of a permanent flood, which occurred on 17 June 2014 when rice was at the 4- to 5-leaf stage. Based on UACES guidelines, N was applied as urea (46% N) in a split application, where Roy J and CLXL729 each received 100 kg N ha⁻¹ and CLXL745 and XL753 each received 135 kg N ha⁻¹ as the first split one day prior to permanent flood establishment [40]. The second split application of N occurred on 10 July 2014 at the beginning of internode elongation for Roy J (50 kg N ha⁻¹) and at the booting growth stage on 23 July 2014 for the three hybrid cultivars (33 kg N ha⁻¹). A floodwater depth of 5 to 10 cm was maintained by use of Polytube (Delta Plastics, Little Rock, AR) inlet irrigation until grain maturity was reached on 3 September 2014, upon which floodwater was released and plots were allowed to dry for harvest, which occurred on 18 September 2014 with a small-plot combine. Plots were regularly scouted and managed to remain below threshold levels of insects and weeds throughout the season according to UACES guidelines [41, 42].

2.4 Soil Sampling

Soil samples were collected prior to N fertilization and flooding using a 2-cm-diameter push probe by combining five cores from the 0- to 10-cm depth in each plot. Composite samples were dried at 70 °C for 48 h and sieved through a 2-mm mesh screen prior to subsamples being analyzed for Mehlich-3 extractable nutrients (i.e., P, K, Ca, Mg, Fe, Mn, Na, S, Cu, and Zn; Spectro Analytical Instruments, Spectro Arcos ICP, Kleve, Germany) using inductively coupled plasma atomic emission spectroscopy [43]. Total N (TN) and total C (TC) concentration were determined from additional subsamples by high-temperature combustion using a VarioMax CN analyzer (Elementar Americas Inc., Mt. Laurel, NJ) [44]. Soil pH and electrical conductivity (EC) were determined potentiometrically on a 1:2 (m:v) soil-to-solution paste. Soil organic matter (OM) concentration was determined by loss on ignition [45]. Bulk density samples were collected from the 0- to 10-cm depth using a slide hammer and 4.7-cm-diameter core chamber with a beveled core tip, dried at 70 °C for 48 h, weighed and ground to pass through a 2-mm mesh screen for particle-size analysis using a modified 12-h hydrometer method [46].
of bulk densities from the plots were used in conjunction with measured TN, TC, and OM concentrations to determine TN, TC, and SOM contents (Mg ha⁻¹).

2.5 Gas Sampling and Analysis

Enclosed headspace chambers, as detailed by Livingston and Hutchinson [47] and similar to those used in previous studies [30, 32, 48, 49], were used for collection of gas samples. This methodology is commonly used for measuring trace gas fluxes [50] and involves the use of 30-cm inner diameter (ID) polyvinyl chloride to create a permanent base collar, several sizes of chamber extensions to accommodate increasing plant growth over time, and a vented sampling cap to sample a portion of each plot. One base collar, which remained in the same place throughout the study, was placed within each plot to contain 40 cm of rice row length in order to duplicate the plant density of the plots. Elevated boardwalks were established at the perimeter of plots prior to flooding in order to access chambers during sampling, while minimizing disturbance of plants and soil surrounding the chambers. Chamber caps contained a 15-cm section of 4.5-mm ID copper tubing as a vent to maintain atmospheric pressure, gas sampling and thermometer ports sealed with gray butyl-rubber septa (Voight Global, part number 73828A-RB, Lawrence, KS), and a 2.5-cm-diameter, 9V-battery-operated fan (Sunon Inc, MagLev, Brea, CA) to mix the headspace air within the chamber during sampling.

Gas sampling throughout the study occurred between 0800 and 1000 hours, similar to previous studies [30, 32, 48, 49, 51, 52, 53], in order to prevent excessive heating within the chambers during sampling. Gas samples for flux measurements occurred on approximately weekly intervals at 7, 14, 21, 28, 35, 43, 49, 56, 63, 71, and 77 days after flooding (DAF) for the flooded duration of the study. Sampling intensity was increased after flood release to 80, 82, 83, 84, and 85 DAF [i.e., 2, 4, 5, 6, and 7 days after flood release (DAFR)] in an attempt to adequately quantify a post-flood-release pulse of CH₄ that has commonly been observed in previous studies [32, 48, 49, 54, 55, 56].

On each sampling date, samples were collected at 20-minute intervals (i.e., 0, 20, 40, and 60 minutes after cap closure) using 20-mL B-D syringes (Becton Dickinss and Co., Franklin Lakes, NJ) and immediately transferred into evacuated 10-mL, crimp-top glass vials (Agilent Technologies, part number 5182-0838, Santa Clara, CA). Field samples were analyzed within 48 hours using an Agilent 6890-N gas chromatograph equipped with a flame ionization detector (FID). Methane fluxes were then calculated based on changes of headspace CH₄ concentrations over time as outlined by Parkin and Venterea [50]. Season-long total CH₄ emissions were determined for each chamber by linear interpolation between flux measurement dates. More detailed descriptions of gas sampling and analysis procedures used in this study have been previously reported [32, 49].

2.6 Plant Sampling and Analyses

Plant samples were collected on 17 September 2014, one day prior to harvest, in order to determine any impact of rice cultivar on total aboveground dry matter accumulation over the growing season as well as to compare aboveground dry matter from within and outside the chambers to determine if the chambers had a negative impact on plant growth. All aboveground biomass from within each chamber and a 1-m row of rice from adjacent to each chamber were cut, dried at 60°C until no further moisture loss occurred, and weighed in order to determine total aboveground dry matter accumulation. A 4-m length of the center five rows of each plot was harvested at physiological maturity using a plot-scale combine. Grain samples were then weighed and analyzed for moisture content so that final grain yields could be reported on a 120 g kg⁻¹ grain moisture content.

2.7 Statistical Analyses

Initial soil physical and chemical properties were analyzed by analysis of variance (ANOVA) in SAS v. 9.4 (SAS Institute, Inc., Cary, NC) using PROC Mixed based on a RCB design in order to evaluate whether inherent differences were present in soil properties among plots planted to each cultivar. Similarly, grain yield was analyzed by ANOVA based on a RCB design in order to determine the impact of rice cultivar on grain yield. An additional ANOVA was performed based on a split-plot RCB design, where cultivar was the whole-plot factor and sampling location (i.e., in-chamber or in-plot) was the
split-plot factor, in order to compare total aboveground dry matter accumulation as affected by cultivar and sampling location.

Methane flux data showed no indication of non-normal distribution based on a visual inspection for normality using normal probability plots of the studentized residuals. Therefore, an ANOVA was performed based on a RCB repeated-measures design, where sampling event was treated as a repeated measure, in order to evaluate cultivar impact on CH$_4$ fluxes over time. Flux data were analyzed separately for the flooded and non-flooded periods of the season due to differences in CH$_4$ transport mechanisms and sampling frequency. Total seasonal CH$_4$ emissions effects among cultivars, expressed as mass-per-area (area scaled) and mass-per-grain-yield (yield scaled), as well as post-flood-release emissions, on an area-scaled basis and as a percentage of total seasonal emissions, were analyzed by ANOVA based on a RCB design. When appropriate, means were separated at the 0.05 level using the Fisher protected least significant difference (LSD).

3 Results and Discussion

3.1 Initial Soil Properties

Table 1. Mean soil physical and chemical properties (n = 4 per cultivar) prior to flood establishment in the top 10 cm of a Dewitt silt loam during the 2014 growing season at the Rice Research and Extension Center near Stuttgart, AR.

| Soil property      | Cultivar         | CLXL729 | CLXL745 | XL753 | Roy J |
|--------------------|------------------|---------|---------|-------|-------|
| pH                 |                  | 6.44    | 6.49    | 6.45  | 6.42  |
| Sand (g g$^{-1}$)  |                  | 0.07    | 0.07    | 0.08  | 0.07  |
| Silt (g g$^{-1}$)  |                  | 0.75    | 0.76    | 0.76  | 0.76  |
| Clay (g g$^{-1}$)  |                  | 0.17    | 0.17    | 0.17  | 0.17  |
| Bulk density (g cm$^{-3}$) |          | 1.34    | 1.35    | 1.34  | 1.34  |
| Electrical conductivity (dS m$^{-1}$) |       | 0.216   | 0.193   | 0.191 | 0.205 |
| Mehich-3 extractable nutrients (mg kg$^{-1}$) | | 44.9    | 50.0    | 43.6  | 52.4  |
| P                  |                  | 136     | 139     | 133   | 148   |
| K                  |                  | 1645    | 1644    | 1636  | 1666  |
| Ca                 |                  | 158     | 158     | 156   | 159   |
| Mg                 |                  | 363     | 365     | 361   | 367   |
| Mn                 |                  | 240ab†  | 240c    | 246abc| 251a  |
| Na                 |                  | 56.4a   | 52.9bc  | 51.6c | 55.6ab|
| S                  |                  | 12.5    | 11.3    | 11.5  | 13.5  |
| Cu                 |                  | 1.32    | 1.31    | 1.30  | 1.32  |
| Zn                 |                  | 5.08    | 5.05    | 3.75  | 5.19  |
| Organic matter (g kg$^{-1}$) |         | 19.6    | 19.9    | 20.1  | 20.2  |
| Organic matter (Mg ha$^{-1}$) |       | 26.2    | 27.0    | 26.9  | 27.0  |
| Total N (g kg$^{-1}$)|             | 0.88    | 0.90    | 0.88  | 0.91  |
| Total N (Mg ha$^{-1}$)|           | 1.17    | 1.22    | 1.17  | 1.21  |
| Total C (g kg$^{-1}$)|             | 9.1     | 8.8     | 8.9   | 8.7   |
| Total C (Mg ha$^{-1}$)|           | 12.2    | 11.9    | 11.9  | 11.6  |
| C:N ratio          |                  | 10.4    | 9.8     | 10.2  | 9.6   |

†Values in the same row followed by different letters are significantly different ($P < 0.05$).

Initial soil physical and chemical properties in the top 10 cm measured prior to flooding did not differ among pre-assigned treatments (Table 1), with the exception of extractable Na ($P = 0.035$) and
extractable Mn ($P = 0.004$). However, the magnitude of differences among pre-assigned treatments amounted to 8.9, and 4.5% of the overall mean values among all four cultivars for extractable soil Na and Mn, respectively (Table 1). These minor differences in the properties of soil upon which each treatment was implemented likely had no practical or agronomic significance in this study. Extractable soil P, K, and Zn were all within the optimum levels of 36 to 50 mg P kg$^{-1}$, 131 to 175 mg K kg$^{-1}$, and $\geq$ 4.1 mg Zn kg$^{-1}$ recommended for optimal rice produced on a silt-loam soil, indicating adequate levels of these nutrients for production of optimum rice yields based on UACES guidelines [40].

### 3.2 Methane Fluxes from Flooding to Flood Release

Table 2. Analysis of variance summary of the effects of cultivar, time, and their interaction on methane (CH$_4$) fluxes from flooding to flood release and following flood release from a silt-loam soil during the 2014 growing season at the Rice Research and Extension Center near Stuttgart, AR.

| Source of variation | Measurement period | $P$ |
|---------------------|--------------------|-----|
|                     | Flooding to flood release | Post-flood release |
| Cultivar            | 0.002              | 0.042 |
| Time                | $< 0.001$          | $< 0.001$ |
| Cultivar × time     | $< 0.001$          | 0.156 |

Figure 1. Methane (CH$_4$) fluxes measured over time from a pure-line cultivar (Roy J) and three hybrid rice cultivars (CLXL729, CLXL745, and XL753) throughout flooded portion of the 2014 growing season at the Rice Research and Extension Center near Stuttgart, AR. Vertical lines on the graph indicate approximate dates of panicle differentiation (PD) and 50% heading (HDG) for the hybrid cultivars (long-dashed lines) and pure-line cultivar (short-dashed lines). The least significant difference for the same cultivar over time is 0.679 mg CH$_4$-C m$^{-2}$ h$^{-1}$ and for different cultivars over time is 0.824 mg CH$_4$-C m$^{-2}$ h$^{-1}$ prior to flood release.

Methane fluxes measured during the flooded portion of the 2014 growing season differed among cultivars over time ($P < 0.001$; Table 2). Methane fluxes did not differ among the four cultivars during the first three weeks after flooding or at 43 days after flooding (DAF), while fluxes were greater from Roy J than from one (CLXL745) and two (CLXL745 and CLXL729) of the hybrid cultivars at 28 and 35 DAF, respectively (Figure 1). The pure-line cultivar, Roy J, however, had greater CH$_4$ fluxes than all three hybrid cultivars during the four weeks following 50% heading. Differences in CH$_4$ fluxes among the three hybrid cultivars were minor with differences occurring at 56 DAF, where fluxes were less from CLXL745 than from the other two hybrids, which did not differ, and at 63 DAF, where fluxes were less from
CLXL745 than from CLXL729, in which both did not differ from XL753 (Figure 1). While the magnitude of fluxes were greater from the pure-line cultivar late in the growing season, all four cultivars exhibited the same general trend where fluxes were all less than 0.14 mg CH$_4$C m$^{-2}$ h$^{-1}$ at 7 DAF, then generally increased over time, peaking shortly after 50% heading at 7.9 mg CH$_4$C m$^{-2}$ h$^{-1}$ for Roy J and 5.3, 5.2, and 4.8 mg CH$_4$C m$^{-2}$ h$^{-1}$ for CLXL729, XL753, and CLXL745, respectively, which did not differ (Figure 1). Fluxes then generally declined for the remainder of the flooded portion of the growing season.

Reduced CH$_4$ fluxes from hybrid cultivars relative to pure-line cultivars have been similarly observed on silt-loam and clay soils by Rogers et al. [30] and Smartt et al. [32], respectively, where fluxes from CLXL745 were significantly less than from two pure-line cultivars (Cheniere and Taggart), especially late in the growing season. Similarly, Simmonds et al. [31] observed a reduction in CH$_4$ emissions from the hybrid CLXL745 relative to the pure-line cultivar, Francis, following heading, while no differences in emissions were observed prior to heading. Although examining cultivars not typically grown in the US, in China, Ma et al. [29] observed reduced dissolved CH$_4$ concentrations in the rhizosphere of hybrid rice as well as a 67% increase in CH$_4$ oxidation potential relative to pure-line cultivars, while no differences in CH$_4$ production potential were observed. This indicates a potential for increased CH$_4$ oxidation with hybrid cultivars, possibly due to greater methanotrophic activity in the rhizosphere of hybrid rice relative to pure-line cultivars. Butterbach-Bahl et al. [23] attributed a difference in CH$_4$ fluxes among cultivars to differences in CH$_4$ transport capacity, as no differences were measured between potential CH$_4$ productions or oxidations among the cultivars. While several studies suggested differences in gas transport capacity or microbial community structure are the major influencing factors on differences in CH$_4$ fluxes among cultivars, additional studies have consistently suggested that differences in root exudation rates among cultivars are the primary factors that influence differences in CH$_4$ fluxes among cultivars [26, 27, 28, 57, 58].

The seasonal pattern of CH$_4$ emissions increasing once the flood is applied, peaking near heading, then declining prior to flood release has been observed in numerous previous studies [30, 32, 49, 51, 53, 59, 60, 61, 62] and suggests that root exudates increase during vegetative growth providing substrate for methanogenesis and decrease again during grain fill as resources are translocated to the filling grains. Research conducted by Denier van der Gon et al. [63] indicated that CH$_4$ emissions are related to allocation of photosynthetically derived C between roots and grains and that decreasing translocation of C to grains (i.e., removing florets prior to grain fill) causes an increase in C translocation to roots and an increase in CH$_4$ emissions. Sass and Cicerone [64] also determined a link between grain filling and methanogenesis, where increasing CH$_4$ emissions occur as conditions become more unfavorable for spikelet formation, thus decreasing the grain-sink for photosynthates. The plant-related peak in CH$_4$ fluxes observed in this study is common, where additional C sources, such as rice residue or green manure, have not been introduced to the system and similar seasonal trends have been observed in root growth [65, 66, 67], root exudation rates [28], and anaerobic root respiration rates [68]. Using $^{13}$C labeling techniques, Watanabe et al. [69] determined that, when no rice straw was incorporated into the system, 80 to 85% of CH$_4$ emissions were derived from growing rice plants, while the remainder originated from soil organic matter. Due to low organic residue inputs in this study and based on results of previous research, it would seem that seasonal trends in CH$_4$ fluxes observed in this study are largely linked to plant activity and that reduced fluxes from hybrid cultivars may be a result of reduced root exudation rates relative to the pure-line cultivar.

### 3.3 Methane Fluxes Following Flood Release

Methane fluxes following flood release, which occurred at 78 DAF, differed among cultivars ($P = 0.042$) and over time ($P < 0.001$; Table 2). Averaged across time, Roy J (4.23 mg CH$_4$C m$^{-2}$ h$^{-1}$) had greater post-flood-release fluxes than CLXL745 and XL753, which did not differ and averaged 2.95 mg CH$_4$C m$^{-2}$ h$^{-1}$, while CLXL729 (3.56 mg CH$_4$C m$^{-2}$ h$^{-1}$) did not differ from any of the other cultivars. While post-flood-release fluxes did not differ between Roy J and CLXL 729, the increased fluxes observed in Roy J relative to the other hybrid cultivars are consistent with CH$_4$ fluxes prior to flood release, where fluxes from Roy J were nearly twice as large as from the hybrid cultivars 1 day prior to flood release.

Averaged across cultivar, the measured post-flood-release CH$_4$ flux was greatest 4 days after flood release (DAFR) at 5.71 mg CH$_4$C m$^{-2}$ h$^{-1}$, which was greater than fluxes of 4.21 and 3.98 mg CH$_4$C m$^{-2}$ h$^{-1}$...
which did not differ and occurred at 2 and 5 DAFR, respectively. Fluxes incrementally decreased again at 6 and 7 DAFR (2.65 and 0.56 mg CH₄-C m⁻² h⁻¹, respectively). The post-flood-release pulse of CH₄ that occurred in this study has been observed in previous studies [30, 32, 48, 49, 53, 54, 55, 56], usually occurring from 3 to 6 DAFR, and is generally attributed to the release of CH₄ from soil pores as they drain and allow transport of previously entrapped gases.

3.4 Aboveground Dry Matter and Grain Yield

Aboveground dry matter measured at physiological maturity differed among cultivars ($P = 0.039$) and between sampling locations ($P = 0.025$). Averaged across sampling location, aboveground dry matter was greater from XL753 (2862 g m⁻²) than from CLXL729 and Roy J, which did not differ and averaged 2488 g m⁻², while aboveground dry matter measured from CLXL745 (2649 g m⁻²) did not differ from any of the other cultivars. Although previous studies have observed a positive correlation between CH₄ emissions and aboveground dry matter [52, 62, 70, 71], data from this study did not support that relationship, where in this case the cultivar exhibiting greater CH₄ fluxes (Roy J) was one of the lowest accumulators of aboveground dry matter. Huang et al. [71] suggested that the relationship between biomass accumulation and CH₄ emissions, although correlated when examining the relationship for a single cultivar, was not strong when evaluating the relationship among different cultivars.

Averaged across cultivar, aboveground dry matter was slightly greater inside the chamber (2733 g m⁻²) than outside the chamber in the bulk plot (2510 g m⁻²). Similar studies that examined the effect of sampling location, however, did not observe a significant difference in aboveground dry matter [30, 32, 48, 53]. The difference observed in this study may simply be a factor of chamber placement, where greater plant densities were inadvertently included within the chambers. While the reason for increased biomass accumulation within chambers, which was consistent among cultivars, was not apparent, it would seem that the chambers did not adversely impact plant growth, and, if it did impact CH₄ fluxes, the result would be a possible approximate 9% overestimation of CH₄ emissions relative to in-plot plant density.

Similar to aboveground dry matter accumulation and as expected, grain yield differed among cultivars ($P < 0.001$). The hybrid cultivar XL753 achieved the greatest grain yield of 12.3 Mg ha⁻¹, while grain yields from the three other cultivars (CLXL745, CLXL729, and Roy J) did not differ and averaged 9.6 Mg ha⁻¹. Grain yields measured in this study were similar to those reported by Rogers et al. [30] in a similar study on a silt-loam soil in Arkansas and to results of Arkansas Rice Performance Trials conducted in 2014, where XL753, CLXL745, CLXL729, and Roy J attained grain yields of 12.7, 10.0, 9.9, and 9.9 Mg ha⁻¹, respectively [38].

3.5 Seasonal Methane Emissions

As expected, based on CH₄ flux measurements throughout the growing season, season-long, area-scaled CH₄ emissions differed among cultivars ($P = 0.001$), where emissions were greater from Roy J (74.8 kg CH₄-C ha⁻¹ season⁻¹) than from the three hybrid cultivars, which did not differ and averaged 52.4 kg CH₄-C ha⁻¹ season⁻¹ (Table 3). The 30% reduction in CH₄ emissions from hybrid rice cultivars relative to a pure-line cultivar in this study is consistent with previous studies where Smartt et al. [32] and Rogers et al. [30] observed reductions of 31 and 37%, respectively, from CLXL745 relative to two pure-line cultivars on a clay and silt-loam soil, respectively. Similarly, Simmonds et al. [31] measured a 25% reduction from CLXL745 relative to pure-line cultivars in Arkansas, while no differences in CH₄ emissions between hybrid and pure-line cultivars were detected in California. The magnitude of CH₄ emissions measured in this study were 56 to 60% less than emissions measured from similar treatments by Rogers et al. [30] on a similar soil. Lower emissions measured in this study relative to those reported by Rogers et al. [30] may be due to a combination of lower sand content or greater extractable soil P, both of which have been shown to result in reduced emissions [72, 73]. Results obtained by Adviento-Borbe et al. [54], where emissions from CLXL745 averaged 44 kg CH₄-C ha⁻¹ season⁻¹, and Simmonds et al. [31], where emissions from CLXL745 were 56 kg CH₄-C ha⁻¹ season⁻¹ and three pure-line cultivars averaged 75 kg CH₄-C ha⁻¹ season⁻¹, however, were consistent with the results of this study.
Table 3. Summary of methane (CH4) emissions as affected by cultivar expressed on season-long, area- and yield-scaled bases, post-flood release area-scaled basis, and post-flood release percentage of total seasonal emissions from a silt-loam soil during the 2014 growing season at the Rice Research and Extension Center near Stuttgart, AR.

| Emissions property                     | Cultivar       | P   |
|----------------------------------------|----------------|-----|
|                                        | CLXL729        | CLXL745 | XL753 | Roy J |     |
| Area-scaled emissions (kg CH4-C ha⁻¹ season⁻¹) | 55.3b†         | 48.9b    | 53.0b  | 74.8a | 0.001|
| Yield-scaled emissions [kg CH4-C (Mg grain)⁻¹] | 5.85b          | 5.08bc   | 4.31c  | 7.85a | < 0.001|
| Post-flood emissions (kg CH4-C ha⁻¹)     | 6.21b          | 5.30b    | 5.18b  | 8.26a | 0.010|
| Post-flood emissions (% total emissions)  | 11.2           | 10.8     | 9.8    | 11.1  | 0.612|

†Values in the same row followed by different letters are significantly different (P < 0.05).

Similar to area-scaled emissions, yield-scaled CH4 emissions differed among cultivars (P < 0.001; Table 3). Yield-scaled emissions from Roy J were greatest at 7.85 kg CH4-C (Mg grain)⁻¹. As a result of a greater grain yield, XL753 (4.31 kg CH4-C (Mg grain)⁻¹) resulted in lower yield-scaled emissions than CLXL729 (5.85 kg CH4-C (Mg grain)⁻¹), while yield-scaled emissions from CLXL745 did not differ from that from either of the other hybrid cultivars (Table 3). Although grain yields were similar between the two studies, yield-scaled emissions from this study were much less than emissions of 11.1 and 20.1 kg CH4-C (Mg grain)⁻¹ from CLXL745 and two pure-line cultivars, respectively, reported by Rogers et al. [30] due to differences in area-scaled CH4 emissions. Yield-scaled emissions in this study were reduced by 25, 35, and 45% from CLXL729, CLXL745, and XL753, respectively, relative to Roy J. Smartt et al. [32] measured a similar reduction in yield-scaled CH4 emissions of 37% from CLXL745 relative to two pure-line cultivars. Rogers et al. [30] observed a reduction in yield-scaled emissions of 45% from CLXL745 relative to two hybrids, which is slightly greater than the reduction from CLXL745 observed here due to a 12% increase in grain yield from the hybrid relative to the two pure-line cultivars, while yields from CLXL745 and Roy J did not differ in this study.

Methane emissions following flood release differed among cultivars on an area-scaled basis (P = 0.010), while no differences were observed when CH4 emissions were expressed as a percentage of total seasonal emissions (P = 0.612) (Table 3). Similar to results of CH4 fluxes and total emissions, post-flood-release emissions were greater from Roy J (8.26 kg CH4-C ha⁻¹) than from the three hybrid cultivars, which did not differ and averaged 5.56 kg CH4-C ha⁻¹ (Table 3). As a percentage of total area-scaled CH4 emissions, however, post-flood-release emissions did not differ among cultivars and averaged 10.7%. Rogers et al. [30] measured post-flood release emissions ranging from 6.8 to 27.4 kg CH4-C ha⁻¹, which were generally greater than those reported here, likely due to greater overall seasonal emissions. The proportion of CH4 emitted following flood release in this study, however, was similar to emissions ranging from 10.5% from CLXL745 to 16% from a semi-dwarf, pure-line cultivar observed by Rogers et al. [30]. Post-flood CH4 release has been observed in numerous previous studies ranging from 3 to 20% of total area-scaled emissions [30, 32, 51, 54, 56, 70, 74]. While the magnitude of post-flood-release CH4 emissions differs among various treatments, it has become apparent that CH4 has the potential to accumulate in saturated soils and be released as the soil dries and macropores become accessible for gas transport.

4 Conclusions

Numerous studies have provided evidence of differences in microbial community structure, CH4 production and oxidation rates, gas transport capacities, and root exudation rates that result in differential CH4 emissions among rice cultivars. The impact of these various factors has not been well studied in US rice production, but evidence has consistently demonstrated a reduction in CH4 emissions from hybrid rice cultivars grown in the US. This study has elaborated on previous works and demonstrated that other hybrid cultivars have a similar capacity to reduce CH4 emissions relative to pure-line cultivars as CLXL745, which has been consistently demonstrated in the mid-southern US. Studies have regularly reported a link between photosynthetically derived C and CH4 emissions, indicating that differences in C partitioning and root exudation among cultivars can greatly impact CH4 emissions. Plant-derived C plays a major role in CH4 emissions from rice, particularly where organic
residue inputs into the system are limited, such as in the mid-southern US, where rice is generally rotated with soybean and crop residues are largely decomposed prior to flooding the subsequent rice crop. The mitigation of CH$_4$ emissions by hybrid rice cultivars demonstrated in this study shows that simple changes like growing high-yielding, low-emitting cultivars, such as the hybrids included in this study, have great potential for reducing CH$_4$ emissions from rice produced in the US. While other mitigation strategies may be difficult to implement, expensive, and cause yield reductions, switching to cultivars such as XL753 shows potential to decrease CH$_4$ emissions, while maintaining or even increasing yields.

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