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Methane Emissions from Direct-Seeded, Delayed-Flood Rice Grown on a Clay Soil

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ABSTRACT

Due to the production of methane (CH_4) under flooded-soil conditions, rice (*Oryza sativa* L.) cultivation is a major contributor to agricultural CH_4 emissions. Studies examining CH_4 emissions from rice have only recently been initiated in Arkansas and no data have been collected from rice produced on clay soils in Arkansas. Therefore, research was conducted in 2012 and 2013 at the Northeast Research and Extension Center in Keiser, Arkansas to examine the factors affecting CH_4 emissions from rice produced on a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts). The objectives of this study were to determine: 1) the effect of vegetation (i.e., no vegetation, low vegetation, and high vegetation) on CH_4 fluxes and season-long emissions from a clay soil, 2) the effect of chamber size (i.e., 15.2-cm and 30-cm inner diameter) on measurements of CH_4 fluxes and emissions, and 3) the impact of previous crop [rice or soybean (*Glycine max* L.)] and cultivar (Cheniere, Taggart, and CLXL745) on CH_4 fluxes and emissions. Total season-long emissions in 2012 were greatest in the high vegetation treatment ($P < 0.01$), amounting to 35.6 kg CH_4-C ha^{-1}, compared to 1.8 and 8.96 kg CH_4-C ha^{-1} in the no vegetation and low vegetation treatments, respectively. Methane fluxes or season-long emissions did not differ between the two chamber sizes evaluated. Season-long emissions in 2013 were 64% lower ($P < 0.01$) following soybean than following rice, amounting to 7.0 and 19.6 kg CH_4-C ha^{-1}, respectively. Season-long emissions were 31% lower ($P = 0.03$) from the hybrid cultivar (CLXL745) than from the pure-line, semi-dwarf cultivar (Cheniere) or the pure-line, standard-stature cultivar (Taggart), which totaled 10.2, 15.5, and 14.2 kg CH_4-C ha^{-1}, respectively. The low emissions measured in this study, coupled with the magnitude of Arkansas rice production and extent of production on clay and clay-loam soils (> 40%) in Arkansas, indicates that CH_4 emissions from mid-southern U.S. rice cultivation may be substantially

overestimated. Further research on mid-southern CH_4 emissions from various locations and cultural practices will be important to more accurately assess current greenhouse gas emissions from rice production and to mitigate potential negative impacts on the environment.

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DEDICATION

I dedicate this thesis to my wife, Kristen, who encouraged me to enroll as an undergraduate student and supported me in all aspects of life allowing me to complete both my BS and MS degrees at the University of Arkansas. Without the tremendous encouragement and support of Kristen, I would not be who I am today and would not be enjoying such a wonderful and eventful life with the children that have kept my life busy and exciting during this part of my life. I would like to also dedicate this thesis to my babies, Juliet and Maddox, as well as to our older girls, Josephine and Emilia. I dedicate this to my mom and dad, Linda and William, who have instilled in me a strong work ethic and a sense of pride in everything I do, allowing me to accomplish my goals, and who have helped pick up the slack through the sacrifices I have made in traveling across the state on a regular basis and spending long hours analyzing samples.

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INTRODUCTION

Methane (CH_4) is a greenhouse gas much more potent, on a mass basis, than carbon dioxide (CO_2) and is produced by anaerobic *Archaea* under anoxic conditions. Due to the anaerobic conditions that form in saturated soils, flooded rice cultivation is one of the leading global agricultural sources of anthropogenic CH_4 emissions. Methane forms as an end product of anaerobic decomposition of labile organic matter, such as previous-crop residues, residue and exudates from the current crop, animal manure applications, and green manure applications. Fermentation of organic matter by a greater consortium of microorganisms, particularly bacteria, provides CO_2 and acetate as substrates for the methanogenic *Archaea* to produce CH_4. Produced CH_4 then must travel by ebullition, diffusion through the floodwater, or diffusion through the rice plant, via aerenchyma tissue, through oxidized zones of the soil surrounding rice roots and near the surface. A group of aerobic bacteria, methanotrophs, exists in these oxidized zones and has the ability to oxidize CH_4 to CO_2 before it enters the atmosphere. It has been repeatedly demonstrated that plant-mediated diffusion is the dominant transport mechanism of CH_4 to the atmosphere, while diffusion through the floodwater is insignificant and ebullition plays only a minor role, particularly early in the season and with high residue inputs.

The amount of CH_4 emitted from a system is the difference between the amount of CH_4 produced and the amount oxidized. Methane production rates are determined largely by the amount of labile organic matter, where, up to a certain point when another factor becomes limiting, increasing amounts of organic matter generally result in increases in CH_4 production. Methane production is also largely affected by the abundance of terminal electron acceptors in the soil, where production is reduced as the content of electron acceptors in the soil increases. Additionally, environmental factors such as soil temperature impact CH_4 production by causing

an increase in production as temperature increases. The proportion of CH_4 oxidized is largely dependent upon soil redox potential (Eh) and soil particle-size distribution. The rate of soil Eh decline after saturation varies from soil to soil and is generally slower in fine- than in coarser-textured soils. Furthermore, the magnitude of soil Eh decline may vary based on soil characteristics and it has been demonstrated that a greater proportion of CH_4 is oxidized at greater soil Eh values. Research has consistently observed greater oxidation rates in clayey compared to coarser-textured soils due to increased tortuosity, slower movement, and greater entrapment of CH_4 by clay soils.

Rice plants themselves greatly modify both the trend of CH_4 fluxes over time as well as the ultimate magnitude of season-long emissions as plant biomass accumulation has often been related to CH_4 fluxes and season-long emissions. Furthermore, rice cultivar selection, while not well understood, has provided potential for mitigation as certain cultivars have demonstrated reduced emissions relative to others.

While many factors have been determined to impact CH_4 emissions from rice cultivation, due to a lack of data, the United States Environmental Protection Agency (USEPA) currently uses a single emission factor for all non-California primary rice crops. This is the first study conducted on a clay soil in eastern Arkansas, and, together with recent research conducted on silt-loam soils in the region, it may be possible to further refine the USEPA's emission factors to reflect differences in emissions resulting from differences in soil properties, residue management, and cultivar selection.

CHAPTER ONE

LITERATURE REVIEW

Global Climate Change

Global climate change is a concept that has evolved over the past several decades as climate and earth sciences have gained an increased understanding of anthropogenic influences on Earth's climate. The idea of global warming is one of the key elements of global climate change and is supported by a 0.74 °C ± 0.18 °C increase in global mean surface temperatures between 1906 and 2005 (Trenberth et al., 2007). An increase in mean surface temperatures does not imply a warming trend over the entire globe, but rather that warming trends are more common and widespread than cooling trends. Compelling evidence of a global warming trend include a decrease in mean annual arctic sea ice extent of $33 \pm 7.4 \times 10^3$ km^2 yr^{-1} since 1978 as well as mass loss of ice caps and glaciers at an estimated rate of 0.5 mm yr^{-1} in sea level equivalents between 1961 and 2004 (Lemke et al., 2007). Total mass loss from the cryosphere has resulted in a mean sea level rise of approximately 18 cm in the last century (Bindoff et al., 2007).

Anthropogenic effects on Earth's climate extend beyond temperature changes. Additional observed effects include increased precipitation over land in temperate regions and decreased precipitation in the tropics since the 1970s, an increase in heavy precipitation events, and longer, more intense droughts resulting from increased temperatures and decreased precipitation (Trenberth et al., 2007). The direct effect of an increase of less than 1 °C may be of little consequence compared to the indirect impact that the increase in temperature has on precipitation and weather patterns that determine global agricultural productivity and the ability to provide food, fuel, and fiber to an expanding global population. It is important to understand and minimize anthropogenic impacts on global climate change in an attempt to prevent or at least reduce the potential negative impacts of anthropogenic-induced climate change.

Radiative Forcing

Radiative forcing (RF) is a concept that is used to quantitatively compare the strengths of both natural and human causes of climate change. Radiative forcing values are reported in units of Watts m^{-2} (W m^{-2}) and calculated as the estimated difference in RF between present day and the beginning of the industrial era (i.e., 1750). Positive RF values result from factors that lead to global surface warming, while negative RF values are related to cooling factors (Forster et al., 2007). Between 1750 and 2005, long-lived greenhouse gases have led to a combined RF of +2.63 W m^{-2} with CO_2 contributing +1.66 W m^{-2}, CH_4 contributing +0.48 W m^{-2}, nitrous oxide (N_2O) contributing +0.16 W m^{-2}, and halocarbons contributing +0.34 W m^{-2} (Forster et al., 2007). Other results of human activities that contribute to positive RF include increased tropospheric ozone (+0.35 W m^{-2}), along with minor contributions from increased stratospheric water vapor, black carbon on snow, and linear contrails. Significant negative RF arises from the direct effect of atmospheric aerosols (-0.5 W m^{-2}) as well as the indirect cloud albedo effect of aerosols (-0.7 W m^{-2}). The overall combined anthropogenic RF is estimated to be +1.6 W m^{-2}, which is more than five times greater than natural processes (Forster et al., 2007). Based on estimated RF, it appears that increased atmospheric concentrations of greenhouse gases play a dominant role in global warming.

The Greenhouse Effect

The greenhouse effect is a mechanism by which certain gases such as CO_2, CH_4, N_2O, and water vapor (H_2O) absorb and release infrared radiation (IR), interfering with the ability of solar radiation to leave Earth's atmosphere. The absorption of thermal radiation by H_2O and CO_2

was discovered through laboratory experiments in 1859 (Tyndall, 1861). However, other gases including CH_4 and N_2O were not recognized as greenhouse gases until the 1970s (Ramanathan, 1975). Global warming potential (GWP) is a metric that allows the warming impact of various greenhouse gases to be quantitatively compared on the same scale. The assignment of GWP values to gases requires knowledge of the contribution to global warming of gas emissions over time based on the amount of radiation per mass that the gas can absorb and emit as well as the atmospheric lifetime of the gas. Global warming potentials are assigned relative to that of CO_2, so the 100-yr GWP of CO_2, CH_4, and N_2O are 1, 25, and 298, respectively (Forster et al., 2007). One kilogram of CH_4 released to the atmosphere is equivalent to 25 kg of CO_2 being released. Global warming potentials allow greenhouse gas emissions to be reported as CO_2 equivalents in order to compare warming effects of various gases on a single scale.

The current climate change problem is not a result of the greenhouse effect itself, but rather from an increasing greenhouse effect resulting from anthropogenic activities that have increased atmospheric concentrations of greenhouse gases. Prior to 1750, the atmospheric CO_2 mixing ratio was about 280 parts per million (ppm) (Indermuhle et al., 1999). Since the beginning of the industrial era, atmospheric CO_2 has risen drastically to 379 ppm in 2005 (Forster et al., 2007) and 395 ppm as of April 2013 (Tans and Keeling, 2013). Between 1750 and 2005, atmospheric CH_4 has increased from about 700 parts per billion (ppb) to 1,774 ppb (Forster et al., 2007). Nitrous oxide was more variable ranging from 180 to 260 ppb prior to 1750, but has similarly increased to a mixing ratio of 319 ppb in 2005 (Forster et al., 2007). While atmospheric N_2O and CO_2 concentrations have increased steadily over the past several decades, the growth rate of atmospheric CH_4 seems to be declining. The growth rate of atmospheric CH_4 has decreased from highs of about 1% per year in the 1970s and 1980s to

nearly zero between 1999 and 2005. However, the decreasing growth rate is poorly understood (Forster et al., 2007).

Greenhouse Gas Emissions

Globally, CO_2 accounted for about 76% of greenhouse gas emissions in 2004, with around 75% of CO_2 emissions resulting from fossil fuel use and much of the remainder from deforestation and biomass decomposition [Intergovernmental Panel on Climate Change (IPCC), 2007]. Methane and N_2O accounted for 14% and 8%, respectively, of estimated global greenhouse gas emissions in 2004. Major CH_4 sources include agricultural activities, waste management, and energy use, while N_2O emissions are primarily a result of agricultural activities, such as fertilizer use and soil management (IPCC, 2007). In the United States in 2011, an estimated 84% of the total greenhouse gas emissions were CO_2, 9% were CH_4, and 5% were N_2O (USEPA, 2013). Major sources of greenhouse gas emissions are the same in the United States as the global sources mentioned above. The major global sectors responsible for greenhouse gas emissions are energy supply (26%), industry (19%), forestry (17%), agriculture (14%), and transportation (13%) (IPCC, 2007). In comparison, major U.S. sectors are energy supply (33%), transportation (28%), industry (20%), commercial and residential (11%), and agriculture (8%) (USEPA, 2013).

Although agricultural activities do not dominate total greenhouse gas emissions, agriculture contributes an estimated 50 and 60% of global anthropogenic emissions of CH_4 and N_2O, respectively (Smith et al., 2007). Agriculture in the U.S. is responsible for an estimated 33% of anthropogenic CH_4 emissions and 75% of anthropogenic N_2O emissions (USEPA, 2013). Enteric fermentation, rice (*Oryza sativa* L.) cultivation, and manure management contribute an estimated

64, 22, and 8%, respectively, to global anthropogenic agricultural CH_4 emissions, while agricultural N_2O emissions are dominated by agricultural soil management (80%) (USEPA, 2006). In comparison, enteric fermentation, rice cultivation, and manure management contribute 70, 3.7, and 26% to U.S. anthropogenic agricultural CH_4 emissions (USEPA, 2014). Although rice cultivation makes up a small portion of CH_4 emissions in the U.S., globally rice cultivation accounts for approximately 11% of total anthropogenic CH_4 emissions.

Methane emissions from U.S. rice cultivation were estimated to be 7.4 Tg CO_2 Eq. in 2012, a reduction from 9.3 Tg CO_2 Eq. in 2010 due to a decline in production area (USEPA, 2014). Arkansas was responsible for 41% of estimated CH_4 emissions from rice cultivation, although the state accounted for 48% of 2012 U.S. rice production. Louisiana was the next leading contributor to CH_4 emissions accounting for 25% of 2012 emissions, while harvesting 13% of 2012 production (USDA NASS, 2013; USEPA, 2014). Louisiana and Texas CH_4 emissions are large relative to production areas due to extensive ratoon cropping, which occurs on 40 and 61%, respectively, of production area in those states (USEPA, 2014). California, Mississippi, and Missouri, none of which reported any ratoon cropping, contributed 14, 3.4 and 4.8%, respectively, to estimated 2012 CH_4 emissions from U.S. rice cultivation (USEPA, 2014).

Separate emission factors of 178 kg CH_4-C ha^{-1} $season^{-1}$ and 585 kg CH_4-C ha^{-1} $season^{-1}$ were used in the inventory estimates for non-California primary rice cropping and ratooned cropping areas, respectively, as is consistent with IPCC (2006), which recommends calculating separate emission factors for as many different factors and cultural practices as is possible. Emission factors for California rice production are 200 and 100 kg CH_4-C ha^{-1} $season^{-1}$ for winter-flooded and non-winter flooded rice, respectively (USEPA, 2014). While it is known that factors such as water management, soil properties, rice cultivar, fertilizer management, and

residue management have strong impacts on CH_4 emissions from rice, data available from U.S. studies limits the further disaggregation of these factors (USEPA, 2014). The non-California primary crop emission factor is based on U.S. studies with emissions ranging from 46 to 375 kg CH_4-C ha^{-1} $season^{-1}$ (Byrd, 2000; Kongchum, 2005; Rogers et al., 2012; Sass et al., 1991a, 1991b, 2002a, 2002b; Yao, 2001) and the ratoon crop factor is based on studies conducted in Louisiana with emissions ranging from 361 to 1118 kg CH_4-C ha^{-1} $season^{-1}$ (Lindau and Bollich 1993; Lindau et al., 1995). The California specific factors include studies with emissions ranging from 47 to 166 kg CH_4-C ha^{-1} $season^{-1}$ for the non-winter flooded and from 98 to 277 kg CH_4-C ha^{-1} $season^{-1}$ for the winter-flooded rice (Bossio et al., 1999; Fitzgerald et al., 2000).

Rice Production

Rice is a semi-aquatic cereal grain that makes up about 21% of total global grain production (USDA FAS, 2013a). The importance of rice is further exemplified by the fact that rice is a staple crop for about half of the global population, with direct human consumption accounting for 85% of rice production compared to 72% of wheat (*Triticum aestivum* L.) and 19% of maize (*Zea mays* L.) production (Chang, 2000; Maclean et al., 2002). In Southeast Asia, 60% of human food intake is provided by rice as well as 35% of food intake in both East Asia and South Asia (Chang, 2000). Rice has the ability to support more people per unit of land than wheat or maize because rice produces, on an average yield basis, more food energy and protein per hectare than wheat or maize (Lu and Chang, 1980).

The two distinct forms, or species, of cultivated rice are known as African rice (*Oryza glaberrima* Steud.) and Asian, or common, rice (*Oryza sativa* L.), while 20 additional wild species of rice are also recognized (Chang, 2003). Common rice is grown throughout much of

the world and comprises the majority of rice production, whereas African rice is confined to West Africa (Chang, 2000). There are three different types, or ecogenetic races, of *O. sativa.* The *indica* type is generally tropical in origin and is characterized by tall plants with profuse tillering that shatter easily. The *japonica* cultivars are the temperate-zone counterpart characterized by short- to medium-statured plants with medium tillering capacity and low shattering. The final type is the *javanica* cultivars, which are characterized by taller, slower growing plants with lower tillering capacity and larger grains compared to the other two types (Chang, 2003). *Indica* cultivars make up approximately 80% of worldwide production, while the United States and other temperate growing regions rely heavily on *japonica* varieties (Mackill, 1995). Cultivars being developed in the United States are trending toward reduced plant height and shorter growth duration with breeding programs focusing on the development of semi-dwarf cultivars, although Arkansas has recently released some taller varieties (Mackill and McKenzie, 2003). The development of *indica/japonica* hybrids is an active area of research where yields greater than conventional varieties can be attained with lower seeding rates due to greater tillering capacity of the hybrids (Mackill and McKenzie, 2003).

The exact center of origin for rice is difficult to pinpoint due to the widespread occurrence of wild *Oryza* species throughout Asia, Africa, Central and South America, and Australia (Chang, 2000). Beginning in the early 1970s, it has generally become accepted that the origin of the genus was the Gondwana supercontinent, where a single wild species became dispersed and genetically differentiated over time to form the wild species that were spread throughout the globe as the land mass broke up and continents became separated (Chang, 2003). Human domestication of rice is also somewhat unclear, although it has been postulated that rice was first domesticated between 8,000 and 10,000 years ago (Greenland, 1997). Rice cultivation

has played a large role in human civilizations and expanded quickly as irrigation technologies were developed and spread circa 300 B.C. during the Iron Age (Randhawa, 1980).

Rice Production Extent

Asian rice is commercially produced in 112 countries worldwide, spanning latitudes from 53°N along the Amur River at the China-Russia border to 35°S in central Argentina (Chang, 2000). In 2012, more than 158 million hectares globally were planted to rice with average yields of 4.43 Mg ha^{-1} for a total global production of 470.2 million metric tons (Tg) of rice. Comparatively, nearly 216 million hectares were planted in wheat in 2012 with average yields of 3.04 Mg ha^{-1} for a total of 656 Tg of global wheat production. More than 174 million hectares were planted in maize in 2012 with an average yield of 4.91 Mg ha^{-1} and a total global production of 857 Tg of maize (USDA FAS, 2013a). Global rice production peaked in 1994 at 534 Tg of rice, with Asia being responsible for 90% of that production (Chang, 2003). The majority of global rice production occurs in East Asia, South Asia, and Southeast Asia, which together accounted for 90% of global production in 2012. Substantial production also occurs in South America (Brazil and Peru), Sub-Saharan Africa (Nigeria and Madagascar), Europe (Italy and Spain), Egypt, and the United States (USDA FAS, 2013a).

China and India currently dominate global rice production accounting for 30 and 22%, respectively, of the total 470.2 Tg of global production in 2012. The third, fourth, and fifth ranked global producers in 2012 were Indonesia (8%), Bangladesh (7%), and Vietnam (6%). The remaining top 10 producers, in order, were Thailand, the Philippines, Burma, Brazil, and Japan, followed by the eleventh ranked U.S., which accounted for 1.3% of global production (USDA FAS, 2013a). The U.S., however, plays a larger role in global exports contributing 9.2% of 2012

global exports and ranking fifth after Thailand (21.4%), India (20%), Vietnam (19.8%), and Pakistan (10.2%). Global exports in 2012 were estimated to be 7.9% of total production, while the U.S. exported 55% of 2012 production (USDA FAS, 2013b). Global rice yields in 2012 were estimated to be 4.43 Mg ha^{-1}, compared to 8.35 Mg ha^{-1} in the U.S., which was second only to Egypt (8.8 Mg ha^{-1}) among major rice-growing countries. The two top rice-producing countries, China and India, had estimated yields of 6.74 Mg ha^{-1} and 3.63 Mg ha^{-1}, respectively (USDA FAS, 2013a).

Nearly 1.1 million ha of rice were planted in the U.S. in 2012 yielding an average of 8.35 Mg ha^{-1} for a total production of 9.05 Tg of rice prior to milling, compared to 23 million ha planted with an average yield of 3.11 Mg ha^{-1} for a total of 62 Tg of wheat production, and over 39 million ha of planted maize with average yields of 7.74 Mg ha^{-1} for a total production of 274 Tg (USDA NASS, 2013). The four major regions that produce rice in the U.S. are the Arkansas Grand Prairie, the Mississippi Delta, which is made up of portions of Arkansas, Missouri, Mississippi, and Louisiana, the Gulf Coast (Texas and Southwest Louisiana), and California's Sacramento Valley. Most U.S. states produce primarily long-grain cultivars, while much of the medium-grain rice and nearly all of the short-grain rice is produced in California (USDA NASS, 2013). Although Oklahoma and Florida are often included as rice producing states, the six previously mentioned states make up essentially all of U.S. production in recent years (USDA NASS, 2013). Arkansas is the leading state in both area of cultivation and total production, contributing 48% of total U.S. rice production in 2012, followed by 23% of production by California and 13% of production by Louisiana (USDA NASS, 2013). Arkansas rice production takes place in the eastern portion of the state with the top five rice-producing counties in 2012

being Poinsett, Lawrence, Arkansas, Greene, and Cross, which made up 35% of the state's production area (Hardke and Wilson, 2013b).

Global Rice Production Practices

Production practices in rice vary globally based on economic, cultural, and climatic factors, each of which show temporal and spatial variability throughout the rice-growing countries. A simple classification or characterization of rice production systems is nearly impossible on a global scale due to the variability of factors that influence production. Classifications of rice production techniques are commonly based upon flood presence (upland or lowland), water source (irrigated or rainfed), and stand establishment technique (transplanting, direct seeding, or water seeding) with many combinations and variations of these techniques occurring throughout the globe (De Datta, 1981). In one of the most recent classification attempts, Chang (1999) classified global rice production into five major agroecosystems; i) irrigated wetland, which made up 53% of global rice production area and had the greatest yield potential at 3 to 5 Mg ha^{-1}, ii) rainfed wetland, making up 26% of global area and yielding 2 to 4 Mg ha^{-1}, iii) flood-prone or tidal swamps, which made up an insignificant area, iv) deep water (1-5 m), making up 8% of global area, and v) dryland, which made up an estimated 13% of global production area with average yield potentials of 1 to 1.5 Mg ha^{-1}.

While a small portion of rice is produced under upland conditions, the majority of rice production requires substantial quantities of water in order to maintain a flood on the semi-aquatic crop. In much of the tropical rice-growing area, particularly South and Southeast Asia, rainfed rice is the main production system where most of the production comes from wet-season harvests and the cropping season is determined by rainfall patterns (De Datta, 1981). In

temperate production areas, rice production must coincide with suitable temperatures for the crop which, coupled with inadequate rainfall, requires that temperate rice be almost entirely irrigated in order to maintain a flood for the duration of the growing season (De Datta, 1981). The utilization of irrigation in temperate areas allows greater control of environmental factors, which ultimately tends to increase yields, while rainfed systems may suffer from droughts and floods that may substantially damage crops and reduce yields (De Datta, 1981).

Direct seeding and transplanting are common establishment techniques in both irrigated wetland and rainfed wetland systems, while direct seeding is the major practice in dryland and deep-water agroecosystems (Chang, 1999). While transplanting does occur in irrigated wetland systems and direct seeding occurs in rainfed wetland systems, it is more common for irrigated systems to utilize direct seeding and for rainfed systems to use transplanting techniques (De Datta, 1981). Transplanting systems involve raising seedlings in a nursery seedbed area at the beginning of the season and transplanting into puddled paddy soils early in the vegetative growth stage. Transplanting is the major establishment system for rainfed rice in tropical Asia with the majority of production in Northeast India, Bangladesh, and Thailand relying upon transplanting techniques (De Datta, 1981). Direct seeding by grain-drilling or broadcasting pre-germinated seeds onto puddled soil is practiced in parts of India, Sri Lanka, Bangladesh, and the Philippines, while drill seeding into dry soil is the most common practice in the U.S. and other mechanized regions such as Australia (De Datta, 1981). Rice seed may be broadcast onto dry or moist soil by airplane followed by harrowing to cover seeds, but this establishment method requires more seed and stand establishment is often poorer than with drill-seeding (De Datta, 1981). Water-seeding is an establishment technique that originated and is practiced in parts of Asia, where pre-germinated seeds are broadcast from an airplane into already flooded fields (De Datta, 1981).

United States Rice Production Practices

Rice production under mechanized U.S. systems requires high temperatures, nearly level land, plentiful water, and soils that inhibit percolation of floodwater, so production is limited to Arkansas, Louisiana, Mississippi, Missouri, Texas, California, and Florida (Street and Bollich, 2003). All U.S. rice is produced using high input, mechanized production practices, but practices also vary somewhat from region to region based on differences in climate, soils, weed proliferation, and other factors that influence production. Essentially all U.S. rice is irrigated and sources of irrigation water include deep or shallow groundwater wells, runoff reservoirs, rivers, bayous, and lakes (Street and Bollich, 2003). It is estimated that between 1000 and 2500 m^3 ha^{-1} of water are required to produce a rice crop in the southern U.S. and generally less than one third of that requirement is met by rainfall (Martin et al., 1976). Levees, which separate fields into bays, or paddies, and control flood depth (by use of gates or spills), are commonly constructed on contours that were surveyed on 3 to 6 cm vertical intervals. This creates winding, contour-shaped levees in fields that are not precision leveled, whereas precision leveling to a uniform grade of 0.2% or less allows the construction of uniformly spaced straight levees and may reduce the number of levees required (Street and Bollich, 2003).

The two stand establishment techniques utilized in the U.S. are dry-seeding and water-seeding. Dry-seeding techniques, particularly drilling, are predominant in most of the U.S., while water-seeding techniques are used extensively in California and to a small degree in Southwest Louisiana as a weed control method (Street and Bollich, 2003). A continuous flooding water-seeding technique is used in California, where pre-germinated seeds are broadcast by airplane into flooded fields and the seedlings grow through a standing flood, while a pinpoint flood water-

seeding technique is used in Louisiana where seeds are broadcast into a flooded field that is drained within a few days and then permanently flooded after drying for 3-5 days (Linscombe et al., 1999; Street and Bollich, 2003). In dry-seeded systems, seed is most often drilled on 15 to 25 cm rows to a depth of 2.5 cm or less into a well-pulverized, firm, and weed-free seedbed. When rice is following a high residue crop (i.e., rice or maize) it is necessary to till the land in the fall or early spring so that decomposition of the residue does not immobilize nutrients after rice is planted, whereas rice following soybean (*Glycine max* L.) may not require as much preparation because residues are not as persistent (Klosterboer and Turner, 1999; Street and Bollich, 2003).

Water management at planting varies across U.S. systems, but a permanent flood is established in all systems usually by the four-leaf growth stage (V4; Moldenhauer et al., 2013). Flush irrigation is used as necessary to promote germination and seedling growth in dry-seeded rice systems prior to establishment of a permanent flood at three to four weeks after emergence (i.e., V4 or V5). Mid-season drainage is typically avoided except for certain mid-season fertilizer applications, to aerate the soil in order to treat or prevent disorders such as straighthead and hydrogen sulfide toxicity, or to apply pesticides. Fields are drained prior to harvest in order to dry the soil enough for operation of harvest equipment (Street and Bollich, 2003). Fields are flooded again within five to seven days after primary-crop harvest in ratoon cropping systems, which are common in Southwest Louisiana, Texas, and Florida, and the flood is maintained until harvest of the ratoon crop (Street and Bollich, 2003).

Crop rotations are important in rice, especially where weedy rice, (*Oryza spp.*) is problematic and difficult to control during rice cropping seasons. In order to suppress weedy rice, nearly all rice in Louisiana is grown either in a 1:1 rotation with soybean or a 1:1:1 rotation where crawfish (*Procambarus clarkia*) are double cropped following rice, with soybean

produced the following season (Street and Bollich, 2003). In 2012, greater than 70% of Arkansas rice was produced in rotation with soybean, with most of the remaining production in a rice-rice rotation (Hardke and Wilson, 2013b). In California, approximately 70% of rice is produced in a rice-fallow or rice-rice rotation (Hill et al., 1992).

Arkansas Rice Production Practices

Arkansas rice production began in 1902 when one acre was planted in Lonoke County. Production increased over time until 1955 when government quotas limited production to 500,000 acres. The limitation was lifted in 1974 and production increased again peaking in 1981 at 1.54 million acres, again in 1999 with 1.65 million harvested acres, and finally in 2010 with 1.79 million acres (Hardke and Wilson, 2013a). In 2012, 1.28 million acres of rice were harvested in Arkansas (USDA NASS, 2013). Rice production in Arkansas is highly mechanized with a heavy dependence upon synthetic fertilizers, chemical pest control, and machinery. Planting of rice in Arkansas generally begins the last week of March and extends into early June with floods typically being established by the end of May or early June. Harvesting operations usually begin in mid-August and peak in early- to mid-September (Hardke and Wilson, 2013a).

Arkansas rice is produced on a wide variety of soils ranging from sandy soils to clay soils with the differing textural classes generally requiring different management, especially with regards to tillage practices and nutrient management (Hardke and Wilson, 2013b; Wilson et al., 2013). Production on sands and sandy loams is minor and has been decreasing from 3.1 and 5.2% of Arkansas acreage, respectively, in 2007 to 0.7 and 3.7%, respectively, in 2012. Arkansas production on clay and clay-loam soils, however, has increased from under 40 to 47.8% between

2007 and 2009, declining to 42.8% in 2012. Production on silt-loam soils has remained fairly steady at 52.1% in 2007 and 52.8% in 2012 (Hardke and Wilson, 2013b; Wilson et al., 2010).

Dry-seeding techniques have always dominated in Arkansas. Water seeding has varied between 2.2 and 8.2% of production area between 2007 and 2012, with an estimated 5.2% of 2012 Arkansas rice acreage being water seeded (Hardke and Wilson, 2013b; Wilson et al., 2010). Approximately 80% of 2012 Arkansas rice acreage was drill seeded, compared to approximately 20% being broadcast seeded (Hardke and Wilson, 2013b). It is recommended that 320 seeds per m^2 (30 seeds per ft^2) are drill seeded using an ideal row spacing of 15- to 20-cm (6- to 8-in) in order to attain optimal stand densities between 110 and 220 plants per m^2 (10 and 20 plants per ft^2) when using conventional cultivars. Stand densities may be reduced to between 65 and 110 plants per m^2 (6 to 10 plants per ft^2) by seeding at rates between 110 and 165 seeds per m^2 (10 and 15 seeds per ft^2) with high-tillering hybrid cultivars that are becoming more widely utilized (Wilson et al., 2013). It is necessary to increase seeding rates for conditions such as no-tillage and early seeding (10% increase), broadcast seeding and clay soils (20%), and water-seeding (30%) in order to achieve good stands (Wilson et al., 2013). Drilling into or broadcasting onto a dry, pulverized seedbed is recommended when daily average 10-cm-depth soil temperatures reach 16°C (60°F). Broadcast seeds are covered by either a final tillage operation after broadcasting or by flushing fields after levees are constructed, while drill seeding may require a rolling operation to ensure good seed-to-soil contact (Wilson et al., 2013). Conventional tillage accounted for over half of Arkansas acreage, while stale-seedbed (tillage and floating in the fall or winter) and no-tillage accounted for 35 and 10% of acreage, respectively, in 2012 (Hardke and Wilson, 2013b). Stale-seedbed and no-tillage are oftentimes utilized on clay soils where

conventional tillage produces a cloddy seedbed with poor seed-to-soil contact (Wilson et al., 2013).

While pinpoint water-seeding techniques do occur in Arkansas, over 90% of Arkansas rice production acreage utilizes a delayed-flood system where permanent floods are not established until the 4-5 leaf growth stage, which generally occurs approximately three to four weeks after emergence (Hardke and Wilson, 2013b). Fields are drained two to three weeks prior to harvest and most fields remain unflooded until the subsequent rice crop is produced, while nearly 20% of Arkansas rice acreage is winter flooded (Hardke and Wilson, 2013b; Street and Bollich, 2003). Over 75% of Arkansas rice is irrigated by groundwater with 10 and 13% of acreage utilizing water stored in reservoirs and from streams/rivers, respectively (Hardke and Wilson, 2013b).

The two methods of nitrogen (N) fertilization in Arkansas are the standard two-way split system, where 65 to 75% of the total N is applied pre-flood with the remainder applied mid-season in one or two applications between beginning internode elongation and half inch internode elongation (R0 to R1), and the single optimum pre-flood system, where a single N application is made immediately prior to flooding. Nitrogen fertilizer rate recommendations have previously been based only on cultivar, soil texture, and previous crop. Implementation of the new N-Soil Test for Rice (N-STaR) enables recommendations to be adapted to the soil's ability to supply N to the rice crop on a field-by-field basis, reducing the likelihood of over and under fertilization of N (Norman et al., 2013). Ammonium-N sources, such as urea and ammonium sulfate, are used in order to prevent N loss through denitrification that occurs with NO_3^--N fertilizers. Phosphorus and potassium are incorporated prior to planting as recommended by

routine soil tests (Norman et al., 2013). Organic amendments are uncommon, although poultry litter is utilized to a small degree, especially in precision-leveled fields.

Flooded Soils

The saturated soils that occur during wetland, or lowland, rice cultivation give rise to a set of physical, chemical, and biological properties that are quite different from upland soils. Rice is the only major row crop produced under flooded-soil conditions and the absence of air-filled pores along with reduced soil-atmosphere interactions results in an almost entirely different set of processes than those occurring in upland cropping systems.

Physical Characteristics of Flooded Soils

The major physical difference between saturated and unsaturated soils involves the availability and rates of movement for gases and solutes. Under aerated conditions, the soil atmosphere contains essentially the same gases as the atmosphere although the proportions of oxygen and CO_2 differ from the atmosphere due to soil respiration (Scott et al., 2003). Carbon dioxide diffuses into the atmosphere from the soil due to production during respiration and oxygen diffuses into the soil as it is consumed during respiration. The saturation and ponding of flooded soils greatly reduce gas transport between the soil and atmosphere compared to aerated soils and plant-mediated transport of gases by diffusion is often the main exchange mechanism between the soil and atmosphere in saturated or flooded systems (Livingston and Hutchinson, 1995). As a flooded soil dries, gases trapped in the soil may escape due to increases in rates of diffusion and convective flow that occur as water escapes the soil pores.

While solute movement by diffusion may be greater in saturated soils due to an increase in water-filled pore space, diffusion of gases through water is roughly 10,000 times slower than diffusion of gases through air (Greenwood, 1961; Hillel, 2004). Both diffusion and convective flow of gases and solutes are related to pore connectivity and tortuosity, so it is expected that movement of gases and solutes are slower in fine-textured soils, such as clays and clay loams, than in coarser-textured soils, such as silt loams and sands, which generally have larger, more connected pores (Hillel, 2004). Convective flow of gases in saturated soils can occur as dissolved gases move with moving soil water, which is dependent largely upon soil texture and structure, and as ebullition, gases escaping as bubbles through ponded water (Hillel, 2004). Generally diffusion dominates gas transport in fine-textured soils, such as clay loams and clays, and diffusion rates typically decrease as particle size decreases, which is due to differences in size, orientation, and shape of soil pore spaces (Livingston and Hutchinson, 1995; Nazaroff, 1992). Soil texture also affects the amount of time it takes a soil to become saturated with infiltration rates in clayey soils estimated to generally be 1-5 mm hr^{-1} compared to 10-20 mm hr^{-1} in soils such as silt loams (Hillel, 2004). The amount of time a soil takes to become saturated has an effect on chemical and biological processes that develop as the system becomes anaerobic.

Soil Redox Potential

Isolation of flooded soils from the atmosphere and depletion of soil oxygen induces biological and chemical reactions that create anaerobic and reducing conditions rather than the aerobic and oxidized conditions that generally occur in upland soils. Organic matter decomposition slows under anaerobic conditions, but as organic matter is oxidized, transformations such as denitrification and manganese (Mn) and iron (Fe) reduction occur as

well as production of gases such as hydrogen sulfide (H_2S) and CH_4. Soil reduction/oxidation (redox) reactions are coupled half-reactions where the oxidation of organic matter, which provides electrons, is coupled with the reduction of elements or compounds that act as electron acceptors (Vepraskas and Faulkner, 2001). Oxygen is the major electron acceptor under aerobic conditions, but as oxygen is depleted, the sequence of electron acceptors shifts to NO_3^-, MnO_2, $Fe(OH)_3$, SO_4^{2-}, and CO_2, which are theoretically reduced in that order based on thermodynamic favorability (Scott et al., 2003; Turner and Patrick, 1968). The reduced forms of the previously mentioned terminal electron acceptors, respectively, are H_2O, N_2, Mn^{2+}, Fe^{2+}, H_2S, and CH_4. Soil redox reactions in a controlled laboratory environment may follow the theoretical sequence, but environmental conditions in the field result in spatial variability of oxidizable organic compounds, electron acceptors, and microorganisms that cause substantial overlap of the terminal electron acceptor sequence (Scott et al., 2003; Vepraskas and Faulkner, 2001).

Soil redox potential (Eh) is a measure of the electrical potential status of a system that results from the tendency of substances in the system to donate or acquire electrons (Brady and Weil, 2008). Soil redox potential is measured in millivolts (mV) using a platinum (Pt) electrode along with a mercury-chloride (HgCl) or silver chloride (AgCl) reference electrode both connected to a voltmeter (Vepraskas and Faulkner, 2001). Combination platinum electrodes are also available that can continuously monitor soil Eh when connected to a logger box. When using AgCl electrodes, a correction factor of +199 mV is added to field-measured voltages in order to adjust measurements to the standard hydrogen electrode (Patrick et al., 1996). In well-aerated soils, soil Eh may be as great as +700 mV, but Eh values near -300 mV may be observed in saturated organic-matter-rich soils (Brady and Weil, 2008). As a system shifts from aerobic to anaerobic and soil redox potential declines, atmospheric oxygen (O_2) is reduced first at +380 to

+320 mV, followed by NO_3^- (+280 to +220 mV), MnO_2 (+220 to +180 mV), $Fe(OH)_3$ (+110 to +80 mV) , SO_4^{2-} (-140 to -170 mV), and CO_2 (-200 to -280 mV), based on measurements by Patrick and Jugsujinda (1992).

Soil pH and Nitrogen Transformations

Soil pH values tend to increase toward neutrality following submergence of acidic soils due to the consumption of protons in the redox reactions that occur in anaerobic soils. Similarly, alkaline soils approach neutrality following saturation, with the decline in pH being attributed to the $CaCO_3$-H_2O-CO_2 system that buffers soil pH (Scott et al., 2003). As a result of these processes, rice soils typically stabilize at pH values near 6.5 to 7.0 after prolonged submergence.

The major forms of N in the soil are organic-N (ON), which consists of N contained in plants, microbes, and soil organic matter, and inorganic-N, which is mostly NO_3^-, and NH_4^+ (Vepraskas and Faulkner, 2001). Under most circumstances, 95 to 99% of soil N is contained in the organic pool, which may then be slowly mineralized to NH_4^+ by soil microbes (Brady and Weil, 2008). Many studies suggest that only 1.5 to 3.5% of the ON of a soil mineralizes annually, however up to 25% of previously immobilized fertilizer N may become available through mineralization (Brady and Weil, 2008). Studies conducted in Japan and the Philippines suggest that 50 to 80% of N requirements of a rice crop may be obtained through mineralization of organic matter (Broadbent, 1979; Koyama, 1975). Decomposition and mineralization of organic matter occurs in both aerobic and anaerobic environments. Anaerobic decomposition is much slower than aerobic decomposition, but anaerobic mineralization retains approximately five times more NH_4^+ than under aerobic conditions where the NH_4^+ may be nitrified and more susceptible to loss mechanisms such as leaching or denitrification (Patrick, 1982).

While rice crops may obtain a portion of their N needs from mineralization of soil organic matter, optimal grain yields require substantial inputs of mineral N-fertilizer, with most U.S. cultivars requiring 135 to 200 kg inorganic-N ha^{-1} (Norman et al., 2003). In order to prevent N-loss by denitrification in lowland rice cultivation, it is recommended to use NH_4^+ or NH_4^+-forming fertilizers, such as urea or ammonium sulfate, and to establish the flood soon after N-application (Norman et al., 2003). A nitrification-denitrification loop exists in which NH_4^+ diffuses into aerobic zones, such as exist at the soil-floodwater interface or surrounding rice-roots, where nitrification occurs, followed by diffusion of the formed NO_3^- back into anaerobic zones of the soil where denitrification can occur (Reddy et al., 1976). This loss mechanism is of minor significance in direct-seeded, delayed-flood systems because pre-flood N is generally taken up within the first three weeks after application (Bufogle et al., 1997; Norman et al., 2003). Mid-season N-applications can be taken up in three to seven days, with up to 80% efficiency, if applied properly (Wilson et al., 1989). Ammonia volatilization is another loss mechanism for NH_4^+, which can be minimized by incorporating urea with a flood within five days after application to a dry soil surface or by using an effective urease inhibitor such as NBPT (Norman et al., 2003).

Methane Emissions from Rice

Methane emissions from a particular ecosystem are governed by the magnitude and balance of microbial CH_4 production (methanogenesis) and oxidation (methanotrophy), which occur by separate microbial communities. The two groups of microorganisms are adapted to different environmental conditions, and, as a result, are affected differently based on the structure and conditions of an ecosystem, which results in variability of CH_4 production and oxidation

potentials across time and space (Conrad, 1989). With low CH_4 production rates or long diffusion pathways, it seems that the majority of produced CH_4 is oxidized. Conversely, in cases where CH_4 production rates are high or diffusion paths are short, less CH_4 is oxidized and a greater portion reaches the atmosphere (Conrad, 1989).

Methane Production and Oxidation

Methane production occurs toward the end of a complex anaerobic decomposition processes in which organic matter is degraded to acetate, hydrogen gas (H_2), and CO_2 by a community of various fermenting microorganisms (mostly bacteria). Methanogenic *Archaea* are then able to split acetate into CH_4 and CO_2 (acetoclastic methanogenesis) or utilize H_2 and CO_2 to produce CH_4 (hydrogenotrophic methanogenesis) (Conrad et al., 2006; Nazaries et al., 2013). Methanogens encompass a large group of strictly anaerobic obligate *Archaea*, which is currently composed of three classes, six orders, 12 families, and 35 genera (Nazaries et al., 2013). Rice Cluster I (RCI) is a specific group of methanogens identified by Grosskopf et al. (1998) that contains enzymes in order to detoxify highly reactive oxygen species, allowing them to survive in aerated soils or oxygenated rhizospheres, and occurs preferentially in environments that undergo transient aerobic conditions, such as rice fields (Conrad et al., 2006; Seedorf et al., 2004). Rice cluster I has been detected in almost all rice field soils tested (Conrad et al., 2008; Ramakrishnan et al., 2001) and occur in great abundance in rice soils and on rice roots, representing up to 50% of total methanogens in rice fields (Kruger et al., 2002). Rice cluster I has been identified as occupying a niche on rice roots by producing CH_4 from photosynthates released as root exudates (Conrad et al., 2006; Lu and Conrad, 2005). Recent research has confirmed that methanogens are ubiquitous in aerobic soils and have the ability to produce CH_4

as soon as anoxic conditions form and substrate is available (Nazaries et al., 2013). Conrad (2002) reported that methanogens isolated from the soil of rice fields were not killed, but only inhibited by high redox potentials or O_2 exposure, allowing them to survive drainage and maintain their population size throughout the year in a state of low activity.

Most methanogens are mesophiles and neutrophiles, with optimal growth occurring between 30 and 40 ˚C and between a pH of 6 and 8 (Conrad, 1989). Methanogens are highly sensitive to variations in temperature and pH and CH_4 production is greatly reduced when soil temperatures are low or in acidic or alkaline soils (Nazaries et al., 2013). Within the optimal temperature range, which is generally the case during the rice growing season, temperature has a positive effect on methanogenesis, causing an increase in CH_4 production as temperature increases (Conrad, 1989; Nazaries et al., 2013).

Methane oxidation is achieved by a group of aerobic *Proteobacteria* known as methanotrophs, which only utilize CH_4 or methanol as a source of C and energy and are currently classified into two phyla, three orders, four families, 21 genera, and 56 species (Nazaries et al., 2013). One group, known as low-affinity methanotrophs, is capable of oxidizing high CH_4 concentrations (> 100 ppm) and exists at oxic-anoxic interfaces, where they consume CH_4 produced in anoxic environments (Nazaries et al., 2013). Another group, known as high-affinity methanotrophs, exists in upland soils and possesses the ability to oxidize CH_4 at low atmospheric levels (< 2 ppm) (Bender and Conrad, 1992). Unlike methanogenesis, methanotrophy is not impacted greatly by temperature, although CH_4 oxidation is decreased below 10 ˚C and above 40 ˚C, or pH, as similar CH_4 oxidation has been observed in soils with pH values ranging from 3.5 to 8 (Nazaries et al., 2013). Due to the differing effect of temperature on methanogenesis and methanotrophy, CH_4 production increases as soil temperatures increase,

while CH_4 oxidation changes little, resulting in an increase in CH_4 emissions as soil temperature increases. This effect has been confirmed in a laboratory incubation of anaerobic soils at various temperatures between 5 and 25 ˚C (Van Winden et al., 2012).

Substrate for Methane Production

Available OM stimulates CH_4 production due to enhanced fermentative production of acetate and H_2/CO_2 and, in principle, CH_4 production could be expected to be proportional to organic C inputs, but the reduction of nitrate (NO_3^-), iron (Fe), manganese (Mn), and sulfate (SO_4^{2-}) all precede methanogenesis and reduce the amount of available C for CH_4 production (Conrad, 1989). Methane production may be stimulated by root exudates (Aulakh et al., 2001a, 2001b; Dannenberg and Conrad, 1999; Lu et al., 2000) or the application of animal manure (Buendia et al., 1998), green manure (Bronson et al., 1997; Denier van der Gon et al., 1996; Shang et al., 2011; Tsutsuki and Ponnamperuma, 1987), or rice straw (Bronson et al., 1997; Dannenberg and Conrad, 1999; Schutz et al., 1989a; Tsutsuki and Ponnamperuma, 1987; Yagi and Minami, 1990), while the application of composted organic C sources does not greatly increase CH_4 production (Denier van der Gon and Neue, 1995; Tsutsuki and Ponnamperuma, 1987; Yagi and Minami, 1990). This indicates that the amount of available OC is more important in determining CH_4 production than total OC (TOC), as composted residue contains lower amounts of degradable C on a mass basis compared to fresh residues (Inoko, 1984). Yagi and Minami (1990) and Wang et al. (1993) confirmed a positive correlation between CH_4 production and readily mineralizable C, while studies have indicated no clear relationship between soil TOC and CH_4 production (Cheng et al., 2007; Huang et al., 2002; Lu et al., 2000; Neue et al., 1997). Research conducted by Denier van der Gon and Neue (1995) determined that increasing fresh

OM inputs would result in increases in CH_4 production up to a point where another factor becomes limiting, however, fresh green manure inputs up to 20 Mg ha^{-1} still indicated OC limitations. In most rice production situations, organic residue inputs are below 20 Mg ha^{-1} and will generally exhibit an increase in CH_4 emissions as organic inputs increase.

Using ^{13}C-labeled rice straw incorporated at 6 Mg ha^{-1}, Watanabe et al. (1999) determined that 42% of season-long CH_4 emissions originated from rice-straw C, 37 to 40% from the rice plant, and 18 to 21% from SOM. The contribution of SOM to CH_4 production was fairly consistent over the growing season, while the contribution from rice straw decreased from nearly 90% at 14 days after transplanting to only 11 to 16% during heading and grain fill. In contrast, the contribution of living rice plants to CH_4 production increased over time and amounted to 65 to 70% during heading and grain fill (Watanabe et al., 1999). Chidthaisong and Watanabe (1997) also observed that the contribution of rice straw to CH_4 production was greatest at 20 to 40 days after flooding, while plant-derived C became increasingly more important as the season progressed. The link between root exudates and CH_4 production has been observed directly by Aulakh et al. (2001b), who showed a positive correlation between TOC in root exudates and CH_4 production. Several others have observed an inverse relationship between grain yield and CH_4 production (Denier van der Gon et al., 2002; Sass and Cicerone, 2002), indicating that lower grain yields are accompanied by greater CH_4 production as a result of greater root exudation, which was confirmed by Aulakh et al. (2001a). Using ^{13}C-labeled CO_2, Minoda and Kimura (1994) and Minoda et al. (1996) observed that photosynthates were a major source of CH_4 and accounted for 3.8 to 52% of CH_4 under field conditions.

Duration and Timing of Methane Production

Methane production occurs some period of time following a period of prolonged saturated conditions and continues until the C substrate becomes limiting or environmental conditions limit methanogenesis (i.e., the soil becomes too cold, hot, or aerated). In flooded soils, the rate of reduction processes is determined by the composition and texture of a soil as well as the content of inorganic electron acceptors (i.e., NO_3, Fe, Mn, SO_4), so the amount of time between flooding a soil and the onset of methanogenesis can vary from several days to several weeks (Ponnamperuma, 1981). From the onset of methanogenesis, CH_4 emissions generally increase over time as the soil becomes more reduced and usually shows one or more of three general peak flux trends. Early-season peak fluxes are generally attributed to decomposition of freshly incorporated residues and generally occur within 20 to 40 DAF (Chidthaisong and Watanabe, 1997; Wassmann et al., 2000) and late-season peaks that are thought to result from decomposition following senescence of rice roots (Lindau et al., 1991; Sass and Fisher, 1997). The other time period of peak fluxes generally occurs near the timing of 50% heading and has been linked to the sink-source relationship of photosynthates in the plant when CH_4 fluxes have been observed to increase during vegetative growth as root exudates increase and decrease following heading as fixed-C is translocated to developing grain. This plant-related peak has been observed by several studies (Huang et al., 2002; Nouchi et al., 1994; Rogers et al., 2014; Sass et al., 1990, 1991a, 1991b, 1992) and similar seasonal trends have been observed in root growth (Beyrouty et al., 1988, 1993; Slaton et al., 1990), root exudation rates (Aulakh et al., 2001a), and anaerobic root respiration rates (Tolley et al., 1986).

Transport Mechanisms

The three mechanisms by which CH_4 is transported from a ponded soil to the atmosphere are diffusion through the floodwater, ebullition, and plant-mediated diffusion. Diffusion of CH_4 through overlying floodwater is minor as diffusion of gases is approximately 10,000 times slower through water than through air (Greenwood, 1961). Ebullition, bubbles forming and forcing their way to the surface, may be a significant transport mechanism early in the season, especially with high OM inputs, soil disturbances, and in coarse-textured soils, but generally plays only a small role in CH_4 transport, which diminishes as plants mature and plant-mediated transport (PMT) increases (Denier van der Gon and Neue, 1995; Schutz et al., 1989b). The majority of CH_4 emissions from a rice system occur through the rice plants via aerenchyma cells, where studies have indicated that about 90% of season-long emissions are released through the rice plants, compared to 8 to 9% released by ebullition and 1 to 2% by diffusion through the floodwater (Butterbach-Ball et al., 1997; Cicerone and Shetter, 1981; Holzapfel-Pschorn et al., 1986; Nouchi et al., 1990; Schutz et al., 1989b).

Based on experiments using artificial atmospheres of various gas compositions, Denier van der Gon and van Breemen (1993) determined that PMT is driven by molecular diffusion and not affected by transpiration or stomatal opening. Others have observed a decreasing CH_4 concentration gradient from the soil to the rice root aerenchyma, shoot aerenchyma, and atmosphere, indicative of a diffusive transport pathway from the soil to the atmosphere through the plant (Butterbach-Bahl et al., 2000; Nouchi et al., 1990). Other studies have also confirmed that CH_4 transport is not related to transpiration and is unaffected by cutting plants just above the water surface (Holzapfel-Pschorn et al., 1986; Nouchi and Mariko, 1993; Nouchi et al., 1990). Hosono and Nouchi (1997), however, determined that PMT was reduced linearly as roots were cut and increased with root growth up to heading, indicating that the surface area of roots in

contact with soil solution is important in determining PMT. Several studies have determined that the most restrictive zone of CH_4 transport through the rice plant is the root-shoot transition zone where dense intercalary meristem cells restrict movement from the root aerenchyma to the shoot aerenchyma (Aulakh et al., 2000; Butterbach-Bahl et al., 1997, 2000; Denier van der Gon and van Breemen, 1993; Groot et al., 2005).

It has been postulated that CH_4 in the gaseous form or dissolved in water enters into root aerenchyma, which forms by degeneration of cortical cells between the exodermis and vascular bundle, where the dissolved CH_4 is gasified and moves by diffusion from the root aerenchyma through the restrictive transition zone into the aerenchyma of the culm and then to the atmosphere (Aulakh et al., 2000; Nouchi and Mariko, 1993; Nouchi et al., 1990). It has been determined that CH_4 is released from the rice plant mainly through the lower leaf sheaths. Examining the cultivar Koshihikari with a scanning electron microscope, Nouchi et al. (1990) and Nouchi and Mariko (1993) observed CH_4 release from 4-µm diameter, hook-shaped micropores arranged regularly approximately 80 µm apart on the abaxial epidermis of leaf sheaths as well as from the connections of leaf sheaths to the culm at nodes. Butterbach-Bahl et al. (2000) also determined that CH_4 is primarily released through the lower leaf sheaths, however, micropores were not observed in the cultivars Roma or Lido. More research is required to determine differences in CH_4 release from various cultivars. It has been determined that rice cultivars have differences in CH_4 transport capacity, likely in relation to differences in aerenchyma morphology and the root-shoot transition zone (Butterbach-Bahl et al., 1997) and that CH_4 transport capacity increases as soil temperatures increase (Hosono and Nouchi, 1997). Research indicates that PMT is the dominant mechanism of CH_4 release from rice soils and that the rate of transport can be influenced by cultivar or environmental conditions.

Factors Affecting Methane Emissions from Rice

Through numerous research efforts since the 1980s, several factors have been determined to affect CH_4 emissions from rice cultivation. Due to the complex balance of methanogenesis and methanotrophy that determines how much CH_4 escapes the rice system to the atmosphere along with the large variety of cultural and environmental conditions around the globe, there is large variability in the impact of different factors across time and space. There are a few soil, environmental, and plant factors, however, that seem to have somewhat consistent impacts on CH_4 emissions from rice.

Soil Factors Affecting Methane Emissions from Rice

Various studies have observed inconsistent results of N fertilizer application on CH_4 emissions including an increase in emissions with added N (Aerts and Ludwig, 1997; Aerts and Toet, 1997; Denier van der Gon et al., 2002; Lindau et al., 1991), a decrease in emissions with added N (Cai et al., 1997; Wang et al., 1992), or no impact of added N on CH_4 emissions (Adviento-Borbe et al., 2014; Rogers et al., 2013; Yagi and Minami, 1990). Banger et al. (2012) conducted a meta-analysis and determined that CH_4 emissions were significantly greater ($P < 0.05$) from N-fertilized rice in 98 out of 155 data pairs, indicating that the increase in plant growth and C fixation resulting from N-fertilization generally increases CH_4 emissions. Wang et al. (1993) postulated that the effect of urea on CH_4 emissions may be impacted by pH, where it was observed that urea may cause a decrease in emissions in alkaline soils as urea hydrolysis increases soil pH, limiting the neutrophilic methanogens. In acidic soils, however, the increase in pH from urea hydrolysis moves the soil pH toward neutral and enhances methanogenesis.

Research has consistently indicated that ammonium sulfate reduces CH_4 emissions relative to urea application (Banger et al., 2012; Bronson et al., 1997; Cai et al., 1997), likely due to the impact of sulfate reduction decreasing available C substrate for methanogenesis. Similarly, other studies have determined that oxidized Fe (Furukawa and Inubushi, 2004; Huang et al., 2002; Mitra et al., 2002; Watanabe and Kimura, 1999; Yu et al., 1997) or NO_3^- (Yu et al., 1997) amendments have the ability to reduce CH_4 emissions. Lu et al. (1999) observed a 19 to 33% reduction in CH_4 emissions with the application of P, due to enhanced root growth and root exudation that was measured in the P-deficient treatment.

Multiple studies have indicated no significant correlations between CH_4 emissions and any stable soil properties (Lu et al., 2000; Neue et al., 1997) or CH_4 emissions and total soil C (Cheng et al., 2007; Huang et al., 2002), while readily mineralizable C has been shown to be positively correlated with CH_4 emissions (Wang et al., 1993; Yagi and Minami, 1990). Particle-size distribution is the main soil property that has been regularly related to CH_4 emissions as emissions have been positively correlated with soil sand content (Huang et al., 2002; Mitra et al., 2002; Sass et al., 1994; Wang et al., 1993; Watanabe and Kimura, 1999) and inversely correlated with soil clay content (Denier van der Gon et al., 1996; Mitra et al., 2002; Sass et al., 1994; Wang et al., 1993; Watanabe and Kimura, 1999). Studies have observed an increase in CH_4 entrapment resulting from increasing clay contents (Denier van der Gon et al., 1996; Wang et al., 1993) and Sass and Fisher (1997) attributed the reduction in CH_4 emissions from clay soils to the entrapment and slow movement of CH_4 that allows more CH_4 to be oxidized in aerated zones surrounding roots and at the soil surface. In a laboratory incubation study, Wang et al. (1993) observed varying degrees of CH_4 entrapment, even among soils with similar sand contents, where the greatest entrapment (98.5%) was measured from a Sharkey clay soil compared to 80.6

and 67.8% entrapment from a Beaumont clay and a Sacramento clay, respectively. This research indicates that clayey soils have the capability of restricting movement of CH_4 to the atmosphere and that other factors, such as type of clay, may impact emissions more than simply the total amount of clay.

Environmental Factors Affecting Methane Emissions from Rice

The two major environmental factors that impact CH_4 emissions from rice are temperature and soil saturation status. Numerous studies have observed increases in CH_4 flux rates in relation to increasing soil temperatures (Hosono and Nouchi, 1997; Schutz et al., 1989a; Wang et al., 1997). A study conducted in Japan observed a 1.6-fold increase in emissions from one year to another under the same management and location resulting from an increase in average air temperature from 24.6 to 26.9 ˚C (Watanabe and Kimura, 1999). Methanotrophic activity changes only slightly between 10 and 40 ˚C, while temperature has a strong influence on methanogenesis (Nazaries et al., 2013), which leads to a decrease in the proportion of CH_4 oxidized and an increase in emissions as soil temperature increases. Van Winden et al. (2012), for example, reported 98% CH_4 oxidation at 5 ˚C compared to 50% oxidation at 25 ˚C.

Soil saturation status has a profound influence on CH_4 emissions through the impact of saturation on soil redox processes, such as methanogenesis. Methane emissions have been observed from soils at an Eh as great as of -100 mV (Hou et al., 2000), while emissions increase as Eh decreases. The amount of time required after saturation to reach low redox potentials conducive to methanogenesis varies based on soil textural and chemical properties (Watanabe and Kimura, 1999), but generally occurs within several days or weeks after flooding. Studies have indicated that a single mid-season drainage can reduce CH_4 emissions by as much as 65%

(Bronson et al., 1997; Cai et al., 1997; Lu et al., 2000; Sass et al., 1992; Yagi and Minami, 1990; Zou et al., 2005), however, the potential for greenhouse gas mitigation is reduced or negated due to an increase in N_2O emissions resulting from the drainage (Bronson et al., 1997; Cai et al., 1997; Kreye et al., 2007; Zou et al., 2005). Further research is needed in order to more adequately understand the balance between CH_4 and N_2O emissions under various water management regimes as well as the impact that N management has on emissions when fields are drained.

Plant Factors Affecting Methane Emissions from Rice

Due to the strong impact of rice plants on CH_4 transport and CH_4 production through root exudates and residue, there are several plant factors that significantly impact emissions from rice cultivation. A strong relationship between plant growth and CH_4 emissions has been observed in many studies (Huang et al., 2002; Nouchi et al., 1994; Rogers et al., 2014; Sass et al., 1990, 1991a, 1991b, 1992), particularly in temperate regions, where much of the previous-crop residue decomposes during the winter. Studies have indicated that CH_4 emissions are up to 20 times greater from soil planted with rice than from unvegetated soil (Dannenberg and Conrad, 1999; Nouchi and Mariko, 1993), indicating the large influence of rice plants on emissions.

One of the major plant factors impacting CH_4 emissions from rice is whether or not a ratoon crop is grown. This impact is reflected in the USEPA emission factors, which are 177 kg CH_4-C ha^{-1} for non-California primary rice crops and an additional 584 kg CH_4-C ha^{-1} when a ratoon crop is produced, based on ratoon crops studied in Louisiana by Lindau and Bollich (1993) and Lindau et al. (1995) (USEPA, 2014). The large increase in emissions from ratoon crops is likely a result of large straw-residue inputs from the harvest of the primary crop in

addition to well-developed root systems that further increase available C for methanogenesis. Lindau et al. (1995) observed a significant positive correlation between rice straw additions from a primary crop and resulting emissions from the following ratoon crop.

Another plant factor that has a substantial impact on CH_4 emissions is biomass accumulation. Huang et al. (1997) determined that CH_4 fluxes measured during the growing season were positively correlated to aboveground and belowground dry matter on the dates of flux measurements. Additional studies have observed positive correlations between season-long CH_4 emissions and aboveground (Cicerone and Shetter, 1981; Huang et al., 1997; Sass et al., 1991a; Shang et al., 2011) and belowground dry matter (Whiting and Chanton, 1993). These studies have indicated a strong relationship between plant growth and CH_4 emissions, which may result from an increase in available substrate as root exudates have been correlated to biomass (Aulakh et al., 2001a).

Cultivar selection has also been shown to be an important plant factor influencing CH_4 emissions from rice. While the mechanisms for cultivar differences in CH_4 emissions have not been extensively studied, it appearss that differences likely arise from variability in CH_4 transport capacity, biomass production, root exudation, and microbial community dynamics among cultivars. Butterbach-Bahl et al. (1997), for example, attributed a 24 to 31% difference in emissions between two pure-line cultivars to differences in CH_4 transport capacities, as no differences were observed between CH_4 production or oxidation. Aulakh et al. (2001b) observed a positive correlation between total OC from root exudates and CH_4 production potential, indicating the potential for cultivar differences in emissions based on variable root exudation rates. Previous studies have reported reduced emissions from semi-dwarf relative to standard-stature cultivars (Lindau et al., 1995; Sass and Fisher, 1997; Sigren et al., 1997). The difference

in CH_4 emissions between semi-dwarf and standard-stature cultivars observed in these studies may be a result of the positive correlation between dry matter and C exudation rates from roots (Aulakh et al., 2001b) or between aboveground dry matter and CH_4 emissions (Cicerone and Shetter, 1981; Huang et al., 1997; Sass et al., 1991a; Shang et al., 2011). While a reduction in emissions from semi-dwarf cultivars is oftentimes linked to reduced dry matter accumulation, Rogers et al. (2014) observed a reduction in aboveground dry matter that was not accompanied by a reduction in emissions. Furthermore, Sigren et al. (1997) measured greater emissions accompanied by greater soil acetate concentrations from a standard stature (Mars) relative to a semi-dwarf cultivar (Lemont), while aboveground dry matter was similar between the two cultivars. Huang et al. (1997) indicated that, while biomass may explain differences in emissions within one cultivar, the intervarietal differences in biomass are small in comparison to differences in emissions, indicating that another factor besides aboveground dry matter impacts intervarietal differences in CH_4 emissions.

Cultivar differences extend beyond the impact of biomass on emissions, however, as Ma et al. (2010) observed a 67% increase in CH_4 oxidation from a hybrid cultivar accompanied by a reduction in emissions and soil CH_4 concentration relative to pure-line *Indica* and *Japonica* cultivars. Additional studies have also identified reduced fluxes from hybrid relative to pure-line cultivars (Simmonds et al., 2015; Rogers et al., 2014; Smartt et al., 2015). This indicates that greater methanotrophic activity in the rhizosphere of hybrid cultivars may reduce CH_4 fluxes by oxidizing a greater proportion of the produced CH_4. It is clear that cultivar selection has potential for mitigation of CH_4 from rice cultivation, however, due to the lack of understanding the mechanisms for differences in emissions, it appears that direct CH_4 flux measurements from various cultivars is necessary in determining emissions differences.

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CHAPTER TWO

INFLUENCE OF VEGETATION AND CHAMBER SIZE ON METHANE EMISSIONS FROM RICE PRODUCTION ON A CLAY SOIL IN ARKANSAS

Abstract

Rice (*Oryza sativa* L.) is the only major cereal crop that is almost exclusively grown under flooded-soil conditions and is one of the main staple crops for much of the world's human population. Rice production systems have a greater global warming potential than upland row crops due to methane (CH_4) emissions resulting from anaerobic conditions of the flooded soils. The main objective of this study was to determine the effect of vegetation (i.e., no, low, and high vegetation) on CH_4 fluxes and growing-season-long emissions from rice produced on a clay soil in Arkansas. A secondary objective was to examine the effect of chamber size (i.e., 15.2- and 30-cm inner diameter) on measurements of CH_4 fluxes and emissions. This study was conducted in 2012 at the Northeast Research and Extension Center in Keiser, Arkansas on a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts). Gas samples were collected from enclosed-headspace gas sampling chambers at 0, 20, 40, and 60 minutes after chamber closure and CH_4 fluxes were calculated from changes in headspace CH_4 concentration over time. Fluxes were determined weekly during the flood retention period and every other day for one week following flood release. Methane fluxes increased during the vegetative growth period in both the high-and low-vegetation rice treatments reaching maximum observed fluxes of 4.8 and 0.94 mg CH_4-C m^{-2} hr^{-1}, respectively, following 50% heading. Methane fluxes then decreased over time in both treatments containing rice and approached 0 mg CH_4-C m^{-2} hr^{-1} at flood release. Methane fluxes from the unvegetated treatment remained near zero throughout the flooded period. Methane fluxes after flood release remained low in all treatments until 5 days after flood release when substantial CH_4 pulses of 0.77, 1.2, and 2.5 mg CH_4-C m^{-2} hr^{-1} were measured in the high-, low-, and no-vegetation treatments, respectively. Total season-long emissions were greatest ($P <$ 0.001) in the high- (35.6 kg CH_4-C ha^{-1}) compared to the no- (1.8 kg CH_4-C ha^{-1}) and low-

vegetation (9.0 kg CH_4-C ha^{-1}) treatments, which did not differ. Chamber size impacted CH_4 flux measurements on only one of 14 sampling dates, which occurred following flood release, and there were no differences in season-long emissions between the two chamber sizes. Estimated CH_4 emissions in this study were approximately 20% of the United States Environmental Protection Agency's (USEPA) reported emission factor, indicating that CH_4 emissions from Arkansas rice production on clay soils may be substantially less than the USEPA estimate. More data are needed in order to accurately quantify CH_4 emissions from Arkansas rice production and evaluate the multitude of factors known to affect CH_4 emissions.

Introduction

Rice (*Oryza sativa* L.) is the only major row crop that is almost exclusively grown under flooded-soil conditions for at least a portion of the growing season and is one of the main staple food crops for much of the world's population, with direct human consumption accounting for 85% of rice production compared to 72% and 19% for wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), respectively (Maclean et al., 2002). However, the global warming potential (GWP) of rice production systems is estimated to be 5.7 and 2.7 times greater than that of wheat and maize systems, respectively (Linquist et al., 2011). The greater GWP of rice systems is primarily due to methane (CH_4) emissions resulting from flooded-soil conditions, with CH_4 contributing ~92% to the GWP in rice systems with nitrous oxide contributing to most of the remainder (Linquist et al., 2012). Methane production occurs in flooded soils after oxygen is depleted and subsequent terminal electron acceptors are used before carbon dioxide (CO_2) is reduced to CH_4 by anaerobic methanogenic archaea at redox potentials less than -150 mV (Masscheleyn et al., 1993). However, aerobic methanotrophic bacteria in oxygenated zones surrounding rice roots and at the soil-water interface can potentially oxidize 58 to 90% of the produced CH_4 (Holzapfel-Pschorn et al., 1985; Sass et al., 1990).

The majority of CH_4 emissions from a rice system occur through the rice plants via aerenchyma cells, where studies have indicated that about 90% of season-long emissions are released through the rice plants, compared to 8 to 9% released by ebullition and 1 to 2% by diffusion through the floodwater (Butterbach-Ball et al., 1997; Cicerone and Shetter, 1981; Holzapfel-Pschorn et al., 1986; Nouchi et al., 1990; Schutz et al., 1989b). Furthermore, studies have indicated increased CH_4 oxidation, accounting for 75 to 97% of produced CH_4, late in the season when plant-mediated transport dominates and peak fluxes occur, compared to < 30%

oxidation early in the season (Sass et al., 1992; Schutz et al., 1989b; Sigren et al., 1997). Methane fluxes during the growing season have been positively correlated to aboveground and root dry matter (Huang et al., 1997), while season-long CH_4 emissions have also been positively correlated with aboveground (Cicerone and Shetter, 1981; Huang et al., 1997; Sass et al., 1991a; Shang et al., 2011) and belowground dry matter (Whiting and Chanton, 1993), indicating a strong relationship between plant growth and CH_4 emissions. Using ^{13}C labeling techniques, Watanabe et al. (1999) reported that 80 to 85% of released CH_4 originated as plant-fixed C when no additional rice straw was incorporated, whereas about 42 and 40% of CH_4 originated from rice straw and plant-fixed C, respectively, when rice straw was incorporated at a rate of 6 Mg ha^{-1}.

As a greenhouse gas, CH_4 has a GWP 25 times greater than CO_2 (Forster et al., 2007). Globally, agriculture accounts for 47% of total anthropogenic CH_4 emissions, with 64% and 22% of agricultural emissions resulting from enteric fermentation and rice cultivation, respectively (Smith et al., 2007; USEPA, 2006). In the United States, 33% of CH_4 emissions result from agricultural activities. Of that 33%, 70% results from enteric fermentation and 3.7% from rice cultivation (USEPA, 2014). A CH_4 emission factor of 178 kg CH_4-C ha^{-1} $season^{-1}$ has been reported by the United States Environmental Protection Agency (USEPA) for estimating CH_4 emissions from a non-California primary rice crop in the U.S. (USEPA, 2014). However, the primary crop emission factor is based on US studies with emissions ranging from 46 to 375 kg CH_4-C ha^{-1} $season^{-1}$ (Byrd et al., 2000; Kongchum, 2005; Rogers et al., 2012; Sass et al. 1991a, 1991b, 2002a, 2002b; Yao et al., 2001), only one of which was conducted in Arkansas, the leading rice-producing state in the US.

Methane emission studies in Arkansas under common cultural practices within the region have only recently been initiated (Brye et al., 2013; Rogers et al., 2012, 2013). Since Arkansas is the leading rice-producing state in the US, contributing roughly 48% of US production (USDA NASS, 2013), it is important to accurately represent CH_4 emissions under Arkansas cultural practices. Methane fluxes and emissions from rice are dependent upon factors such as soil texture, cultivar, residue management, and flood management (Lindau et al., 1995; Sass et al., 1991a, 1992, 1994; Wassmann et al., 1993). Therefore it is important to quantify and compare emissions measured from a variety of the factors known to affect CH_4 emissions.

Considering CH_4 is a greenhouse gas with a global warming potential 25 times more potent than CO_2, increasing atmospheric concentrations of greenhouse gases and global atmospheric temperatures have led to a global need for mitigation of greenhouse gas, such as CH_4, concentrations in the atmosphere. In addition to the need for mitigation of greenhouse gas concentrations, there is a general lack of data for CH_4 emissions as affected by various cultural practices associated with rice production. An emission factor of 178 kg CH_4-C ha^{-1} $season^{-1}$ for primary rice crops is used to estimate non-California U.S. emissions; however, research studies used to establish the estimated emission factor are limited and many factors, such as residue management, soil properties, and fertilizer management, are not being accounted for (USEPA, 2014). A more accurate estimate of US and global emissions can only be made after accounting for differences resulting from various cultural practices and differences in production area under a larger variety of environments, soils, and cultural practices.

Only in the past few years have there been any studies conducted to evaluate CH_4 emissions from Arkansas rice production, with those studies being conducted on silt-loam soils. Recently, Arkansas rice has been widely produced on clay and clay-loam soils, increasing from

38.7% in 2006 to 47.8% in 2009, while declining to 43.2% in 2013 (Hardke, 2014; Wilson et. al., 2009, 2010). The recent increase in rice production on clay soils makes it even more important to quantify CH_4 emissions from clay soils as well as from silt-loam soils, which together accounted for nearly 95% of the total rice production in Arkansas in 2013 (Hardke, 2014). Therefore, the overall goal of this study was to characterize CH_4 fluxes and quantify CH_4 emissions from drill-seeded, delayed-flood rice grown on a clay soil in Arkansas. The main objective in satisfying this goal was to examine the effect of rice vegetation (i.e., no, low, and high vegetation) on CH_4 fluxes and emissions from a clay soil. A secondary objective of the study was to determine the influence of chamber size (i.e., 15.2- and 30-cm inner diameter) on measurements of CH_4 fluxes and emissions.

Studies have shown that CH_4 emissions are less from fine- than from coarse-textured soils (Chen et al., 1993; Neue and Sass, 1994; Parashar et al., 1991; Sass et al., 1994). This is likely due to differences in the time required to achieve saturation and differences in gas diffusion through the soil. Clay soils generally require more time to become saturated, due to inherently lower infiltration rates and hydraulic conductivities, so that it takes more time for the soil to reach the redox potential required for CH_4 production (i.e., ~ -150 to -200 mV), effectively reducing the time that CH_4 production is able to occur in fine- compared to coarse-textured soils (Masscheleyn et al., 1993; Reddy and DeLaune, 2008). Furthermore, diffusion of CH_4 and other gases through the soil is generally slower in clays than in silt loams due to the smaller pores and greater tortuosity in clays, which may allow more of the CH_4 to be oxidized by methanotrophic bacteria in the partially aerated root zone of the soil (Livingston and Hutchinson, 1995; Nazaroff, 1992). Consequently, it was hypothesized that CH_4 fluxes and season-long CH_4

emissions from a clay soil will be less than those measured from silt-loam soils in Arkansas under similar management.

Methane emissions from rice are greatly influenced by the plants themselves, so emissions are related to plant growth and biomass production, which in turn is affected by fertility (Lindau et al., 1991). Similar emissions from N-fertilized (high vegetation) and non-N-fertilized (low vegetation) rice can be expected on a yield basis because yield is affected by N fertility in the same way that plant biomass is. When compared to a low-vegetation treatment, the addition of fertilizer-N (i.e., to achieve a high-vegetation treatment) was hypothesized to result in greater total CH_4 emissions on an area basis, but comparable emissions on a yield basis. It was also hypothesized that CH_4 emissions will be least from the bare soil (no vegetation) due to the strong influence of rice plants on CH_4 transport from the soil to the atmosphere. Finally, it was hypothesized that there will be no difference in CH_4 emissions between the two chamber sizes because of equal proportions of row length per chamber area will be present inside both chamber sizes.

Materials and Methods

Site Description

Research was conducted in 2012 at the University of Arkansas Northeast Research and Extension Center (NEREC) in Keiser, Arkansas (35°40’ N 90°05’ W) on a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts; Soil Survey Staff, NRCS-USDA, 2012). The study site is in Major Land Resource Area (MLRA) 131A (Southern Mississippi River Alluvium), which is composed of approximately 70% cropland and contains much of the rice production area in Arkansas. The study location has been managed in a rice-soybean rotation for greater

than 15 years. Crop residues are typically disked into the soil to a depth of 15 cm followed by land floating in the fall (i.e., stale-seedbed tillage) in order to achieve a flat seedbed to plant into in the spring.

Treatments and Experimental Design

This study involved two separate objectives that required separate treatments and treatment structures. Based on the main objective of this study, the effects of rice vegetation on CH_4 fluxes and growing-season-long emissions were evaluated by examining non-N-fertilized bare soil (unvegetated), non-N-fertilized rice (low vegetation), and optimally N-fertilized rice (high vegetation) using 30-cm inside diameter chambers. A randomized complete block design was used where four blocks were created, each containing all three randomly assigned treatments, for a total of four replications of each treatment combination. The secondary objective was to compare CH_4 fluxes and growing-season-long emissions from bare soil and optimally N-fertilized rice measured with 15.2- and 30-cm inside diameter chambers. In order to accomplish this, 15.2-cm inner diameter chambers were installed in addition to the 30-cm inner diameter chambers in the no vegetation and high vegetation plots associated with the main objective. A split-plot design was used for the secondary objective, with the whole-plot factor being vegetation (rice or no rice) and the split-plot factor being chamber size (15.2- or 30-cm). Methane fluxes for both objectives were determined once per week while the flood was in place and every other day for one week following flood release, which resulted in time being an additional experimental factor that was treated as a repeated measure.

Plot Management

Residue management at the study site involved incorporation of crop residue in the fall using tillage and disking to a depth of 15 cm. Land floating followed tillage in the fall and crops were planted into a stale seedbed in the spring after weeds were controlled using burn-down herbicide applications. Plots 1.6 m wide by 5.0 m long were seeded independently with nine rows of rice using an 18-cm row spacing at a rate of 112 kg ha^{-1} in early April (Table 1). The standard stature, pure line, long-grain rice cultivar 'Taggart' (Moldenhauer et al., 2008) was used in this study due to its high yield potential and frequent use throughout the region. Levees were constructed after planting and study bays were flushed as necessary prior to establishment of a permanent flood, which occurred at the V4 to V6 stage (Moldenhauer et al., 2013) (Table 1). Based on University of Arkansas Cooperative Extension Service (UACES) guidelines, N was applied to N-fertilized plots in the form of urea (46% N) at 150 kg N ha^{-1} within one day prior to establishment of the permanent flood. An additional application of 50 kg N ha^{-1} as urea was made at the beginning of internode elongation (R1) to plots requiring N fertilization. A flood depth of 5 to 10 cm was maintained on the plots until the flood was released at grain maturity in late August. Plots were scouted regularly and managed to remain insect and weed free for the duration of the growing season according to University of Arkansas Cooperative Extension Service (UACES) guidelines, and were hand harvested for yield at a gravimetric grain moisture content of approximately 20%.

Soil Sampling and Analyses

Soil samples to be analyzed for soil-fertility-related properties, total carbon (TC), and total N (TN) were collected prior to flooding using a 2.5-cm-diameter push probe by combining six or seven cores from the 0 to 10 cm depth in each plot. Samples were dried in an oven at 70

°C for 48 hours and sieved through a 2-mm mesh screen prior to being analyzed for Mehlich-3 extractable nutrients (i.e., P, K, Ca, Mg, Fe, Mn, Na, S, Cu, and Zn) by ICP-AES (Spectro Analytical Instruments, Spectro Arcos ICP, Kleve, Germany; Tucker, 1992). Soil pH and electrical conductivity (EC) were measured potentiometrically on a 1:2 soil mass:solution volume paste. Total N and TC (g kg^{-1}) were determined on dried, sieved soil by high-temperature combustion using a VarioMax CN analyzer (Elementar Americas Inc., Mt. Laurel, NJ; Nelson and Sommers, 1996). Organic matter concentration was determined by loss-on-ignition.

One soil sample for bulk density was collected prior to flooding from the 0 to 10 cm depth in each plot using a slide hammer and core tip with a 4.7-cm diameter core chamber. Samples were dried in an oven at 70 °C for 48 hours prior to being weighed. Bulk densities were calculated as mass of dry soil (g) per volume of soil collected. After determining bulk densities, the same samples were ground and sieved through a 2-mm mesh screen and analyzed for particle-size distribution using the 12-hr hydrometer method (Gee and Or, 2002). Measured TN, TC, and organic matter concentrations were transformed to contents (Mg ha^{-1}) using measured bulk densities and the 10-cm soil sample depth.

Trace Gas Sampling and Analysis

Similar to that used by Rogers et al. (2012, 2013), enclosed-headspace gas sampling chambers, a methodology commonly used for measuring fluxes of trace gases (Parkin and Venterea, 2010), were used for collection of CH_4 samples in this study. This method involves installing permanent base collars into the soil and using various sizes of extensions and a cap to enclose rice plants prior to sampling the enclosed headspace with a syringe inserted through a septum in the cap.

Schedule 40 polyvinyl chloride (PVC) pipes with inside diameters (ID) of 15.2 cm and 30 cm were used to construct small and large chambers, respectively. Sections of PVC pipe were cut to a length of 30 cm to create the base collars. The 30-cm sections were beveled to a 45° angle on the bottom in order to be driven into the soil and 12.5 mm (0.5 inch) holes were drilled 12 cm from the beveled bottom to allow floodwater to enter and exit the chamber. Holes were plugged with gray butyl-rubber septa (Voigt Global, part # 73828A-RB, Lawrence, KS) during sampling after flood release. Chamber extensions were cut to lengths of 40 cm and 60 cm, in order to adjust the chamber height to accommodate the growth of the rice plants inside the base collars over the course of the growing season. Chamber extension sections were then covered with reflective aluminum tape (CS Hyde, Mylar metalized tape, Lake Villa, IL) to minimize temperature fluctuation inside the chamber during chamber deployment. Ten centimeter cross-sections of tire inner tube were also taped near the bottom of extensions and were used to secure and seal extensions to base collars and to additional extensions when required.

Chamber caps were fabricated using 10-cm tall pieces of 15.2- and 30-cm PVC, each with a 5-mm thick flat sheet of PVC glued to the top. Caps were then covered with reflective aluminum tape (CS Hyde, Mylar metalized tape, Lake Villa, IL) and 10-cm cross-sections of tire inner tube were taped near the bottom in order to secure and seal the caps to the extensions or base collars. A 15-cm section of 4.5-mm ID copper tubing was installed in the side of each cap as a vent that allowed the chamber to maintain atmospheric pressure during deployment. Sampling and thermometer ports were created on each cap by installing gray butyl-rubber septa (Voigt Global, part # 73828A-RB, Lawrence, KS) into 12.5-mm (1/2 inch) holes drilled in the top of each cap. A small (2.5-cm diameter), battery-operated (9V) fan (Sunon Inc., MagLev,

Brea, CA) was installed on the underside of each cap to slowly circulate and mix enclosed air during CH_4 sampling.

Planking was installed using untreated 5- by 30-cm pine (*Pinus* spp.) lumber laid down upon 20- by 20- by 40-cm light-weight concrete blocks prior to installation of base collars. The planking allowed access to chambers in each plot after flood establishment with minimal plant and soil disturbance. Permanent base collars were then installed within reach of the planks by driving them into the soil to a depth of approximately 11 cm where the water-movement holes were just above the soil surface. A 45-cm level was used to insure that base collar rims were level. Base collars were installed one week prior to sampling in locations that approximated the plant density of the whole plot. Using the row spacing in the plots and the surface areas of the chambers, it was calculated that 10 and 40 cm of row length of plants should be included within the small (15.2-cm ID) and large chambers (30-cm ID), respectively, in plots with rice present.

Chamber extensions were placed upon base collars and heights were adjusted to accommodate increasing plant heights as the season progressed. No extensions were used for the bare soil chambers, where caps were secured directly to base collars. Rice plants within each base collar were carefully bundled together with plant-tie wire and chamber extensions were slipped over each bundle and secured to collars approximately 16 hours prior to sampling. Tie wires were carefully removed from the rice in each chamber immediately after extension deployment in order to minimize potential plant disturbance during sampling. Caps were not secured and sealed to the chamber extensions until immediately prior to beginning sampling.

Chamber headspace gas samples were collected using a 20-mL B-D syringe (Beckton Dickson and Co., Franklin Lakes, NJ) inserted into each chamber through a gray butyl-rubber septa (Voigt Global, part # 73828A-RB, Lawrence, KS). Samples were immediately transferred

into previously evacuated 10-mL, crimp-top glass vials (Agilent Technologies, part # 5182-0838, Santa Clara, CA). Gas samples were collected in 20-minute intervals, beginning when each cap was sealed onto each chamber (0 minutes) and ending at 60 minutes after chamber closure for a total of four headspace samples per chamber for each sampling event. Gas measurements were collected weekly from all chambers during the flooded-soil period beginning 11 days after flood establishment. Samples were collected every other day for one week after flood release beginning the first day after flood release.

Gas sampling began in the morning between 0800 and 0830 in order to minimize temperature elevation inside the chamber and required approximately 80 minutes to complete. The air temperature, 10-cm soil temperature, chamber temperature, relative humidity, and barometric pressure were recorded throughout the sampling period. Chambers were sampled in sets of 12 due to time constraints; thus two people were required to complete the full set of 24 chambers. Syringes were flushed two times with ambient air prior to collecting each sample. Time was kept with a stopwatch and the zero-minute time (T0) for each chamber began when the cap was secured and a sample was immediately pulled through the septum and transferred to an evacuated vial. Chamber fans were turned on immediately after each chamber was sampled for the first time. The second chamber in each set was sampled at exactly one minute after the first chamber and each successive chamber was sampled at one minute intervals so that the last chamber (chamber 12 of each set) was sampled at 11 minutes after the first chamber sampling. Chambers were sampled in the same order at exactly 20, 40, and 60 minutes after each chamber was initially enclosed and sampled. Chamber heights from the top of each chamber to the flood-water surface were measured after each sampling event in order to calculate chamber volumes.

Two sets of CH_4 gas standards of 1, 2, 10, 20, and 50 µL L^{-1} were collected from tanks in the field immediately following sampling and were used to make calibration curves for each sampling event. Standards were collected using a 20-mL syringe and immediately transferred to evacuated 10-mL crimp-top glass vials.

Field samples and field standards were analyzed directly from the 10-mL, crimp-top glass vials within 48 hours after sampling using an Agilent 6890-N gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a flame-ionization detector (FID) and utilizing a 30-m-long HP-Plot-Q capillary column (Agilent Technologies, Santa Clara, CA). Laboratory standards of 1, 2, 10, and 50 µL L^{-1} were also analyzed immediately after being collected from standard tanks and compared to field standards in order to ensure that none of the field sample was lost due to leakage. Peak-area response from the chromatograms of the field standards were used to create calibration curves for each sampling event and the curves were then used to calculate CH_4 concentrations (µL L^{-1}) in the field samples.

Changes in headspace CH_4 concentration (ppm or µL L^{-1}) of field samples for each sampling event of each chamber were plotted over time (minutes) and the slopes of the resulting best-fit lines were multiplied by the chamber volume (L) and divided by the surface area (m^2) for fluxes calculated in units of µL CH_4 m^{-2} min^{-1} (Parkin and Venterea, 2010). Resulting units of µL CH_4 were then converted to µmol CH_4 using the Ideal Gas Law (PV=nRT), where P is pressure in atmospheres (atm), which was recorded at the time of each sampling, V is volume (L), n is number of moles of gas, R is the gas law constant (0.08206 L atm Mol^{-1} K^{-1}), and T is chamber temperature in Kelvin, which was recorded at the time of each sampling. The molar mass of CH_4 was then used to convert µmol CH_4 to mass of CH_4 for final units of mass $area^{-1}$

time^{-1} (i.e., mg CH_4 m^{-2} day^{-1}) (Parkin and Venterea, 2010). Season-long CH_4 emissions were calculated on a chamber-by-chamber basis by linear interpolation between measurement dates.

Soil Redox Potential and Soil Temperature Monitoring

Soil redox potential sensors (Sensorex, Model S65OKD-ORP, Garden Grove, CA) with Ag/AgCl reference solution and thermocouples were installed in the soil to a depth of 7.5 cm four days after flood initiation (12 June, 2012) and connected to dataloggers (Campbell Scientific Inc., Model CR 1000, Logan, UT). Redox potential sensors were checked for accuracy prior to installation using Sensorex ORP Calibration Solution Standard (Part No. B225) at 225 mV. Soil redox potential sensors were installed between rice rows in plots that contained rice as well as in each of the bare-soil plots. Thermocouples were installed in each of the 12 plots providing four replicates for each of the three treatment combinations (i.e., no, low, and high vegetation). Soil temperature and soil redox potential measurements were made at 15-minute intervals and means were output every four hours. Data were collected by uploading to a handheld computer and daily means were reported for the duration of sensor deployment (12 June to 30 August, 2012). Soil redox potential data were corrected to the standard hydrogen electrode (SHE) by adding 199 mV as is common in research investigating soil redox potential in near-neutral soils (Patrick et al., 1996).

Plant Sampling and Analyses

Plant samples were collected at physiological maturity in order to determine yield from each plot. Additional plant samples were collected at physiological maturity in order to compare

total aboveground biomass between the low- and high-vegetation treatments as well as to compare total aboveground biomass inside each of the two chamber sizes to the biomass outside of the chambers. Panicles from three, 1-m row lengths of rice were hand-harvested in each of the eight plots containing rice. Rice from each plot was threshed by hand, weighed, and adjusted to 12% grain moisture. One, 1-m row length of rice was collected by hand from each plot containing rice by cutting the plants at the soil level and removing all aboveground biomass. Total aboveground biomass was also collected by hand for each of the chambers containing rice. Biomass samples were dried at 60 °C for 96 hours, when no further moisture loss was measured, and weighed for total aboveground dry matter.

Statistical Analyses

Initial soil property data collected prior to fertilizer application (i.e., particle-size distribution, bulk density, Mehlich-III extractable nutrients, EC, and pH) were analyzed by analysis of variance (ANOVA) in SAS (version 9.2, SAS Institute, Inc., Cary, NC) using PROC Mixed based on a randomized complete block (RCB) design with three levels of vegetation (i.e., no, low, and high vegetation). Similarly, grain yield and yield-scaled emissions were subjected to ANOVA based on a RCB design with two levels of vegetation, where the unvegetated treatment and plots were omitted. Aboveground dry matter was analyzed by ANOVA based on a split-plot design [i.e., the whole-plot factor was vegetation and the split-plot factor was location (in-chamber or in-plot)]. Additionally, with regards to the chamber-size comparison, aboveground dry matter was analyzed by ANOVA based on a RCB design with three sampling locations (i.e., 15.2-cm chambers, 30-cm chambers, and in-plot).

There was no indication of a non-normal distribution for the flux data based on visual inspection for normality using normal probability plots of the studentized residuals. Therefore, an ANOVA was conducted to evaluate the effect of vegetation (no, low, and high vegetation) on CH_4 fluxes over time based on a RCB repeated-measures design. A second ANOVA based on a split-plot repeated-measures design (i.e., the whole-plot factor was vegetation, the split-plot factor was size, and time was treated as a repeated measure) was conducted to evaluate the effects of vegetation and chamber size on CH_4 fluxes over time. In both of the previously mentioned analyses, CH_4 flux data were analyzed separately from flooding to flood release and following flood release due to differences in sampling interval and mechanisms of CH_4 release.

Total area-scaled, post-flood-release, and post-flood-release emissions expressed as a percentage of total emissions were analyzed by ANOVA based on an RCB design with three levels of vegetation for the first objective and based on a split-plot design (i.e., vegetation was the whole-plot factor and size was the split-plot factor) for the chamber size comparison. When appropriate, means were separated using the Fisher protected least significant difference at an alpha level of 0.05. Linear correlation and regression analyses were conducted using Minitab (version 16, Minitab, Inc., State College, PA) to evaluate the relationship between aboveground biomass and growing-season emissions.

Results and Discussion

Initial Soil Physical and Chemical Properties

With a few exceptions, most of the initial soil physical and chemical properties measured in the surface 10 cm prior to flood establishment did not differ ($P > 0.05$) among the three vegetation treatments (Table 2). Mean extractable sodium (Na) concentration was greatest ($P =$

0.031) in the high- and lower in the low- and no-vegetation treatments, which did not differ, however, the difference of < 6 mg kg^{-1} likely had no practical or agronomic significance in this study. Additionally, total N concentration was greater (P = 0.028) in the no-vegetation (1.27 g kg^{-1}) than in the low- (1.21 g kg^{-1}) and high-vegetation (1.19 g kg^{-1}) treatments, which did not differ. However, when transformed to TN content using measured bulk densities, there were no differences among treatments. Extractable soil phosphorus (P) and potassium (K) were both above optimum levels of > 51 and > 175 mg kg^{-1}, respectively, recommended for rice and zinc (Zn) fell within the medium level (2.6 to 4.0 mg kg^{-1}) indicating adequate native soil levels of these nutrients for rice production based on University of Arkansas recommendations (Norman et al., 2013).

Methane Fluxes from Flooding to Flood Release

Methane fluxes measured during the flooded portion of the 2012 growing season differed among the three vegetation treatments over time (P < 0.001) (Table 3). Fluxes did not differ among treatments for the first three sample dates, with all fluxes starting at < 0.03 mg CH_4-C m^{-2} h^{-1} on the first sample date, 11 days after flooding (DAF) (Figure 1). By 32 DAF, fluxes were greater in the high-vegetation than the other two treatments, which did not differ, and remained greater for the remainder of the flooded portion of the season. Fluxes increased rapidly over time after 25 DAF in the high-vegetation treatment and exhibited greater fluxes from week to week compared to the previous week up to a peak flux of 4.8 mg CH_4-C m^{-2} h^{-1} at 60 DAF just following 50% heading (58 DAF), followed by a rapid decline to 1.1 mg CH_4-C m^{-2} h^{-1} just before flood release. A similar trend with a peak flux of 0.94 mg CH_4-C m^{-2} h^{-1} at 53 DAF (4 days after 50% heading) occurred in the low-vegetation treatment, although this peak did not

differ from the fluxes measured in the two weeks prior to or the week following this numerical peak. Fluxes from the low-vegetation treatment were only greater than fluxes from the unvegetated treatment from 39 to 60 DAF, which corresponded to the reproductive portion of the season when peak fluxes occurred in both of the vegetated treatments. Fluxes from the unvegetated treatment remained at or below 0.06 mg CH_4-C m^{-2} h^{-1} for the entire flooded portion of the season and never differed significantly from a flux of zero over time.

The general trend of fluxes in the vegetated treatments increasing during vegetative growth, peaking during reproductive growth, and declining during grain fill has observed in several other studies (Huang et al., 2002; Nouchi et al., 1994; Rogers et al., 2014; Sass et al., 1990, 1991a, 1991b, 1992). This pattern has generally been attributed to the link between plant growth and CH_4 emissions, where root exudates, which provide substrate for methanogenesis, increase during vegetative growth and decrease again during grain fill as resources are translocated to the panicle. The link between CH_4 fluxes and plant growth observed in this study is supported by studies conducted in Arkansas that have consistently observed rapid, linear root growth during the vegetative stage with maximum root length occurring from early vegetative to heading stages, followed by a decline in root growth until after grain fill among cultivars grown on different soils (Beyrouty et al., 1988; Slaton et al., 1990) and cultivars that differ in maturity (Beyrouty et al., 1993). Other studies have shown similar seasonal trends in root exudation rates (Aulakh et al., 2001) and anaerobic root respiration rates (Tolley et al., 1986). Fluxes observed in this study do not show characteristics of early season peak fluxes, which are attributed to decomposition of freshly incorporated residues (Chidthaisong and Watanabe, 1997; Wassmann et al., 2000) or late-season peaks that are thought to result from decomposition following senescence of rice roots (Lindau et al., 1991; Sass and Fisher, 1997). The relationship between

the magnitude of CH_4 fluxes and amount of vegetation present, as well as the seasonal tend in fluxes from the vegetated treatments, suggest a strong association between CH_4 fluxes and plant growth and biomass.

Similar studies conducted on clay and silty-clay soils have reported maximum CH_4 fluxes from fertilized rice ranging from 2.1 to 25 mg CH_4-C m^{-2} h^{-1}, with all of the greater fluxes observed in direct-seeded, delayed-flood rice along the gulf coast in Texas (Bossio et al., 1999; Cicerone et al., 1992; Sass et al., 1991a,b). Cicerone et al. (1992) conducted a similar study on a Capay silty clay (fine, smectitic, thermic Typic Haploxererts) in California and measured maximum CH_4 fluxes of 0.9, 1.3, and 4.3 mg CH_4-C m^{-2} h^{-1} from unfertilized bare soil, unfertilized rice, and fertilized rice, respectively. On a silt-loam soil under Arkansas production practices, Rogers et al. (2012) measured maximum fluxes of 11.6, 13.9, and 22.6 mg CH_4-C m^{-2} h^{-1} for unfertilized bare soil, unfertilized rice, and fertilized rice, respectively. The two studies previously mentioned, as well as others, have given strong evidence that CH_4 fluxes are less in fine-textured clay soils than in more coarse-textured soils such as silt loams (Sass et al., 1994; Sass and Fisher, 1997). Furthermore, greater emissions from unvegetated relative to vegetated soil in coarser-textured soils indicates that plant-mediated-transport mechanisms may be more important in fine-textured soils, while ebullition and diffusion through the floodwater may play a larger role in emissions from silt-loam and sandy soils (Schutz et al., 1989a).

Soil redox potential (Eh) measurements recorded for the duration of flooding were consistent with observed CH_4 fluxes (Figure 2). There were only minor differences in Eh between the three vegetation treatments in the first three weeks of flooding, after which the high-vegetation treatment generally had lower redox values, indicative of more reduced conditions and greater potential for methanogenesis, than the low-vegetation treatment. The no-vegetation

treatment consistently had the greatest redox potentials, which is consistent with the low fluxes measured. Average daily soil temperatures measured throughout the growing season varied between 25 and 30 °C for the first nine weeks following flooding before a minor decrease toward the end of the season and averaged 27.01, 27.59, and 27.63 ^{0}C, in the high-, low-, and no-vegetation treatments, respectively, for the duration of the flooded portion of the season (Figure 3). However, there were no apparent links between season-long soil temperature trends and CH_4 flux trends. The increasing difference in soil temperature between the three vegetation treatments as the season progressed indicated the increasing effect of rice vegetation shading and cooling the soil as the canopy developed over time. Studies have shown, however, that increasing soil temperature increases CH_4 production and emissions (Hosono and Nouchi, 1997), so the effect of canopy shading on CH_4 production in this study was clearly overpowered by the positive effect of vegetation on CH_4 fluxes.

Chamber size did not have a significant impact during the flooded period on measured fluxes from the unvegetated or high-vegetation treatments, although vegetation differences were apparent over time (Table 3). The observation of greater fluxes from the vegetated treatments throughout most of the growing season was consistent with the findings detailed above. While no direct comparisons of measured CH_4 fluxes from rice among differing chamber sizes have been reported, Livingston and Hutchinson (1995) suggested that chamber areas typically range from 175 cm^2 (i.e., 15.2-cm diameter) to 1 m^2, and that chamber areas of 500 to 900 cm^2 (i.e., 25- to 34-cm diameters, respectively) are most common. Larger chambers tend to mask spatial variability by integrating measurements over a larger soil area, while smaller chambers may be desirable in studies examining environmental gradients (Livingston and Hutchinson, 1995; Parkin and Venterea, 2010). Over the growing season, none of the flux measurements from

either chamber size exhibited any indication of a non-linear CH_4 concentration increase over time (i.e., increasing CH_4 concentrations within the chambers did not substantially affect the molecular diffusion gradient), indicating that both 15.2- and 30-cm diameter chambers appear suitable for measuring CH_4 fluxes from flooded rice.

Methane Fluxes Following Flood Release

Soil CH_4 fluxes in all three vegetation treatments approached zero prior to flood release (74 DAF) and remained low until 5 days after flood release (DAFR) when increased ($P < 0.001$; Table 3) CH_4 fluxes were measured from all three treatments, followed by a decrease thereafter in all vegetation treatments (Figure 4). Vegetation did not affect ($P = 0.488$) CH_4 fluxes after flood release (Table 3). Methane fluxes measured by 7 DAFR indicated negligible CH_4 release from the soil. A similar trend was observed by Cicerone et al. (1992) where CH_4 fluxes had neared zero immediately prior to flood release, peaked at 4 DAFR, then became negligible a few days later. Studies have indicated that post-flood-release pulses of CH_4 occur as soil macropores drain and become aerated during drying, releasing entrapped CH_4, and can account for up to 10% of total CH_4 released during the growing season (Bossio et al., 1999; Denier van der Gon et al., 1996; Rogers et al., 2013; Yagi et al., 1996). While vegetation did not affect CH_4 fluxes over time ($P = 0.065$; Table 3) following flood release, the numeric differences among treatment means supported the idea that plant-mediated CH_4 transport dominated in this study. The post-flood-release CH_4 flux peak was 55% greater from the low-vegetation treatment and 3.3 times greater from the unvegetated treatment than from the high-vegetation treatment, which indicated greater CH_4 accumulation in the soil where little or no vegetation was present to enhance CH_4 release to the atmosphere. Cicerone et al. (1992) observed a similar trend where the post-flood-

release pulse was greatest from bare soil and least from fertilized rice with CH_4 fluxes comparable to maxima observed during flood retention from bare soil and unfertilized rice. This trend may be an indication that CH_4 emissions from rice produced on clay soils are limited by the amount of CH_4 capable of moving through the rice plants, resulting in significant CH_4 accumulation in the soil where plants are absent or sparse. Numerous studies have observed greater CH_4 concentrations, indicating accumulation due to limited transport, in unvegetated plots than in plots containing rice, while fluxes were greater from vegetated plots (Dannenberg and Conrad, 1999; Holzapfel-Pschorn et al., 1986; Kruger et al., 2002; Nouchi and Mariko, 1993). The intermediate pulse from the low- indicated a limitation in CH_4 transport capacity relative to the high-vegetation treatment, which resulted in some build-up of CH_4 in the soil over the growing season that was able to escape to the atmosphere after flood release and subsequent soil drying.

When averaged across chamber size, a significant post-flood-release CH_4 pulse occurred in the unvegetated treatment at 5 DAFR ($P = 0.007$), while no pulse was evident in the vegetated treatment (Table 3). These findings were consistent with those detailed above. Averaged across vegetation treatments CH_4 fluxes differed between chamber sizes over time ($P = 0.007$), where the 30-cm chambers had a greater measured flux at 5 DAFR than the 15.2-cm chambers. This result was likely due to the sampling frequency, which may have resulted in missing the pulse from the 15.2-cm chambers due to a possible difference in soil-drying time and time of the pulse release between the two chamber sizes.

Aboveground Dry Matter and Grain Yield

Aboveground dry matter measured at harvest did not differ ($P = 0.793$) based on sampling location (i.e., within the chamber or within the plot), which indicated that the chambers did not affect plant growth throughout the season (Table 4). However, vegetation level significantly impacted aboveground dry matter ($P = 0.012$), where, averaged across sampling location, the low- only accumulated 38% of the aboveground dry matter that accumulated in the high-vegetation treatment (Table 5). In addition, vegetation level did not affect aboveground dry matter between sampling locations ($P = 0.920$). Chamber size did not impact aboveground dry matter as the 15.2-cm chambers accumulated 2.71 kg aboveground dry matter m^{-2}, compared to 2.77 and 2.83 kg m^{-2} for the 30-cm chambers and plots, respectively ($P = 0.951$).

Grain yields differed ($P = 0.023$) between vegetation levels, with the low- yielding only 45% of that from the high-vegetation treatment (10.8 Mg ha^{-1}). The high-vegetation treatment's yield was slightly greater than the yield of 10.2 Mg ha^{-1} reported for Taggart at NEREC in the 2012 Arkansas Rice Performance trials (Hardke et al., 2013).

Seasonal Methane Emissions

Season-long area-scaled CH_4 emissions differed ($P < 0.001$) by vegetation level, where the high-vegetation treatment resulted in 35.6 kg CH_4-C ha^{-1} $season^{-1}$, compared to 8.94 and 1.75 kg CH_4-C ha^{-1} $season^{-1}$ from the low-vegetation and no-vegetation treatments, respectively, which did not differ from each other (Table 6). A similar result was obtained by Cicerone et al. (1992) on a Sacramento clay in California, where the bare-soil (8.85 kg CH_4-C ha^{-1} $season^{-1}$) and low-vegetation (10.5 kg CH_4-C ha^{-1} $season^{-1}$) treatments did not differ substantially, but were less than the 21.6 kg CH_4-C ha^{-1} $season^{-1}$ released from the high-vegetation treatment. Other studies, however, have indicated no difference in area-scaled emissions between unfertilized

(i.e., low vegetation) and N-fertilized (i.e., high vegetation) rice on a clay soil in California (Adviento-Borbe et al., 2014) and on a silt-loam soil in Arkansas (Rogers et al., 2013). Wang et al. (1993) also observed no impact of N application on CH_4 emissions and other studies have even reported decreased emissions with N application (Cai et al., 1997; Wang et al., 1992), however a meta-analysis by Banger et al. (2012) showed that CH_4 emissions were significantly greater when N fertilizer was applied in 98 out of 155 data pairs, indicating that the increase in plant growth and C supply from plants due to the added N generally stimulates methanogenesis to a greater extent than the addition of N stimulates methanotrophy.

Further evidence supporting the impact of added N on plant growth and emissions in this study was indicated by a positive correlation between aboveground dry matter (i.e., measured within chambers) and season-long CH_4 emissions, both excluding ($P < 0.001$; $r^2 = 0.815$; Figure 5A) and including unvegetated chambers ($P < 0.001$; $r^2 = 0.892$; Figure 5B). Results indicate that the greater the aboveground dry matter accumulation by the end of the growing season the greater the CH_4 emissions, which has also been previously reported (Cicerone and Shetter, 1981; Huang et al., 1997; Sass et al, 1991a; Shang et al., 2011). The strong impact of rice plants on CH_4 emissions discussed here is consistent with flux results presented in this study, which indicated a relationship between the partitioning of photosynthates in the plant and CH_4 release, which likely reflects the rate of root exudation throughout the season.

Yield-scaled emissions did not differ between vegetated treatments ($P = 0.080$), although emissions from the high- [3.5 kg CH_4-C (Mg grain)$^{-1}$] were nearly double those from the low-vegetation treatment (Table 6). This result was attributed to a 2-fold increase in yield with N application accompanied by a 4-fold increase in area-scaled emissions, which may indicate N limitations in the methanogenic community that were alleviated by N application, resulting in

greater emissions than would be expected based on grain yield alone. The decrease in aboveground dry matter was greater than the decrease in yield when no N was applied, possibly indicating greater partitioning of photosynthates to filling grains relative to those lost through the roots, which would result in lower yield-scaled emissions in the low-vegetation treatment. While attaining similar yields in non-N-fertilized (4.5 Mg ha^{-1}) and slightly lower yields in N-fertilized (8.8 Mg ha^{-1}) rice, Rogers et al. (2013) observed no difference in yield-scaled emissions, although emissions were much greater than in this study at 34.9 kg CH_4-C (Mg grain)$^{-1}$ for the non-N-fertilized and 27.6 kg CH_4-C (Mg grain)$^{-1}$ for the N-fertilized rice. Adviento-Borbe et al. (2014) also observed no significant impact of N fertilization on yield-scaled CH_4 emissions on a clay soil in California.

The season-long CH_4 emissions measured in this study were only 20% of the USEPA's reported emission factor for non-California, primary rice production (178 kg CH_4-C ha^{-1} season^{-1}) and were even 22% lower than the lowest measured emissions used in the most recent inventory (USEPA, 2014). This shows that, when compared to other domestic studies under typical management conditions, emissions from direct, drill-seeded, delayed flood rice grown on a Sharkey clay soil in eastern Arkansas are quite low. A study conducted on a silt-loam soil in eastern Arkansas under similar management observed emissions of 195 kg CH_4-C ha^{-1} season^{-1}, which were close to the USEPA emission factor, but substantially greater than the 35.6 kg CH_4-C ha^{-1} season^{-1} (Rogers et al., 2013). The difference between emissions from a clay and silt-loam soil in eastern Arkansas can largely be attributed to an inverse correlation between soil clay content and CH_4 emissions observed in several studies (Mitra et al., 2002; Sass et al., 1994; Watanabe and Kimura, 1999). Another factor which may influence differences between the two Arkansas soils is a greater S concentration (19 mg kg^{-1}) in the clay soil than in the silt-loam soil

(9.2 mg kg^{-1}), which theoretically could result in greater oxidation of soil organic matter as sulfate is reduced. This would reduce the organic substrate available for methanogenesis as well as lengthening the time required for the redox potential to reach levels low enough for methanogenesis to occur, which would reduce CH_4 production potential (Conrad, 1989). Whether the effect is due to greater S concentrations or a textural effect, redox potentials reached lower levels faster and were generally lower in the silt-loam (Rogers et al., 2013) than in the clay soil, which is consistent with lower emissions from the clay soil.

Methane emissions measured from this study were similar to results from other similar-textured soils when a hurricane caused a levee breech, temporarily draining the field, and resulting in emissions of 34 kg CH_4-C ha^{-1} $season^{-1}$ (Sass et al., 1990) or when residue from the previous crop was burned resulting in emissions of 21.6 kg CH_4-C ha^{-1} $season^{-1}$ (Cicerone et al., 1992), 22.5 kg CH_4-C ha^{-1} $season^{-1}$ (Bossio et al., 1999), and 16.4 to 67.1 kg CH_4-C ha^{-1} $season^{-1}$ (Fitzgerald et al., 2000). All of these studies showed significantly greater emissions from unburned straw or when supplemental rice straw was incorporated prior to flooding. Evidence from previous studies suggests that the low emissions observed in this study may be a result of low organic residue inputs or that a greater proportion of produced CH_4 was oxidized in the Sharkey clay compared to other soils that have been studied. A study conducted by Rogers et al. (2014) reported that emissions were reduced by 31% when following soybean compared to following rice as a previous crop. These results were consistent with reduced emissions in this study as this study followed soybean as the previous crop, while previous studies generally followed rice or fallow from the preceding season. Assuming similar residue inputs prior to flooding between the clay and silt-loam soil (Rogers et al., 2013) in eastern Arkansas, with both studies following soybean as a previous crop, results indicate that the soil textural effect

outweighs the effect of reducing the C input from previous crop residue. Further evidence for reduced C inputs to the soil lies in the above-optimum soil P level in this study. Lu et al. (1999) observed enhanced root growth and exudation in low-P soils accompanied by 19 to 33% greater CH_4 emissions compared to a P-fertilized treatment. Additionally, several studies have observed decreased emissions accompanied by increased yields (Denier van der Gon et al., 2002; Sass et al., 1991a, 1991b; Sass and Cicerone, 2002) and Aulakh et al. (2001) reported that increasing grain yield has a negative effect on root exudation. High yields achieved in this study (10.8 Mg ha^{-1}) compared to average yields of 7.5 Mg ha^{-1} for studies conducted on Beaumont clays in Texas (Sass and Fisher, 1997), for example, may be accompanied by a decrease in C release from the roots and a reduction in CH_4 emissions.

Organic C inputs prior to flooding may have been less in this study than in other comparable studies, however, evidence suggests that greater CH_4 oxidation in this study was more likely the cause of lower observed emissions. Studies have consistently reported emissions to be lower from fine- than from coarser-textured soils, which may be explained by increased tortuosity and decreased diffusivity limiting CH_4 movement in fine-textured soils (Livingston and Hutchinson, 1995; Nazaroff, 1992). Sass and Fisher (1997) postulated that clayey soils may exhibit reduced CH_4 emissions due to entrapped CH_4 that may be oxidized prior to release, due to slow movement through the oxidized zones of the soil. Studies have indicated an increase in the proportion of entrapped CH_4 and a decrease in emissions as clay content increases (Denier van der Gon et al., 1996; Wang et al., 1993). In a laboratory incubation study with soils from all over the world, Wang et al. (1993) observed the greatest CH_4 entrapment (98.5%) from a Sharkey clay soil, even when compared to a Beaumont clay soil with nearly the same sand and clay fractions (80.6% entrapment) or a Sacramento clay soil with greater clay and lower sand

fractions (67.8% entrapment). The magnitude of CH_4 entrapment and resulting oxidation in a Sharkey clay soil may explain the low emissions observed in this study compared to those measured from Beaumont clay soils in Texas (averaging 102 kg CH_4-C ha^{-1} $season^{-1}$; Sass and Fisher, 1997) or Sacramento clays in California (Cicerone et al., 1992). Other evidence supporting greater CH_4 oxidation in this study, relative to previous studies, lies in the generally greater redox potentials measured in this study compared to those measured by Rogers et al. (2013) and Bossio et al. (1999). Kludze et al. (1993) reported that 56% of CH_4 was oxidized at -300 mV compared to 74% at -200 mV, indicating a greater proportion of oxidation at greater redox potentials.

Post-flood-release CH_4 emissions did not differ among vegetation treatments (Table 6), although the relative magnitude of treatment means is indicative of greater CH_4 accumulation in the soil when plants are absent or sparse. When comparing post-flood-release emissions as a percentage of total seasonal emissions, the unvegetated treatment had greater emissions with 63.5% of the total following flood release, compared to an average of 5.1% for the vegetated treatments. The average proportion of post-flood-release emissions observed from the vegetated treatments in this study was similar to that reported by Rogers et al. (2013) (5.2%). However, the large proportion of emissions following flood release from unvegetated soil in this study has not been reported in previous studies (Cicerone et al., 1992; Rogers et al., 2013) and provides evidence for the occurrence of large amounts of CH_4 accumulation or entrapment in this study. Furthermore, the low percentage of post-flood-release emissions in the high-vegetation treatment (1.6%) relative to previous studies (Bossio et al., 1999; Denier van der Gon et al., 1996; Rogers et al., 2012; Yagi et al., 1996) may indicate greater CH_4 oxidation in this study resulting from reduced CH_4 movement in the soil.

Chamber size did not affect any CH_4 emission properties, while vegetation influenced both area-scaled emissions and post-flood-release emissions as a proportion of total emissions (Table 7). The differences in emissions observed between unvegetated and vegetated treatments were consistent with results discussed above (Table 8). Total area-scaled CH_4 emissions were 32.6 and 35.6 kg CH_4-C ha^{-1} $season^{-1}$ from the 15.2- and 30-cm chambers, respectively, and the similarity indicates that the 15.2-cm diameter chambers adequately facilitated the quantification of CH_4 emissions in this study.

Summary and Conclusions

Methane fluxes and subsequent emissions were measured from drill-seeded, delayed-flood rice grown on a clay soil in eastern Arkansas using enclosed-headspace chambers. Methane fluxes from vegetated treatments generally increased throughout the vegetative and early reproductive stages, peaking just after 50% heading, and declining again thereafter until following flood release, where a pulse of CH_4 emissions occurred in all treatments at 5 DAFR. Fluxes remained below 0.06 mg CH_4-C m^{-2} h^{-1} in the unvegetated treatment for the duration of flooding prior to having the largest post-flood-release pulse of CH_4. Total season-long emissions were greatest in the high-vegetation treatment, but amounted to less than 20% of emissions measured from a similar study conducted on a silt-loam soil in eastern Arkansas and about 20% of the USEPA emission factor for non-California, primary-rice production. Based on results presented in this study, CH_4 fluxes and emissions appeared to be closely associated with plant growth and development. Post-flood-release pulses and emissions, in addition to low measured fluxes, indicated a large degree of entrapment or accumulation of CH_4 in this soil. Studies have shown a positive correlation between entrapment and clay content and one study reported

entrapment from a Sharkey clay, the same soil from this study, to be greater than any other soils studied, even with similar sand and clay fractions. The large degree of entrapment observed in this study was likely indicative of increased gas tortuosity and slow diffusion rates, which are believed to cause an increase in oxidation of CH_4 by methanotrophs. In addition to high oxidation rates, reduced C inputs in this study may help explain the low measured CH_4 emissions. The low emissions observed in this study, in combination with the degree of production on clay and clay-loam soils in Arkansas, indicate that CH_4 emissions from Arkansas rice may be substantially overestimated. A more accurate estimate of U.S. and global emissions can only be made after accounting for differences resulting from various cultural practices and differences in production area under a larger variety of environments, soils, and cultural practices. The only difference in CH_4 fluxes between 15.2- and 30-cm chambers occurred following flood release, where the post-flood-release pulse was apparently missed using the 15.2-cm chambers. However, there was no difference in season-long emissions between the two chamber sizes. Results from this study indicate that 15.2-cm diameter chambers were adequate in measuring CH_4 fluxes and emissions.

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Appendices

Appendix 1: Example SAS program and relevant data for analyzing flooded CH_4 fluxes for the vegetation objective.

```
title 'Methane Field Study - Seasonal Flooded Fluxes (12 inch) 2012 -  Alden Smartt / Chris W. Rogers';

title2 'Clay Soil Data Methane 2012 ANOVA';

data floodedfluxes2012;

infile 'C:\Users\adsmartt\Documents\SAS input files\Fluxes over time(Flood to FR)-12in.prn' firstobs=2;

input time chamber treatment $ block flux;
 run;

proc sort data=floodedfluxes2012; by time chamber treatment;
quit;

title3 'INITIAL DATA LISTING AND DATA PLOT';

proc print data=floodedfluxes2012 noobs; by time;
  id time;
  var chamber treatment block flux;
  run;

proc sort; by treatment time;
proc means;
class treatment time;
var flux;
run;
quit;


title3 'ANALYSIS OF VARIANCE';
proc mixed data=floodedfluxes2012 method=type3;
class block treatment time;
model flux = treatment time treatment*time / ddfm=kr;
random block block*treatment;
lsmeans treatment*time / diff;
run;
quit;
```

DAF	Chamber	Treatment	Block	mg CH4-C/m2/h
11	1	ZeroN	1	0.02348
11	2	BareSoil	3	0.00306
11	4	OptN	1	0.02477
11	7	OptN	3	0.03391
11	10	BareSoil	1	0.00163
11	12	ZeroN	3	0.03193
11	13	OptN	2	0.03186
11	16	OptN	4	0.01633
11	19	ZeroN	2	0.01982
11	20	BareSoil	4	0.01751
11	22	BareSoil	2	0.00746
11	24	ZeroN	4	0.02497
18	1	ZeroN	1	0.13915
18	2	BareSoil	3	0.07137
18	4	OptN	1	0.28205
18	7	OptN	3	0.38236
18	10	BareSoil	1	0.03575
18	12	ZeroN	3	0.05242
18	13	OptN	2	0.09103
18	16	OptN	4	0.11399
18	19	ZeroN	2	0.17741
18	20	BareSoil	4	0.09417
18	22	BareSoil	2	0.04399
18	24	ZeroN	4	0.06449
25	1	ZeroN	1	0.50565
25	2	BareSoil	3	0.00821
25	4	OptN	1	0.48127
25	7	OptN	3	0.69958
25	10	BareSoil	1	0.02891
25	12	ZeroN	3	0.1721
25	13	OptN	2	0.17725
25	16	OptN	4	0.2844
25	19	ZeroN	2	0.34563
25	20	BareSoil	4	0.02494
25	22	BareSoil	2	0.003
25	24	ZeroN	4	0.10071
32	1	ZeroN	1	0.68152
32	2	BareSoil	3	0.01319
32	4	OptN	1	1.96737
32	7	OptN	3	1.55999
32	10	BareSoil	1	0.01612
32	12	ZeroN	3	0.22577

32	13	OptN	2	0.85878
32	16	OptN	4	0.91599
32	19	ZeroN	2	0.60886
32	20	BareSoil	4	0.02493
32	22	BareSoil	2	0.01903
32	24	ZeroN	4	0.26677
39	1	ZeroN	1	1.03152
39	2	BareSoil	3	0.03342
39	4	OptN	1	3.45877
39	7	OptN	3	3.32443
39	10	BareSoil	1	0.05853
39	12	ZeroN	3	0.46699
39	13	OptN	2	2.45855
39	16	OptN	4	1.88654
39	19	ZeroN	2	0.83648
39	20	BareSoil	4	0.02421
39	22	BareSoil	2	0.02437
39	24	ZeroN	4	0.47599
46	1	ZeroN	1	1.15951
46	2	BareSoil	3	0.01965
46	4	OptN	1	4.02114
46	7	OptN	3	4.05725
46	10	BareSoil	1	0.01902
46	12	ZeroN	3	0.54553
46	13	OptN	2	3.75773
46	16	OptN	4	2.40412
46	19	ZeroN	2	1.10542
46	20	BareSoil	4	0.02419
46	22	BareSoil	2	0.01678
46	24	ZeroN	4	0.69885
53	1	ZeroN	1	1.26404
53	2	BareSoil	3	0.00755
53	4	OptN	1	5.00751
53	7	OptN	3	4.60332
53	10	BareSoil	1	0.02637
53	12	ZeroN	3	0.55918
53	13	OptN	2	4.59209
53	16	OptN	4	3.12759
53	19	ZeroN	2	1.07224
53	20	BareSoil	4	0.06282
53	22	BareSoil	2	0.02283
53	24	ZeroN	4	0.84805
60	1	ZeroN	1	1.07343

60	2	BareSoil	3	0.02808
60	4	OptN	1	5.95942
60	7	OptN	3	5.04958
60	10	BareSoil	1	0.1341
60	12	ZeroN	3	0.51453
60	13	OptN	2	5.29518
60	16	OptN	4	2.76699
60	19	ZeroN	2	0.8439
60	20	BareSoil	4	0.02228
60	22	BareSoil	2	0.03151
60	24	ZeroN	4	0.91799
67	1	ZeroN	1	0.57782
67	2	BareSoil	3	0.01505
67	4	OptN	1	2.99206
67	7	OptN	3	2.52014
67	10	BareSoil	1	0.04853
67	12	ZeroN	3	0.25441
67	13	OptN	2	3.03545
67	16	OptN	4	1.45057
67	19	ZeroN	2	0.47223
67	20	BareSoil	4	0.01835
67	22	BareSoil	2	0.01741
67	24	ZeroN	4	0.48868
74	1	ZeroN	1	0.33141
74	2	BareSoil	3	0.00291
74	4	OptN	1	1.49432
74	7	OptN	3	1.06854
74	10	BareSoil	1	0.04384
74	12	ZeroN	3	0.10494
74	13	OptN	2	1.23751
74	16	OptN	4	0.72799
74	19	ZeroN	2	0.23719
74	20	BareSoil	4	0.02464
74	22	BareSoil	2	0.01068
74	24	ZeroN	4	0.25373

Appendix 2: Example SAS program and relevant data for analyzing post-flooded CH_4 fluxes for the vegetation objective.

```
title 'Methane Field Study - Seasonal Post-flooded Fluxes (12 inch) 2012 -  Alden Smartt / Chris W. Rogers';

title2 'Clay Soil Data Methane 2012 ANOVA';

data floodedfluxes2012;

infile 'C:\Users\adsmartt\Documents\SAS input files\Fluxes over time(FR to Harvest)-12in.prn' firstobs=2;

input time chamber treatment $ block flux;
 run;

proc sort data=floodedfluxes2012; by time chamber treatment;
quit;

title3 'INITIAL DATA LISTING AND DATA PLOT';

proc print data=floodedfluxes2012 noobs; by time;
  id time;
  var chamber treatment block flux;
  run;

proc sort; by treatment time;
proc means;
class time;
var flux;
run;
quit;


title3 'ANALYSIS OF VARIANCE';
proc mixed data=floodedfluxes2012 method=type3;
class block treatment time;
model flux = treatment time treatment*time / ddfm=kr;
random block block*treatment;
lsmeans time / diff;
run;
quit;
```

DAF	Chamber	Treatment	Block	mg CH4-C/m2/h
77	1	ZeroN	1	0.28735
77	2	BareSoil	3	0.00281
77	4	OptN	1	0.52992
77	7	OptN	3	0.33722
77	10	BareSoil	1	0.00626
77	12	ZeroN	3	0.08275
77	13	OptN	2	0.56877
77	16	OptN	4	0.07019
77	19	ZeroN	2	0.17674
77	20	BareSoil	4	0.02109
77	22	BareSoil	2	0.02696
77	24	ZeroN	4	0.193
79	1	ZeroN	1	0.4927
79	2	BareSoil	3	0.01841
79	4	OptN	1	0.5083
79	7	OptN	3	0.26413
79	10	BareSoil	1	0.00399
79	12	ZeroN	3	0.13454
79	13	OptN	2	0.13071
79	16	OptN	4	0.05026
79	19	ZeroN	2	0.1757
79	20	BareSoil	4	0.09463
79	22	BareSoil	2	0.03271
79	24	ZeroN	4	0.17583
81	1	ZeroN	1	2.09199
81	2	BareSoil	3	0.39451
81	4	OptN	1	1.51437
81	7	OptN	3	0.95013
81	10	BareSoil	1	3.06059
81	12	ZeroN	3	0.71898
81	13	OptN	2	0.28882
81	16	OptN	4	0.32165
81	19	ZeroN	2	0.9486
81	20	BareSoil	4	5.48169
81	22	BareSoil	2	1.2235
81	24	ZeroN	4	1.01468
83	1	ZeroN	1	0
83	2	BareSoil	3	0
83	4	OptN	1	0.04797
83	7	OptN	3	0
83	10	BareSoil	1	0.06988
83	12	ZeroN	3	0.02601

83	13	OptN	2	0
83	16	OptN	4	0.01088
83	19	ZeroN	2	0.05986
83	20	BareSoil	4	0.19389
83	22	BareSoil	2	0.05743
83	24	ZeroN	4	0.0331

Appendix 3: Example SAS program and relevant data for analyzing flooded CH_4 fluxes for the chamber-size comparison objective.

```
title 'Methane Field Study - Seasonal Flooded Fluxes (Size Comp.) 2012 -  Alden Smartt / Chris W. Rogers';

title2 'Clay Soil Data Methane 2012 ANOVA';

data floodedfluxes2012;

infile 'C:\Users\adsmartt\Documents\SAS input files\Fluxes over time(Flood to FR)-SizeComp.prn' firstobs=2;

input time chamber vegetation $ size $ block flux;
 run;

proc sort data=floodedfluxes2012; by time chamber vegetation size;
quit;

title3 'INITIAL DATA LISTING AND DATA PLOT';

proc print data=floodedfluxes2012 noobs; by time;
  id time;
  var chamber vegetation size block flux;
  run;

proc sort; by vegetation size time;
proc means;
class vegetation time;
var flux;
run;
quit;


title3 'ANALYSIS OF VARIANCE';
proc mixed data=floodedfluxes2012 method=type3;
class block vegetation size time;
model flux = vegetation size vegetation*size time time*vegetation time*size
time*vegetation*size / ddfm=kr;
random block block*vegetation block*vegetation*size;
lsmeans vegetation*time / diff;
run;
quit;
```

DAF	Chamber	Vegetation	Size	Block	mg CH4-C/m2/h
11	2	NoRice	12	3	0.00306
11	3	NoRice	6	3	0.00954
11	4	Rice	12	1	0.02477
11	5	Rice	6	1	0.05746
11	7	Rice	12	3	0.03391
11	8	Rice	6	3	0.02909
11	10	NoRice	12	1	0.00163
11	11	NoRice	6	1	0.00408
11	13	Rice	12	2	0.03186
11	14	Rice	6	2	0.02242
11	16	Rice	12	4	0.01633
11	17	Rice	6	4	0.0179
11	20	NoRice	12	4	0.01751
11	21	NoRice	6	4	0.00385
11	22	NoRice	12	2	0.00746
11	23	NoRice	6	2	0.00069
18	2	NoRice	12	3	0.07137
18	3	NoRice	6	3	0.02624
18	4	Rice	12	1	0.28205
18	5	Rice	6	1	0.20299
18	7	Rice	12	3	0.38236
18	8	Rice	6	3	0.1167
18	10	NoRice	12	1	0.03575
18	11	NoRice	6	1	0.03322
18	13	Rice	12	2	0.09103
18	14	Rice	6	2	0.02359
18	16	Rice	12	4	0.11399
18	17	Rice	6	4	0.10505
18	20	NoRice	12	4	0.09417
18	21	NoRice	6	4	0.00465
18	22	NoRice	12	2	0.04399
18	23	NoRice	6	2	0.02661
25	2	NoRice	12	3	0.00821
25	3	NoRice	6	3	0.01261
25	4	Rice	12	1	0.48127
25	5	Rice	6	1	0.24934
25	7	Rice	12	3	0.69958
25	8	Rice	6	3	0.12069
25	10	NoRice	12	1	0.02891
25	11	NoRice	6	1	0.01245
25	13	Rice	12	2	0.17725
25	14	Rice	6	2	0.09301

25	16	Rice	12	4	0.2844
25	17	Rice	6	4	0.39866
25	20	NoRice	12	4	0.02494
25	21	NoRice	6	4	0.00777
25	22	NoRice	12	2	0.003
25	23	NoRice	6	2	0.01321
32	2	NoRice	12	3	0.01319
32	3	NoRice	6	3	0.01575
32	4	Rice	12	1	1.96737
32	5	Rice	6	1	0.49186
32	7	Rice	12	3	1.55999
32	8	Rice	6	3	0.59785
32	10	NoRice	12	1	0.01612
32	11	NoRice	6	1	0.01956
32	13	Rice	12	2	0.85878
32	14	Rice	6	2	0.47013
32	16	Rice	12	4	0.91599
32	17	Rice	6	4	1.13149
32	20	NoRice	12	4	0.02493
32	21	NoRice	6	4	0.02943
32	22	NoRice	12	2	0.01903
32	23	NoRice	6	2	0.01903
39	2	NoRice	12	3	0.03342
39	3	NoRice	6	3	0.02566
39	4	Rice	12	1	3.45877
39	5	Rice	6	1	1.17155
39	7	Rice	12	3	3.32443
39	8	Rice	6	3	1.84579
39	10	NoRice	12	1	0.05853
39	11	NoRice	6	1	0.03006
39	13	Rice	12	2	2.45855
39	14	Rice	6	2	1.08057
39	16	Rice	12	4	1.88654
39	17	Rice	6	4	3.27452
39	20	NoRice	12	4	0.02421
39	21	NoRice	6	4	0.03105
39	22	NoRice	12	2	0.02437
39	23	NoRice	6	2	0.03699
46	2	NoRice	12	3	0.01965
46	3	NoRice	6	3	0.02571
46	4	Rice	12	1	4.02114
46	5	Rice	6	1	2.52986
46	7	Rice	12	3	4.05725

46	8	Rice	6	3	2.5224
46	10	NoRice	12	1	0.01902
46	11	NoRice	6	1	0.05291
46	13	Rice	12	2	3.75773
46	14	Rice	6	2	2.21434
46	16	Rice	12	4	2.40412
46	17	Rice	6	4	5.40123
46	20	NoRice	12	4	0.02419
46	21	NoRice	6	4	0.04598
46	22	NoRice	12	2	0.01678
46	23	NoRice	6	2	0.02939
53	2	NoRice	12	3	0.00755
53	3	NoRice	6	3	0.01734
53	4	Rice	12	1	5.00751
53	5	Rice	6	1	3.65327
53	7	Rice	12	3	4.60332
53	8	Rice	6	3	3.67883
53	10	NoRice	12	1	0.02637
53	11	NoRice	6	1	0.01099
53	13	Rice	12	2	4.59209
53	14	Rice	6	2	3.81099
53	16	Rice	12	4	3.12759
53	17	Rice	6	4	7.13277
53	20	NoRice	12	4	0.06282
53	21	NoRice	6	4	0.02771
53	22	NoRice	12	2	0.02283
53	23	NoRice	6	2	0.03805
60	2	NoRice	12	3	0.02808
60	3	NoRice	6	3	0.02595
60	4	Rice	12	1	5.95942
60	5	Rice	6	1	4.51148
60	7	Rice	12	3	5.04958
60	8	Rice	6	3	2.59848
60	10	NoRice	12	1	0.1341
60	11	NoRice	6	1	0.04982
60	13	Rice	12	2	5.29518
60	14	Rice	6	2	3.3048
60	16	Rice	12	4	2.76699
60	17	Rice	6	4	8.21974
60	20	NoRice	12	4	0.02228
60	21	NoRice	6	4	0.03272
60	22	NoRice	12	2	0.03151
60	23	NoRice	6	2	0.05272

67	2	NoRice	12	3	0.01505
67	3	NoRice	6	3	0.03576
67	4	Rice	12	1	2.99206
67	5	Rice	6	1	2.59871
67	7	Rice	12	3	2.52014
67	8	Rice	6	3	1.72699
67	10	NoRice	12	1	0.04853
67	11	NoRice	6	1	0.04537
67	13	Rice	12	2	3.03545
67	14	Rice	6	2	1.99906
67	16	Rice	12	4	1.45057
67	17	Rice	6	4	3.57127
67	20	NoRice	12	4	0.01835
67	21	NoRice	6	4	0.0161
67	22	NoRice	12	2	0.01741
67	23	NoRice	6	2	0.04432
74	2	NoRice	12	3	0.00291
74	3	NoRice	6	3	0.00116
74	4	Rice	12	1	1.49432
74	5	Rice	6	1	1.49455
74	7	Rice	12	3	1.06854
74	8	Rice	6	3	0.86986
74	10	NoRice	12	1	0.04384
74	11	NoRice	6	1	0.04443
74	13	Rice	12	2	1.23751
74	14	Rice	6	2	1.15983
74	16	Rice	12	4	0.72799
74	17	Rice	6	4	2.40792
74	20	NoRice	12	4	0.02464
74	21	NoRice	6	4	0.01401
74	22	NoRice	12	2	0.01068
74	23	NoRice	6	2	0.05259

Appendix 4: Example SAS program and relevant data for analyzing post-flooded CH_4 fluxes for the chamber-size comparison objective.

```
title 'Methane Field Study - Seasonal Post-Flooded Fluxes (Size Comp.) 2012 -  Alden Smartt / Chris W. Rogers';
title2 'Clay Soil Data Methane 2012 ANOVA';
data floodedfluxes2012;
infile 'C:\Users\adsmartt\Documents\SAS input files\Fluxes over time(FR to Harvest)-SizeComp.prn' firstobs=2;

input time chamber vegetation $ size $ block flux;
 run;

proc sort data=floodedfluxes2012; by time chamber vegetation size;
quit;

title3 'INITIAL DATA LISTING AND DATA PLOT';

proc print data=floodedfluxes2012 noobs; by time;
  id time;
  var chamber vegetation size block flux;
  run;

proc sort; by vegetation size time;
proc means;
class vegetation time;
var flux;
run;
quit;

proc sort; by vegetation size time;
proc means;
class size time;
var flux;
run;
quit;

title3 'ANALYSIS OF VARIANCE';
proc mixed data=floodedfluxes2012 method=type3;
class block vegetation size time;
model flux = vegetation size vegetation*size time time*vegetation time*size
time*vegetation*size / ddfm=kr;
random block block*vegetation block*vegetation*size;
lsmeans vegetation*time size*time / diff;
run;
quit;
```

DAF	Chamber	Vegetation	Size	Block	mg CH4-C/m2/h
77	2	NoRice	12	3	0.00281
77	3	NoRice	6	3	0.00716
77	4	Rice	12	1	0.52992
77	5	Rice	6	1	1.9816
77	7	Rice	12	3	0.33722
77	8	Rice	6	3	0.18064
77	10	NoRice	12	1	0.00626
77	11	NoRice	6	1	0.01127
77	13	Rice	12	2	0.56877
77	14	Rice	6	2	0.57519
77	16	Rice	12	4	0.07019
77	17	Rice	6	4	1.96581
77	20	NoRice	12	4	0.02109
77	21	NoRice	6	4	0.00965
77	22	NoRice	12	2	0.02696
77	23	NoRice	6	2	0.01201
79	2	NoRice	12	3	0.01841
79	3	NoRice	6	3	0.01001
79	4	Rice	12	1	0.5083
79	5	Rice	6	1	0.11655
79	7	Rice	12	3	0.26413
79	8	Rice	6	3	0.4117
79	10	NoRice	12	1	0.00399
79	11	NoRice	6	1	0.00673
79	13	Rice	12	2	0.13071
79	14	Rice	6	2	0.07128
79	16	Rice	12	4	0.05026
79	17	Rice	6	4	0.2832
79	20	NoRice	12	4	0.09463
79	21	NoRice	6	4	0.09857
79	22	NoRice	12	2	0.03271
79	23	NoRice	6	2	0.00789
81	2	NoRice	12	3	0.39451
81	3	NoRice	6	3	0.17213
81	4	Rice	12	1	1.51437
81	5	Rice	6	1	0.28851
81	7	Rice	12	3	0.95013
81	8	Rice	6	3	0.83703
81	10	NoRice	12	1	3.06059
81	11	NoRice	6	1	0.18553
81	13	Rice	12	2	0.28882
81	14	Rice	6	2	0.19045

81	16	Rice	12	4	0.32165
81	17	Rice	6	4	0.32329
81	20	NoRice	12	4	5.48169
81	21	NoRice	6	4	0.15354
81	22	NoRice	12	2	1.2235
81	23	NoRice	6	2	1.40958
83	2	NoRice	12	3	0
83	3	NoRice	6	3	0.25984
83	4	Rice	12	1	0.04797
83	5	Rice	6	1	0.01564
83	7	Rice	12	3	0
83	8	Rice	6	3	0
83	10	NoRice	12	1	0.06988
83	11	NoRice	6	1	0.10383
83	13	Rice	12	2	0
83	14	Rice	6	2	0
83	16	Rice	12	4	0.01088
83	17	Rice	6	4	0
83	20	NoRice	12	4	0.19389
83	21	NoRice	6	4	0.25856
83	22	NoRice	12	2	0.05743
83	23	NoRice	6	2	0.14564

Appendix 5: Example SAS program and relevant data for analyzing seasonal CH_4 emissions for the vegetation objective.

```
title 'Methane Field Study - Emissions (12 inch) 2012 -  Alden Smartt / Chris W. Rogers';

title2 'Clay Soil Data Methane 2012 ANOVA';

data emissions2012;

infile 'C:\Users\adsmartt\Documents\SAS input files\Season-Long Emissions(2).prn' firstobs=2;

input chamber treatment $ block kgha PFRkgha PFRpercent FtoFRkgha;
 run;

proc sort data=emissions2012; by chamber treatment block kgha;
quit;

title3 'INITIAL DATA LISTING AND DATA PLOT';

proc sort; by block treatment;
proc print data=emissions2012 noobs;
  id block;
  var treatment kgha PFRkgha PFRpercent FtoFRkgha;
  run;

title3 'Seasonal Emissions in kgha ANALYSIS OF VARIANCE';
proc mixed data=emissions2012 method=type3;
class block treatment;
model kgha = treatment / ddfm=kr;
random block;
lsmeans treatment / diff;
run;

title3 'PFR Emissions in kgha ANALYSIS OF VARIANCE';
proc mixed data=emissions2012 method=type3;
class block treatment;
model PFRkgha = treatment / ddfm=kr;
random block;
lsmeans treatment / diff;
run;

title3 'PFR %age Emissions in % ANALYSIS OF VARIANCE';
proc mixed data=emissions2012 method=type3;
class block treatment;
model PFRpercent = treatment / ddfm=kr;
random block;
```

```
lsmeans treatment / diff;
run;

title3 'Flooding to flood release in kg C/ha ANALYSIS OF VARIANCE';
proc mixed data=emissions2012 method=type3;
class block treatment;
model FtoFRkgha = treatment / ddfm=kr;
random block;
lsmeans treatment / diff;
run;
quit;
```

Chamber	Treatment	Block	kg CH4-C/ha/season	PFR emissions	PFR%	FtoFR emissions
1	ZeroN	1	12.68	1.35	10.61	11.33
2	BareSoil	3	0.54	0.2	36.91	0.34
4	OptN	1	43.79	1.18	2.69	42.61
7	OptN	3	39.46	0.7	1.79	38.75
10	BareSoil	1	2.17	1.5	68.96	0.68
12	ZeroN	3	5.35	0.45	8.39	4.9
13	OptN	2	36.17	0.41	1.12	35.76
16	OptN	4	22.91	0.21	0.91	22.7
19	ZeroN	2	10.18	0.63	6.14	9.55
20	BareSoil	4	3.32	2.76	82.92	0.57
22	BareSoil	2	0.97	0.63	65.4	0.34
24	ZeroN	4	7.55	0.65	8.66	6.89

Appendix 6: Example SAS program and relevant data for analyzing seasonal CH_4 emissions for the chamber-size comparison objective.

```
title 'Methane Field Study - Emissions (Size Comp.) 2012 -  Alden Smartt / Chris W. Rogers';

title2 'Clay Soil Data Methane 2012 ANOVA';

data emissions2012;

infile 'C:\Users\adsmartt\Documents\SAS input files\Season-Long Emissions-SizeComp.prn'
firstobs=2;

input chamber vegetation $ size $ block kgha PFRkgha PFRpercent kgMggrain;
 run;

proc sort data=emissions2012; by chamber vegetation size block kgha;
quit;

title3 'INITIAL DATA LISTING AND DATA PLOT';

proc sort; by block vegetation size;
proc print data=emissions2012 noobs;
  id block;
  var vegetation size kgha PFRkgha PFRpercent kgMggrain;
  run;

title3 'Seasonal Emissions in kgha ANALYSIS OF VARIANCE';
proc mixed data=emissions2012 method=type3;
class block vegetation size;
model kgha = vegetation size vegetation*size / ddfm=kr;
random block block*vegetation;
lsmeans vegetation / diff;
run;

title3 'PFR Emissions in kgha ANALYSIS OF VARIANCE';
proc mixed data=emissions2012 method=type3;
class block vegetation size;
model PFRkgha = vegetation size vegetation*size / ddfm=kr;
random block block*vegetation;
lsmeans vegetation size / diff;
run;

title3 'PFR %age Emissions in % ANALYSIS OF VARIANCE';
proc mixed data=emissions2012 method=type3;
class block vegetation size;
model PFRpercent = vegetation size vegetation*size  / ddfm=kr;
```

```
random block block*vegetation;
lsmeans vegetation / diff;
run;
quit;
```

Chamber	Vegetation	Size	Block	kg CH4-C/ha/season	PFR emissions	PFR%	kg CH4-C/Mg grain
2	NoRice	12	3	0.5402	0.1994	36.9069	.
3	NoRice	6	3	0.5174	0.1837	35.5067	.
4	Rice	12	1	43.7898	1.1799	2.6944	4.4322
5	Rice	6	1	29.2103	0.9142	3.1296	2.9565
7	Rice	12	3	39.4586	0.7048	1.7862	3.6401
8	Rice	6	3	24.0192	0.665	2.7684	2.2158
10	NoRice	12	1	2.1747	1.4996	68.9575	.
11	NoRice	6	1	0.6259	0.1338	21.3816	.
13	Rice	12	2	36.1686	0.4065	1.1238	4.1813
14	Rice	6	2	23.7614	0.333	1.4013	2.747
16	Rice	12	4	22.9061	0.2079	0.9075	1.6708
17	Rice	6	4	53.5539	0.9996	1.8665	3.9062
20	NoRice	12	4	3.3241	2.7562	82.9161	.
21	NoRice	6	4	0.5733	0.2177	37.9801	.
22	NoRice	12	2	0.9692	0.6339	65.3997	.
23	NoRice	6	2	1.2429	0.7377	59.356	.

Table 1. Summary of dates for major agronomic activities involved in management of CH_4 emissions plots for the 2012 rice growing season at the University of Arkansas Northeast Research and Extension Center in Keiser, Arkansas.

Activity	Date
Planting	2 April, 2012
Emergence	30 April, 2012
Pre-flood N fertilizer application	8 June, 2012
Flood establishment	8 June, 2012
Mid-season N fertilizer application	10 July, 2012
Flood release	23 August, 2012
Harvest	11 September, 2012

Table 2. Mean soil properties (n = 12) prior to flood establishment from a Sharkey clay during the 2012 growing season at the University of Arkansas Northeast Research and Extension Center in Keiser, Arkansas.

Soil property	Mean (± SE)
pH	7.6 (0.02)
Sand (g g^{-1})	0.15 (0.003)
Silt (g g^{-1})	0.35 (0.003)
Clay (g g^{-1})	0.50 (0.004)
Bulk density (g cm^{-3})	0.83 (0.03)
Electrical conductivity (dS m^{-1})	0.30 (0.02)
Mehlich-3 extractable nutrients (mg kg^{-1})	
P	80 (3.7)
K	346 (6.3)
Ca	4671 (34)
Mg	857 (4)
Fe	412 (5.6)
Mn	70 (3)
Na	65 (1.3)
S	19 (1.1)
Cu	5 (0.05)
Zn	3.6 (0.05)
NO_3-N (mg kg^{-1})	5.5 (0.55)
NH_4-N (mg kg^{-1})	11.9 (1.4)
Total N (g kg^{-1})	1.2 (0.01)
Total N (Mg ha^{-1})	1.0 (0.05)
Total C (g kg^{-1})	14 (0.3)
Total C (Mg ha^{-1})	11.4 (0.6)
C:N ratio	11.2 (0.1)
Organic matter (g kg^{-1})	34 (0.5)
Organic matter (Mg ha^{-1})	29 (1.2)

Table 3. Analysis of variance summary of the effects of vegetation, time, and their interaction (vegetation objective) as well as vegetation, chamber size, time, and their interactions (chamber-size comparison objective) on CH_4 fluxes from flooding to flood release and following flood release from a clay soil during the 2012 growing season at the Northeast Research and Extension Center in Keiser, Arkansas.

CH_4 flux property/effect	Flooding to flood release	Post-flood release
Vegetation		
Vegetation	0.001	0.487
Time	< 0.001	< 0.001
Vegetation × time	< 0.001	0.065
Chamber size		
Vegetation	< 0.001	0.897
Chamber size	0.788	0.320
Vegetation × chamber size	0.795	0.147
Time	< 0.001	0.001
Vegetation × time	< 0.001	0.007
Chamber size × time	0.773	0.007
Vegetation × chamber size × time	0.768	0.183

Table 4. Analysis of variance summary of the effects of vegetation (low- and high-vegetation), sampling location (within chamber and within plot), and their interaction as well as the effect of chamber size (within 15.2-cm chambers, within 30-cm chambers, and within plots) on aboveground dry matter accumulation and the effect of vegetation (low- and high-vegetation) on grain yield from a clay soil during the 2012 growing season at the Northeast Research and Extension Center in Keiser, Arkansas.

Property/effect	*P*
Aboveground dry matter (kg m^{-2})	
Vegetation	
Vegetation	0.012
Location	0.793
Vegetation × location	0.920
Chamber size	
Location	0.951
Grain yield (Mg ha^{-1})	
Vegetation	0.023

Table 5. Mean aboveground dry matter from the vegetated treatments and chambers and mean yields from N-fertilized (high vegetation) and non-N-fertilized (low vegetation) rice collected at harvest (11 September, 2012) at the Northeast Research and Extension Center in Keiser, Arkansas. Different letters following values within the same row indicate a significant difference ($P < 0.05$).

Property/effect	Low Vegetation	High Vegetation
Aboveground Dry matter (kg m^{-2})		
Chamber	1.04 a[†]	2.77 a
Field plot	1.07 a	2.83 a
Mean	1.06 b	2.80 a
Grain yield (Mg ha^{-1})	4.9 b[†]	10.8 a

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

Table 6. Summary of the effect of vegetation on seasonal CH_4 emissions and emissions means for unvegetated, low-vegetation, and high-vegetation treatments from a clay soil during the 2012 growing season at the Northeast Research and Extension Center in Keiser, Arkansas. Different letters following values within the same row indicate a significant difference ($P < 0.05$).

Emissions property	*P*	No vegetation	Low vegetation	High vegetation
Area-scaled emissions (kg CH_4-C ha^{-1} $season^{-1}$)	< 0.001	1.75 b[†]	8.94 b	35.6 a
Yield-scaled emissions [kg CH_4-C (Mg grain)$^{-1}$]	0.080	--	1.9 a	3.5 a
Post-flood emissions (kg CH_4-C ha^{-1})	0.447	1.27 a	0.77 a	0.62 a
Post-flood emissions (% total emissions)	< 0.001	63.5 a	8.5 b	1.6 b

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

Table 7. Summary of the effects of vegetation, chamber size, and their interaction on CH_4 emissions from a clay soil during the 2012 growing season at the Northeast Research and Extension Center in Keiser, Arkansas.

Emissions property	Vegetation	Chamber Size	Vegetation × Chamber Size
		P	
Area-scaled emissions (kg CH_4-C ha^{-1} $season^{-1}$)	< 0.001	0.737	0.869
Post- flood emissions (kg CH_4-C ha^{-1})	0.723	0.249	0.164
Post-flood emissions (% of total seasonal emissions)	0.005	0.096	0.083

Table 8. Seasonal CH_4 emissions for unvegetated and high-vegetation treatments measured with 15.2- and 30-cm diameter chambers from a clay soil during the 2012 growing season at the Northeast Research and Extension Center in Keiser, Arkansas. Different letters following values within the same row indicate a significant difference ($P < 0.05$).

Property/Effect	No vegetation	High vegetation	Mean
Area-scaled emissions (kg CH_4-C ha^{-1} $season^{-1}$)			
15.2-cm	0.74	32.6	16.7 A
30-cm	1.75	35.6	18.7 A
Mean	1.25 b[†]	34.1 a	
Post-flood emissions (kg CH_4-C ha^{-1})			
15.2-cm	0.32	0.73	0.53 A
30-cm	1.27	0.62	0.95 A
Mean	0.80 a[†]	0.68 a	
Post-flood emissions (% total emissions)			
15.2-cm	38.6	2.3	20.5 A
30-cm	63.5	1.6	32.6 A
Mean	51.0 a[†]	2.0 b	

[†] Different lower-case letters within a row for a measured property indicate differences among cultivars and different upper-case letters within a column for a measured property indicate differences between previous crop treatments ($P < 0.05$).

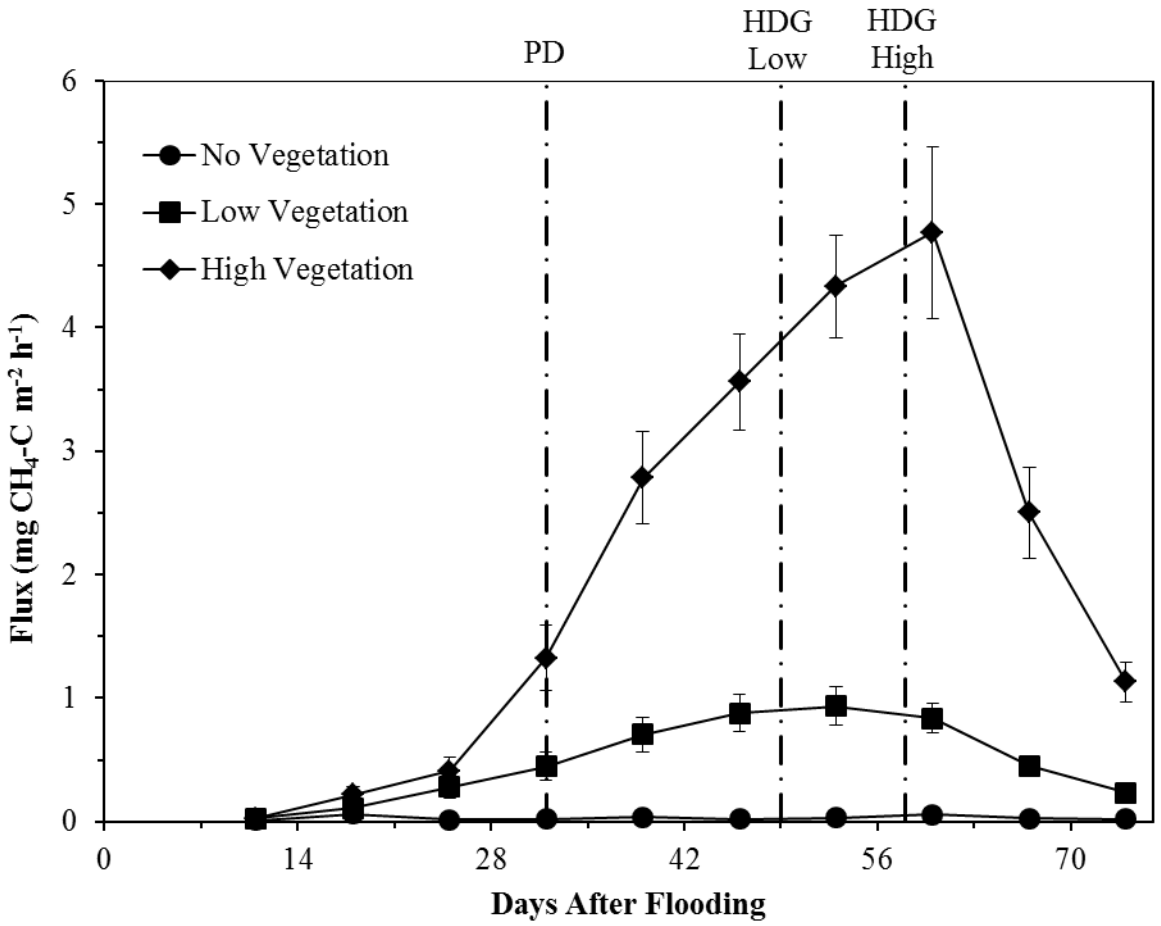


Figure 1. Methane fluxes over time throughout the 2012 growing season from the unvegetated, low-, and high-vegetation treatments at the Northeast Research and Extension Center in Keiser, Arkansas. The vertical dashed lines represent panicle differentiation (PD) and 50% heading dates for low- (HDG Low) and high-vegetation (HDG High) treatments. Flood release occurred on 76 days after flooding. Least significant difference for the same vegetation treatment = 0.404 mg CH_4-C m^{-2} $hr^{-1}$and for different vegetation treatment = 0.637 mg CH_4-C m^{-2} hr^{-1}. Error bars indicate standard errors for the treatment means (n = 4).

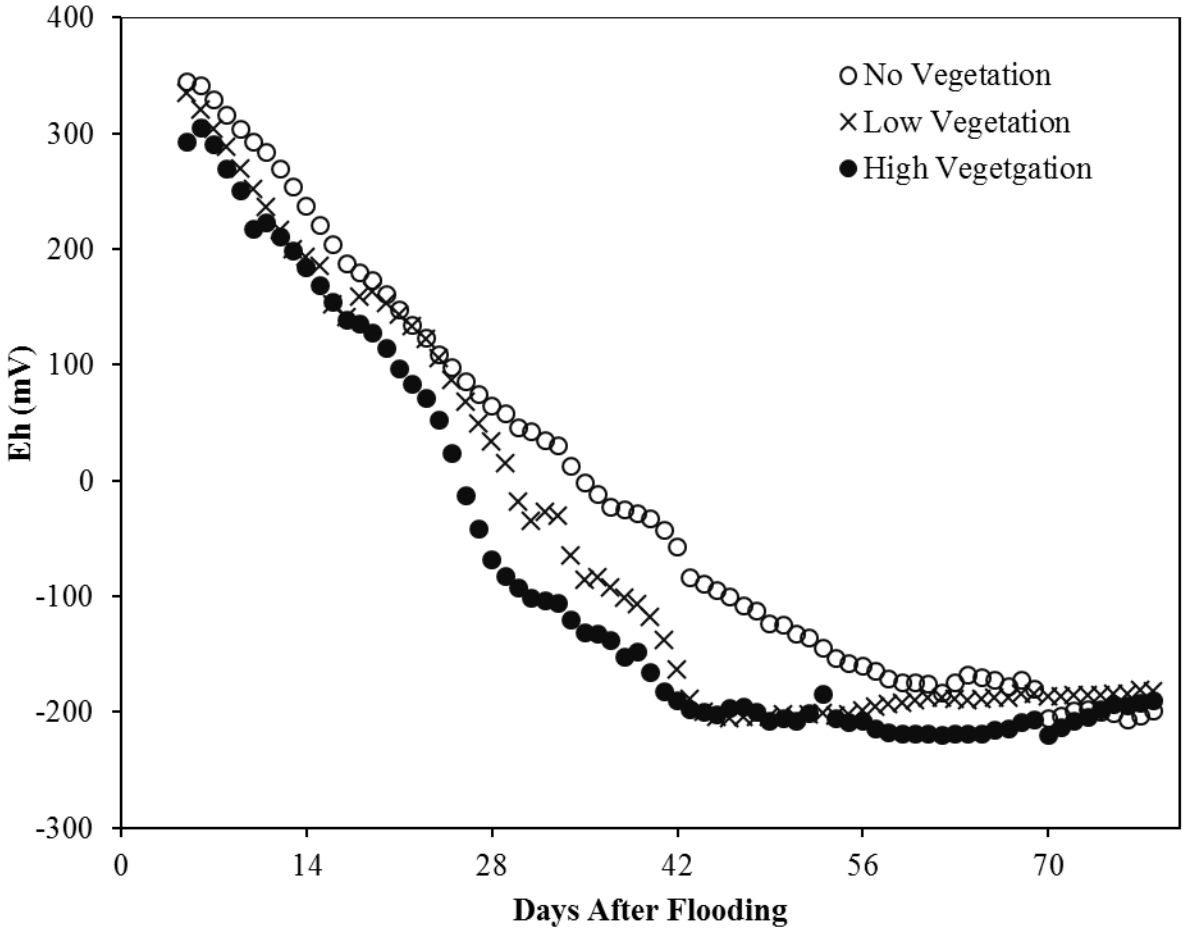


Figure 2. Soil oxidation-reduction potential (Eh) over the flooded portion of the 2012 growing-season for unvegetated, low-, and high-vegetation treatments at the Northeast Research and Extension Center in Keiser, Arkansas.

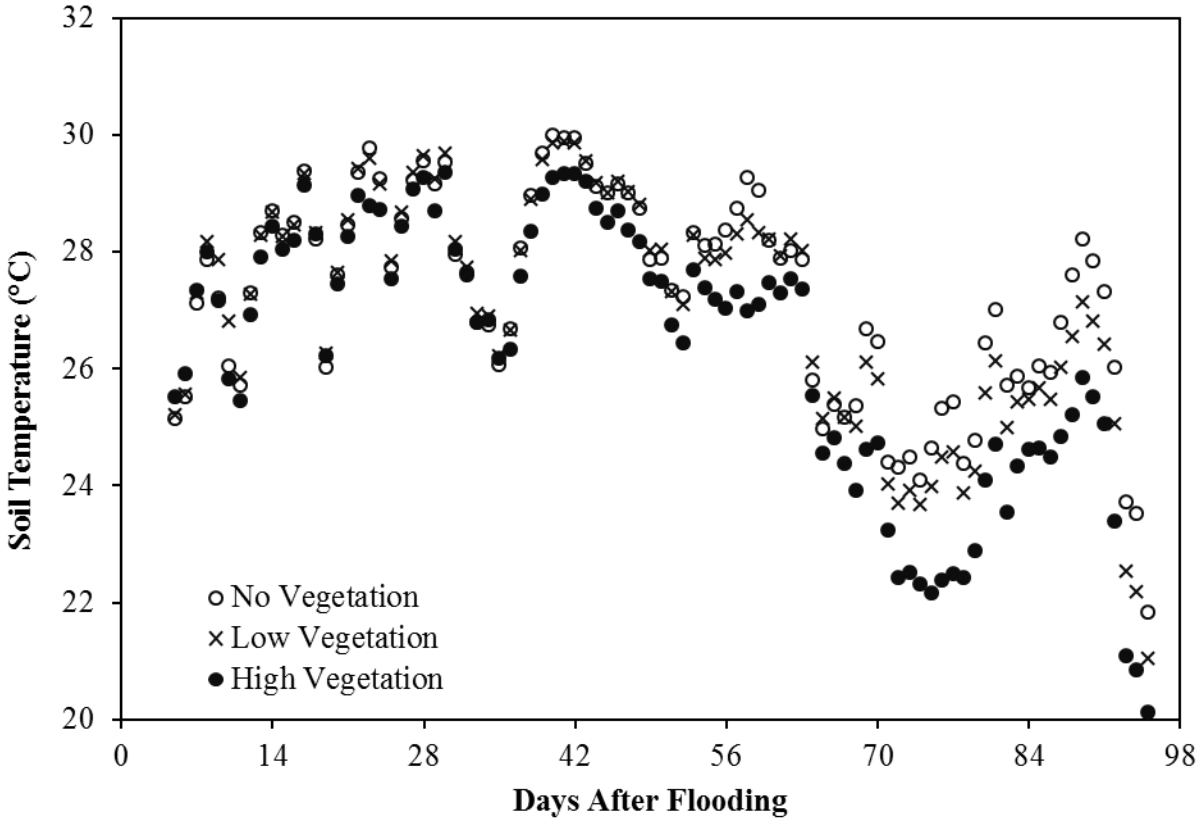


Figure 3. Daily mean soil temperature over the 2012 growing-season for unvegetated, low-, and high-vegetation treatments at the Northeast Research and Extension Center in Keiser, Arkansas.

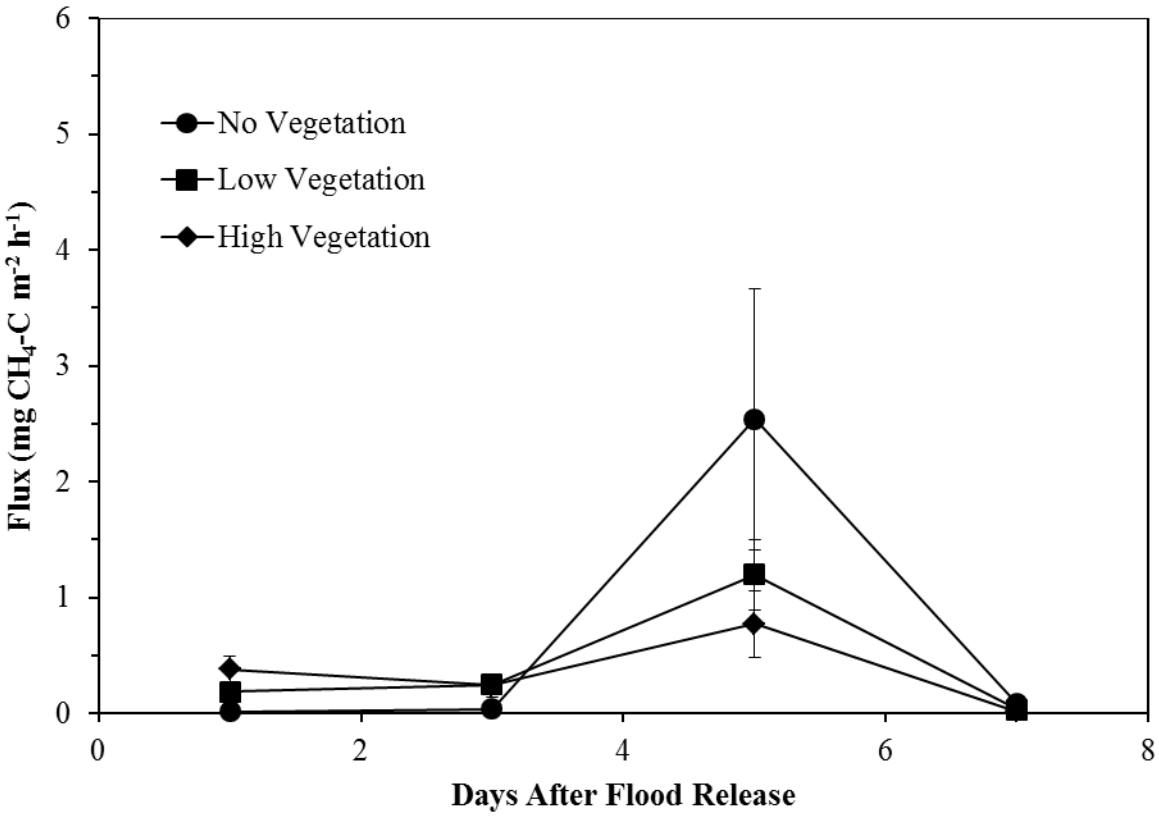
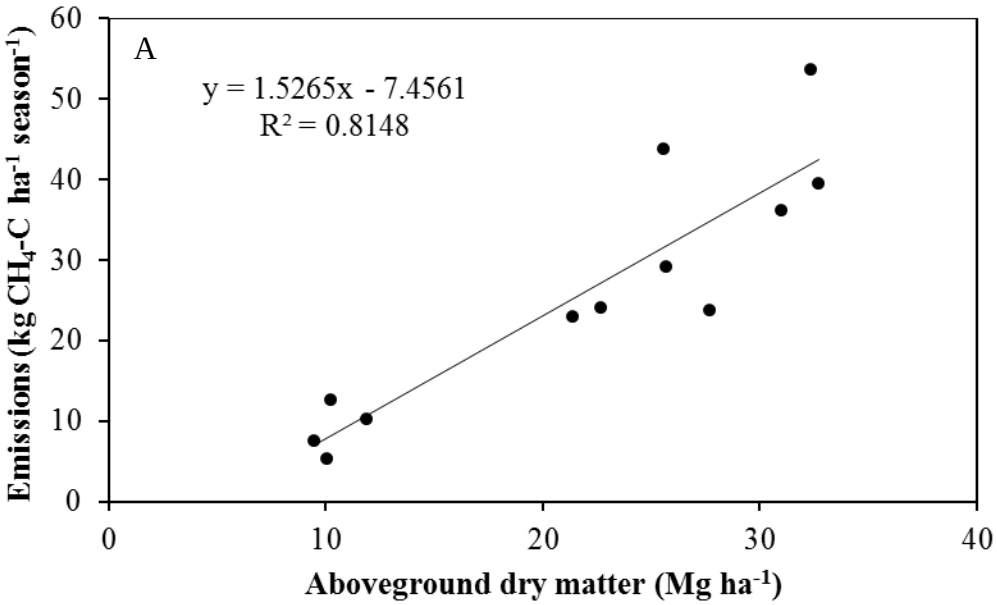


Figure 4. Methane fluxes over time following flood release at the end of the 2012 growing season from the unvegetated, low-, and high-vegetation treatments at the Northeast Research and Extension Center in Keiser, Arkansas. Error bars indicate standard errors for the treatment means (n = 4).



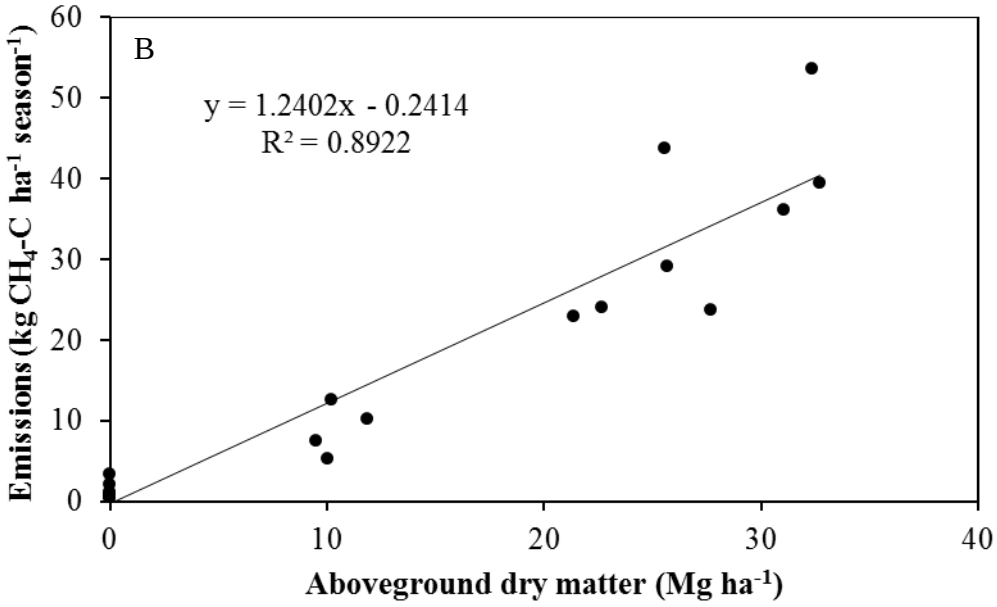


Figure 5. Linear relationship between aboveground dry matter and season-long CH_4 emissions measured during 2012 within chambers of low-, and high-vegetation treatments (A) and all treatments (B) at the Northeast Research and Extension Center in Keiser, Arkansas.

CHAPTER THREE

PREVIOUS CROP AND CULTIVAR EFFECTS ON METHANE EMISSIONS FROM DRILL-SEEDED, DELAYED-FLOOD RICE GROWN ON A CLAY SOIL

Abstract

Methane (CH_4) is one of the major greenhouse gases and has a global warming potential 25 times greater than carbon dioxide (CO_2). Methane production occurs as a specific group of *Archaea*, methanogens, decompose organic matter under anoxic conditions. Due to the anaerobic conditions that develop in soils used for flooded rice (*Oryza sativa* L.), along with the large global extent of rice production, it is estimated that rice cultivation is responsible for 11% of global anthropogenic CH_4 emissions. The current U.S. estimates of CH_4 emissions from rice are based on data from all of the major rice-growing regions; however, there is a general lack of data representing Arkansas' cultural practices. In order to adequately estimate and mitigate CH_4 emissions from rice cultivation, it is important to have data representing the range of regional, climatic, and cultural variability throughout the nation. The objective of this study was to determine the effect of previous crop and cultivar on CH_4 fluxes and seasonal emissions from rice grown on a clay soil in the direct-seeded, delayed-flood production system in eastern Arkansas. This study was conducted during the 2013 growing season at the Northeast Research and Extension Center (NEREC) in Keiser, Arkansas on a Sharkey clay (very-fine, smectitic, thermic, Chromic Epiaquerts). A split-plot design was used where the whole-plot factor was previous crop [rice or soybean (*Glycine max* L.)] and the split-plot factor was cultivar [CLXL745 (hybrid), Taggart (pure-line standard-stature), and Cheniere (pure-line semi-dwarf)]. Gas samples were collected at 20-minute intervals from 30-cm diameter, enclosed headspace chambers and fluxes were calculated from the change in headspace CH_4 concentration over time. Averaged across cultivar, rice following rice as a previous crop had greater ($P < 0.01$) fluxes than rice following soybean on seven of 10 measurement dates resulting in growing-season emissions differences ($P < 0.01$) of 19.6 and 7.0 kg CH_4-C ha^{-1}, respectively. Averaged across

previous crop, CH_4 fluxes from Cheniere and Taggart only differed on one measurement date, while fluxes from CLXL745 were generally lower than Cheniere prior to heading and lower than Taggart following heading ($P < 0.01$). As a result, season-long emissions from CLXL745 (10.2 kg CH_4-C ha^{-1}) were less ($P = 0.03$) than emissions from Cheniere or Taggart (15.5 and 14.2 kg CH_4-C ha^{-1}, respectively) which did not differ. Results of this study indicate that CH_4 emissions from mid-southern rice grown on a clay soil may be substantially overestimated by the emission factor used by the United States Environmental Protection Agency (USEPA; 178 kg CH_4-C ha^{-1}). Furthermore, Arkansas practices, such as growing rice in rotation with soybean (> 70% of production) and planting hybrid cultivars (> 40% of production), may further reduce CH_4 emissions from Arkansas rice production. Data from studies such as this will enable the USEPA to further refine CH_4 emission factors to account for additional variables, such as soil texture, previous crop, and cultivar, but it is important to continue research on emissions from mid-southern U.S. rice in order to more accurately assess the problem and to mitigate potential negative environmental aspects of rice cultivation.

Introduction

Agricultural practices around the globe are estimated to account for nearly half of anthropogenic methane (CH_4) emissions, and rice (*Oryza sativa* L.) cultivation is one of the leading agricultural sources of CH_4, accounting for 22% of global anthropogenic agricultural emissions, second only to enteric fermentation (Smith et al., 2007; USEPA, 2006). Rice is the only major row crop grown under flooded soil conditions and the anoxic environment leads to the production and emission of CH_4, a greenhouse gas with a global warming potential 25 times stronger than carbon dioxide (CO_2) (Forster et al., 2007). The global warming potential (GWP) of rice cultivation has been estimated to be 2.7 and 5.7 times greater than that of maize (*Zea mays* L) and wheat (*Triticum aestivum* L.) systems, respectively, with CH_4 contributing more than 90% of the GWP of rice systems (Linquist et al., 2011, 2012). Methane production occurs in anaerobic soils as a specific group of *Archae*, collectively known as methanogens, utilize acetate or hydrogen gas (H_2) and CO_2, which are formed as fermentation products of a greater consortium of anaerobic bacteria, as substrates for methanogenesis (Nazaries et al., 2013). A portion of the CH_4 produced during methanogenesis, however, is oxidized by a group of aerobic bacteria, known as methanotrophs, as CH_4 moves through oxidized portions of soil surrounding rice roots (Armstrong, 1971; Conrad and Rothfuss, 1991) and near the soil surface (Rothfuss and Conrad, 1998; Schutz et al., 1989b). Studies have shown that up to 90% of CH_4 produced in the soil of rice systems is oxidized prior to entering the atmosphere, greatly reducing the proportion of produced CH_4 that is released from the soil (Butterbach-Bahl et al., 1997; Holzapfel-Pschorn et al., 1985, 1986; Sass et al., 1990, 1992; Schutz et al., 1989b).

Studies have indicated that the majority of CH_4 released from rice fields occurs through the aerenchyma tissues of rice plants, with the plant-mediated transport accounting for about

90% of emissions, compared to around 8 and 2% of emissions from ebullition and diffusion through the floodwater, respectively (Butterbach-Bahl et al., 1997; Holzapfel-Pscorn et al., 1986; Nouchi et al., 1990; Schutz et al., 1989b). Furthermore, several studies have identified a positive correlation between CH_4 emissions and both aboveground and belowground dry matter accumulation (Huang et al., 1997; Sass et al., 1991a; Shang et al., 2011; Whiting and Chanton, 1993), which may result from an increase in available substrate as root exudates have been correlated to biomass (Aulakh et al., 2001), or due to differences in CH_4 transport capacity (MTC) between cultivars. Butterbach-Bahl et al. (1997), for example, attributed a 24 to 31% difference in emissions between two pure-line cultivars to differences in MTC, as no differences were observed between CH_4 production or oxidation. Several studies have observed increased emissions from standard-stature relative to semi-dwarf cultivars, which is consistent with the positive effect of biomass on CH_4 emissions (Lindau et al., 1995; Sass and Fisher, 1997; Sigren et al., 1997). Cultivar differences extend beyond the impact of biomass on emissions, however, as Ma et al. (2010) observed a reduction in emissions and soil CH_4 concentration accompanied by a 67% increase in CH_4 oxidation from a hybrid cultivar relative to pure-line *Indica* and *Japonica* cultivars.

While Rogers et al. (2014) is the only known study to have directly compared emissions from rice following rice or soybean previous crops, and who observed a 31% reduction in emissions following soybean compared to following rice, several other studies have reported reductions in emissions when previous crop residue was burned (Bossio et al., 1999; Cicerone et al., 1992; Fitzgerald et al., 2000) or when growing rice following a tillage-suppressed fallow period (Sass et al., 1991a, 1994). Furthermore, it has been suggested that promoting aerobic decomposition of residues or growing rice in rotation with upland crops may provide a means of

CH_4 mitigation as composted residues reportedly resulted in a six-fold decrease in available substrate for methanogenesis relative to rice straw or green manure (Denier van der Gon and Neue, 1995; Yagi et al., 1997). Although it has not been studied greatly, the impact of residue management and rotation with upland crops, such as soybean, has shown potential for mitigation of CH_4 emissions.

While temperature may not be a major determinant of seasonal emissions patterns (Sass et al., 1991b; USEPA, 2014), evidence has consistently indicated a positive link between CH_4 fluxes and soil temperature (Holzapfel-Pschorn and Seiler, 1986; Hosono and Nouchi, 1997; Schutz et al., 1989a; Wang et al., 1997; Yagi and Minami, 1990), indicating the potential for differences in CH_4 emissions resulting from differences in temperature between locations or years. It has been observed that temperature has a stronger impact on methanogenesis than it does on methanotrophy, meaning that CH_4 production increases more, relative to CH_4 oxidation, as temperature increases, resulting in smaller proportions of CH_4 being oxidized as soil temperature increases (Nazaries et al., 2013; Van Winden et al., 2012). Tsutsuki and Ponnamperuma (1987) observed that it took three times longer to reach a redox potential (Eh) of -200 mV at 20 ˚C than it did at 35 ˚C (i.e., one week compared to three weeks of flooding), indicating a greater potential for methanogenesis in warmer conditions. Although it has not been reported often, Watanabe and Kimura (1999) attributed a 1.6-fold increase in CH_4 emissions from one year to another to a difference in average air temperatures, which were 24.6 ˚C in the year of low fluxes and 26.9 ˚C in the year of greater fluxes.

Currently, CH_4 emissions budgets are created by summing contributions from identifiable homogeneous areas, such that average measured fluxes of representative factors are used in estimates on a regional or national basis (IPCC, 2006; Sass et al., 1999). With limited data, the

USEPA is currently using one emission factor (178 kg CH_4-C ha^{-1} $season^{-1}$) for all non-California primary rice crops, while separate factors are used when ratooning or for winter-flooded and non-winter flooded rice in California (USEPA, 2014). As more data become available, CH_4 budgets and models can be further refined to account for factors such as soil texture, previous crop, and cultivar. This is particularly important for Arkansas, which accounts for nearly 50% of national rice production and contains a large portion of production following soybean (71%) and planted with hybrid cultivars (> 40%), both of which have been shown to reduce emissions and could be used to create more accurate, lower CH_4 emission factors for mid-southern rice production (Hardke, 2014b).

The impact of rice cultivation on greenhouse gas emissions coupled with the intense management involved in rice production allows for potential mitigation strategies based on various practices that are known to reduce CH_4 emissions, such as increasing the use of high-yielding cultivars that have shown potential for reduced emissions. It is necessary to study the impacts of various practices on CH_4 emissions in a wide array of soils and climates in order to adequately understand the extent of the problem and to direct management practices toward mitigation of the greenhouse gas, while maintaining high yields and profitability.

While research on CH_4 emissions from rice has recently been conducted in Arkansas (Adviento-Borbe et al., 2014; Brye et al., 2013; Rogers et al., 2012, 2013, 2014; Simmonds et al., 2015), no study has examined the influence of previous crop and cultivar selection on direct-seeded, delayed-flood rice production on a clay soil in Arkansas. Direct measurements of CH_4 fluxes and emissions from field studies are necessary to further refine the USEPA emission factors, which currently use a single factor for all non-California primary rice crops. Therefore, the objective of this study was to assess the impact of previous crop (i.e., rice and soybean) and

cultivar (i.e., standard-stature, semi-dwarf, and hybrid) on CH_4 fluxes and season-long emissions from drill-seeded, delayed-flood rice produced on a clay soil in eastern Arkansas.

It was hypothesized that CH_4 fluxes and emissions would be greater when following rice as a previous crop due to the more recalcitrant nature of the rice straw residue, compared to the more labile soybean residue. More C is likely available from rice residue than soybean residue at the beginning of the growing season due to greater breakdown of soybean residue in the fall and winter. The only study to directly compare rice and soybean as previous crops observed a 31% reduction in CH_4 emissions when following soybean on a silt-loam soil (Rogers et al., 2014). It was also hypothesized that the hybrid cultivar would result in lower CH_4 fluxes and emissions than the two pure-line cultivars due to increased methanotrophic activity and CH_4 oxidation observed in hybrid cultivars, especially after heading (Ma et al., 2010). Furthermore, it was hypothesized that CH_4 fluxes and emissions would be less from the semi-dwarf cultivar than from the standard-stature cultivar as was observed by Lindau et al. (1995). Lower aboveground dry matter accumulation in the semi-dwarf cultivar is likely to correspond to reduced emissions, as many studies have observed a positive correlation between biomass accumulation and CH_4 emissions (Cicerone and Shetter, 1981; Huang et al., 1997; Sass et al., 1991a; Shang et al., 2011).

Materials and Methods

Site Description

This study was conducted during the 2013 growing season at the University of Arkansas Northeast Research and Extension center in Keiser, Arkansas (35°40’ N 90°05’ W). Research plots were located on a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts), which

makes up 31% of the Mississippi County soil survey area (Soil Survey Staff, NRCS-USDA, 2012). The study site is located within the Southern Mississippi River Alluvium Major Land Resource Area (MLRA 131A), which is located along the Mississippi River from the southern tip of Illinois to the Gulf Coast and is composed of approximately 70% cropland. The location of the study has been cropped in a rice-soybean rotation for greater than 15 years and crop residues are typically incorporated in the fall by disking to a depth of 15 cm. Mean annual precipitation at this site is 126 cm, ranging from an average of 6.8 cm in August to an average of 14.1 cm in May (NOAA, 2002). The mean annual air temperature is 15.5°C, while the mean minimum and maximum temperatures occur in January (-2.4°C) and July (33.3°C), respectively (NOAA, 2002).

Treatments and Experimental Design

The purpose of this study was to examine the impacts of previous crop (rice or soybean) and rice cultivar (standard-stature, semi-dwarf, or hybrid) on CH_4 fluxes and season-long emissions from rice grown on a clay soil in Arkansas. Cultivars were selected in an attempt to represent rice commonly produced in Arkansas with various growth characteristics and breeding lines. The cultivar 'Cheniere', developed at Louisiana State University (Linscombe et al., 2006), was selected as a pure-line, semi-dwarf cultivar. Cheniere is an early season, long-grain rice cultivar with an average height of 97 cm and average grain yield of 8.9 Mg ha^{-1} based on Arkansas performance trials (Hardke et al., 2013). The standard-stature, pure-line cultivar 'Taggart', developed at the University of Arkansas (Moldenhauer et al., 2008), was also selected due to its high yield potential. Taggart is a mid-season, long-grain cultivar with an average grain yield of 10.0 Mg ha^{-1} and an average height of 117 cm (Hardke et al., 2013). The final cultivar

selected for use in this study was the hybrid 'CLXL745' (RiceTec, Inc.), which is a very early season, long-grain cultivar averaging 114 cm in height and achieving an average yield of 10.1 Mg ha^{-1} in Arkansas (Hardke et al., 2013). The hybrid cultivar CLXL745 was the most popular cultivar in Arkansas in 2012 and 2013, accounting for 28 and 22% of total production, respectively, in those years (Hardke, 2014b).

Research plots were 1.6 m wide by 5 m long and arranged in a split-plot design. Previous crop was the whole-plot factor, which was arranged as a randomized complete block with four replicates of each previous crop. The split-plot factor was rice cultivar and each of the three cultivars were randomly located within each of the previous-crop, whole-plot units. Due to the repeated nature of sampling, sampling date was treated as a repeated measure in analyzing CH_4 flux data.

Plot Management

Previous crop residues, which were left standing in the field following harvest, were incorporated one week prior to planting by disking to a depth of 15 cm. Research plots were independently seeded in late May (Table 1) with nine rows of rice drill-seeded using 18-cm row spacing. The two pure-line cultivars, Cheniere and Taggart, and the hybrid, CLXL745, were seeded at rates of 112 kg ha^{-1} and 34 kg ha^{-1}, respectively (Hardke, 2014a). Levees were constructed following seeding and plots were irrigated with groundwater by flushing as necessary prior to permanent flood establishment, which occurred at the V4 to V6 growth stage (Moldenhauer et al., 2013). Based on University of Arkansas Cooperative Extension Service (UACES) guidelines, nitrogen (N) was applied as urea (46% N) in a split application with the pure-line cultivars and hybrid cultivar receiving 151 kg N ha^{-1} and 168 kg N ha^{-1}, respectively,

one day prior to permanent flood establishment. The second application of N occurred at the beginning of internode elongation (R1) for the pure-line cultivars (50 kg N ha^{-1}) and at booting (R2) for the hybrid cultivar (33 kg N ha^{-1}), amounting to a total of 201 kg N ha^{-1} for all cultivars (Norman et al., 2013). A floodwater depth of 5 to 10 cm was maintained until grain maturity, after which the floodwater was released and plots were allowed to dry prior to harvest. Plots were scouted regularly and managed to remain insect- and weed-free during the growing season according to UACES guidelines (Lorenz and Hardke, 2013; Scott et al., 2013).

Soil Sampling and Analyses

Composite soil samples from six, 2-cm diameter soil cores were collected prior to flooding and N fertilization from the top 10 cm of each plot. Composite samples were then oven-dried at 70 ˚C for 48 hours and passed through a 2-mm mesh screen sieve prior to subsamples being analyzed for Mehlich-3 extractable nutrients (i.e., P, K, Ca, Mg, Fe, Mn, Na, S, Cu, and Zn) using spectrophotometry (Spectro Analytical Instruments, Spectro Arcos ICP, Kleve, Germany; Tucker, 1992). Additional dried, sieved subsamples were analyzed for total N (TN) and total C (TC) concentrations by high-temperature combustion using a VarioMax CN analyzer (Elementar Americas Inc., Mt. Laurel, NJ; Nelson and Sommers, 1996) and analyzed for soil pH and electrical conductivity (EC) potentiometrically in a 1:2 (m:v) soil-to-solution paste. Soil organic matter (OM) concentration was determined by weight-loss-on-ignition.

Additional soil samples were collected from the top 10 cm in each plot prior to flooding using a slide hammer and 4.7-cm diameter core chamber with a beveled core tip. Bulk densities were determined after samples were oven dried for 48 hours at 70 °C. Samples were then ground and sieved through a 2-mm mesh screen and analyzed for particle-size distribution using a

modified 12-hour hydrometer method (Gee and Or, 2002). Bulk densities measured from each plot were then used in combination with measured TN, TC, and OM concentrations to determine total contents (Mg ha^{-1}) of each in the top 10 cm of soil.

Trace Gas Sampling and Analysis

Non-steady-state, enclosed headspace gas sampling chambers, similar to those used by Rogers et al. (2012, 2013) and detailed by Livingston and Hutchinson (1995), were used for collection of gas samples for CH_4 in this study. This methodology is common in measuring trace gas fluxes (Parkin and Venterea, 2010) and involves installing permanent base collars into the soil and using various sized chamber extensions along with a vented cap in order to accommodate increasing plant growth throughout the season. Base collars, chamber extensions, and chamber caps were constructed using schedule 40 polyvinyl chloride (PVC) pipe with an inside diameter of 30 cm. Chamber base collars were cut to a length of 30 cm with one beveled edge for driving into the soil and four 12.5 mm holes placed 12 cm from the bottom to allow free movement of the floodwater. Chamber extensions cut to lengths of 40 and 60 cm, in order to accommodate growing plants while minimizing chamber volume, and 10-cm caps were covered with reflective aluminum tape (CS Hyde, Mylar metalized tape, Lake Villa, IL) in order to reduce temperature elevation during sampling. Cross-sections of tire inner tubes cut to a width of 10 cm were adhered to the bottom of chamber extensions and caps in order to seal the separate pieces together. Chamber caps also included a 15-cm section of 4.5-mm ID copper tubing as a vent to maintain atmospheric pressure, sampling and thermometer ports with gray butyl-rubber septa (Voigt Global, part # 73828A-RB, Lawrence, KS) inserted into 12.5-mm holes, and a 2.5-

cm diameter, battery operated (9V) fan (Sunon Inc., MagLev, Brea, CA) to slowly circulate and mix air within the chamber during sampling.

Boardwalks were established between plots prior to flooding in order to access chambers for sampling, while minimizing damage to plants and soil disturbance during sampling. Permanent base collars were installed within each plot to a depth of 11 cm, where the four holes were just above the soil surface, and were situated to contain 40 cm of row length in order to match the plant density of the plots. Plants were carefully bundled with plant tie wire in order to deploy chamber extensions during each sampling event without damaging plants, but ties were removed immediately after extension placement as to not affect the plants during sampling. Headspace gas samples were collected weekly for the duration of flooding [i.e., 7, 14, 28, 36, 42, 49, 56, 63, 71, and 77 days after flooding (DAF)], with the exception of during the third week after flooding when poor weather conditions did not permit sampling, and every other day following flood release [i.e., 1, 3, 5, and 7 days after flood release (DAFR)].

Chamber headspace gas sampling occurred between 0800 and 1000 h, similar to previous studies (Adviento-Borbe et al., 2014; Rogers et al., 2012, 2013; Shang et al., 2011), in order to reduce excessive chamber heating during sampling, while sampling during a time of near-average soil temperatures. Samples were collected at 20-minute intervals (i.e., 0, 20, 40, and 60 minutes after sealing) using 20-mL B-D syringes (Beckton Dickson and Co., Franklin Lakes, NJ) and immediately transferred to previously evacuated 10-mL, crimp-top glass vials (Agilent Technologies, part # 5182-0838, Santa Clara, CA). Chamber air temperature, 10-cm soil temperature, barometric pressure, and relative humidity were recorded throughout each sampling event and chamber volumes were calculated by measuring each chamber's height above the floodwater. Duplicate sets of CH_4 standards (i.e., 1, 2, 5, 10, 20, and 50 μL L^{-1}) were collected in

the field into evacuated glass vials and an additional set of laboratory standards was again collected immediately prior to sample analysis in order to ensure that sample integrity was maintained as samples were transported from the field to the laboratory.

Field headspace samples, field standards, and laboratory standards were analyzed within 48 hours after each sampling event using an Agilent 6890-N gas chromatograph with a 30-m-long by 0.53-mm-diameter HP-Plot-Q capillary column (Agilent Technologies, Santa Clara, CA) and equipped with a flame-ionization detector (FID). Methane concentrations of field samples were determined based on calibration curves for each sampling event created from peak-area responses from known sample concentrations. Methane fluxes (μL CH_4 m^{-2} min^{-1}) were calculated for each chamber by using changes in headspace CH_4 concentration (μL L^{-1}; y axis) regressed against time (min; x axis) and multiplying the resulting best-fit line from that regression by chamber volume (L) and dividing by chamber surface area (m^2) as outlined by Parkin and Venterea (2010). Fluxes were then converted to mass-based units (i.e., mg CH_4 m^{-2} min^{-1}) using the Ideal Gas Law. Season-long total CH_4 emissions were determined for each chamber by linear interpolation between flux measurement dates.

Soil Redox Potential and Soil Temperature Monitoring

Soil oxidation/reduction (redox) potential (Eh) was monitored throughout the flooded portion of the growing season using redox potential sensors (Sensorex, Model S650KD-ORP, GardenGrove, CA) with Ag/AgCl reference solution and a built-in reference electrode that were installed to a soil depth of 7.5 cm immediately prior to flooding. Additionally, chromel-constantan thermocouples were installed immediately prior to flooding to a soil depth of 7.5 cm in order to monitor soil temperature. Due to equipment limitations, soil Eh and temperature

readings were only conducted in two of the four replicates of Cheniere and CLXL745 following each of the previous crops. Soil redox potential and temperature measurements were recorded at 4-hour intervals using a datalogger (CR 1000, Campbell Scientific Inc., Logan, UT) contained within an environmental enclosure. Soil redox potential measurements were corrected to the standard hydrogen electrode (SHE) by adding 199 mV to the raw measurements in order to facilitate comparison to previous studies.

Plant Sampling and Analyses

Plant samples were collected at physiological maturity in order to determine any impact of previous crop and cultivar on aboveground dry matter accumulation as well as to compare aboveground dry matter from within and outside the chambers to investigate the impact of the chamber on plant growth. All biomass from within each chamber and a 1-m row of rice from adjacent to each chamber was cut at the soil surface, 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 (October 24) using a plot-scale combine. Grain samples were then weighed and analyzed for moisture content so that final grain yields could be adjusted to and reported at 12% moisture content.

Statistical Analyses

Initial soil chemical and physical properties from the top 10 cm prior to N application and flooding were analyzed by analysis of variance (ANOVA) in SAS 9.2 (SAS Institute, Inc., Cary, NC) using PROC Mixed based on a split-plot design (i.e., the whole-plot factor was previous crop and the split-plot factor was cultivar) in order to determine any differences in soil properties

among treatment combinations. Similarly, grain yield was analyzed by ANOVA based on a split-plot factor (i.e., the whole-plot factor was previous crop and the split-plot factor was cultivar) in order to determine the impact of previous crop and cultivar on grain yields. An additional ANOVA was performed based on a split-split-plot design (i.e., the whole-plot factor was previous crop, the first split-plot factor was cultivar, and the second split-plot factor was sampling location) in order to compare total aboveground dry matter accumulation as affected by sampling location (i.e., in-chamber or in-plot), previous crop, and cultivar.

Methane flux data showed no indication of a non-normal distribution based on an inspection for normality using normal probability plots of the studentized residuals. Consequently, an ANOVA was performed based on a split-plot, repeated measures design (i.e., previous crop was the whole-plot factor, cultivar was the split-plot factor, and time was a repeated measure) to evaluate the impact of previous crop, cultivar, and their interaction on CH_4 fluxes over time. Flux data were analyzed separately for the duration of flooding and following flood release due to differences in CH_4 transport mechanisms and sampling intervals. Seasonal total CH_4 emissions, calculated based on 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 split-plot design (i.e., previous crop was the whole-plot factor and cultivar was the split-plot factor). When appropriate, means were separated at the $P < 0.05$ level using the Fisher protected least significant difference (LSD). Linear correlation and regression analyses were performed using Minitab (version 16, Minitab, Inc., State College, PA) in order to evaluate the relationships between soil sand content and growing-season emissions, soil clay content and emissions, and aboveground dry matter and emissions.

Results and Discussion

Initial Soil Physical and Chemical Properties

Several soil physical and chemical properties in the top 10 cm differed based on previous crop ($P < 0.05$), however, soil properties were unaffected by cultivar or the interaction of previous crop and cultivar (Table 2). The most notable differences were in soil particle-size distribution, where sand content was 4% greater and clay content was 4.7% less in the treatments where rice was the previous crop compared to soybean as a previous crop. The difference in particle-size distribution was due to natural spatial variability within the study site as the two groups of previous-crop treatments were separated by approximately 15 m. While these differences likely had no agronomic significance, several studies have observed an inverse correlation between soil clay content and CH_4 emissions as well as a positive correlation between soil sand content and CH_4 emissions (Sass et al., 1994; Wang et al., 1993; Watanabe and Kimura, 1999). The effect of particle-size distribution on CH_4 emissions could potentially result in slightly greater emissions from cultivars following rice as a previous crop in this study, due to differences in sand and clay content based on the spatial orientation of the previous-crop treatments.

In addition to having a slightly greater clay content, the treatment combinations following soybean as a previous crop had greater extractable calcium (Ca), magnesium (Mg), and zinc (Zn), as well as a greater OM concentration (Table 2). The differences in Ca, Mg, and Zn concentrations, however, were minor in comparison to the relatively high concentrations of these nutrients in both previous crop treatments, and likely posed no practical significance in this study. There were no differences in OM, TN, or TC contents among treatments when

concentrations were transformed to contents using measured bulk densities (Table 2), meaning available substrate for methanogenesis was similar among all treatment combinations prior to flooding. Extractable soil phosphorus (P) was greater ($P < 0.05$) following rice as a previous crop and was within the above-optimum level (≥ 51 mg kg^{-1}), while the P concentration following soybean was within the optimum level (36 to 50 mg kg^{-1}), indicating adequate native soil P in both previous crop treatments based on University of Arkansas recommendations (Norman et al., 2013). Extractable soil potassium (K) was unaffected by previous crop and was within the above-optimum level (≥ 174 mg kg^{-1}). Extractable soil zinc (Zn) was also unaffected by previous crop and was within the medium level (2.6 to 4.0 mg kg^{-1}) recommended for rice, indicating adequate levels of both K and Zn for rice production (Norman et al., 2013).

Methane Fluxes from Flooding to Flood Release

Methane fluxes measured during the flooded portion of the 2013 growing season differed between previous crops over time ($P < 0.001$) and differed among cultivars over time ($P < 0.001$) (Table 3). Averaged across cultivar, CH_4 fluxes did not differ among previous crops on the first two or the final sampling dates (i.e., 7, 14, and 77 DAF), while fluxes were greater when the previous crop was rice for the remainder of the sampling dates (Figure 1). Both previous-crop treatments exhibited the same trend, where fluxes generally increased from less than 0.02 mg CH_4-C m^{-2} h^{-1} at 7 DAF to peaks of 2.15 and 0.81 mg CH_4-C m^{-2} h^{-1} that occurred at 56 DAF in the rice following rice and rice following soybean treatments, respectively. After peak CH_4 fluxes occurred near the time of 50% heading, fluxes decreased over time to 0.46 and 0.23 mg CH_4-C m^{-2} h^{-1} following rice and soybean as previous crops, respectively, at 77 DAF, the final sampling date of the flooded portion of the season.

The only other known study to have directly compared CH_4 fluxes from rice and soybean as previous crops, Rogers et al. (2014), observed similar results on a silt-loam soil where fluxes were consistently reduced when soybean was the previous crop and average peak fluxes of approximately 16.8 and 12.8 mg CH_4-C m^{-2} h^{-1} following rice and soybean, respectively, occurred near 50% heading. Other studies examining the impact of residue management on CH_4 fluxes have observed reduced fluxes when previous rice crop residue was burned as opposed to soil-incorporated (Bossio et al., 1999; Cicerone et al., 1992; Fitzgerald et al., 2000) or when following fallow, suppressed by occasional disking, rather than rice as a previous crop (Sass et al., 1991a, 1994). While various studies have observed an early season CH_4 peak attributed to rice straw decomposition (Chidthaisong and Watanabe, 1997; Holzapfel-Pschorn et al., 1986; Sass et al., 1991b; Wassmann et al., 2000), an early season peak did not occur in this study, which was more consistent with studies that have observed greater fluxes from rice straw additions throughout the entire growing season without influencing the timing of flux trends (Nouchi et al., 1994; Sass et al., 1991a).

Averaged across previous crop, CH_4 fluxes did not differ among cultivars on the first two sample dates (i.e., 7 and 14 DAF), where all fluxes were less than 0.15 mg CH_4-C m^{-2} h^{-1}, or on the final sample date, where all fluxes were less than 0.44 mg CH_4-C m^{-2} h^{-1} (i.e., 77 DAF) (Figure 2). All three cultivars exhibited a general increase in CH_4 fluxes up to a peak of approximately 1.5 mg CH_4-C m^{-2} h^{-1}, which occurred near 50% heading in all cultivars (i.e., 56 DAF for Cheniere and CLXL745 and 63 DAF for Taggart) and did not differ, followed by a decrease in fluxes up to the time of flood release. Methane fluxes from Cheniere and Taggart only differed at 42 DAF, where fluxes were greater from Cheniere, while the hybrid cultivar,

CLXL745, had significantly lower fluxes than Cheniere on four of six sampling dates prior to heading and lower fluxes than Cheniere and Taggart on two of three dates following heading.

Reduced CH_4 fluxes from CLXL745 relative to Cheniere and Taggart were similarly observed by Rogers et al. (2014) especially toward the end of the season where fluxes from CLXL745 decreased much more rapidly than those from the pure-line cultivars. Additional studies have also observed reduced fluxes from hybrid relative to pure-line cultivars (Ma et al., 2010; Simmonds et al., 2015; Smartt et al., 2015). Ma et al. (2010) measured lower dissolved CH_4 concentrations and increased methanotrophic bacteria in the rhizosphere of hybrid rice accompanied by a 67% increase in CH_4 oxidation potential relative to pure-line cultivars, while all cultivars had similar CH_4 production potentials. This indicates that reduced CH_4 fluxes from hybrid cultivars may be due to differences in microbial community structure, where greater methanotrophic activity reduces CH_4 transport to the atmosphere by oxidizing a greater proportion of the produced CH_4. Similar to this study, Rogers et al. (2014) observed only minor differences in CH_4 fluxes between Cheniere and Taggart, while previous studies have reported reduced fluxes from semi-dwarf relative to standard-stature cultivars (Lindau et al., 1995; Sass and Fisher, 1997; Sigren et al., 1997). The similarity in CH_4 fluxes from a semi-dwarf and standard-stature cultivar observed in this study and by Rogers et al. (2014) may be inconsistent with other studies due to cultivar differences, which needs to be further studied, as all of the other previous studies used different cultivars.

The general trend of CH_4 fluxes observed in all treatments, where fluxes begin increasing within a couple of weeks after flooding to a peak flux occurring near the time of 50% heading and decrease during grain fill, has been observed in several studies (Brye et al., 2013; Nouchi et al., 1994; Rogers et al., 2014; Sass et al., 1990, 1991a, 1991b, 1992; Smartt et al., 2013). This

pattern in seasonal CH_4 fluxes has generally been attributed to a strong link between plant growth and CH_4 emissions, with the suggestion that root exudates, which act as a substrate for CH_4 production, increase over time until the plant begins translocating photosynthates to developing grains during grain fill, which consequently reduces root exudation and CH_4 production toward the end of the growing season. Research conducted in Arkansas and Texas has consistently observed a similar pattern of root growth, where rapid linear growth occurs during vegetative development, with maximum root length occurring near heading and declining again until after grain fill (Beyrouty et al., 1988, 1993; Sass et al., 1990; Slaton et al., 1990). Beyrouty et al. (1993) reported this general trend to be consistent among cultivars that differ in maturity, however, it was observed that tall plants produced less total root length during vegetative growth and more during ripening relative to shorter cultivars, which is consistent with this study where CH_4 fluxes were numerically lower from Taggart than Cheniere prior to heading and numerically greater following heading. Additional studies reported similar seasonal trends in root exudation rates among 10 cultivars (Aulakh et al., 2001) and in anaerobic root respiration rates (Tolley et al., 1986). Research conducted in Japan verified that the MTC of rice increased with age through vegetative growth then gradually decreased following heading and, by cutting and removing portions of roots, observed that MTC was strongly related to root contact with CH_4 in soil solution (Hosono and Nouchi, 1997; Nouchi et al., 1994). The seasonal trends in CH_4 fluxes observed in this study indicate that rice plants themselves greatly impact fluxes, resulting in the same general trend that is modified within a site-year combination by the residue carried over from the previous crop, which may modify the availability and concentration of CH_4 in the soil. Furthermore, seasonal flux trends are affected by cultivar selection, which may result in differences in root growth and exudation, aerenchyma development and MTC.

While the relative treatment differences and flux trends measured in this study were consistent with previous studies, the magnitude of peak fluxes was on the low end of peaks measured from similar studies conducted on clay and clay-loam soils, which ranged from 2.1 to 25 mg CH_4-C m^{-2} h^{-1} (Bossio et al., 1999; Cicerone et al., 1992; Fitzgerald et al., 2000; Sass et al., 1991a, 1991b). On a silt-loam soil in Arkansas under similar management and methodology to this study, Rogers et al. (2014) observed peak fluxes at heading ranging from 8.3 mg CH_4-C m^{-2} h^{-1} for CLXL745 following soybean to 18.7 mg CH_4-C m^{-2} h^{-1} for CLXL745 following rice. The low magnitude of fluxes observed in this study is likely largely due to the high clay content, as several studies have indicated greater fluxes from coarse- than from fine-textured soils (Brye et al., 2013; Sass et al., 1994; Sass and Fisher, 1997). The low fluxes measured in this study may have also been partially due to soil Eh trends.

Soil Eh did not differ substantially between rice or soybean previous crops (data not shown), however, when averaged across previous crop, soil Eh was consistently lower from Cheniere than CLXL745 until approximately 60 DAF, after which soil Eh did not appear to differ much among cultivars and stabilized near -200 mV (Figure 3). Lower soil Eh values prior to heading were indicative of more reduced conditions and greater potential for methanogenesis from Cheniere relative to CLXL745, which is consistent with CH_4 flux observations. The reason for the difference in soil Eh is not well-understood, but may be related to a difference in root development and exudation as a degradable C supply for redox reactions, or a difference in aerenchyma development or root structure that may result in greater oxygenation in the rhizosphere of CLXL745. Similar soil Eh values at heading were consistent with observed fluxes, however, the greater reduction in fluxes from CLXL745 following heading was not reflected in soil Eh measurements, but may have been a result of greater methanotrophic activity

in the rhizosphere of the hybrid as observed by Ma et al. (2010). The general stabilization of soil Eh following heading may be a result of the decrease in root exudation observed in previous studies, which would limit the supply of degradable C and suppresses a further drop in soil Eh as well as cause a reduction in methanogenesis.

In addition to soil Eh, low fluxes measured in this study may have also been partially the result of soil temperature variations. Average daily soil temperatures at the 7.5-cm depth ranged from 22 to 28.6 ˚C for the first 10 weeks of flooding, before dropping as low as 18.7 ˚C in the last few weeks of the growing season, and over the flooded portion of the growing season averaged 24.5 and 23.9 ˚C following rice and soybean, respectively (Figure 4). While the seasonal trend in soil temperature did not appear to drive CH_4 flux trends, the unexpected decrease in fluxes at 49 DAF may largely be due to uncharacteristically low temperatures in the week prior to 49 DAF. This period of low soil temperatures reduced fluxes at 49 DAF and likely caused a reduction in peak fluxes, which otherwise would have likely followed the pre-heading trend of continually increasing fluxes, as well as a reduction in fluxes following heading. Beyrouty et al. (1996) observed an influence of soil temperature on root growth, suggesting that the low temperatures prior to 49 DAF may have slowed root growth and enhanced the effect of low temperatures reducing fluxes as observed in other studies (Hosono and Nouchi, 1997; Wang et al., 1997). Average daily soil temperatures were consistently lower following soybean than rice, especially early in the season, as a result of faster canopy development when soybean was the previous crop, which provided greater shading to the soil. The difference in canopy between rice following previous crops lessened over the growing season and had little impact by maturation. Greater soil temperatures following rice may partially explain greater fluxes following rice, however, the differences in temperature were small compared to the difference in

fluxes, indicating that fluxes are more strongly influenced by the quantity and quality of previous crop inputs.

Methane Fluxes Following Flood Release

After the flood was released, CH_4 fluxes differed over time ($P = 0.002$), while previous crop and cultivar had no impact on the magnitude of fluxes (Table 3). Methane fluxes in all treatment combinations were below 0.46 mg CH_4-C m^{-2} h^{-1} on the last sampling date prior to flood release and did not change substantially by 1 DAFR, averaging 0.29 mg CH_4-C m^{-2} h^{-1} (Figure 5). Averaged across all treatment combinations, post-flood-release CH_4 fluxes were greatest at 3 DAFR, averaging 0.65 mg CH_4-C m^{-2} h^{-1}, while mean fluxes on the remaining sampling dates did not differ from each other, but achieved a minimum value of 0.05 mg CH_4-C m^{-2} h^{-1} on the last sampling date (7 DAFR). The post-flood-release CH_4 pulse observed in this study has been recorded by numerous other studies (Adviento-Borbe et al., 2014; Rogers et al., 2013, 2014; Simmonds et al., 2015; Smartt et al., 2013, 2015), generally occurring from 3 to 6 DAFR, and is thought to result from the drying of soil macropores which release entrapped CH_4 (Denier van der Gon et al., 1996; Neue et al., 1997). Similar to results obtained prior to flood release, CH_4 fluxes measured following flood release were lower than those observed in similar studies on silt-loam soils in Arkansas, which reported post-flood-release peak CH_4 fluxes ranging from 4.5 to 15 mg CH_4-C m^{-2} h^{-1} (Rogers et al., 2013, 2014). This difference is likely a result of soil textural differences as it has been demonstrated that clayey soils can result in increased CH_4 oxidation due to greater tortuosity and slower diffusion of gases through clay soils (Sass and Fisher, 1997).

Aboveground Dry Matter and Grain Yield

Sampling location (i.e., in-chamber or in-plot) had no effect ($P = 0.845$) on aboveground dry matter measured at the end of the growing season, indicating that the chambers did not adversely affect plant growth (Table 4). Aboveground dry matter differed, however, between previous crops among cultivars ($P = 0.032$). Averaged across sampling location, aboveground dry matter following rice as the previous crop was greater from CLXL745 than from Cheniere or Taggart, which did not differ and averaged about 20% lower than CLXL745 (Table 5). Following soybean as a previous crop, aboveground dry matter was also about 20% lower from Cheniere than from CLXL745 and Taggart, which did not differ.

Similar to aboveground dry matter, grain yield differed between previous crops among cultivars ($P = 0.044$; Table 4). Grain yields from CLXL745 and Taggart were greater when following soybean than when following rice, while grain yields from Cheniere did not differ based on previous crop (Table 5). When rice was the previous crop, CLXL745 had a greater grain yield than Cheniere, while Taggart did not differ from either. Similarly, following soybean, grain yields differed in all cultivars, with CLXL745 attaining the greatest yield and Cheniere the lowest. Grain yields achieved in this study, ranging from 9.3 Mg ha^{-1} from Cheniere following rice to 11.0 Mg ha^{-1} from CLXL745 following soybean, were similar to 3-yr means reported for Arkansas Rice Performance trials, which amounted to 8.9 Mg ha^{-1} for Cheniere (2010 to 2012), 9.5 Mg ha^{-1} for CLXL745 (2011 to 2013), and 10.3 Mg ha^{-1} for Taggart (2011 to 2013) (Hardke et al., 2013, 2014).

Seasonal Methane Emissions

As expected, based on CH_4 flux measurements, season-long area-scaled CH_4 emissions differed by both previous crop ($P = 0.003$) and by cultivar ($P = 0.034$) (Table 6). Averaged across cultivar, area-scaled CH_4 emissions were greater following rice as a previous crop (19.6 kg CH_4-C ha^{-1} $season^{-1}$) than following soybean (7.0 kg CH_4-C ha^{-1} $season^{-1}$) (Table 7). Many studies have reported an increase in CH_4 emissions with increasing additions of rice straw, indicating the importance of residue inputs on emissions (Bronson et al., 1997; Ma et al., 2007; Sass et al., 1991b; Schutz et al., 1989a; Yagi and Minami, 1990). Based on the only other known study to have compared emissions from rice and soybean previous crops, Rogers et al. (2014) reported a similar trend, although emissions were only reduced by 31% following soybean compared to following rice, whereas a reduction of 64% was measured in this study. Burning rice straw (Bossio et al., 1999; Cicerone et al., 1992; Fitzgerald et al., 2000) or growing rice following a tillage-suppressed fallow period (Sass et al., 1991a, 1994) are residue management methods that have also indicated a reduction in CH_4 emissions by reducing previous crop residue inputs. Similarly, Lindau et al. (1995) observed a significant positive correlation between rice straw additions from a primary crop and resulting emissions from the following ratoon crop. Furthermore, based on a study examining emissions from residue of varying decomposition states, Yagi et al. (1997) postulated that rotation with upland crops has the potential for mitigation of CH_4 emissions by facilitating aerobic decomposition and stabilization of residue prior to flooding. Tsutsuki and Ponnamperuma (1987) observed a substantial decrease in potential CH_4 production from composted residues compared to equal amounts of rice straw or green manure due to lower amounts of degradable C in the compost. The observations made in this study are consistent with results indicating reduced fluxes resulting from a reduction in residue inputs prior to flooding. Reduced fluxes when following soybean as a previous crop are

likely a result of lower residue inputs as well as increased decomposition of the more labile soybean residue under aerobic conditions.

The reduction in CH_4 emissions from rice following soybean in this study (64%) was greater than the 31% reduction previously reported by Rogers et al. (2014). This difference is likely exaggerated by greater sand content and lower clay content following rice as a previous crop in this study (Table 2), whereas Rogers et al. (2014) observed no difference in soil particle-size distribution among treatment combinations. Numerous studies have identified an inverse correlation between soil clay content and CH_4 emissions, indicating a reduction in emissions as clay content increases (Denier van der Gon et al., 1996; Mitra et al., 2002; Sass et al., 1994; Watanabe and Kimura, 1999). Regression analyses indicated significant ($P < 0.05$) inverse correlations ($r > 0.77$) between area-scaled CH_4 emissions and clay content for all data combined as well as data for the three cultivars separately (Figure 6). Similarly, area-scaled emissions were positively correlated ($P < 0.05$; $r > 0.77$) to soil sand content for all data combined as well as for data for Cheniere and Taggart separately (Figure 7). Emissions data separated by previous crop did not yield significant correlations to soil sand or clay content (data not shown). Although the slope was not significant at the $P < 0.05$ level, emissions measured following soybean in this study were reduced by an average of 29.6% compared to predicted emissions following rice [emissions = 2.3429 (% sand) - 12.201; $R^2 = 0.193$; $P = 0.153$]. While CH_4 emissions differences measured in this study may appear inflated due to differences in soil particle-size distribution between previous crop treatments, data presented here as well as by Rogers et al. (2014) indicate that an approximate 30% reduction in emissions following soybean is likely more accurate than the 64% reduction measured in this study.

Averaged across previous crop, season-long area-scaled CH_4 emissions from CLXL745 (10.2 kg CH_4-C ha^{-1} $season^{-1}$) were reduced by 31% relative to Cheniere and Taggart, which did not differ and averaged 14.9 kg CH_4-C ha^{-1} $season^{-1}$ (Table 7). The reduction in emissions from CLXL745 is consistent with previous studies that have reported a 37% reduction from CLXL745 relative to Cheniere and Taggart (Rogers et al., 2014), a 25% reduction from CLXL745 relative to Francis and Jupiter (Simmonds et al., 2015), and a 30% average reduction from three hybrid cultivars (CLXL729, CLXL745, and XL753), which did not differ, relative to the standard-stature, pure-line cultivar Roy J (Smartt et al., 2015). While little research has focused on determining how emissions are reduced from hybrid cultivars, Ma et al. (2010) observed an increase in methanotrophic bacteria and CH_4 oxidation from hybrid rice relative to pure-line cultivars, which is consistent with greater redox potentials observed in the rhizosphere of CLXL745 in this study (Figure 3). Butterbach-bahl et al. (1997) attributed a 24 to 31% reduction in emissions from one cultivar relative to another (both pure lines) to differences in transport capacity between the cultivars. Aulakh et al. (2001) observed a positive correlation between total organic C from root exudates and CH_4 production potential, indicating the potential for cultivar differences in emissions based on variable root exudation rates. While the impacts of variable CH_4 oxidation rates, transport capacities, and root exudation rates are not well-understood, evidence has consistently demonstrated a reduction in CH_4 emissions from hybrid cultivars grown in the US, particularly from CLXL745 (Rogers et al., 2014; Simmonds et al., 2015; Smartt et al., 2015).

Similar to this study, Rogers et al. (2014) observed no difference in area-scaled emissions between Cheniere and Taggart, while several previous studies have reported reduced emissions from semi-dwarf relative to standard-stature cultivars (Lindau et al., 1995; Sass and Fisher, 1997;

Sigren et al., 1997). The difference in fluxes between semi-dwarf and standard-stature cultivars in previous studies may be due to a positive correlation between plant biomass and C exudation rates from roots (Aulakh et al., 2001) or between aboveground dry matter and CH_4 emissions (Cicerone and Shetter, 1981; Huang et al., 1997; Sass et al., 1991a; Shang et al., 2011). While a reduction in emissions from semi-dwarf cultivars is oftentimes linked to reduced dry matter accumulation, this study as well as Rogers et al. (2014) observed a reduction in aboveground dry matter that was not accompanied by a reduction in emissions. Furthermore, Sigren et al. (1997) measured greater emissions accompanied by greater soil acetate concentrations, indicative of increased root exudation, from a standard stature (Mars) relative to a semi-dwarf cultivar (Lemont), while aboveground dry matter was similar between the two cultivars. Huang et al. (1997) indicated that, while biomass may explain differences in emissions within one cultivar, the intervarietal differences in biomass are small in comparison to differences in emissions, indicating that another factor besides aboveground dry matter impacts intervarietal differences in CH_4 emissions. Aboveground dry matter was unable to account for differences in CH_4 emissions in this study as no significant ($P > 0.05$) correlations were identified for emissions based on aboveground dry matter for all data combined or separately among any of the possible treatment combinations (data not shown).

As was the case with area-scaled emissions, yield-scaled emissions varied based on both previous crop ($P = 0.004$) and cultivar ($P = 0.017$), while the interaction was not significant (Table 6). Yield-scaled emissions, averaged across cultivar, were reduced by 67% following soybean [0.7 kg CH_4-C (Mg grain)$^{-1}$] compared to following rice as a previous crop [2.1 kg CH_4-C (Mg grain)$^{-1}$] and, averaged across previous crop, emissions from CLXL745 [1.0 kg CH_4-C (Mg grain)$^{-1}$] were reduced by 36% relative to Cheniere and Taggart, which did not differ and

averaged 1.6 kg CH_4-C (Mg grain)$^{-1}$ (Table 7). While the difference in yield-scaled emissions following soybean compared to rice as a previous crop is greater than previously reported (i.e., 31% reduction following soybean), the reduction in emissions from CLXL745 is fairly consistent with an average reduction of 44% relative to Cheniere and Taggart reported by Rogers et al. (2014). Yield-scaled emissions measured in this study, however, were only about 10% of those reported by Rogers et al. (2014), which ranged from 11.1 to 20.5 kg CH_4-C (Mg grain)$^{-1}$, indicating a strong suppression of CH_4 emissions from a clay relative to a silt-loam soil under similar management and production practices. Although not formally statistically analyzed, there appeared to be an inverse relationship between grain yields (Table 5) and seasonal area-scaled emissions (Table 7), as greater yields following soybean and from CLXL745 corresponded to reduced emissions, while lower yields and greater emissions were measured following rice and from Cheniere. Similar research has also reported an inverse relationship between grain yield and CH_4 emissions (Denier van der Gon et al., 2002; Sass and Cicerone, 2002) and root exudation (Aulakh et al., 2001), indicating the importance of the source-sink relationship of plant-fixed C on CH_4 emissions.

Methane emissions following flood release were unaffected ($P > 0.05$) by previous crop, cultivar, or their interaction both on an area-scaled basis and as a percentage of total emissions (Table 6). Averaged across all treatment combinations, post-flood emissions amounted to 0.6 kg CH_4-C ha^{-1}, which was equivalent to an average of 4.5% of total season-long, area-scaled emissions. The proportion of CH_4 emitted following flood release in this study was much less than post-flood-release emissions of 10.5, 13, and 16% from CLXL745, Taggart, and Cheniere, respectively, reported by Rogers et al. (2014) from a silt-loam soil, which may be a result of greater CH_4 oxidation in the clay soil reducing the amount of CH_4 built-up that is released upon

soil drying. Post-flood-release CH_4 emissions observed in this study, however, were similar to the 5.1% reported by Smartt (2015) and the 5.2% reported by Rogers et al. (2013). Additional studies have reported post-flood-release emissions ranging from 7 to 20% of total area-scaled emissions (Denier van der Gon et al., 1996; Wassmann et al., 1994; Yagi et al., 1997). While the magnitude and fraction of post-flood-release emissions vary, it is apparent that under certain conditions, CH_4 builds up in the soil and is rapidly released in a pulse as the soil dries and macropores become open for gas movement.

Season-long, area-scaled emissions measured in this study (Table 7) only amounted to 4 to 11% of the current USEPA emission factor for non-California, primary rice crops (178 kg CH_4-C ha^{-1}) and were substantially less than the lowest reported emissions used in calculating that factor (i.e., emissions ranged from 46 to 375 kg CH_4-C ha^{-1}), many of which were measured on clay soils in Texas (USEPA, 2014). Similarly, emissions measured in this study were only about 10% of those measured from a similar study on a silt-loam soil in eastern Arkansas (Rogers et al., 2014). Studies in California, however, have reported emissions of similar magnitudes (i.e., 6.7 to 14 kg CH_4-C ha^{-1}) from a Capay silty clay (48% clay) and from a Clear Lake clay (59% clay; 9.2 to 19 kg CH_4-C ha^{-1}), while also reporting emissions ranging 58 to 69 kg CH_4-C ha^{-1} from another site with 47% clay (Adviento-Borbe et al., 2014; Simmonds et al., 2015).

While emissions have been shown to be quite variable, even within studies on clay soils, it is likely that a textural effect is largely the cause for low emissions observed in this study, as several studies have indicated an inverse correlation between clay content and emissions. This is likely due to the impact of increasing clay content causing an increase in tortuosity and a decrease in diffusivity, effectively limiting CH_4 movement out of fine-textured soils (Livingston

and Hutchinson, 1995; Nazaroff, 1992). Multiple studies have observed an increase in CH_4 entrapment and decrease in emissions resulting from increasing clay contents (Denier van der Gon et al., 1996; Wang et al., 1993) and Sass and Fisher (1997) attributed the reduction in CH_4 emissions from clay soils to the entrapment and slow movement of CH_4 that allows more CH_4 to be oxidized in aerated zones surrounding roots and at the soil surface. Wang et al. (1993) observed varying degrees of CH_4 entrapment, even among soils with similar sand contents, where the greatest entrapment (98.5%) was measured from a Sharkey clay soil compared to 80.6 and 67.8% entrapment from a Beaumont clay and a Sacramento clay, respectively. These results suggest that more than simple particle-size distribution affects CH_4 emissions and that the low emissions measured in this study likely reflect a large magnitude of CH_4 entrapment and oxidation in the Sharkey clay soil investigated.

Additional evidence suggesting large CH_4 oxidation rates in this study is provided by an examination of the soil Eh and temperature recorded in this study. Soil Eh decreased more slowly, while attaining a similar final Eh, in this study compared to a similar study conducted at the same site in 2012, which reported emissions of 35.6 kg CH_4-C ha^{-1} (Smartt, 2015). Similarly, Rogers et al. (2013) and Bossio et al. (1999) reported faster decreases and lower Eh values, even reaching as low as -275 mV, accompanied by greater emissions than that observed in this study. While lower soil Eh is likely to result in increased CH_4 production, Kludze et al. (1993) also confirmed that a smaller proportion of CH_4 is oxidized by methanotrophs as soil Eh decreases, which supports greater oxidation rates in this study. Furthermore, the difference in average flooded-period soil temperature measured here (i.e., 23.9 ˚C following soybean) relative to the same site in 2012 (i.e., 27.0 ˚C; Smartt, 2015) likely explains the reduction in emissions from 2012 to 2013, and is supported by the observation of reduced fluxes at 49 DAF following a

period of cool soil temperatures. Similarly, a study in Japan measured a 1.6-fold difference in emissions from one year to another under the same management and location and attributed the difference to an increase in average air temperature from 24.6 to 26.9 ˚C (Watanabe and Kimura, 1999). Several studies have observed an increase in emissions related to an increase in soil temperature (Hosono and Nouchi, 1997; Schutz et al., 1989a; Wang et al., 1997). Temperature has a strong influence on methanogenesis, while methanotrophs are less sensitive to temperature changes between 10 and 40 ˚C (Nazaries et al., 2013), which leads to a decrease in the proportion of CH_4 oxidized as soil temperature increases. Van Winden et al. (2012), for example, reported an increase in CH_4 production and oxidation as temperature was increased, although CH_4 production increased to a greater extent, resulting in 98% CH_4 oxidation at 5 ˚C compared to 50% oxidation at 25 ˚C.

Summary and Conclusions

Methane fluxes and resulting growing-season emissions were measured from three rice cultivars (CLXL745, Cheniere, and Taggart) grown following two previous crops (soybean and rice) on a clay soil, using enclosed-headspace gas sampling chambers, in the drill-seeded, delayed-flood rice production system in eastern Arkansas. Methane fluxes from all treatment combinations exhibited a general increase over time, except at 49 DAF when fluxes were reduced due to low soil temperatures, peaking near 50% heading, and then decreasing prior to flood release. Following flood release, a pulse of CH_4 occurred at 3 DAFR in all treatments, but did not differ in magnitude among treatments. For the duration of flooding, fluxes only differed between Cheniere and Taggart on one sampling date, while fluxes from CLXL745 were consistently less than from Cheniere prior to heading and less than Taggart following heading.

Fluxes were greater following rice than soybean as a previous crop on seven of 10 sampling dates, which led to emissions of 19.6 and 7.0 kg CH_4-C ha^{-1}, respectively. Season-long emissions did not differ between the two pure-line cultivars, Cheniere and Taggart, which averaged 14.9 kg CH_4-C ha^{-1}, while emissions from CLXL745 were reduced by 31%, which is likely a result of greater CH_4 oxidation in the rhizosphere of the hybrid cultivar. The 64% reduction in emissions following soybean was likely exaggerated by slightly lower sand and greater clay contents in plots following soybean and a reduction closer to 30% is likely more accurate. Emissions measured in this study only amounted to 4 to 11% of the current USEPA emission factor (178 kg CH_4-C ha^{-1}), and were lower than most previous studies. The large reduction in emissions here, relative to other studies, is likely a result of a large degree of CH_4 entrapment in the Sharkey clay, which slows CH_4 transport by increasing tortuosity and decreasing diffusion rates, resulting in a large degree of CH_4 oxidation by methanotrophs. Low emissions measured in this study were also likely partially attributable to lower soil temperatures as emissions were reduced substantially from those reported for the same site and management the previous season. Based on low emissions from clay soils, in combination with reductions when following soybean as a previous crop and from hybrid cultivars, it appears that emissions from rice in the mid-southern U.S. may be significantly overestimated. Data collected from studies such as this are necessary in order to more adequately estimate emissions from the large range of cultural practices, environments, and soils involved in U.S. rice production and will allow future USEPA estimates to account for such factors.

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Appendices

Appendix 1: Example SAS program and relevant data for analyzing flooded CH_4 fluxes.

```
title 'Methane Field Study 2013(flooded fluxes) - Alden Smartt Chris W. Rogers';
title2 'Seasonal Methane 2013 ANOVA';

data methane2013;

  infile 'C:\Users\cwrogers\Documents\PHD RICE\Research Studies\2013\2013-Methane Clay
soils\Data\SAS input files\Fluxes(Flooding to FR)-NEREC2013.prn' firstobs=2;

  input time chamber variety $ rotation $ block flux;
 run;

proc sort data=methane2013; by time chamber variety rotation;
quit;

title3 'INITIAL DATA LISTING AND DATA PLOT';

proc print data=methane2013 noobs; by time;
  id time;
  var chamber variety rotation block flux;
  run;

proc sort; by rotation variety time;
proc means;
class rotation time;
var flux;
run;
quit;
proc sort; by rotation variety time;
proc means;
class variety time;
var flux;
run;
quit;
ods rtf file='Methane2013.rtf' bodytitle style=journal;

title3 'ANALYSIS OF VARIANCE';
proc mixed data=methane2013 method=type3;
class block variety rotation time ;
model flux = variety rotation variety*rotation time time*variety time*rotation
time*variety*rotation/ddfm=kr;
random block block*rotation block*variety*rotation;
lsmeans variety*time rotation*time/diff;
```

```
quit;

ods rtf close;
ods graphics off;
run;
```

DAF	Chamber	Cultivar	Previous Crop	Block	mg CH4-C/m2/hr
7	3	Taggart	Soybean	4	-0.0008
7	4	Cheniere	Soybean	3	0.039
7	5	CLXL745	Soybean	4	0.0184
7	6	CLXL745	Soybean	3	-0.0038
7	7	Cheniere	Soybean	4	0.0069
7	8	Taggart	Soybean	3	0.0006
7	12	CLXL745	Soybean	2	0.0225
7	13	Taggart	Soybean	1	0.0089
7	14	Taggart	Soybean	2	0.0077
7	15	Cheniere	Soybean	1	0
7	16	Cheniere	Soybean	2	-0.0031
7	17	CLXL745	Soybean	1	0.0061
7	20	Taggart	Rice	2	-0.0019
7	21	CLXL745	Rice	1	0.0065
7	22	CLXL745	Rice	2	0.0167
7	23	Cheniere	Rice	1	-0.0137
7	24	Cheniere	Rice	2	0.0108
7	25	Taggart	Rice	1	0.0233
7	29	Cheniere	Rice	3	0.0023
7	30	CLXL745	Rice	4	0.0214
7	31	Taggart	Rice	3	0.04
7	32	Cheniere	Rice	4	0.0276
7	33	CLXL745	Rice	3	0.0187
7	34	Taggart	Rice	4	0.0415
14	3	Taggart	Soybean	4	0.0509
14	4	Cheniere	Soybean	3	0.1978
14	5	CLXL745	Soybean	4	0.0516
14	6	CLXL745	Soybean	3	0.0483
14	7	Cheniere	Soybean	4	0.1038
14	8	Taggart	Soybean	3	0.0059
14	12	CLXL745	Soybean	2	0.035
14	13	Taggart	Soybean	1	0.0029
14	14	Taggart	Soybean	2	0.0692
14	15	Cheniere	Soybean	1	0.0442
14	16	Cheniere	Soybean	2	0.1094
14	17	CLXL745	Soybean	1	0.0113
14	20	Taggart	Rice	2	0.0942
14	21	CLXL745	Rice	1	0.0368
14	22	CLXL745	Rice	2	0.0778
14	23	Cheniere	Rice	1	0.15
14	24	Cheniere	Rice	2	0.1144

14	25	Taggart	Rice	1	0.0446
14	29	Cheniere	Rice	3	0.1389
14	30	CLXL745	Rice	4	0.1152
14	31	Taggart	Rice	3	0.1471
14	32	Cheniere	Rice	4	0.3041
14	33	CLXL745	Rice	3	0.139
14	34	Taggart	Rice	4	0.2718
28	3	Taggart	Soybean	4	0.3338
28	4	Cheniere	Soybean	3	0.2733
28	5	CLXL745	Soybean	4	0.2021
28	6	CLXL745	Soybean	3	0.0993
28	7	Cheniere	Soybean	4	0.2418
28	8	Taggart	Soybean	3	0.0786
28	12	CLXL745	Soybean	2	0.1826
28	13	Taggart	Soybean	1	0.0252
28	14	Taggart	Soybean	2	0.2166
28	15	Cheniere	Soybean	1	0.0795
28	16	Cheniere	Soybean	2	0.2236
28	17	CLXL745	Soybean	1	0.0161
28	20	Taggart	Rice	2	0.7735
28	21	CLXL745	Rice	1	0.4242
28	22	CLXL745	Rice	2	0.5053
28	23	Cheniere	Rice	1	0.7666
28	24	Cheniere	Rice	2	1.3701
28	25	Taggart	Rice	1	0.4066
28	29	Cheniere	Rice	3	1.3009
28	30	CLXL745	Rice	4	0.5475
28	31	Taggart	Rice	3	0.8758
28	32	Cheniere	Rice	4	1.1887
28	33	CLXL745	Rice	3	0.8921
28	34	Taggart	Rice	4	1.7601
36	3	Taggart	Soybean	4	0.3464
36	4	Cheniere	Soybean	3	0.301
36	5	CLXL745	Soybean	4	0.1187
36	6	CLXL745	Soybean	3	0.1949
36	7	Cheniere	Soybean	4	0.2833
36	8	Taggart	Soybean	3	0.2561
36	12	CLXL745	Soybean	2	0.3263
36	13	Taggart	Soybean	1	0.1163
36	14	Taggart	Soybean	2	0.2851
36	15	Cheniere	Soybean	1	0.1997
36	16	Cheniere	Soybean	2	0.4078
36	17	CLXL745	Soybean	1	0.1219

36	20	Taggart	Rice	2	1.2079
36	21	CLXL745	Rice	1	0.5023
36	22	CLXL745	Rice	2	0.8364
36	23	Cheniere	Rice	1	0.9855
36	24	Cheniere	Rice	2	2.5978
36	25	Taggart	Rice	1	0.6806
36	29	Cheniere	Rice	3	1.5805
36	30	CLXL745	Rice	4	0.4882
36	31	Taggart	Rice	3	1.0992
36	32	Cheniere	Rice	4	1.2964
36	33	CLXL745	Rice	3	1.023
36	34	Taggart	Rice	4	1.692
42	3	Taggart	Soybean	4	0.6389
42	4	Cheniere	Soybean	3	0.6893
42	5	CLXL745	Soybean	4	0.3921
42	6	CLXL745	Soybean	3	0.3217
42	7	Cheniere	Soybean	4	0.5485
42	8	Taggart	Soybean	3	0.5881
42	12	CLXL745	Soybean	2	0.6463
42	13	Taggart	Soybean	1	0.1186
42	14	Taggart	Soybean	2	0.5227
42	15	Cheniere	Soybean	1	0.2668
42	16	Cheniere	Soybean	2	0.6658
42	17	CLXL745	Soybean	1	0.1809
42	20	Taggart	Rice	2	1.7802
42	21	CLXL745	Rice	1	0.846
42	22	CLXL745	Rice	2	1.1978
42	23	Cheniere	Rice	1	1.349
42	24	Cheniere	Rice	2	3.7609
42	25	Taggart	Rice	1	1.0021
42	29	Cheniere	Rice	3	2.222
42	30	CLXL745	Rice	4	1.1715
42	31	Taggart	Rice	3	1.4261
42	32	Cheniere	Rice	4	1.7049
42	33	CLXL745	Rice	3	1.8116
42	34	Taggart	Rice	4	2.1659
49	3	Taggart	Soybean	4	0.627
49	4	Cheniere	Soybean	3	0.6737
49	5	CLXL745	Soybean	4	0.4104
49	6	CLXL745	Soybean	3	0.4991
49	7	Cheniere	Soybean	4	0.5213
49	8	Taggart	Soybean	3	0.6731
49	12	CLXL745	Soybean	2	0.6233

49	13	Taggart	Soybean	1	0.1851
49	14	Taggart	Soybean	2	0.5387
49	15	Cheniere	Soybean	1	0.2753
49	16	Cheniere	Soybean	2	0.6287
49	17	CLXL745	Soybean	1	0.1792
49	20	Taggart	Rice	2	1.7079
49	21	CLXL745	Rice	1	0.6398
49	22	CLXL745	Rice	2	1.0727
49	23	Cheniere	Rice	1	1.186
49	24	Cheniere	Rice	2	2.8682
49	25	Taggart	Rice	1	0.9378
49	29	Cheniere	Rice	3	1.684
49	30	CLXL745	Rice	4	0.9777
49	31	Taggart	Rice	3	1.4019
49	32	Cheniere	Rice	4	1.3862
49	33	CLXL745	Rice	3	1.6976
49	34	Taggart	Rice	4	1.4837
56	3	Taggart	Soybean	4	0.8441
56	4	Cheniere	Soybean	3	0.9147
56	5	CLXL745	Soybean	4	0.878
56	6	CLXL745	Soybean	3	1.2981
56	7	Cheniere	Soybean	4	0.7512
56	8	Taggart	Soybean	3	1.0513
56	12	CLXL745	Soybean	2	1.3259
56	13	Taggart	Soybean	1	0.3089
56	14	Taggart	Soybean	2	0.748
56	15	Cheniere	Soybean	1	0.4384
56	16	Cheniere	Soybean	2	0.8471
56	17	CLXL745	Soybean	1	0.3667
56	20	Taggart	Rice	2	2.5212
56	21	CLXL745	Rice	1	1.1668
56	22	CLXL745	Rice	2	2.113
56	23	Cheniere	Rice	1	1.6026
56	24	Cheniere	Rice	2	2.9542
56	25	Taggart	Rice	1	1.2803
56	29	Cheniere	Rice	3	2.4522
56	30	CLXL745	Rice	4	2.0158
56	31	Taggart	Rice	3	1.8749
56	32	Cheniere	Rice	4	2.0331
56	33	CLXL745	Rice	3	3.5692
56	34	Taggart	Rice	4	2.2284
63	3	Taggart	Soybean	4	1.0289
63	4	Cheniere	Soybean	3	0.7002

63	5	CLXL745	Soybean	4	0.3789
63	6	CLXL745	Soybean	3	0.7868
63	7	Cheniere	Soybean	4	0.5439
63	8	Taggart	Soybean	3	1.5811
63	12	CLXL745	Soybean	2	0.5138
63	13	Taggart	Soybean	1	0.484
63	14	Taggart	Soybean	2	0.9468
63	15	Cheniere	Soybean	1	0.3544
63	16	Cheniere	Soybean	2	0.736
63	17	CLXL745	Soybean	1	0.3197
63	20	Taggart	Rice	2	2.3458
63	21	CLXL745	Rice	1	0.396
63	22	CLXL745	Rice	2	1.4332
63	23	Cheniere	Rice	1	1.2749
63	24	Cheniere	Rice	2	2.5601
63	25	Taggart	Rice	1	1.2671
63	29	Cheniere	Rice	3	1.7862
63	30	CLXL745	Rice	4	1.1916
63	31	Taggart	Rice	3	1.8505
63	32	Cheniere	Rice	4	1.4843
63	33	CLXL745	Rice	3	1.5817
63	34	Taggart	Rice	4	2.2343
71	3	Taggart	Soybean	4	0.6466
71	4	Cheniere	Soybean	3	0.6498
71	5	CLXL745	Soybean	4	0.3284
71	6	CLXL745	Soybean	3	0.3247
71	7	Cheniere	Soybean	4	0.3891
71	8	Taggart	Soybean	3	1.3199
71	12	CLXL745	Soybean	2	0.5158
71	13	Taggart	Soybean	1	0.3533
71	14	Taggart	Soybean	2	0.7514
71	15	Cheniere	Soybean	1	0.3454
71	16	Cheniere	Soybean	2	0.5754
71	17	CLXL745	Soybean	1	0.2026
71	20	Taggart	Rice	2	1.7198
71	21	CLXL745	Rice	1	0.184
71	22	CLXL745	Rice	2	0.4069
71	23	Cheniere	Rice	1	0.7937
71	24	Cheniere	Rice	2	1.5456
71	25	Taggart	Rice	1	0.6289
71	29	Cheniere	Rice	3	1.3502
71	30	CLXL745	Rice	4	0.4943
71	31	Taggart	Rice	3	1.4361

71	32	Cheniere	Rice	4	1.1134
71	33	CLXL745	Rice	3	0.5153
71	34	Taggart	Rice	4	1.2612
77	3	Taggart	Soybean	4	0.3636
77	4	Cheniere	Soybean	3	0.2949
77	5	CLXL745	Soybean	4	0.1439
77	6	CLXL745	Soybean	3	0.0995
77	7	Cheniere	Soybean	4	0.2195
77	8	Taggart	Soybean	3	0.4039
77	12	CLXL745	Soybean	2	0.2422
77	13	Taggart	Soybean	1	0.1619
77	14	Taggart	Soybean	2	0.3653
77	15	Cheniere	Soybean	1	0.1477
77	16	Cheniere	Soybean	2	0.279
77	17	CLXL745	Soybean	1	0.0675
77	20	Taggart	Rice	2	0.4693
77	21	CLXL745	Rice	1	0.1094
77	22	CLXL745	Rice	2	0.1581
77	23	Cheniere	Rice	1	0.4733
77	24	Cheniere	Rice	2	1.0086
77	25	Taggart	Rice	1	0.3231
77	29	Cheniere	Rice	3	0.6409
77	30	CLXL745	Rice	4	0.2707
77	31	Taggart	Rice	3	0.5542
77	32	Cheniere	Rice	4	0.445
77	33	CLXL745	Rice	3	0.4437
77	34	Taggart	Rice	4	0.5838

Appendix 2: Example SAS program and relevant data for analyzing post-flooded CH_4 fluxes.

```
title 'Methane Clay Field Study 2013 - Alden Smartt and Chris W. Rogers';
title2 'Seasonal Methane 2013 ANOVA';

data methane2013;

  infile 'C:\Users\cwrogers\Documents\PHD RICE\Research Studies\2013\2013-Methane Clay
soils\Data\SAS input files\Fluxes(FR to harvest)-NEREC2013.prn' firstobs=3;

  input time chamber variety $ rotation $ block flux;

 run;

proc sort data=methane2013; by time chamber variety rotation;
quit;

proc sort; by rotation variety time;
proc means n mean std;
class rotation variety time;
var flux;
run;
quit;

title3 'INITIAL DATA LISTING AND DATA PLOT';

proc print data=methane2013 noobs; by time;
  id time;
  var chamber variety rotation block flux;
  run;

proc sort; by rotation variety time;
proc means;
class rotation time;
var flux;
run;

proc sort; by rotation variety time;
proc means;
class variety time;
var flux;
run;

ods rtf file='MethaneRelease2013.rtf' bodytitle style=journal;
```

```
title3 'ANALYSIS OF VARIANCE';
proc mixed data=methane2013 method=type3;
class block variety rotation time ;
model flux = variety rotation variety*rotation time time*variety time*rotation
time*variety*rotation / ddfm=kr;
random block block*rotation block*variety*rotation;
lsmeans variety rotation time /diff  ;
quit;

ods rtf close;
ods graphics off;
run;
```

DAF	Chamber	Cultivar	Previous Crop	Block	mg CH4-C/m2/hr
84	3	Taggart	Soybean	4	0.3395
84	4	Cheniere	Soybean	3	0.2458
84	5	CLXL745	Soybean	4	0.194
84	6	CLXL745	Soybean	3	0.1159
84	7	Cheniere	Soybean	4	0.1854
84	8	Taggart	Soybean	3	0.6316
84	12	CLXL745	Soybean	2	0.294
84	13	Taggart	Soybean	1	0.2422
84	14	Taggart	Soybean	2	0.4575
84	15	Cheniere	Soybean	1	0.1585
84	16	Cheniere	Soybean	2	0.1208
84	17	CLXL745	Soybean	1	0.086
84	20	Taggart	Rice	2	0.505
84	21	CLXL745	Rice	1	0.0566
84	22	CLXL745	Rice	2	0.0548
84	23	Cheniere	Rice	1	0.3324
84	24	Cheniere	Rice	2	0.3949
84	25	Taggart	Rice	1	0.2981
84	29	Cheniere	Rice	3	0.5419
84	30	CLXL745	Rice	4	0.137
84	31	Taggart	Rice	3	0.4964
84	32	Cheniere	Rice	4	0.343
84	33	CLXL745	Rice	3	0.2326
84	34	Taggart	Rice	4	0.586
86	3	Taggart	Soybean	4	0.6411
86	4	Cheniere	Soybean	3	0.2263
86	5	CLXL745	Soybean	4	0.5927
86	6	CLXL745	Soybean	3	0.5159
86	7	Cheniere	Soybean	4	0.5884
86	8	Taggart	Soybean	3	0.4468
86	12	CLXL745	Soybean	2	0.3309
86	13	Taggart	Soybean	1	0.1994
86	14	Taggart	Soybean	2	0.567
86	15	Cheniere	Soybean	1	0.2742
86	16	Cheniere	Soybean	2	0.2644
86	17	CLXL745	Soybean	1	0.1484
86	20	Taggart	Rice	2	0.5142
86	21	CLXL745	Rice	1	0.3798
86	22	CLXL745	Rice	2	0.2227
86	23	Cheniere	Rice	1	0.3265
86	24	Cheniere	Rice	2	0.4803

86	25	Taggart	Rice	1	0.9397
86	29	Cheniere	Rice	3	0.4916
86	30	CLXL745	Rice	4	0.2674
86	31	Taggart	Rice	3	0.4212
86	32	Cheniere	Rice	4	1.1757
86	33	CLXL745	Rice	3	5.0015
86	34	Taggart	Rice	4	0.5437
88	3	Taggart	Soybean	4	0.0854
88	4	Cheniere	Soybean	3	0.2098
88	5	CLXL745	Soybean	4	0.0319
88	6	CLXL745	Soybean	3	0.0737
88	7	Cheniere	Soybean	4	0.1742
88	8	Taggart	Soybean	3	0.051
88	12	CLXL745	Soybean	2	0.2361
88	13	Taggart	Soybean	1	0.051
88	14	Taggart	Soybean	2	0.0583
88	15	Cheniere	Soybean	1	0.0719
88	16	Cheniere	Soybean	2	0.3169
88	17	CLXL745	Soybean	1	0.0367
88	20	Taggart	Rice	2	0.4186
88	21	CLXL745	Rice	1	0.0902
88	22	CLXL745	Rice	2	0.0167
88	23	Cheniere	Rice	1	0.4952
88	24	Cheniere	Rice	2	0.9345
88	25	Taggart	Rice	1	0.1304
88	29	Cheniere	Rice	3	1.4368
88	30	CLXL745	Rice	4	0.2749
88	31	Taggart	Rice	3	0.431
88	32	Cheniere	Rice	4	0.3893
88	33	CLXL745	Rice	3	0.2394
88	34	Taggart	Rice	4	0.4947
90	3	Taggart	Soybean	4	0.0308
90	4	Cheniere	Soybean	3	0.0391
90	5	CLXL745	Soybean	4	0.0023
90	6	CLXL745	Soybean	3	0.0365
90	7	Cheniere	Soybean	4	0.0769
90	8	Taggart	Soybean	3	0.056
90	12	CLXL745	Soybean	2	-0.0019
90	13	Taggart	Soybean	1	0.0182
90	14	Taggart	Soybean	2	0.0594
90	15	Cheniere	Soybean	1	-0.0076
90	16	Cheniere	Soybean	2	0.0102
90	17	CLXL745	Soybean	1	-0.0535

90	20	Taggart	Rice	2	0.0352
90	21	CLXL745	Rice	1	0.0298
90	22	CLXL745	Rice	2	-0.0268
90	23	Cheniere	Rice	1	0.0786
90	24	Cheniere	Rice	2	0.1399
90	25	Taggart	Rice	1	0.1238
90	29	Cheniere	Rice	3	0.2364
90	30	CLXL745	Rice	4	0.0593
90	31	Taggart	Rice	3	0.1018
90	32	Cheniere	Rice	4	0.0265
90	33	CLXL745	Rice	3	0.0425
90	34	Taggart	Rice	4	0.1298

Appendix 3: Example SAS program and relevant data for analyzing season-long CH_4 emissions.

```
title 'Methane Clay Field Study 2013 Seasonal Emissions - Alden Smartt  Chris W. Rogers';
title2 'seasonal emissions ANOVA';
data seasonal2013;
  infile 'C:\Users\cwrogers\Documents\PHD RICE\Research Studies\2013\2013-Methane Clay
soils\Data\SAS input files\Emissions-NEREC2013.prn' firstobs=2;
  input rep var $ rotat $ block kgha postkgha postpercent ;
* yield = yield based emission, kgha= emissions based on kg CH4-c/ha, postkgha = same but
only post flood, postpercent= percent of total
  seasonal from days after flood release;
run;

proc sort data=seasonal2013; by rep var rotat block kgha;

quit;

title3 'INITIAL DATA LISTING AND DATA PLOT';

proc sort; by block var rotat;
proc print data=seasonal2013 noobs;
id block;
var var rotat kgha postkgha postpercent yield ;
run;

title3 'Emissions in kg/ha ANALYSIS OF VARIANCE Emissions Based on Area';
proc mixed data=seasonal2013 method=type3;
class block var rotat;
model kgha = rotat var var*rotat;
random block block*rotat;
lsmeans var rotat/diff;
run;

title3 'PostFlood Emissions in kg/ha ANALYSIS OF VARIANCE Emissions Based on Area';
proc mixed data=seasonal2013 method=type3;
class block var rotat;
model postkgha = rotat var var*rotat;
random block block*rotat;
lsmeans var*rotat;
run;

title3 'PostFlood as Percent ANALYSIS OF VARIANCE Emissions Based on Area';
proc mixed data=seasonal2013 method=type3;
class block var rotat;
model postpercent = rotat var var*rotat;
```

```
random block block*rotat;
lsmeans var rotat;
run;
```

Chamber	Cultivar	Previous Crop	Block	kg CH4-C/ha/season	PFR emissions	PFR%
7	Cheniere	Soybean	4	6.9491	0.4604	6.6259
5	CLXL745	Soybean	4	5.6363	0.3704	6.5725
3	Taggart	Soybean	4	9.2916	0.482	5.1877
8	Taggart	Soybean	3	11.1027	0.4865	4.3817
6	CLXL745	Soybean	3	6.7706	0.3379	4.9904
4	Cheniere	Soybean	3	8.8311	0.3119	3.5317
12	CLXL745	Soybean	2	8.1956	0.378	4.6128
14	Taggart	Soybean	2	8.5665	0.4862	5.6761
16	Cheniere	Soybean	2	8.2088	0.3262	3.9739
13	Taggart	Soybean	1	3.4035	0.2139	6.2846
15	Cheniere	Soybean	1	4.0582	0.2232	5.5007
17	CLXL745	Soybean	1	2.6874	0.1198	4.4579
25	Taggart	Rice	1	12.3769	0.6655	5.3772
23	Cheniere	Rice	1	15.9951	0.5424	3.391
21	CLXL745	Rice	1	7.9118	0.2567	3.2449
24	Cheniere	Rice	2	33.8694	0.8717	2.5736
22	CLXL745	Rice	2	13.8657	0.1346	0.971
20	Taggart	Rice	2	23.0404	0.6422	2.7873
29	Cheniere	Rice	3	24.9375	1.2058	4.8354
31	Taggart	Rice	3	19.9115	0.6244	3.1358
33	CLXL745	Rice	3	23.3146	2.6147	11.2147
30	CLXL745	Rice	4	13.273	0.3309	2.4934
32	Cheniere	Rice	4	20.8972	0.8842	4.2313
34	Taggart	Rice	4	26.0834	0.7562	2.899

Table 1. Summary of dates of major agronomic activities involved in the management of CH_4 emissions plots for the 2013 rice growing season at the University of Arkansas Northeast Research and Extension Center in Keiser, Arkansas.

Activity	Date
Planting	28 May, 2013
Emergence	9 June, 2013
Pre-flood N fertilizer application	1 July, 2013
Flood establishment	2 July, 2013
Mid-season N fertilizer application	
Cheniere and Taggart	30 July, 2013
Boot N fertilizer application	
CLXL745	20 August, 2013
Flood release	23 September, 2013
Harvest	24 October, 2013

Table 2. Mean soil properties (n = 12) prior to flood establishment from a Sharkey clay during the 2013 growing season at the University of Arkansas Northeast Research and Extension Center in Keiser, Arkansas.

	Previous crop	
Soil property	Rice	Soybean
pH	7.06 a[†]	7.13 a
Electrical conductivity (dS m^{-1})	0.216 a	0.202 a
Sand (g g^{-1})	0.14 a	0.10 b
Silt (g g^{-1})	0.34 a	0.33 a
Clay (g g^{-1})	0.52 b	0.57 a
Bulk density (g cm^{-3})	1.17 a	1.09 a
Mehich-3 extractable nutrients (mg kg^{-1})		
P	55.9 a	46.3 b
K	387 a	390 a
Ca	4147 b	4570 a
Mg	867 b	919 a
Fe	467 a	445 a
Mn	51.1 a	53.9 a
Na	54.5 a	59.3 a
S	17.6 a	12.3 a
Cu	4.0 a	4.7 a
Zn	3.4 b	3.5 a
Organic matter (g kg^{-1})	37.7 b	39.6 a
Organic matter (Mg ha^{-1})	44.0 a	43.1 a
Total N (g kg^{-1})	1.4 a	1.4 a
Total N (Mg ha^{-1})	1.6 a	1.5 a
Total C (g kg^{-1})	15.0 a	14.8 a
Total C (Mg ha^{-1})	17.4 a	16.1 a
C:N ratio	10.8 a	10.5 a

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

Table 3. Analysis of variance summary of the effects of previous crop, cultivar, time, and their interaction on CH_4 fluxes from flooding to flood release and following flood release from a clay soil during the 2013 growing season at the Northeast Research and Extension Center in Keiser, Arkansas.

	Measurement period	
Source of variation	Flooding to flood release	Post-flood release
	P	
Previous crop	0.004	0.131
Cultivar	0.027	0.962
Previous crop × cultivar	0.099	0.770
Time	< 0.001	0.002
Previous crop × time	< 0.001	0.369
Cultivar × time	< 0.001	0.270
Previous crop × cultivar × time	0.639	0.537

Table 4. Analysis of variance summary of the effects of previous crop, cultivar, sampling location (in-chamber and in-plot), and their interaction on aboveground dry matter accumulation and the effect of previous crop, cultivar, and their interaction on grain yield from a clay soil during the 2013 growing season at the Northeast Research and Extension Center in Keiser, Arkansas.

Property/Source of variation	*P*
Aboveground dry matter (kg m^{-2})	
Previous crop	0.319
Cultivar	0.001
Previous crop × cultivar	0.032
Location	0.845
Previous crop × location	0.216
Cultivar × location	0.960
Cultivar × previous crop × location	0.257
Grain yield (Mg ha^{-1})	
Previous crop	0.012
Cultivar	< 0.001
Previous crop × cultivar	0.044

Table 5. Mean aboveground dry matter and yields collected at harvest (24 October, 2013) from Cheniere, CLXL745, and Taggart following previous crops of rice and soybean at the Northeast Research and Extension Center in Keiser, Arkansas.

Previous crop	Cheniere	CLXL745	Taggart
	Aboveground dry matter (kg m^{-2})		
Rice	1.76 bA[†]	2.17 aA	1.77 bA
Soybean	1.83 bA	2.23 aA	2.26 aA
	Grain yield (Mg ha^{-1})		
Rice	9.3 bA[†]	9.9 aB	9.7 abB
Soybean	9.5 cA	11.0 aA	10.4 bA

[†] Different lower-case letters within a row indicate differences among cultivars and different upper-case letters within a column indicate differences between previous crop treatments ($P < 0.05$).

Table 6. Summary of the effect of previous crop, cultivar, and their interaction on seasonal CH_4 emissions from a clay soil during the 2013 growing season at the Northeast Research and Extension Center in Keiser, Arkansas.

Emissions property	Previous crop	Cultivar	Previous crop × cultivar
		P	
Area-scaled emissions (kg CH_4-C ha^{-1} $season^{-1}$)	0.003	0.034	0.122
Yield-scaled emissions [kg CH_4-C $(Mg\ grain)^{-1}$]	0.004	0.017	0.111
Post-flood emissions (kg CH_4-C ha^{-1})	0.139	0.968	0.781
Post-flood emissions (% total emissions)	0.367	0.877	0.841

Table 7. Season-long CH_4 emissions as affected by previous crop and rice cultivar expressed on an area- and yield-scaled basis from the 2013 growing season on a clay soil at the Northeast Research and Extension Center in Keiser, Arkansas.

Property/effect	Cheniere	CLXL745	Taggart	Mean
Area-scaled emissions (kg CH_4-C ha^{-1} $season^{-1}$)				
Rice	23.9	14.6	20.4	19.6 A
Soybean	7.0	5.8	8.1	7.0 B
Mean	15.5 a†	10.2 b	14.2 a	
Yield-scaled emissions [kg CH_4-C $(Mg\ grain)^{-1}$]				
Rice	2.59	1.45	2.11	2.05 A
Soybean	0.74	0.53	0.78	0.68 B
Mean	1.66 a†	0.99 b	1.45 a	

† Different lower-case letters within a row for a measured property indicate differences among cultivars and different upper-case letters within a column for a measured property indicate differences between previous crop treatments ($P < 0.05$).

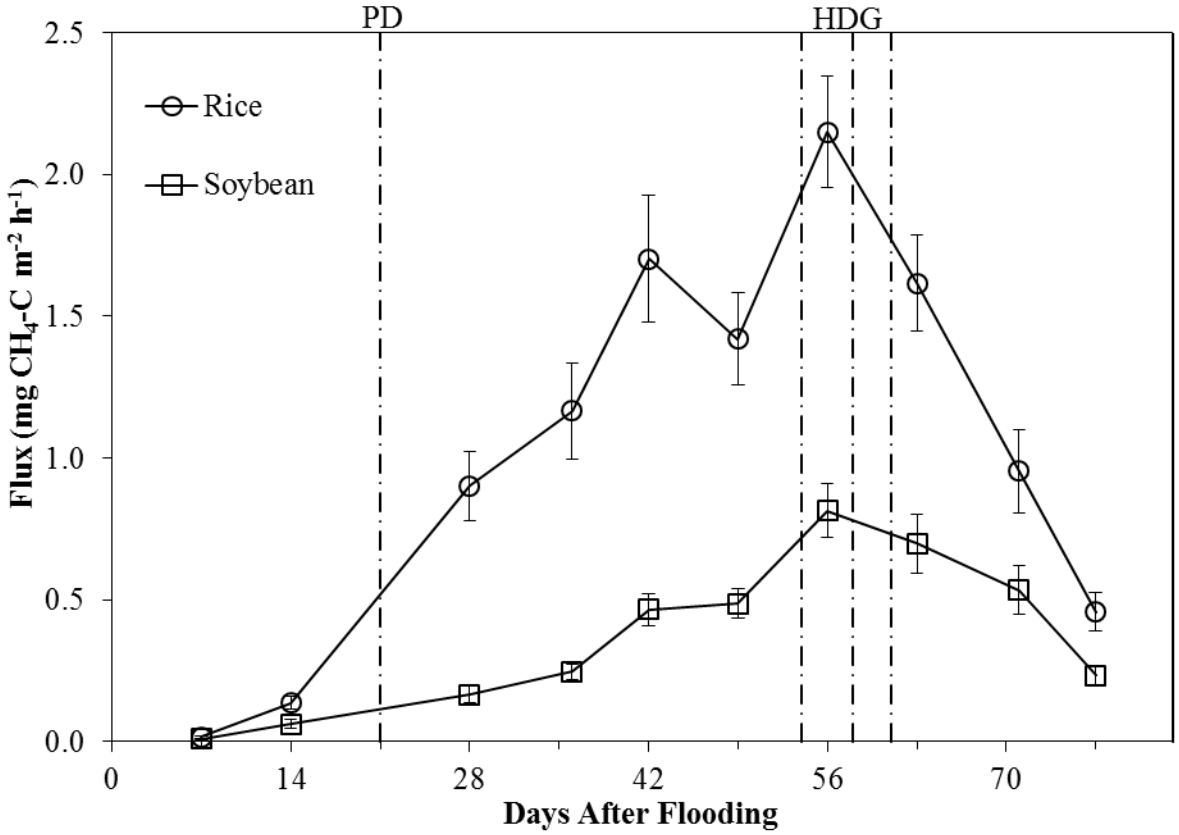

Figure 1. Methane fluxes over time throughout the 2013 growing season from previous crop treatments averaged across cultivar at the Northeast Research and Extension Center in Keiser, Arkansas. The vertical dashed lines represent panicle differentiation (PD) and 50% heading dates for CLXL745, Cheniere, and Taggart at 54, 58, and 61 days after flooding, respectively. Flood release occurred on 83 days after flooding. Least significant difference for the same previous crop treatment = 0.197 mg CH_4-C m^{-2} $hr^{-1}$and for different previous crop treatment = 0.309 mg CH_4-C m^{-2} hr^{-1}. Error bars indicate standard errors for the treatment means (n = 12).

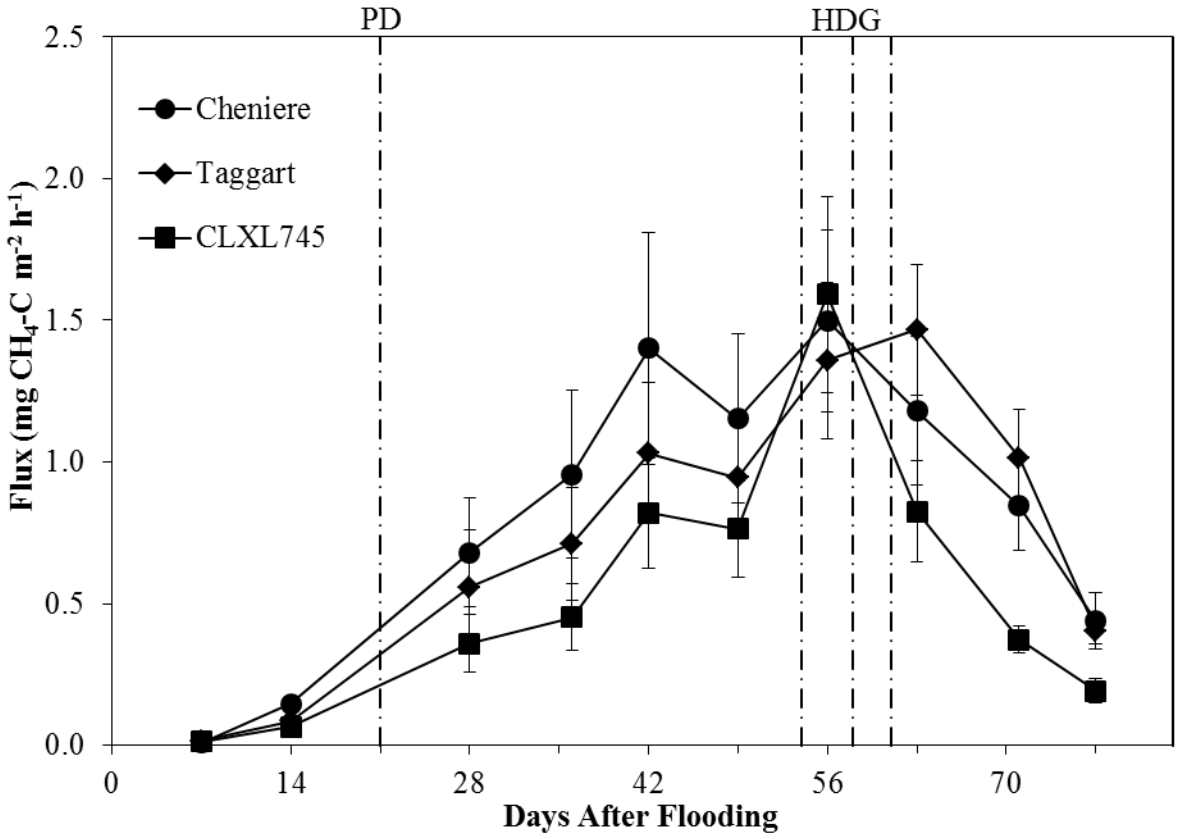

Figure 2. Methane fluxes over time throughout the 2013 growing season from CLXL745, Cheniere, and Taggart averaged across previous crop treatment at the Northeast Research and Extension Center in Keiser, Arkansas. The vertical dashed lines represent panicle differentiation (PD) and 50% heading dates for CLXL745, Cheniere, and Taggart at 54, 58, and 61 days after flooding, respectively. Flood release occurred on 83 days after flooding. Least significant difference for the same cultivar = 0.241 mg CH_4-C m^{-2} $hr^{-1}$and for different cultivars = 0.307 mg CH_4-C m^{-2} hr^{-1}. Error bars indicate standard errors for the treatment means (n = 8).

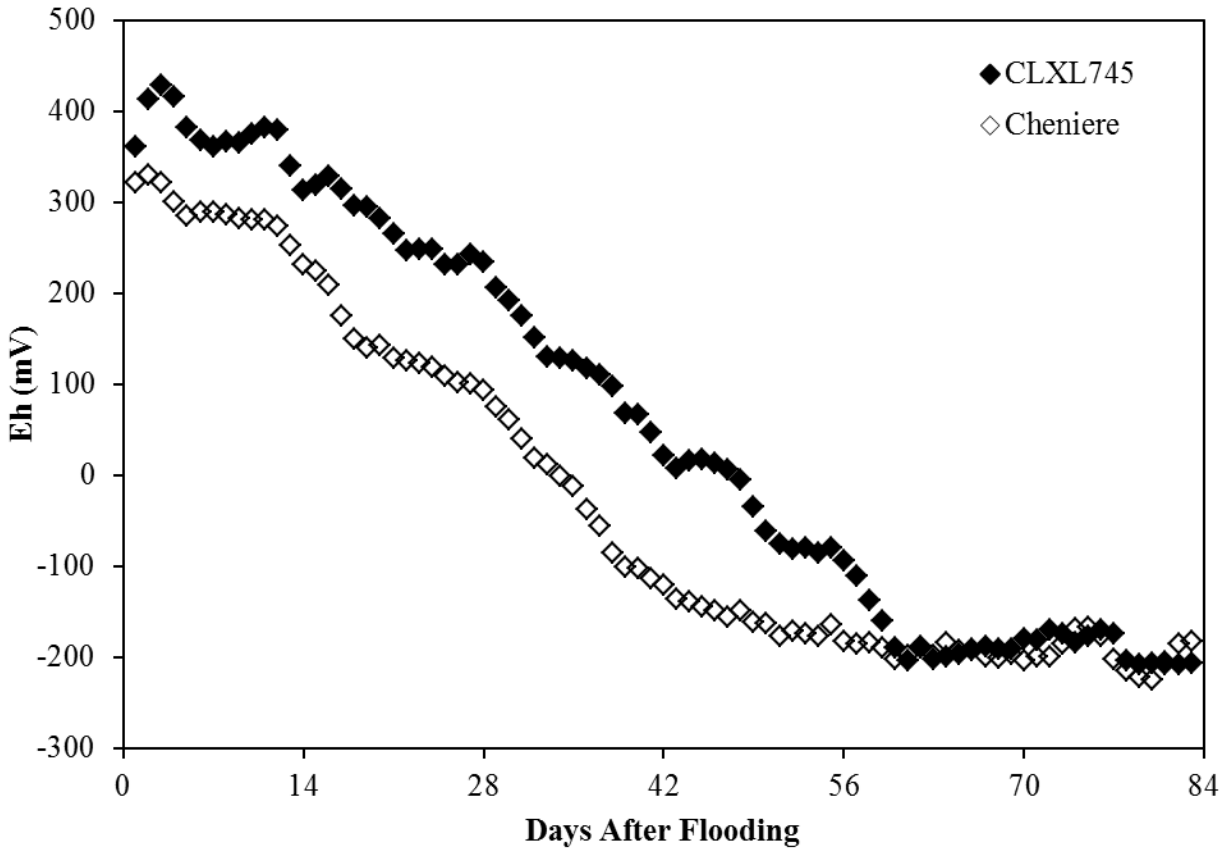

Figure 3. Soil oxidation-reduction potential (Eh) at the 7.5-cm depth over the flooded portion of the 2013 growing-season for CLXL745 and Cheniere at the Northeast Research and Extension Center in Keiser, Arkansas.

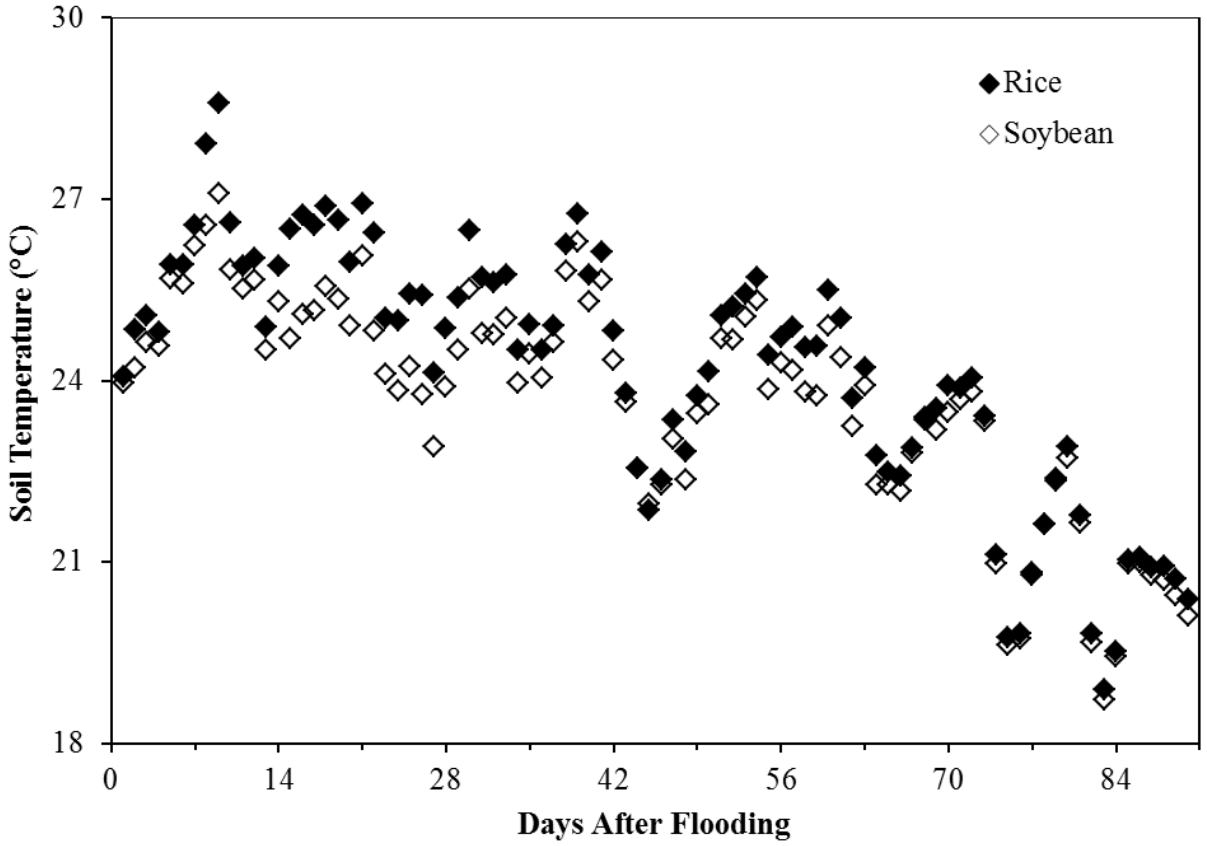

Figure 4. Daily mean soil temperature at the 7.5-cm depth over the flooded portion of the 2013 growing-season for rice and soybean previous crop treatments at the Northeast Research and Extension Center in Keiser, Arkansas.

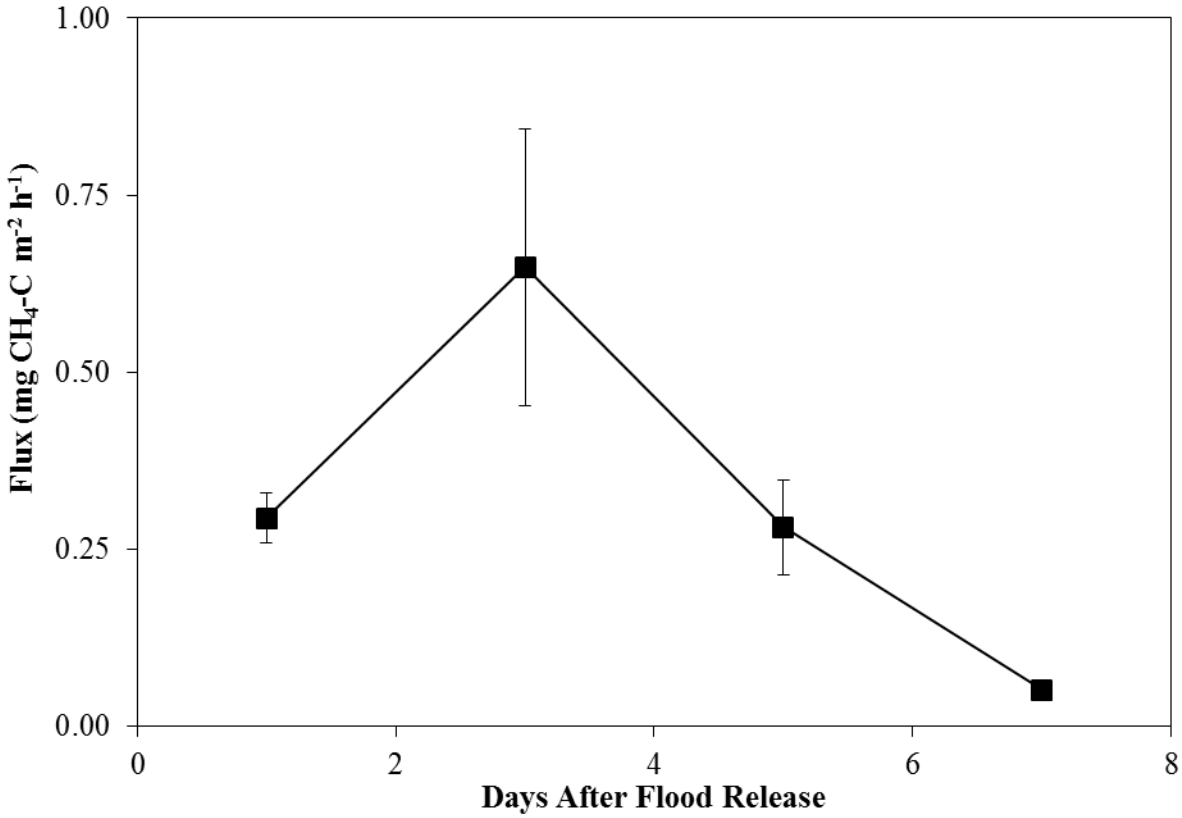

Figure 5. Methane fluxes over time following flood release at the end of the 2013 growing season averaged across all treatments at the Northeast Research and Extension Center in Keiser, Arkansas. Error bars indicate standard errors for the treatment means (n = 24).

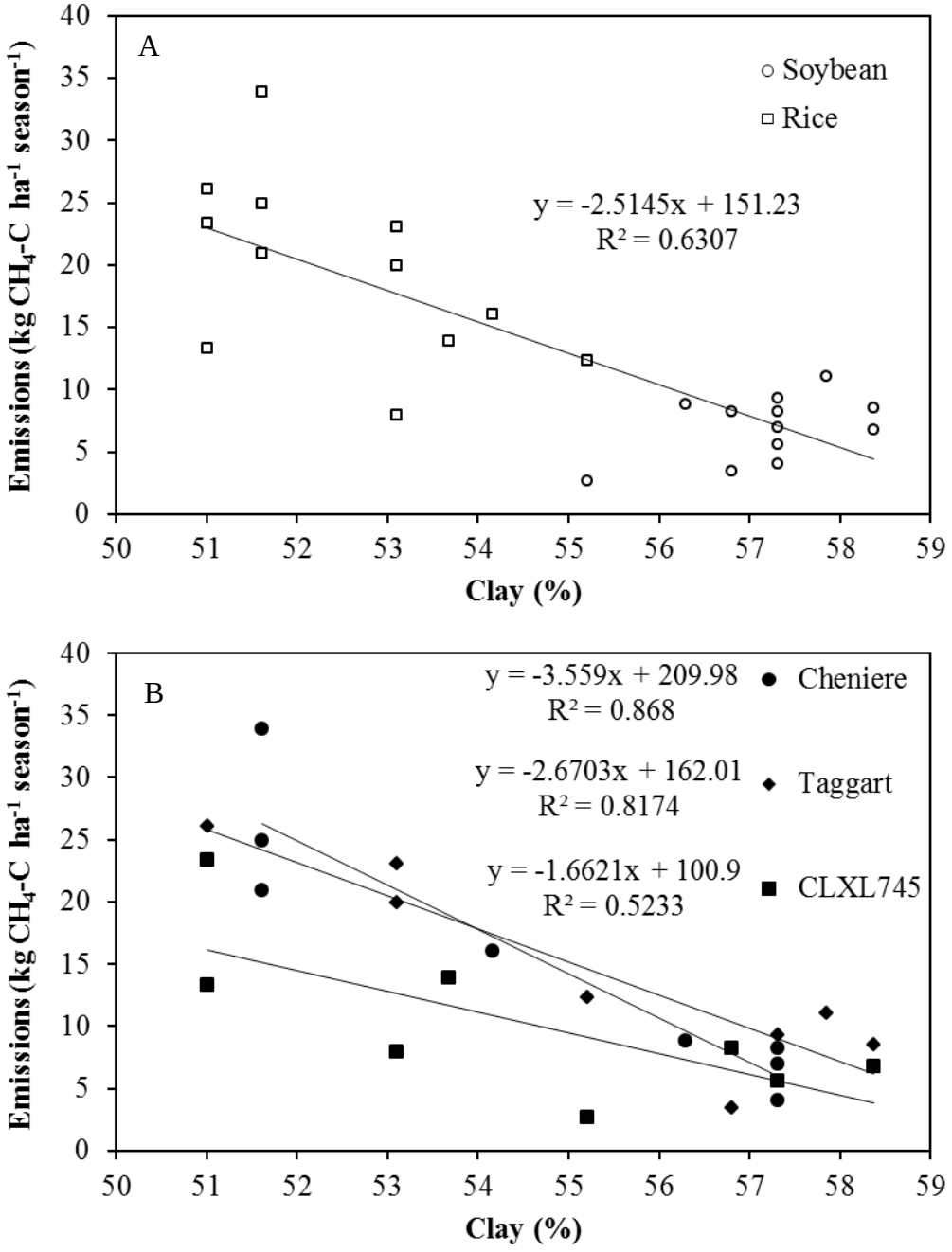

Figure 6. Linear relationship between soil clay content and season-long CH_4 emissions for all data (A) and by cultivar (B) measured during the 2013 growing season at the Northeast Research and Extension Center in Keiser, Arkansas.

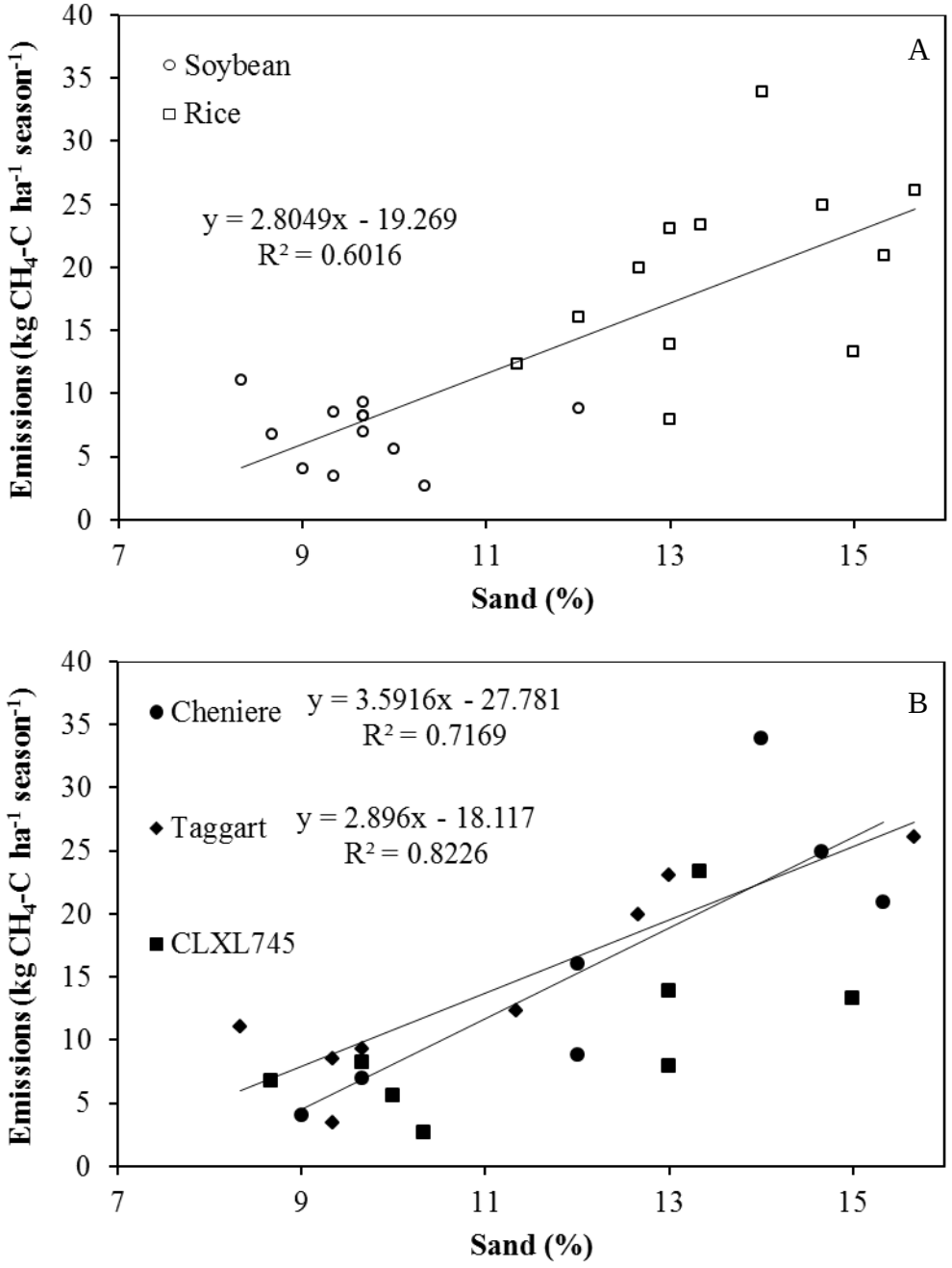

Figure 7. Linear relationship between soil sand content and season-long CH_4 emissions for all data (A) and by cultivar (B) measured during the 2013 growing season at the Northeast Research and Extension Center in Keiser, Arkansas.

CONCLUSIONS

Through the measurement of methane (CH_4) fluxes and emissions over two growing seasons using enclosed-headspace chambers, this research has provided useful insight into the factors and mechanisms affecting emissions from direct-seeded, delayed-flood, rice produced on a clay soil in eastern Arkansas. The focus of the first year of research (2012) was to determine the impact of vegetation on CH_4 fluxes and emissions in an initial characterization of CH_4 emissions from a clay soil in Arkansas, as well as to compare measurements collected from two chamber sizes. Peak CH_4 fluxes measured during the two years of this study ranged from 13 to 21% of peak fluxes and season-long CH_4 emissions ranged from 11 to 18% of those measured from similar studies conducted on silt-loam soils in eastern Arkansas. Methane fluxes and emissions measured in 2012 were strongly impacted by vegetation, as fluxes and emissions increased from near zero for the unvegetated treatment to intermediate values for the low-vegetation treatment and the greatest values in the high-vegetation treatment. Furthermore, data collected in 2012 indicated a positive correlation between aboveground dry matter and season-long CH_4 emissions, indicating a strong connection between plant growth and CH_4 emissions. There were no differences in emissions between the two chamber sizes and only minor differences in flux measurements, indicating that 15- or 30-cm diameter chambers were both adequate in estimating CH_4 emissions.

The focus of the second year of research (2013) was to determine the impact of previous crops of rice or soybean in combination with the effect of three cultivars (Cheniere, Taggart, and CLXL745). All vegetated treatments in both years exhibited the same plant-dominated flux trends, where peak fluxes occurred near the time of 50% heading, followed by a decrease in fluxes until a substantial post-flood-release CH_4 pulse occurred between 3 and 5 d after the flood

was released. Methane emissions measured in 2013 were affected by previous crop residue as well as by cultivar. Measured emissions were reduced by 64% following soybean compared to following rice as a previous crop, and were reduced by 31% from the hybrid cultivar, CLXL745, relative to Cheniere and Taggart, which did not differ. Dry matter was not correlated to CH_4 emissions in 2013, however, soil sand content and clay content resulted in positive and inverse correlations, respectively, to season-long CH_4 emissions.

Overall, CH_4 emissions measured during the two years of this study were far below the USEPA's emission factor, amounting to 20% or less of the factor in 2012 and 4 to 11% of the factor in 2013. While the reasoning behind the low emissions are not fully understood, it is likely that a large degree of CH_4 entrapment, which occurs in clay soils and is particularly strong in Sharkey clays, limits the transport of CH_4 from the soil to the atmosphere. Soil temperature also appears to have impacted emissions as a reduction in average soil temperature from 2012 to 2013 was accompanied by a decrease in emissions, even within similar treatments. Furthermore, lower than expected fluxes measured 49 d after flooding in 2013 were likely a result of cool soil temperatures in the week prior to sampling and provided additional evidence for the dependence of CH_4 emissions on soil temperature. Based on low emissions measured during the two years of this study as well as the magnitude of Arkansas production on clay soils, it appears that CH_4 emissions are presently being severely overestimated in Arkansas and in general in the mid-southern United States. Only after additional data are published can the USEPA further refine emission estimates to reflect the large variety of soils, climates, and cultural practices throughout the United States.