METABOLIC GAS EMISSIONS FROM
RETICULITERMES FLAVIPES (KOLLAR)
(ISOPTERA: RHINOTERMITIDAE)

By

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Bachelor of Science in Biology

Oklahoma State University

Stillwater, Oklahoma

2008

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2016

METABOLIC GAS EMISSIONS FROM

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ACKNOWLEDGMENTS

I would like to thank my wife and family for their support over the last four years and understanding my limited time at home. I especially want to thank my thesis advisor, Brad Kard, for his support and willingness to allow me to develop my project. I have developed better skills as a researcher under his guidance and mentoring. I also want to thank my committee for generosity with their time, and guidance, expertise and willingness to be there when I had questions. I also want extend a special thank you to Bob Hamilton, Director of the Nature Conservancy's Tallgrass Prairie Preserve, for allowing us to conduct research on the prairie. I also thank Cade Morris, Kenneth Maslowski, and Krista Pike for technical help as well as other Entomology and Plant Pathology graduate students, faculty, and staff.

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University

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Date of Degree: July 2016

Title of Study: METABOLIC GAS EMISSIONS FROM *RETICULITERMES FLAVIPES* (KOLLAR) (ISOPTERA: RHINOTERMITIDAE)
And CO_2 EMISSIONS FROM SOIL ON OKLAHOMA'S TALLGRASS PRAIRIE PRESERVE IN THE PRESENCE OR ABSENCE OF TERMITES (ISOPTERA: RHINOTERMITIDAE)
And CO_2 AND CH_4 EMISSIONS FROM DEFINED GROUPS OF SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)
And CO_2 AND CH_4 EMISSIONS FROM PRAIRIE SOIL AND FROM FORAGING SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)

Major Field: ENTOMOLOGY AND PLANT PATHOLOGY (ENTOMOLOGY OPTION)

Abstract:
Production of metabolic gases from many species of mound-building subterranean termites have been well studied in Africa, Australia and South America. However, native North American subterranean termites, *Reticulitermes* spp., metabolic gas emissions have not been well defined in natural settings. This is due in part to termites not being evenly distributed in soil. Carbon dioxide (CO_2) and methane (CH_4) are produced from multiple sources in the soil, including subterranean termites. This was demonstrated in a field study conducted on the Joseph H. Williams Tallgrass Prairie Preserve (TGPP) in northeast Oklahoma, where gas measurements attempted to distinguish termite contributions of CO_2 by studying gases emanating from soils where termites were either present or absent. It was determined that amounts of CO_2 produced from overall soil biological processes obscures the smaller gas amounts produced by termites. When metabolic gases from laboratory-reared *R. flavipes* were studied in a controlled environment, subtle but significant changes in CO_2 and CH_4 emissions where measured emanating from as few as 50 termites overtime, demonstrating that termite-produced CO_2 and CH_4 can be detected. Using this information collected from these controlled experiments, a protocol by which direct measurements of subterranean termite metabolic gases can be made was developed for field use. Field work on the TGPP during 2014 and 2015 established that foraging *R. flavipes* produced significant amounts of CO_2 and CH_4, depending on their population density. Soils emitted no CH_4 but emitted significant amounts of CO_2 during the same period compared with controls. It was also established that termite activity and soil biological processes are governed by atmospheric temperature, soil temperature, and soil moisture content. Therefore, termites comprise one group of many contributors of a large and diverse community of soil organisms producing CO_2. Termites appear to be the main TGPP soil arthropod group producing detectable CH_4. More sensitive detection equipment and improved soil gas monitoring methods are needed to better determine accurate amounts of metabolic gases that subterranean termites contribute to total normal baseline gas emissions on the TGPP.

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CHAPTER I

INTRODUCTION

The past three centuries have seen dramatic increases in global atmospheric levels of carbon dioxide (CO_2) and methane (CH_4). Air samples collected from bubbles in Vostok ice at different depths in eastern Antarctica suggest that atmospheric CH_4 concentrations were ~0.350 ppm 160,000 years ago, and ~0.70 ppm 200-300 years ago, 20 to 40% of what Chappellaz et al. (1990) estimated at 1.74 ppm. Recent data show that atmospheric CH_4 concentrations have leveled off since the early 1990's to present day estimates of ~1.75 ppm (Wuebbles and Hayhoe 2002; Dlugokencky et al. 2003). Similarly, Karl and Trenberth (2003) demonstrated that from pre-industrial times CO_2 levels have increased 32% from 280 ppm to more than 370 ppm. These authors also attributed this change to anthropogenic means, primarily burning fossil fuels.

There are natural sources of CO_2 and CH_4. Soil respiration is the primary path by which CO_2 fixed by plants returns to the atmosphere (Schlesinger and Andrews 2000). For example, CO_2 produced from soils on the Konza Tallgrass Prairie in eastern Kansas is produced by plant roots and soil heterotrophs, releasing as much as 15.1 μmol CO_2 m^{-2} s^{-1} (Knapp et al. 1998). Soil microorganisms are responsible for decomposition and transformation of organic matter from above and below ground plant residues. This microbial activity accounts for the majority of CO_2 released into the atmosphere from soil (Ananyeva et al. 2008). Both CH_4 and CO_2 are emitted from rice paddy fields where CH_4 released into the atmosphere is a product of photosynthesis, and CO_2 is released into the soil-water matrix and subsequently into the atmosphere (Minoda and Kimura 1996). While soils are a significant source of CO_2, swamps, bogs, and marshes are

significant sources of CH_4 due to anaerobic actions of methanogens on decaying matter in these water-saturated soils (Seabacher et al. 1985; Bridgham et al. 1995). Ruminant livestock are another source of atmospheric CH_4, producing 250 to 500L of methane per day per individual cow (Johnson and Johnson 2014). Ruminant ability to digest cellulosic materials is due to actions of ruminal microflora. Digestion is regulated by factors such as feed type and amount of feed intake, type of carbohydrates, the way the feed is processed, the addition of lipids, and ionophores (Johnson and Johnson 2014). Because of their superficial similarity to ruminant livestock in digesting cellulosic material, termites produce significant amounts of CO_2 and CH_4 depending on species (Breznak 1982).

Some researchers have indicated that termites contribute significant amounts of CO_2 and CH_4 to the overall global atmospheric gas budget. Zimmerman et al. (1982), demonstrated through laboratory tests that *Reticulitermes tibialis* Banks, (Isoptera: Rhinotermitidae) and, *Gnathamitermes perplexus* Banks, (Isoptera: Termitidae), had the potential to produce 0.111- and 0.310-mg CO_2 per day, and 0.794- and 0.397-mg CH_4 per day, respectively. Fraser et al. (1986) estimated that the overall termite contribution to atmospheric budget of CH_4 was less than 5% globally because of the extreme variability in feeding preferences demonstrated by termites across many species. Most research on termite contributions to overall global budgets of both CO_2 and CH_4 have been conducted using mound-building termites (Darlington et al. 1996). They estimated *Macrotermes jeanneli* Grasse (Isoptera: Termitidae), produced 800 to 1,500L CO_2 d^{-1}, and 0.5 to 1.31L CH_4 d^{-1} per mound in Kenya, east Africa.

Shelton and Appel (2001) estimated gas emissions in field experiments with *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitidae) and *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), widespread pests in the USA. *Reticulitermes flavipes* produced 0.507-ml CO_2

g^{-1} h^{-1} while *C. formosanus* produced 0.310-ml CO_2 g^{-1} h^{-1}. More recently, CH_4 produced by four species of mound-building termites, *Microcerotermes nervosus* Hill (Isoptera: Termitidae), *M. serratus* Froggatt (Isoptera: Termitidae), *Tumulitermes pastinator* Hill (Isoptera: Termitidae), and *Amitermes darwini* Hill, (Isoptera: Termitidae), was found to be dependent on diurnal and seasonal variations in temperature, soil moisture, and termite biomass (Jamali et al. 2011a, Jamali et al. 2011b, Jamali et al. 2013).

Hydrogen (H_2), CO_2, and CH_4 are by-products of bacterial and protozoan degradation of cellulose (Sugimoto et al. 1998). In lower termites such as *R. flavipes,* H_2 and CO_2 produced are converted to acetate and CH_4, and catalyzed by the methanogenic bacteria through the reaction $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$, while acetate is produced by reduction of CO_2 by other microbes that catalyze the reaction $4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$ (Leadbetter and Breznak 1996). Diet has a significant impact in acetate versus CH_4 production. In wood-feeding termites such as *R. flavipes*, acetogenesis out-produces methane production. However, in fungal-feeding or soil-feeding termites, methane out-produces acetogenesis (Brauman et al. 1992).

Globally, termites constitute one of the largest animal aggregate biomasses, demonstrating their ability to contribute to the global atmospheric budget of CO_2 and CH_4 (Brauman et al. 1992). Subterranean termites-are cryptic by nature and not evenly distributed in soils, making *in situ* observations difficult (Shelton and Appel 2001). Studying their metabolic gases requires direct measurements in natural settings to estimate their impact on the soil atmosphere (Sugitmoto 2000). To complicate matters in accurately determining termite emissions of greenhouse gases, soils alone produce significant amounts of CO_2, and in some ecosystems CH_4 as well, making it difficult to separate termite gas emissions from overall baseline gas output. Aerobic soil microbes, plant roots, and soil-dwelling arthropods are the main sources of CO_2 in

prairie-grassland ecosystems (Amunderson and Davidson 1990, Bouma 1997, Kuzykov 2006). The studies described in this thesis were conducted to examine termite-produced CO_2 and CH_4 on the Joseph H. Williams Tallgrass Prairie Preserve (TGPP), and aid our understanding of overall soil dynamics within which termites thrive.

Objectives

1. Determine CO_2 flux from soils on The TGPP in the presence or absence of termites.
2. Determine amounts of CO_2 and CH_4 emitted from defined groups of *Reticulitermes flavipes* over time in a laboratory setting.
3. Determine amounts of CO_2 and CH_4 produced by subterranean termites feeding on bait wood within flux chambers on the TGPP, and compare these with gasses emitted from natural TGPP soils with known termite activity.

Thesis Format

This thesis is formatted for the Journal of Environmental Entomology. Sections include: Abstract, Key Words, Introduction (no heading), Materials and Methods, Results and Discussion, Acknowledgments, and References Cited. Chapters III and V describe results of two field studies examining gas emissions from soils and *Reticulitermes flavipes* Kollar. Chapter IV details results from a laboratory study examining the differences in CO_2 and CH_4 emissions from different group sizes of *R. flavipes* over time. Chapter I is a general introduction followed by Chapter II, a review of literature. Chapter VI summarizes experimental results.

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CHAPTER II
REVIEW OF LITERATURE

Biodiversity on The Oklahoma Tallgrass Prairie Preserve. The Tallgrass Prairie Preserve (TGPP) is a 15,700-ha natural area located in northeastern Oklahoma (36º50'N, 96º25'W). It is owned and managed by The Nature Conservancy. The cornerstone property, the historic 11,800-ha Barnard Ranch, was purchased by the Conservancy in 1989, while adjacent properties were purchased later to bring the preserve to its current size (Hamilton 2007). It is located in in Osage County and encompasses a variety of grassland, forested, wetlands, and disturbed habitats. The known flora for these areas consists of 763 species in 411 genera and 109 families, with 12% of these being non-native to Oklahoma. Approximately 90% of the native flora consists of Big bluestem (*Andropogon gerardii*), Indian grass (*Sorghastrum nutans*), Tall dropseed (*Sporobolus compositus*), Switchgrass (*Panicum virgatum*), and Little bluestem (*Schizachyrium scoparium*) (Palmer 2007).

The TGPP is home to wide variety of micro- and macro-fauna including large mammals such as bison (*Bison bison*) and white tail deer (*Odocoileus virginianus*), and small mammals that include deer mice (*Peromyscus maniculatus*), white-footed mice (*Peromyscus leucopus*), and the western harvest mouse (*Reithrodontomys, megalotis*) (Kaufman et al. 1989). A number of snake species also call the TGPP home, including but not limited to the Prairie rattlesnake (*Crotalus viridis viridus*), Racer (*Coluber constrictor foxii*), Western fox snake (*Elaphe vulpina vulpine*), Western hognose snake (*Heterodon nasicus nasicus*), green snake (*Liochlorophis vernalis*), Brown snake (*Storeria dekayi*), and Garter snakes (*Thamnophis sirtalis sirtalis* and *T. radix*

radix). Garter snakes *Thamnophis* spp., account for approximately for 78% of total snakes on the prairie (Cagle 2008).

The TGPP is an important wintering area for the Red-tailed hawk, *Buteo jamaicensis* Root, an important predator of small mammals and snakes. Prairie avians are not greatly diverse, comprising less than 10 species, not including raptors. This includes upland sand pipers (*Bartramia longicauda*), sparrows from families Emberizidae and Passeridae, Northern bobwhites (*Colinus virginianus*), Killdeer (*Charadrius vociferous*), Common yellowthroat (*Charadrius vociferus*), House wren (*Troglodytes aedon*), and Red-winged blackbird (*Agelaius phoeniceus*) (Zimmerman 1992; Lish et al. 1996).

Arthropods are diverse and abundant, occupying many ecological niches. This diversity consists of herbivores and detritivores that influence root dynamics and rhizosphere nutrient cycling, often interacting with above-ground groups through plant responses and detrital pathways. Other organisms include microbes, nematodes, and earthworms (Whiles and Charlton 2006). Soil microbes are important in nitrogen and carbon fixation, while nematodes are bacterivorous and omnivorous, interacting with plants and soil microbes. Earthworms, both native and introduced species, are known for their ability to increase soil organic matter through digestion of dead plant material (Knapp and Seastedt 1986; Seastedt et al. 1988; James 1991). Prescribed fires in prairie ecosystems are also important to conservation of small insects (Panzer 2006).

Subterranean termites are important in consumption of cellulosic materials below and above ground. As detritivores they actively feed on decomposing plant material. Digestion is made possible by their association with microbial symbionts in their gut (Holt and Lepage 2000). Termites use soil, saliva and feces to construct their nests. Nests may be subterranean, stand-

alone mounds (tropical), or workings attached to the outside of shrubs or trees and man-made structures. Some termite nests are simple constructions and their internal microclimate is not much different from that in the soil (Pascal et al. 2011). Their foraging and excavation processes are responsible for redistribution of soil from one horizon to the next as they tunnel (Wood 1988). Termite foraging activity also contributes to heterogeneity of soil microbes and carbon structure through fecal deposition (Lavelle et al. 1992). In general, termites are important in causing physical and chemical alterations of soil (Nutting et al. 1985).

The TGPP is home to few species of subterranean termites, the two most common being *Reticulitermes flavipes* often found in grasslands, and *R. hageni* (Isoptera: Rhinotermitidae) found within the cross timber areas adjacent to the prairie proper as well as in the tallgrass habitat. Smith et al. (2012) estimated *R. flavipes* foraging populations at 103,193 (±7,081) to 422,780 (±19,297), and *R. hageni* foraging populations at 44,170 (±4,879) to 207,141 (±9,190). Brown et al. (2008) estimated foraging populations from 10,000 to 180,000 foragers on the Tallgrass Prairie proper with estimated foraging territories encompassing 9.0 to 92.3 m^2. However, termites are cryptic by nature and not evenly distributed in soils, making *in situ* observations of behavior or physiological processes difficult (Shelton and Appel 2001; Dawes-Gramadzki 2003). Sugimoto et al. (2000) suggested that direct measurement of termite metabolic gases, whether in large subterranean colonies or mounds, is crucial for accurately determining their contributions of global atmospheric gases.

Termite CO_2 and CH_4 Emissions. Previous research indicates that termites are a potentially large source of greenhouse gases. The first comprehensive study on termite-produced greenhouse gases (CH_4; CO_2; CO; H_2) suggested that termites were a significant source of the overall budget of these trace gases in the atmosphere (Zimmerman et al. 1982). They estimated

from laboratory experiments that *Reticulitermes tibialis* Banks, *Gnathamitermes perplexus*, had the potential to produce 0.111 and 0.310mg CO_2 d^{-1} per termite, respectively. They also determined that *R. tibialis* produced 0.794mg CH_4 d^{-1} per termite, while *G. perplexus* produced 0.397mg CH_4 d^{-1} per termite. They also estimated that both species combined contributed 1.5×10^{14}g CH_4 and 5×10^{16}g of CO_2 y^{-1} to the atmosphere. Similarly, Rasmussen and Khalil (1983) estimated that methane produced by termites is at least 50 times greater than that reported by Zimmerman. Their calculations estimated that production of CH_4 by termites is probably <15% of overall natural global yearly emissions.

Sieler et al. (1984) studied gas flux from nests of six genera, including *Hodotermes*, *Macrotermes*, *Odontotermes*, *Trinervitermes*, *Cubitermes*, and *Amitermes* from a broadleaf savannah in South Africa. Flux rates from mound nests of these species varied according to genera, ranging from 0.5 to 19.3, and 1.4 to 5.8mg CH_4 $nest^{-1}h^{-1}$. CO_2 from the same genera ranged from 0.023 to 0.042 and 3.2 to 25.6mg CO_2 $nest^{-1}h^{-1}$. Fraser et al. (1986) estimated that termites produce <5% of the global CH_4+CO_2 combined budget, based on the high degree of variation in termite feeding preferences. They also determined that *Coptotermes formosanus* Shiraki produced no detectable CH_4. Wood feeding *Nasutitermes* spp. in Amazon forests produced $3.0 \pm 1.3 \mu g$ CH_4 h^{-1} g^{-1}termites. This volume varied from one location to the next, but still represented about 5% of the total global methane produced from other sources (Martius et al. 1993, 1996).

Darlington et al. (1996) measured CH_4 and CO_2 from intact field mound nests of *Macrotermes jeanneli* Grassé, (Isoptera: Termitidae) and compared these measurements with laboratory studies using separated intact nest components. Measurements of intact field mounds nests consisted of sampling outflow gases within the mound's chimney from seven mounds.

Intact nest CO_2 and CH_4 outflow is comprised of total gases combined from *M. jeanneli*, plant roots, bacteria, and arthropods within the mound (Darlington et al. 1996). They estimated total emissions CO_2 from a single mound ranged from 800 to 1,500L d^{-1} and CH_4 from 0.5 to 1.31L d^{-1}. In laboratory experiments, Darlington et al. (1996) separated nest components by groups consisting of intact fungus combs, and termites from three mounds. They determined that of the 800 to 1,500L d^{-1} CO_2, average totals emitted from three mounds, termites and fungus combs contributed on average bet 175.8 and 287.1L d^{-1}, respectively. In the laboratory they determined different castes emit both CO_2 and CH_4 at significantly different rates.

Shelton and Appel (2001) tested the effects of caste, colony mass, and activity level on CO_2 release from *Coptotermes formosanus* and *Reticulitermes flavipes*. *Reticulitermes flavipes* released significantly more CO_2 compared with *C. formosanus*. They estimated that *R. flavipes* produced 0.507ml CO_2 $g^{-1}h^{-1}$ compared with *C. formosanus* that produced 0.310ml CO_2 g^{-1} h^{-1}. Termite activity, colony mass, and caste did not have significantly different effects on CO_2 production for either species.

Environmental factors such as temperature and soil moisture play a key role in termite production of CO_2 and CH_2. Wheeler et al. (1996) described respiration rates of 13 Nearctic species, measuring their O_2 consumption, and CO_2 and CH_4 production. They determined that in some species, respiration decreased as O_2 decreased and CO_2 increased. They also observed that species dependent on moisture, such as subterranean termites, had lower respiration rates compared with drywood termites, and that biomass significantly influenced respiration rates of all termite species investigated. Larger normal body mass termites generally had higher respiration rates. According to Wheeler et al. (1996), temperature was an important factor in respiration for the drywood termites *Cryptotermes cavifrons* Banks, *Incisitermes tabgae* Snyder,

and *Incisitermes minor* Hagen. Shelton and Appel (2000) determined that at temperatures ranging from 20 to 40°C, *I. minor, I. tagogae*, and *C. cavifrons* produced 1.92ml, 1.66ml, and 1.62ml CO_2 g^{-1} h^{-1}, respectively.

More recently, CH_4 flux from four species of mound building termites (*Microcerotermes nervosus*, *M. serratus, Tumulitermes pastinator* and *Amitermes darwini*) can be directly correlated with diurnal temperature and soil moisture, as well as and seasonal temperature and soil moisture changes on tropical savannas of the Northern Territory of Australia (Jamali et al. 2011). Gas emissions from these termites were species dependent as well as temperature dependent. *Microcerotermes nervosus* Hill, the most dominant species in their study sites, demonstrated the lowest production of CO_2 and CH_4 of the four species. Diurnal and seasonal changes in air temperature and soil moisture must be taken into account when estimating CH_4 flux from termites (Jamali et al. 2013). Termite biomass in soil or mounds is directly related to soil moisture, which in the case of *M. nervosus* was 3.4 times greater during the wet season on tropical savannas (Jamali et al. 2011). The annual gas flux from termite mounds and surrounding savanna soil is dominated by CO_2, with large variations between mounds. CH_4 flux from mounds was generally 5- to 46-times smaller than the concurrent CO_2 measurements (Jamali et al. 2013). However, Williams et al. (1994) demonstrated that *Nasutitermes walkerii* (Hill) is able to diminish some CO_2 within the mound via acetogenesis, and that CH_4 detected was through the reduction of CO_2 though anaerobic respiration.

Termite Gut Symbionts and Greenhouse Gas Emissions. Termites have been called the smallest bioreactors because of their ability to digest cellulosic material and create by-products of CO_2 and CH_4 (Brune 1998). Digestion of cellulosic material by termites begins with salivary gland secretions that include the enzymes endo-β-1,4-glucanase, β-glucosidase, endo-β-1,4-

xylanase, and β-xylosidase in *Reticulitermes speratus* Holmgren, and continuing until final digestion takes place in the hindgut (Breznak 1975; Inoue et al. 1997; Lo et al. 2006).

Termites are a complex assemblage of species that are divided into 'higher' and 'lower' groups. Lower termite hindguts contain flagellated protozoa and prokaryotes, which facilitate wood feeding. Higher termites lack flagellated protozoa but contain prokaryotes and exhibit a variety of feeding habits (Veiver et al. 1982; Ohkuma 2008). Cook (1932) was the first to postulate that a gas, which he hypothesized as methane, was produced by degradation of cellulosic material by symbiotic prokaryotes in the hindgut. It is because of these symbionts that the termite gut resembles that of ruminants such as cattle and sheep, with the exception that the ruminant gut contains fungi that aid in digestion of cellulose (Johnson and Johnson 1995). *Reticulitermes flavipes* gut epithelial cells are densely populated with methanogenic bacteria, resulting in methane accumulation in the hindgut and its subsequent release into the atmosphere (Brune 1998).

As cellulose is decomposed by the gut microorganisms, methane is produced by the fermentation of glucose. The CO_2 and H_2 produced are further converted to CH_4 and acetate by anaerobic bacteria (Sugimoto et al. 1998). Methane arises from members of the methanogenic Archaea that catalyze the reaction $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$. Other microbes are responsible for acetogenesis and catalyze the reaction $4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$ that occurs in the hindgut of termites. Competition occurs for the same H_2 molecules required for both reactions, and feeding habits of termites determine along which pathway the H_2 molecules are utilized most (Leadbetter and Breznak 1996). In wood-feeding termites such as *R. flavipes*, both reactions occur. However, it was determined that CO_2-reducing acetogenic bacteria out-produce methanogenic bacteria during the fermentation process in the hindgut, thereby reducing H_2 and

CO_2 to acetate. Thus, *R. flavipes* produces relatively less methane compared with fungus-growing and soil-feeding termites (Brauman et al. 1992; Konaté 2003).

The community of protists within the termite gut is species specific. In a study by Lewis and Forschler (2004) the gut communities of *R. flavipes, R. virginicus*, and *R. hageni*, were comprised of different densities of various protists, with *R. flavipes* having relatively greater abundance of *Dinenympha gracilis*, thereby distinguishing it from *R. virginicus* and *R. hageni. Reticulitermes virginicus* contains relatively greater abundance of *Trichonympha agilis* compared with *R. flavipes* and *R. hageni*, and *Reticulitermes hageni* is distinguished by having the greatest abundance of both *Dinenympha fimbriata* and *Perosonympha minor*. Regardless of protist species, they are responsible for efficient digestion of cellulose and overall health of the termite (Lewis and Forschler 2004). This is in contrast to gut symbionts in the lower termites from Australia, *Mastotermes darwiniensis* (Mastotermitidae) and *Cryptotermes primus* (Kalotermitidae) that contain *Streptococcus* spp. Guts of four species of Rhinotermitidae, *Heterotermes ferox*, *Coptotermes acinaciformis*, *C. lacteus*, and *Schedorhinotermes intermedius* contained *Enterobacter* spp. Three species of Termitidae, *Nasutitermes exitiosus*, *N. graveolus,* and *N. Walkerii* contained *Staphylococcus* spp. However, *Streptococcus* is a minor bacterium found in *H. ferox, C. Lacteus*, and *S. intermedius intermedius*. Another minor bacterium, *Enterobacter* sp., was found in *M. darwineinsis*, *C. primus*, and *N. graveolus*. *Bacillus* spp. was found in *C. acinaciformis* and *S. intermedius intermedius*, and *M. darwineinsis* contained *Flavobacterium* spp. (Eutick et al. 1978).

Globally, termites constitute one of the largest aggregate biomasses. Estimating their contribution to the global atmospheric budget of CO_2 and CH_4 can be refined by knowing the feeding habits of various termite species, such as wood feeding versus fungus feeding (Brauman

et al. 1992). Khalil et al. (1990) estimated that globally, termites as a biomass produce 4×10^{15}g CO_2 yr^{-1} and 12×10^{12}g CH_4 yr^{-1}, based primarily on CO_2 emissions from termite mounds.

Soil CO_2 Production. CO_2 concentrations in soil are primarily the product of biological processes mediated by microbial respiration. Amunderson and Davidson (1990) demonstrated that once produced these gases diffuse from one soil layer to the next overlying layer. This gas movement has been described by Fick's law. Within different ecosystems worldwide, CO_2 concentrations in the upper few centimeters of soil vary between 0.04 to 13.0% by volume. The higher percentage of CO_2 in certain ecosystems highlights the importance of organic layers and the gas breakdown by soil microbes (Amunderson and Davidson 1990). The release of CO_2 by aerobic respiration is a non-linear function of temperature over a wide range of soil water content. However, aerobic respiration becomes a function of moisture content as the soil becomes drier. When water tables are lowered in some ecosystems, CO_2 levels increase and CH_4 concentrations decrease. When water tables are raised, CO_2 levels decrease and CH_4 levels increase through anaerobic respiration (Smith et al. 2003).

There are five biogenic sources of CO_2 efflux from soils that have been previously described. These sources include root respiration, rhizomicrobial respiration, decomposition of plant residues, the priming effect induced by root exudation (or the addition of plant residues), and the basal respiration by microbial decomposition of soil organic matter (SOM). These five processes can be combined with each other to form root-derived CO_2, plant-derived CO_2, SOM-derived CO_2 from rhizosphere respiration, heterotrophic microbial respiration, and autotrophic respiration (Bouma 1997, Kuzykov 2006). Soil microorganisms are responsible for the decomposition and transformation of organic matter from above- and below-ground plant

residues. This accounts for the majority of CO_2 released into the atmosphere from soil (Ananyeva et al. 2008).

Some CO_2 in soil occurs in geological formations that result in the formation of CO_2 springs, which can cause significant, localized concentrations of this gas in the soil and its subsequent release into the atmosphere (Kern and Johnson 1993). These CO_2 concentrations have been determined to reduce microbial and plant growth in the areas containing above normal CO_2 concentrations (Pierce and Sjörgersten 2009). Amount of CO_2 in soil is also governed by soil organic carbon, root biomass, and microbial populations in prairie soils. Soil moisture content is a major factor in CO_2 emissions on the Northern Great Plains. During cold months, soils produced 86g CO_2-Carbon m^{-2}, while the same soils during warm months produced 728g CO_2-Carbon m^{-2}. Monthly, there was a rise and fall of CO_2 emissions from the soil, with the lowest emissions occurring in January and February, then increasing in March and peaking in June and July, then declining again in the fall months (Frank et al. 2002). Similarly, Pumpanen et al. (2003) investigated a Scotts pine forest in southern Finland, and determined that both CO_2 concentrations within soils and flux from soils were seasonally driven according to soil moisture content and temperature. Emissions created followed a bell-shaped flux curve during the year, with highest CO_2 evolving in June and July, and lowest during winter months.

Yuste et al. (2007) determined that the amount of CO_2 produced by soil microbes was influenced by moisture and temperature that regulated microbe metabolism, but was also dependent upon the carbon inputs into the microbes produced by decomposition of plant exudates. Liu et al. (2002) determined that water alone does not drastically change CO_2 flux from soils on a tallgrass prairie. The tallgrass prairie system of Texas presents another situation where the prairie is often warm and dry. Over several years there was little difference between

average daily temperatures, but the amount of soil water each year varied depending on the amount of rainfall. However, CO_2 flux fluctuated, with 26% of this variation dependent on soil water content, while 46% of flux variation was due to temperature (Meilnick and Dugas 2000). On the TGPP, rainfall is the primary contributor to soil water and is essential for microbial and root growth as well as termite survival (Orchard and Cook 1983; Suiter et al. 2009; Cook and Orchard 2008).

In another study, soil in a Sitka pine forest showed significant correlation between soil temperature and CO_2, while soil moisture content showed no obvious effect on CO_2 efflux. Temperature alone demonstrated the most significant impact on CO_2 efflux (Fang and Moncrieff 2001).

In a UK study, three types of manure and two types of inorganic fertilizer were applied to grassland soils in southeast Scotland. These amendments resulted in significantly different CO_2 efflux among the treatments, with manure acting as a CO_2 sink as opposed to releasing CO_2. When cattle slurry was applied to the soil CH_4 flux increased slightly, but this effect was temporary (Jones et al. 2005).

Fires have also been shown to dramatically increase CO_2 emissions for up to a year after a burn in tropical savannahs (Poth et al. 1995). Fire on the Konza Prairie in Kansas elicited significant increases in CO_2 efflux when compared with non-burned sites. Non-burned areas produced 10.3μmol CO_2 $m^{-2}s^{-1}$, whereas burned areas produced 15.1μmol CO_2 $m^{-2}s^{-1}$. However, it was also shown that the time of year the burn took place significantly influenced change in CO_2 efflux, with the greatest change coming soon after spring burns (Knapp et al. 1998).

There are several natural sources for CO_2 accumulation in soil. Soil respiration is the primary pathway by which CO_2 previously fixed by plants returns to the atmosphere (Schlesinger and

Andrews 2000). Up to 20 to 30% of carbon taken in by grasses is transferred to the soil, and approximately one-third of this uptake is transferred to soil as CO_2 through root respiration (Kuzykov and Domanski, 2000). Roots of mature Slash pine are the single largest contributor of CO_2 in soils from within *Pinus elliottii* plantations in Florida. It was estimated that pine roots contributed up to 51% of the CO_2 evolved in the soils of nine-year-old plantation, and 62% in a 29-year-old plantation (Ewel et al. 1987). Prairie and grassland soils provide suitable habitats for root biomass and microbial growth, with plant roots contributing to CO_2 emissions (Larionova et al. 2006).

Among soil invertebrates that contribute to greenhouse gas emissions, ants are known to facilitate increased microbial and fungal activity in soils (Bender and Wood 2003). Soil invertebrates are soil ecosystem engineers that have significant effects on soil fertility and water holding capacity (Jouquet et al. 2006). As such, many soil-dwelling invertebrates deposit carbon into the soil that is then utilized by microbes and subsequently released as CO_2 (Hendrix et al. 2006). To assess soil in laboratory experiments, it's important to properly collect and store samples prior to experimental use. Storage temperatures of 4.4°C must be maintained to preserve bacterial and fungal organisms (Paetz and Wilkes 2005).

Weather data were acquired from the Foraker Mesonet station in NE Oklahoma to evaluate the effects of soil and air temperature and relative humidity on soil gas flux and possible effects on termite foraging within TGPP soil (Oklahoma Climatological Survey 2014, 2015). Globally, there is a direct correlation between vegetation and soil respiration, and soil moisture and soil temperature (Raich and Potter 1997; Raich and Tufekcioglu 2000). Emission of CO_2 from soils is dependent on several variables including the amount of organic material deposited into the soil as well as the soil temperature and moisture content (Rustad et al. 2007; Frouz and Jilkova

2008). Soil temperature can account for 80% of the seasonal variation in CO_2 flux (Davidson et al. 1998).

Methane Oxidation and Production. Methanothrophs and microaerophilic organisms utilize methane to derive energy, converting it to CO_2 then methanol. Factors that regulate methane oxidation include soil diffusivity and water potential, which exert their influence through interactions with methane monooxygenase, the enzyme that catalyzes the reaction converting methane to methanol, which is the first step in the oxidation process ultimately leading to conversion to CO_2 (Mancinelli 1995).

Chan and Parkin (2001) demonstrated that methane flux from soil-to-atmosphere is controlled by two microbial processes, methanogenesis and oxidation. Land use has a profound impact on the activities of these two processes, governing the amounts of CH_4 released into the atmosphere and the amount oxidized in the soil. Methane production in agricultural soils tends to be low except where soil is kept saturated with water, creating an anaerobic environment as is the case of rice fields. Drier agricultural areas tend to demonstrate high oxidation rates of CH_4. On the other hand, prairie soils show high CH_4 oxidation rates under ambient atmospheric CH_4 levels, but less compared with agricultural systems. This demonstrates that the two microbial processes controlling methanogenesis and oxidation vary depending on the type of ecosystem and its characteristics (Chan and Parkin 2001). Similar observations were made in *Pinus radiata* plantations, shrub lands, and pastures in New Zealand, where there was little difference in the CH_4 uptake between these different land uses. CO_2 production and microbial populations were lower in pine plantation soils as opposed to shrub lands or pasture lands (Tate et al. 2007).

Seiler et al. (1984) demonstrated the ability of tropical and subtropical soils to decompose methane produced by termites. They demonstrated that these soils consumed methane at 21 x 10^{13}g yr^{-1}, well above the estimated 2 to 5 x 10^{13}g yr^{-1} produced by termites.

The highest CH_4 consumption rates are observed in soils where methanogenesis is extensive, such as rice fields, swamps or landfills and where CH_4 concentrations are or have been much higher than in the atmosphere (Le Mer and Roger 2001). Well drained non-agricultural soils mediate the oxidation of CH_4 directly from the atmosphere, which contributes to 5 to 10% of the global CH_4 sink. This process is enabled by the activity of two methanotrophic microbial populations. One population is active at high atmospheric CH_4 concentrations, and is referred to as low affinity bacteria. The second populations are active at low atmospheric CH_4 levels. These are designated 'high affinity' and are the predominant microorganisms responsible for atmospheric methane oxidation (Bull et al. 2000)

Chamber-Based Methods of Gas Collection. Parkin and Venterea (2010), as a part of the Greenhouse Gas Reduction through Agricultural Carbon Enhancement network (GRACEnet) project, established minimum guidelines for the construction of flux chambers. These requirements are: **1).** The chambers must be fabricated from non-reactive material, such as stainless steel, aluminum, PVC, polypropylene, polyethylene, or plexiglass. **2).** Materials should be white or coated with a reflective material such as either Mylar or paint. **3).** Chambers should be large enough to cover at least 182cm^2 of the soil surface and have a target height of 15cm (height can be adjusted lower to increase sensitivity or raised to accommodate plants). **4).** Chambers should contain a stainless steel tubing vent tube at least 10-cm long and 6.35mm in diameter (0.25 inch). **5).** Chambers must have a sampling port to enable the removal of gas samples. The recommended design dictates that a chamber should have two parts. **1).** a

permanent anchor (a base) driven into the ground on which, **2).** a chamber lid will tightly fit (Parkin and Venterea 2010). There are several methods described in the literature for the collection, storage and analysis of CO_2 and CH_4 flux measurements, including four basic principles flux chamber design to measure CO_2 soil flux. These include closed dynamic chamber systems, closed static chamber systems, open chamber systems, and eddy covariance systems (Norman et al. 1997; Hutchinson et al. 2001; Livingston et al. 2006). In one form or another, each principle has been applied to the systems listed below.

In closed dynamic chamber systems, air is circulated between the chamber and the external infra-red gas analyzer (IRGA) with a pump so the CO_2 concentration increase is a function of time and is proportional to the CO_2 flux. Closed static chambers are of a simpler design, in having no mechanical circulation of air. They consist of only the chamber with a sampling port and collar, with samples taken via syringe and analyzed in a laboratory with a gas chromatograph or portable IGRA. Open chambers have a continuous flow of air so that the difference between CO_2 concentration entering and exiting the system (gas flow rate) and enclosed soil surface area are used to calculate fluxes (Norman et al. 1997). Eddy covariance systems analyze the exchange rate of CO_2 across the interface between the atmosphere and plant canopy by measuring the covariance between fluctuations in vertical wind velocity (Baldocci 2003). A few commercial gas collection and analyses systems are marketed as follows.

LI-6200 System. This is the most widely used system. It is a closed gas exchange system with air circulated between a small chamber that is placed on the soil. It uses an IRGA, and gas flux is measured by the increase in CO_2 concentration over time (Norman et al. 1997).

Crill System. This system utilizes a 71L static closed flux chamber that tightly fits into a grooved base collar that was inserted into the soil to 4cm, which was sealed with water. The

chamber is made of welded aluminum and painted black. It is equipped with a thermoresistor to measure the enclosed air temperature and a battery operated brushless fan to mix the air within the chamber prior to sampling (Crill 1991).

Savage System. This system utilizes an 8L chamber with air mixing done by pumping a 60ml syringe twelve times before sampling. Closed chambers are made of steel or other sturdy material, such as Polyvinylchloride (PVC) (Parkin and Ventera 2010). Both Crill and Savage Systems involve placing the chamber on a collar that has been previously inserted into the ground. These systems can be manually sampled with gas-tight syringes at 10, 20, and 30 minutes, or can have an LI-6200 analyzer installed (Norman et al. 1997). Wu et al. (2010) utilized a modified version of this process by using a chamber made of PVC and sealed with a metal lid. Their chamber incorporated a small fan to circulate the air for complete mixing. The head space of their chambers were approximately 1.8L and five samples were taken at 0, 15, 30, 45, and 60 minutes after the lid was placed on the chamber.

Rayment System. In this system air is passed through a chamber enclosing the soil and any change in the CO_2 concentration of air leaving the chamber and through the analyzer (LI-6252; LiCOR, Lincoln, NE) is compared with the ambient air drawn in from outside the chamber, to determine CO_2 efflux. This method is also used in conjunction with an LI-6200 IRGA. The flux chamber is seated onto a collar previously inserted into the soil. Air samples are taken at two or three diurnal cycles, after which the chamber is moved and placed over another in-ground collar and the process is repeated (Rayment and Jarvis 1997).

Striegl System. The Striegl method incorporates paired metal chambers, each with a volume of 34.3L, installed approximately 10-m apart in the field. These chambers contain fans or pumps, and are connected to a series of stainless steel and nylon tubing buried at depths of 2.0cm

and 100cm, from which gas samples are extracted. Methane was injected into the tubing, with degradation being measured as function of soil depth. Gas samples were taken using a gastight syringe, and analyses were performed with a gas chromatograph equipped with a flame ionization detector (FID) (Striegl et al. 1992).

Baldocchi Eddy Correlation System. This system measures CO_2 concentrations within and above a tree canopy using non-dispersive IRGAs. One analyzer, (Model LI-6252 from LiCor, Lincoln, NE) measured CO_2 concentrations at 4.3, 10.4, 16.3 and 20.7m above ground level. Each sensor was connected to the Model LI-6252 via tubing, and a computer-controlled switch between samples at each height directed air to the gas analyzer. CO_2 Levels were sampled for 20 seconds every two minutes. A second analyzer (model LI-6251, LICOR, Lincoln, NE) measured CO_2 concentrations at 1.8m above the forest floor during the course of the experiment (Baldocci 2003).

Gas Sampling and Storage. The ability to accurately sample, store and transport intact gas samples from the field to the laboratory is key in assessing gas flux from any source. There are as many ways of sampling, storing, and transporting gas samples as there are chamber methods. As with the different chamber methods there is no set way of sampling, storage, or transporting gas samples. Unless using a portable gas analyzer such as the LI-6251, syringes and vials are the most common way of sampling, storage, and transporting (Rochette and Bertrand 2003). Rochette and Bertrand (2003) also noted the importance of protecting the integrity of the sample, and the type of syringe and vial utilized can have a significant negative or positive impact on this process. They demonstrated that polypropylene syringes can absorb at least 5.8% of gasses into the plastic walls of the syringe and that most syringes lost up to 16% of gas from leakage in 24 h, even with the needle capped with a septum, thus making them not reliable for sample storage.

Glass is non-absorbent and is ideal for gas storage, but if vials are capped with red butyl septa they tend to lose vacuum over time (up to 89% after 136 days) and create contamination in the vial. Grey butyl and silicone septa tended to create less contamination, and the hole created by a needle resealed more efficiently, retaining 98% of the vacuum after 136 days (Rochette and Bertrand 2003). However, Wu et al. (2010) employed 20 ml plastic syringes that were equipped with three-way valves that prevented leakage or degradation of the gases. In addition, these samples were analyzed within a few minutes of being taken, which also reduced the potential of degradation of the sample. Parkin and Venterea (2010) recommended the use of crimp-top vials with butyl septa, but stressed the importance of making sure the crimping tool applied adequate, even pressure to ensure an airtight seal. To increase the efficacy of the seal the authors recommended testing several vials by applying vacuum and evaluating the remaining vacuum one week prior to the next sampling event, and adjusting the crimping tool accordingly (Brooks 1993; Parkin and Venterea 2010). The use of a septa system in the design of the chamber is crucial for gas extraction (Mehra et al. 2013). In order to calculate the flux from chambers, sampling times must be taken at regular intervals, from time zero minutes to an hour, e.g., 0, 30, 60 minutes, or 0, 20, 40, 60 minutes (Mosier et al. 2006, Parkin and Venterea 2010).

Trace Gas Analysis. By far the most user friendly system for any field gas analysis is a portable device, similar to the various Li-COR IRGA models, which can accurately determine the concentration of CO_2 flux from soils. One limitation to the LiCOR instruments is that only CO_2 analysis can be performed, as methane requires a GC equipped with a flame ionization detector (FID) (Christensen et al. 2000). Another method of analysis is to use laboratory-based gas chromatographs (GC) and similar analytical instruments. Wheeler et al. (1996) suggested using two GCs, one equipped with an FID for methane, and a second equipped with a thermo-

conductivity detector (TCD) for CO_2. A gas volume of 25μl is injected onto a Hewlett Packard(HP) 5890A GC for CO_2 analysis, and the injector and detector set at a temperature of 30ºC and 100ºC, respectively. The oven temperature program is started at 30ºC for five minutes, then increased to 60ºC at 15ºC min^{-1} intervals. The column used was a 7.6m by 0.5mm diameter stainless-steel-packed HayeSepD (Alltech Associates, Inc. Deerfield, IL). For CH_4 analysis, Christensen et al. (2000) injected 100μl air sample into a HP 5890 series II, equipped with a split-splitless injector set at 100ºC, with an FID set at 220ºC. The column used in this GC was a wide-bore fused silica Poraplot Q (0.53mm outside diameter by 10m length) that was subjected to isothermal oven temperature of 200ºC for four minutes (Wheeler et al. 1996). Jamali et al. (2011) followed a similar method for methane analysis for CH_4 flux from termite mounds in Australia using a Shamadzu 17A-GC equipped with a FID and a 1.8m Poropak Q separation column. Seiler et al. (1984) employed a combined system to study CO_2 and CH_4 emissions from termite nests in South Africa by using a Mini Shimadzu GC and a BINOs IR detector. The GC utilized an FID for CH_4 while the BINOs IR detector was used for CO_2. Atmospheric samples were passed through a stainless steel molecular sieve (13x, 60/80 mesh). Both systems were installed in a van and used on site (Seiler et al. 1984).

Wu et al. (2010) used GCs from two different companies to analyze soil gases from a semi-arid grassland in Inner Mongolia, China. The first GC was a Shimadzu 14A equipped with an FID for CH_4 analysis, and the second GC was a Perkin-Elmer equipped with a TCD for CO_2 analysis.

According to ice core samples taken from Volostov, east Antarctica, methane levels 160,000 years ago varied from ~0.350 to 0.50 ppm, with concentrations of 1.50ppm present between 1700 and 1900 A. D. (Chappellaz et al.1990; Wuebbles and Hayhoe 1990). Current models

show that average CO_2 levels are at 380 to 400ppm compared with ~280ppm during pre-industrial times (Karl and Trenberth 2003; NOAA 2014-2015). Since the 1980s, CH_4 has increased from 1.63ppm to ~1.74ppm in 2002. The greatest increase in CH_4 occurred from 1984 to 1999, but this increase has leveled off at ~1.74ppm (Dlugokencky et al. 2003).

Methods for laboratory studies involving termite bioassays followed Kard et al. (2003) and Konemann et al. (2014). This literature review describes several areas that are involved in either soil or termite greenhouse gas analyses. It also describes the processes in soil and termites on how these gases are produced. The literature also describes the environmental influences on production of these gases. Just as important, the gas collection methods and analytical methods are described. There are many types of chamber-based methods for facilitating gas flux research. From this array of information, a small portion was actually used to design and implement the research presented in this thesis.

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CHAPTER III

CO_2 EMISSIONS FROM SOIL ON OKLAHOMA'S TALLGRASS PRAIRIE PRESERVE IN THE PRESENCE OR ABSENCEOF TERMITES (ISOPTERA: RHINOTERMITIDAE)

Abstract CO_2 flux from soil is a significant component of total atmospheric carbon as well as an indicator of soil biological health. Gas flux is a useful tool for evaluating the impact of agricultural practices on productivity, and a key variable relevant to global climate change. A year-long study was conducted to evaluate subterranean termite contribution to soil CO_2 flux on Oklahoma's Joseph H. Williams Tallgrass Prairie Preserve. Gas samples were extracted from metal flux chambers located within soil plots containing naturally occurring populations of foraging subterranean termites, and from two different types of control plots without termites. Results indicate that CO_2 flux during January, March, April, June, July, and December was similar among all three treatments. However, CO_2 flux in control plots during May was significantly greater compared with termite-active plots. Overall CO_2 flux from both termite-infested and termite-free plots was great enough to obscure CO_2 contributions from subterranean termites alone. Therefore, although total normal baseline CO_2 emissions from soil were measured, the specific amount of CO_2 that termites alone contributed to this flux could not be accurately determined.

Key Words CO_2 flux, greenhouse gas, *Reticulitermes flavipes*, subterranean termite

Termites are important recyclers of cellulosic materials, whereby they influence soil moisture and fertility and overall composition and type classification (Nutting et al. 1985). Similar to other soil-dwelling invertebrates such as earthworms and ants, termites are soil engineers because they tunnel within and excavate soil, altering its physical characteristics (Jouquet et al. 2006). Termites influence soil structure and properties because they excavate and move deep soil particles to the surface, thereby increasing aeration as well as improving water percolation and retention. Termite foraging activity also contributes to heterogeneity of soil microbes and carbon structure through fecal deposition (Lavelle et al. 1992).

Subterranean termites within the Joseph H. Williams Tallgrass Prairie Preserve (TGPP) in northeast Oklahoma are keystone reducer-decomposers of ligno-cellulosic plant material, a capability attributed to the digestive actions of their symbiotic gut micro-fauna, and cellulases produced in their salivary glands (Brune 1998; Lo et al. 2006). In addition, laboratory studies have demonstrated that the number of termites present, including their caste ratio, influences the amount of CO_2 they produce (Shelton and Appel 2001).

Termite gas emissions have been collected and measured by 'tenting' over colonies of tropical mound-building termites (Konaté et al. 2003). Some studies enclosed mounds within a plastic tent configured with gas extraction ports for collecting 'head space' samples (Seiler 1984). Khalil et al. (1990) estimated that globally, termites as a biomass produce 4×10^{15}g CO_2 yr^{-1} and 12×10^{12}g CH_4 yr^{-1}, based primarily on CO_2 emissions from termite mounds.

Similar to mound-building termites, non-mound-building, wood-feeding subterranean Nearctic termites produce CO_2 by digesting cellulose through hydrolysis, and fermenting glucose to form acetate and CO_2, which is reduced to CH_4 that is subsequently released into the soil. Notably, some CO_2 is not reduced to CH_4, but is released directly into the soil (Brauman et al.

1992). However, without a mound to locate colony workings, termite gas emissions from soil in this study were collected using mechanical devices that sampled only a small part of a colony's possible foraging territory.

The amount of CO_2 released by termites in a field environment is of interest from an ecological and climate change perspective. Zimmerman et al. (1982) was one of the first to determine that termites have the potential to be major contributors to normal atmospheric concentrations of CO_2, CH_4, and H_2. They estimated termites produce between 0.75×10^{14}g yr^{-1} and 3.1×10^{14}g yr^{-1} of combined trace gases worldwide, and that variation of food sources in different ecosystems influences the amount of trace gases released. Current models estimate that normal atmospheric CO_2 levels range from 380 to 400mg kg^{-1} (ppm; NOAA 2014). These concentrations are partially attributed to anthropogenic sources such as burning fossil fuels (Karl and Trenberth 2003).

Approximately 10% of atmospheric CO_2 originates from soil (Pierce and Sjogersten 2009). This amount is ten times greater than CO_2 produced by burning fossil fuels (Raich and Potter 1995). Prairie soils in temperate climates of North America contain an abundance of CO_2-producing microbes that utilize carbon deposits from soil-dwelling invertebrates such as earthworms and micro- and macro-arthropods, including subterranean termites (Panzer 2002; Hendrix et al. 2006). Soil microbes contribute CO_2 through digestion organic matter, increasing the total normal amount of gases released into the atmosphere (Kern and Johnson 1993; Kuzyakov 2006).

The TGPP is a unique study site that contains an abundance of foraging subterranean termite populations and diverse flora (Palmer 2007; Brown et al. 2008; Smith et al. 2012). Brown et al. (2008) estimated that horizontal foraging areas for individual colonies of *R. flavipes* (Kollar) on

the TGPP range from 9.0 to 92.3 m^2 and contain 10,357 to 183,495 foragers, making this an ideal study location. Measuring CO_2 emissions from soil with and without termites present will enhance our understanding of normal metabolic gas flux into the atmosphere from tallgrass prairie soil, thereby increasing our knowledge of baseline background gas emissions. If CO_2 concentrations in the atmosphere change, known normal baseline emissions from soil can be used to help evaluate such changes. Gas emissions measured in this study will allow future measurement of possible effects of atmospheric CO_2 changes on gas emissions from TGPP soil. In addition, determining subterranean termite contributions to these emissions will add to our understanding of their role in TGPP soil ecology. Therefore, the study objective was to determine CO_2 baseline emissions from TGPP soil where actively foraging subterranean termites were present and compare these with CO_2 emissions from soil where termites were eliminated.

Materials and Methods

Study Site. The TGPP is located between 36.37° and 39.90°N latitude, and 96.32° and 96.49°W longitude in northeast Oklahoma, and encompasses 15,410 ha, 90% consisting of grasslands populated predominately by *Andropogon gerardii*, *Sorghastrum nutans*, *Panicum virgatum,* and *Schizachyrium scoparium*. A linear band of mixed hardwood forest cross-timbers originating in Kansas and ending in Texas, consisting predominately of two oak species, *Quercus stellata* and *Quercus marilandica*, runs north-to-south across eastern TGPP (Palmer 2007). The study location was open tallgrass prairie near TGPP headquarters.

Plot Design. There were 15 field plots and three treatments, with five plots assigned to each treatment. Each of three study areas encompassed 305 m^2 of prairie grassland and contained five randomly-placed plots for each of the three treatments. Each plot measured 3.1- x 3.1-m square

and contained nine termite-monitoring stations inserted into the soil and arranged on a 3 x 3 grid pattern on 1.5-m centers (Fig. 1).

Treatments. All treatment plots were installed in close proximity on tallgrass sites with consistent soil type, vegetation cover, and climate factors. Treatment A plots were normal TGPP grass areas containing actively foraging termites that were monitored using in-ground, cylindrical termite-monitoring stations containing cellulose food matrix but no toxicant. Treatment B (termite-free plots) monitoring stations initially contained food matrix only and were regularly inspected until foraging termites infested them. Then, a bait cartridge containing lethal insect growth regulator (IGR) was placed into each termite-active station. Termites fed on the IGR bait matrix, resulting in their elimination from these plots within a few weeks. Because Treatment B plots were open to possible termite re-infestation, IGR bait cartridges were renewed as needed to ensure these plots remained termite free. Treatment C, a vertical 15-cm-wide by 75- to 90-cm-deep trench was dug downward and into the prairie bedrock around the plot perimeter. A stainless steel, fine mesh continuous barrier was inserted vertically edgeways into the trench completely around each plot and down into the bedrock. Trenches were backfilled to return soil up to the original surface level (Fig. 2). Bait stations were then emplaced and provisioned with an active IGR cartridge to eliminate termites, and monitored to validate continuing absence of termite activity. Treatments B and C were designed to evaluate changes in CO_2 flux by comparing pre- and post-termite-elimination gas emissions, with Treatment C ensuring continuous complete exclusion of termites from these mesh-enclosed plots. The active bait matrix is contained in a cylindrical plastic bait station with narrow ‘slits’ cut into its side to allow for entrance of termites but exclude most other soil-dwellers. Therefore, worms, crickets, ants, and other macro-organisms were not affected by the IGR.

Flux Chambers. Flux chambers were modified from Parkin and Venterea (2010), and constructed from 3.8-liter metal paint cans measuring 18.8-cm-tall by 16-cm diameter, with an epoxy coating on the interior surfaces to prevent corrosion. Chamber bottoms were removed to allow for influx of gases from the soil. Lids were fitted with a rubber sampling septum to facilitate interior atmosphere sampling (Diksjtra et al. 2009; Mehra et al. 2013). Each septum was installed by punching a 1.97-cm-diameter hole in the lid and inserting a 2.0-cm-diameter septum (Hutchinson and Livingston 2001; Butnor et al. 2005; Livingston et al. 2006). A vent tube was inserted through the lid to prevent pressure build-up, while also allowing for increases in interior CO_2 and CH_4 concentrations (Parkin and Venterea 2010). The vent tube was constructed using 0.95-cm outside diameter (0.63-cm inside diameter) 15.2-cm-long flexible copper tubing inserted through a hole in the lid and secured using a 0.95-cm-diameter bulkhead-fitting rubber O-ring to seal the hole. The tubing was bent to form a double-inverted curved 'J' shape in a manner to allow insertion into the bulkhead fitting and through the O-ring. The open ends of the tube were approximately 5-mm above and below the lid surfaces.

To minimize disturbing the soil, flux chambers were inserted into the soil by tracing the bottom edge of the cylindrical chamber onto the soil surface and vertically cutting into the soil along the traced edge with a curved, serrated-edge trowel to a depth of 7 cm. Each chamber was then inserted into the cut in the soil by gently tapping on the chamber top edge with a rubber mallet until the chamber bottom edge reached 7-cm deep. Soil was then firmly packed against both the interior and exterior walls of the chamber at the soil surface to create a firm seal.

Gas Collection. Methods for sampling and storage of collected gases were modified from Rochette and Bertrand (2003). Glass containers (15-ml volume) used for extracting gas samples consisted of a BD Vacutainer® (A. Daigger and Company, Vernon Hills, IL) pre-vacuumed,

negative internal pressure blood collection vial without either anti-blood clotting additive or a silicone coating on the inside surface. However, to increase accuracy of CO_2 samples and remove possible trace internal contamination, Vacutainers were prepared as follows. The rubber stopper sealing each Vacutainer top was removed and these vials were flushed with nitrogen for 10 seconds, then the stopper was replaced while nitrogen continued flowing into the vial. The resulting air and nitrogen in the collection vials was evacuated for 1.0 minute using a laboratory bench vacuum pump. Vials were used in conjunction with a two-way, dual-point Vacutainer multi-sample needle attached to a disposable plastic needle blood collection vial holder. The dual-point needle contains a valve that allows the user to insert this needle through the septum and into the flux chamber without venting internal gases. The valve automatically opens as the second needle penetrates the vial's rubber septum, allowing the vial to draw air from inside the flux chamber.

Gas Flux Determination. The first group of gas samples was extracted one month after initial field placement of the flux chambers. Gas fluxes from the soil were determined by taking gas samples from each flux chamber in sequence at 0-, 30-, and 60-minute timed intervals. Time-0 sample was taken immediately after the lid was placed on the flux chamber, followed by time 30- and 60-min samples (Parkin and Venterea 2010; Mosier et al. 2006). Vacutainer vials were returned to the lab within 24 to 48 hours where their atmospheres were analyzed using a gas chromatograph (GC). Gas samples in the Vacutainer collection vials can be stored for up to 5 days at 2°C prior to analysis without degradation (Rochette and Bertrand 2003).

Gas Chromatography. Gas samples (5.0-ml vial) were analyzed with a Varian®450 GC that incorporated a flame ionization detector (FID) for CH_4 analysis and a thermal-conductivity detector (TCD) for CO_2. Gases are separated by an 80/100-mesh-packed column (Mosier et al.

2006; Parkin and Venterea 2010). The GC interfaced with a Dell OptiPlex desktop computer with Windows XP® Office operating system. Varian's Galaxie® data collection software controls all GC functions including run time, and injector, oven, and detector temperatures. Galaxie software also integrates data and automatically calculates CO_2 concentration (mg kg^{-1}). Injector temperature was set at 135°C, FID at 300°C, and TCD at 120°C. Samples were analyzed using an isothermal oven temperature program at 50°C for 7.0 minutes followed by a 1.0-minute stabilizing time, totaling 8.0 minutes.

Flux Analyses. GC analysis provided the area[1] under target peaks for CO_2. Dividing the area under the target peak by the area under the standard peak provides a percentage measurement of the target peak in ppm.[2] Linear regression determined slope by ppm change over time (0 min; 30 min; 60 min). Taking the slope and multiplying by the flux chamber's headspace volume, then dividing by the soil surface area (m^2) within the chamber results in μl CO_2 m^{-2} min^{-1} (Parkin and Venterea 2010). Conversion of μl CO_2 m^{-2} min^{-1} to mg CO_2 m^{-2} h^{-1} was done according to Nicoloso et al. (2013).

[1] Area under peak = (peak height) x (width at ½ peak height)

[2] ppm = [(area under Target peak)/(area under Standard peak)] x (ppm of Standard)

Statistics. SAS Version 9.3 was used for all statistical analyses (SAS Institute, Cary, NC). Analysis of variance methods (PROC MIXED) were used to assess the combined effect of treatment and time on the response variables ppm and mg CO_2 m^{-2} min^{-1}. A repeated measures model with an autoregressive period 1 covariance structure was used with treatment as the main factor and time as the repeated factor. Simple effects of treatment given time, and time given treatment, were assessed with planned contrasts (SLICE option in an LSMEANS statement). Protected pairwise comparisons for simple effects were done (*P*=0.05). Treatments were

compared within and across all months to evaluate significance of changes in mg CO_2 m^{-2} min^{-1} emissions over time.

Results

Gas Flux Determination. During 2013, January through April CO_2 emissions were relatively low compared with May through September. In April and May, CO_2 output increased within all three treatments and peaked in June and July. Emissions began to decline in September and steadily decreased through December. However, each treatment demonstrated variability in CO_2 output (Table 1; Fig. 3). Variable emission amounts demonstrate the seasonality of CO_2 fluxes from soils and soil fauna, including possible emissions from subterranean termites. Means provided in Table 1 correlate with the trends delineated on Figure 3, and provide exact amounts of CO_2 emissions for cross-reference.

Flux Analysis. Within individual months, CO_2 flux during January, March, April, June, July, and December was not significantly different among the three treatments (P=0.2045; Table 1). However, fluxes during May, September, and October were different within months. Notably, termite-free plots enclosed within a stainless steel mesh barrier emitted the greatest amounts of CO_2 compared with termite-free baited plots and termite active plots during these three months. In addition, during May and September, both baited and stainless-steel-enclosed plots emitted significantly more CO_2 compared with termite-active plots. This indicates that cumulative CO_2 emissions due to soil microbe and other soil-dwelling fauna respiration are great enough to both exceed and obscure termite CO_2 output. For example, CO_2 fluxes during May in termite-free Treatments B and C were 16.92±2.44mg and 17.80±2.25mg CO_2 m^{-2} h^{-1}, respectively, both significantly greater than Treatment A plots at 11.6±1.78mg CO_2 m^{-2} h^{-1} that contained actively foraging termites (P=0.027). Similarly, during September, CO_2 emissions from Treatments B

and C were also significantly greater compared with Treatment A (*P*=0.0025; Table 1). However, this trend did not occur on the other sampling dates. Data from this study follow a trend set forth by Yuste et al. (2007), who demonstrated that soil carbon content as well as soil temperature and moisture significantly influence CO_2 emissions. During this study each treatment displayed an initial increase followed by a decrease of CO_2 from January through December (Fig. 3). However, these differences were generally not significant within each individual monthly evaluation.

When evaluating the nine greatest CO_2 emission readings taken during the year, Treatment C exhibited the highest CO_2 output four times (April, May, September, October), Treatment B the greatest output three times (January, March, June), and Treatment A with actively foraging termites only twice (July, December).

Comparison of CO_2 flux from termite-infested soil compared with termite-free soil did not always determine differences between treatments. Results did not clearly elucidate CO_2 contributions from termites alone. The CO_2 contributed by subterranean termites to total flux within the TGPP study sites was too small to be separately determined from overall cumulative gas emissions.

Discussion

Throughout the study, termites were active in Treatment A with the exception of May after a prescribed burn that occurred in April but returned to these plots by June. Treatments B and C remained termite-free throughout the study. However, several ant species, primarily acrobat ants, *Crematogaster* sp., (Hymenoptera: Formicidae) were observed occupying some bait stations in these treatments. Acrobat ants established a nest in one flux chamber in Treatment A, one in B, and two in C. Infested chambers were removed, cleaned, and randomly placed in a

different location within their plot. Ants could possibly affect CO_2 flux, and are known to influence greenhouse gas emissions by changing physical properties of soil by facilitating increased microbial and fungal activity (Raich and Tufekcioglu 2000; Bender and Wood 2003; Frouz and Jilkova 2008). All three treatment plots contained a few insect-free stations where the cellulosic matrix was being degraded by fungi, causing it to be less palatable to termites. Degraded matrix was replaced as needed.

Termite-free field plots emitted more CO_2 on seven of nine monthly collections, with termite-active plots exhibiting the greatest CO_2 output only twice. This result seems counter-intuitive, as plots containing foraging termites would be expected to emit comparatively greater CO_2. However, the termite-free bait plots, and the stainless steel mesh-enclosed plots, often had greater CO_2 emissions compared with plots containing termites, although these differences were usually not significant (Table 1). These differences could possibly be due to termites foraging at deeper depths at different times depending on soil moisture and temperature fluctuations. Termite CO_2 that is emitted at the deepest foraging depths below topsoil could dissipate before reaching the surface, resulting in less gas emitted into the atmosphere. This result is not clearly understood and requires further testing and evaluation.

When evaluating CO_2 emissions over the entire study period, significant differences were apparent between early-to-late winter compared with spring and summer. Overall, CO_2 emissions were greatest during relatively warm, summer months. Emissions declined to their lowest levels from October through April. During April and May, emissions began to increase until reaching significantly greater output in June, July and September (Table 1; Fig 3).

Another consideration is that Oklahoma tallgrass prairie management practices include periodic prescribed burns to aid in control of invasive weeds (Hamilton 2007). Poth et al. (1995)

showed that prescribed burning affects CO_2 amounts released from soils, and determined that CO_2 flux increases for up to a year post-burn. They also noted that termite activity decreased due to burning and loss of above-ground food resources. Knapp et al. (1998) demonstrated that spring prairie fires increased soil CO_2 emissions by 20 to 55% compared with non-burned sites on The Konza Tallgrass Prairie of Kansas. Monthly, 10.3μmol CO_2 m^{-2} s^{-1} was emitted in non-burned sites compared with burned sites at 15.1μmol CO_2 m^{-2} s^{-1}. Similarly, the area utilized for our study was intentionally burned in spring 2013, which may have influenced CO_2 emissions. Similar termite behavior was seen in our active termite plots where termites were present in monitoring stations prior to burning and absent immediately after. It required one-to-two months for termites to re-establish themselves in previously active monitoring stations. On the TGPP, naturally occurring, lightning-initiated range fires are also a regular occurrence.

Soil respiration is a significant contributor to atmospheric CO_2. Prairie and grassland soils provide suitable habitats for root biomass and microbial growth, with plant roots contributing to CO_2 emissions from soil (Larionova et al. 2006). Frank et al. (2003) noted a correlation between soil temperature, soil moisture, microbial populations, and CO_2 flux. Seasonal changes showed that CO_2 fluxes were low in early spring, peaked during June and July, and steadily decreased during fall and winter months. These observations corroborate our measurements, which exhibited a similar trend. As TGPP soil and air temperatures increased and then decreased, CO_2 fluxes followed a similar pattern.

Due to their soft bodies, termites are susceptible to desiccation and rely on moisture-rich environments for survival and proliferation. On the TGPP, rainfall is the primary contributor to soil water and is essential for microbial and root growth as well as termite survival (Suiter et al. 2009; Cook and Orchard 2008). Typically on the TGPP, April, May and June experience the

greatest rainfall, whereas October through March receives the least. However, during this 2013 study, uncharacteristically high rainfall occurred, with the wettest months being May, July, August, and October, averaging of 24.2, 22.6, 10.5, and 12.0cm, respectively. Above average rainfall could partially explain increasing CO_2 emissions during warm, wet months (Oklahoma Climatology Survey 2014). In addition, although termites were not individually counted, they were observed in larger than usual numbers (100s) in Treatment A monitoring stations when evaluations during these four wettest months were accomplished. Treatment A plots should have seen increases in CO_2 production compared with Treatments B and C. However, such increases were not apparent, suggesting other larger factors were the main contributors to CO_2 flux.

These results demonstrate that CO_2 emissions from soil originate from multiple naturally occurring sources. Many soils, whether non-grazed prairie soils, soils tilled for agriculture, or grazing pasture land, produce significant amounts of CO_2 (Sims and Bradford 2001; Mosier et al. 2006). Emission of CO_2 from soils is dependent on several variables including the amount of organic material deposited into the soils as well as the soil temperature and moisture content (Rustad et al. 2000; Yuste et al. 2007).

This study demonstrates that prairie soil respiratory dynamics, which include respiration from plant roots, micro- and macro-organisms, diverse flora and fauna, and chemical abiotic processes, produce steady, measurable CO_2 emissions. In our study the amount termites produce appears to be relatively low compared with overall CO_2-producing soil processes. Subterranean termites are one of many invertebrates that comprise arthropod diversity in TGPP soils. Because of their continuous foraging and dispersal throughout soil within a large surface area (Brown et al. 2008) and their non-homogeneous distribution in soil, accurately detecting their individual contribution alone to gas emissions from soil is difficult. Also, foraging activity may suppress

natural termite respiratory dynamics to a greater extent than the CO_2 they normally produce. This phenomenon has been shown in other soil dwelling insects, which breathe discontinuously to avoid O_2 toxicity (Hetz and Bradely 2006). This is true with subterranean termites that have O_2 sensitive anaerobic microbiota in their hindgut (Nunes et al. 1997). Measurements from this study provide normal baseline CO_2 flux emitted from TGPP grassland soil. Further studies with large flux chambers placed over known termite foraging populations and clearly delineated colony territories are needed to accurately measure termite CO_2 emissions from TGPP soil.

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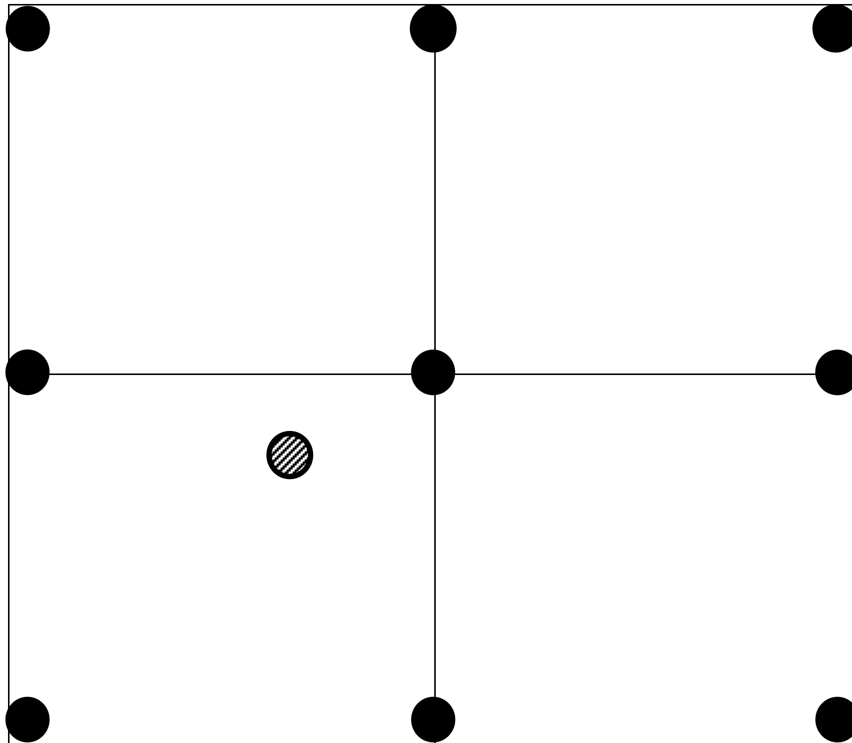

Figure 3.1. Field plot design: 15 plots total, each 3.1- x 3.1-m square. Nine cylindrical bait stations and one randomly-placed flux chamber are vertically inserted into the soil within each plot.

= Bait Station

= Flux Chamber

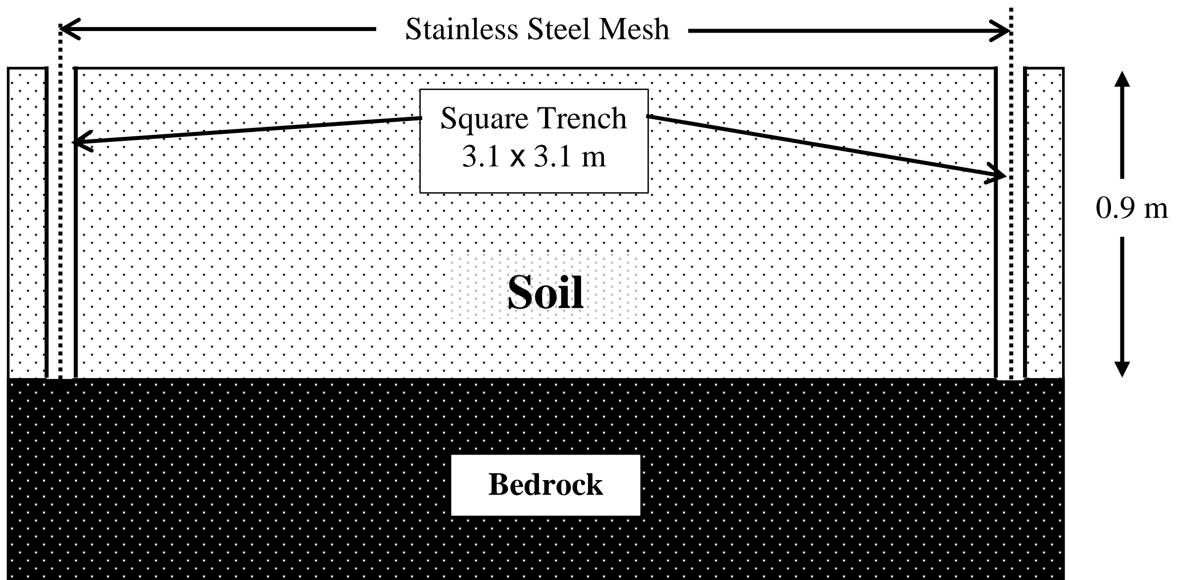

Figure 3.2. Stainless-steel-mesh-enclosed square field plot, lateral aspect.

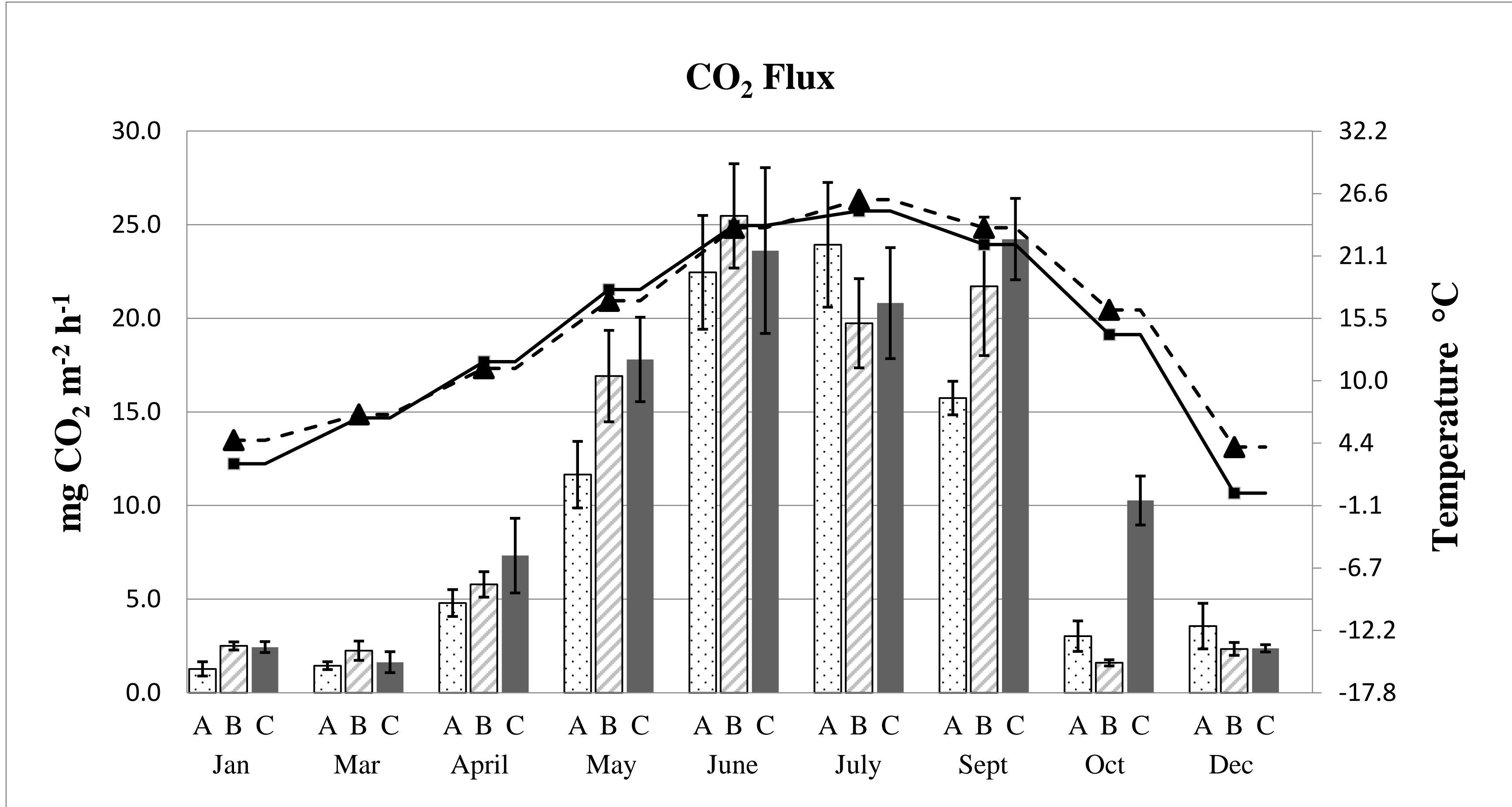

Figure 3.3. Trends in CO_2 flux emitted from tallgrass prairie soil, and average air and soil temperatures (January-December 2013)

A=Termite-active plots; **B**=Termite-free baited plots; **C**=Termite-free stainless-steel-mesh-enclosed plots
▲= Average monthly soil temperature at 5.0-cm depth
■ = Average monthly air temperature (10.0°C = 50.0°F)
Note: Soil and air temperature data source: Oklahoma Foraker Mesonet field site, located ~150 m from plots

Table 3.1. CO_2 flux emitted from tallgrass prairie soil over 12 months. Mean ± SEM.

Month	Treatment*	†mg CO_2 m^{-2} h^{-1}	
January	A-Active	1.27 ± 0.36	a
	B-Baited	2.51 ± 0.22	ab
	C-Stainless steel	2.44 ± 0.29	a
March	A-Active	1.45 ± 0.21	a
	B-Baited	2.25 ± 0.51	a
	C-Stainless steel	1.63 ± 0.56	a
April	A-Active	4.80 ± 0.72	ab
	B-Baited	5.79 ± 0.68	abc
	C-Stainless steel	7.33 ± 1.99	bcd
May	A-Active	11.66 ± 1.78	de
	B-Baited	16.92 ± 2.44	fg
	C-Stainless steel	17.80 ± 2.25	fgh
June	A-Active	22.45 ± 3.03	hij
	B-Baited	25.47 ± 4.24	j
	C-Stainless steel	23.62 ± 4.43	ij
July	A-Active	23.93 ± 3.33	ij
	B-Baited	19.74 ± 2.39	fghi
	C-Stainless steel	20.82 ± 2.97	ghij
September	A-Active	15.74 ± 0.90	ef
	B-Baited	21.71 ± 3.70	hij
	C-Stainless steel	24.23 ± 2.17	ij
October	A-Active	3.03 ± 0.82	ab
	B-Baited	1.61 ± 0.16	a
	C-Stainless steel	10.27 ± 1.30	cd
December	A-Active	3.56 ± 1.22	ab
	B-Baited	2.34 ± 0.34	a
	C-Stainless steel	2.38 ± 0.19	a

*A =Termite active plots; B = Termite-free baited plots; C = Termite-free stainless-steel-mesh-enclosed plots

† Means correlate with trends delineated in Fig. 3, and provide exact amounts of CO2 emissions for cross-reference. Means annotated with the same letter are not significantly different ($P = 0.05$)

CHAPTER IV

CO_2 AND CH_4 EMISSIONS FROM DEFINED GROUPS OF SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)

Abstract. In previous research we were not able to determine differences in carbon dioxide (CO_2) and methane (CH_4) emissions from soils on Oklahoma's Joseph H. Williams Tallgrass Prairie Preserve (TGPP) when subterranean termites, *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitidae), were present compared with termite absence from the soil. The experiment presented here was designed to determine CO_2 and CH_4 emissions from groups of specific numbers (densities) of *R. flavipes*, primarily to measure gas output from these groups foraging within enclosed laboratory test arenas. We used three groups of laboratory maintained *R. flavipes* collected from three field colonies at densities of 0, 50, 100, 150, 200, 250, or 300 individuals, and analyzed their gas output over time (t-0, t-20, t-40, t-60 min). Results show that across all sampling intervals, 300 termites demonstrated the greatest significance (P <0.0001) for CO_2 and CH_4 emissions compared with the smaller number groups at each sampling event. Similar to CO_2 emissions over time, 300 termites at time-60 emitted significantly more CH_4 than the smaller groups combined. Other groups of 50, 100, 150, 200, or 250 showed similar increasing cumulative gas emissions over time. Sampling events occurred at each of four periods, and gas emission amounts for each sample and group size were measured. Analyses showed that emissions of CO_2 and CH_4 from different group sizes at specific sampling times were significantly different. Carbon dioxide emissions at times 20-, 40-, 60-minutes were significant (P<0.0001). Time-0 groups also showed significant differences (P=0.0207).

Methane emissions between groups and sampling times were also significantly different ($P<0.0001$). Results show there are clearly measurable differences in CO_2 and CH_4 emissions between different size groups of *R. flavipes*.

Key Words: carbon dioxide, greenhouse gas, methane, *Reticulitermes flavipes*

Subterranean termites, specifically *Reticulitermes* spp. of North America, are important structural pests. However, in nature they are beneficial primary decomposers of leaf and wood litter (Su and Scheffrahn 1990; Gentry and Whitford 1982). In native grasslands such as prairies or savannas, the primary food source for subterranean termites is plant detritus. To digest this material, lower termites in the family Rhinotermitidae depend on symbiotic protozoa and bacteria in their hindgut (Inoue et al. 1997).

In general, termites have a digestion similar to terrestrial ruminants in that they can digest cellulosic material with the aid of gut symbionts, which has the potential to produce large quantities of methane (Johnson and Johnson 1995). Cook (1932) was the first to postulate that a non-identified gases found during respiratory experiments with *Zootermopsis* (*Termopsis*) *nevadensis* Hagen, (Isoptera: Termopsidae) was possibly methane or hydrogen produced by gut fauna. We now know that many termites produce CO_2, methane (CH_4), and molecular hydrogen (H_2) through digestion of cellulose by symbiotic gut bacteria/protozoa and cellulases found in salivary glands (Nunes et al. 1997; Sugimoto et al. 1998; Brune 1999; Lo et al. 2011). Carbon dioxide and acetate are formed by hydrolysis, fermenting glucose, whereas CH_4 is produced by reduction of CO_2, which are released into the atmosphere (Brauman et al. 1992).

Research by others have shown that termites, regardless of diet variations, produce CO_2 and CH_4. This includes species like *Ancistrotermes cavithorax* Silvestri (Isoptera: Termitidae) and *Odontotermes nr pauperans* (Silvestri) (Isoptera: Termitidae), both fungus-growing termites of

Africa. The *Anoplotermes* group (Isoptera: Termitidae), and *Foraminitermes* group (Isoptera: Termitidae), which are soil-feeding termites in the Danum Valley Conservation Area of East Malaysia, produce CO_2 and CH_4 (Eggleton et al. 1999; Konaté et al. 2003). However, Eggleton et al. (1999) noted that soil-feeding and soil-wood interface feeders tended to produce more methane than fungus-growing termites.

Breznak (1975) demonstrated that termites in general produce measurable CH_4, while Zimmerman et al. (1982) were the first to attempt to determine the amounts of CO_2 and CH_4 that *Reticulitermes tibialis*, a close relative to *Reticulitermes flavipes*, and *Gnathamitermes perplexus* (Isoptera: Termitidae) contribute to the atmospheric budget. Fraser et al. (1986) determined that there were many uncertainties when trying to estimate the total contribution of methane to the atmosphere by termites. The primary uncertainties are based on biomass densities and ecological region variabilities (Fraser et al. 1986).

Sugimoto et al. (2000) suggested that direct measures of termite greenhouse gases in natural settings, whether emanating from mounds or soils, offer the most reliable data. To demonstrate this most field studies on termite gas emissions have been conducted with mound-building termites in Kenya and the Northern Territory of Australia (Darlington et al. 1996; Jamali et al. 2013). Subterranean termites are cryptic in nature and not evenly distributed in soil, making it difficult to conduct in situ observations of behavioral or physiological processes (Shelton and Appel 2001; Dawes- Gromadzki 2003).

Regression analysis is commonly used to evaluate CO_2 and CH_4 flux from soils and termite mounds in South Sudan savannas of Burkina Faso in West Africa as well as Kenya (Darlington et al. 1997; Brummer et al. 2009). The objective of this laboratory experiment was to compare CO_2 and CH_4 emission rates over time from different specific group numbers of *Reticulitermes*

flavipes Kollar that originated from three colonies. Our second objective was to develop a model of termite CO_2 and CH_4 emissions using regression analysis.

Materials and Methods

Insects. This experiment utilized three colonies of field-collected subterranean termites, *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitidae), reared in 18.9-liter (5.0-gallon) galvanized steel trash containers. Termites were provisioned with eight pieces of *Pinus radiata* D. Don measuring 1.9-cm thick x 14-cm wide x 20.3-cm long, with a sand/vermiculite artificial substrate maintained at 26% moisture (Kard et al. 2003; Konemann et al. 2014). *Pinus radiata* boards were submerged in laboratory R/O water for 10-minutes, removed and allowed to air dry for one hour prior to placing in the rearing container. Average relative humidity (Rh) in the containers was 82.3%. The total number of termites needed from each colony was 4,200 mature workers and 140 soldiers.

Treatments. Six test groups from each colony consisted of 50, 100, 150, 200, 250, and 300 worker termites plus five soldiers each. Each set of six groups was replicated four times for each colony. Time was also used as a treatment, which included collecting gases at 0-, 20-, 40-, and 60-minute events for each replicate.

Foraging Substrate. An artificial foraging substrate was made using a 10:1 ratio of well-mixed clean white sand and vermiculite. The dry mixture was sterilized in an autoclave for 30 min prior to adding water. Sterile deionized water was added at 350ml per 1,000g of dry mixture, which yielding 26% (w:w) moisture content. This mixture provides an ideal foraging environment. Actual moisture content was determined to be 25.5% ± 0.17% via the oven drying method (Konemann et al. 2014).

Food Source. Clean *Pinus radiata* sawdust was collected from table saw waste. The table saw was thoroughly cleaned prior to cutting wood that produced sawdust. The sawdust was brought to the lab and passed through a series of sieves with apertures of 2.0-mm (#10), 1.4-mm (#14), and 0.6-mm (#30) (ASTM E11-15, 2014). Only the material that passed through the 2.0-mm and collected on the 1.4-mm sieve was used, ensuring uniformity of food source.

Bioassay Arenas. Twenty-eight 0.71L (1.5 pints) wide-mouth canning jars with lids and sealing rings were needed for the bioassay. Lids were modified to incorporate red butyl septa by punching a 12-mm hole into the lid center, and inserting the septa into the hole. Air-tightness of the septa was tested by applying positive air pressure to the jar with the lid and ring emplaced and tightened. A solution of water and dish soap was applied to the septa and inspected for bubble. If the soapy solution bubbled, the lid was discarded and replaced with one that passed the air-tight test.

Glass jars were autoclaved for 20 minutes to reduce bacterial and fungal contaminates. Once jars cooled to room temperature, 2.5g of *P. radiata* sawdust was added to each jar, and covered with 100 g of foraging substrate. The substrate was not compacted, but evenly spread over the *P. radiata* sawdust to a uniform depth of 3.5mm. The substrate remained non-compacted to enable gases produced by the termites to flow to the surface with minimal restriction. Appropriate numbers of termites were added to each arena. The arenas with termites added were placed in the experimental room where the air temperature and humidity were maintained at 23.3^{o}C and 75% Rh, respectively. Prior to gas sampling, the termites were allowed to acclimate and begin actively foraging in the bioassay arenas for seven days.

Gas Sampling Vials. Gases from *R. flavipes* was collected using 20-ml crimp top vials (Thermo Scientific part # 60180-506; Rockwood, TN) capped with 20-mm grey butyl stopper (Thermo Scientific Part # 60180-744) and 20-mm aluminum crimp ring (SUN-SRI part # 500-334;

Rockwood, TN), then crimped using a Wheaton™ 11-mm crimper (Fisher Scientific Part # 06-451-456). Once a vial was crimped, air was evacuated using a Duo-Seal vacuum pump (Model # 1405; Sargent-Welch Scientific Company, Skokie, IL). This method was modified from Rochette and Bertrand (2003)

Gas Sampling. Gas samples were extracted at 0, 20, 40, and 60 minutes. To ensure that samples were taken at the exact specified time, sampling was started at the sampling period (0, 20, 40, 60), then staggered two minutes for each of the populations. For example, Time 20 was taken at 20 minutes for zero termites, 22 minutes for the 50 termite group, 24 minutes for the 100 termite group, 26 minutes for 150 termite group, 28 minutes for 200 termite group, 30 minutes for 250 termite group, and 32 minutes for 300 termite group. This left eight minutes remaining to set up vials for the 40-minutes sampling period, then each gas sampling time was staggered like the 20-minutes sampling period. This was then repeated for the 60-minute sampling period (Table 1.). This procedure guaranteed samples were taken exactly 20-minutes between each termite group size for each sampling period.

Sampling vials were placed vertically at a 90° angle in plastic holders made from a 50ml centrifuge tube, over a two-way Eclipse blood collection needle (Part #EF 2392A; Diagger Scientific, Vernon Hill, IL). One end of the needle was inserted into the bioassay arena lid's septa while the opposite end was supported by the plastic holder directly under the center of the vial septa. This end had a silicone covering that acted as a valve, creating a seal until the sample was extracted. Sampling was conducted by depressing each vial onto the silicone covered end of the blood collection needle and held for 10 seconds. After removing the vial from the holder, the needle's silicone covering reformed a seal over the needle, preventing further gas loss. Once the sampling was complete, the vials were transferred to a gas chromatograph for-analysis.

Gas Chromatography. Analysis was conducted using a Varian 450 gas chromatograph (GC), dedicated to greenhouse gas analysis. Each gas sample was injected on the GC column (WCOT fused silica, 10M x 0, 53mm i.d., CP-SIL, 5CB Coating) via auto-sampler. The GC consisted of three detectors, each for a specific gas. Methane was analyzed via a flame ionization detector (FID), CO_2 was analyzed by thermos-coupled detector (TCD), and NO_2 (not recorded for this experiment) could be analyzed using an electron-capture detector (ECD) when needed.

Statistics. Statistical analyses were conducted with SAS Version 9.4 (SAS Institute, Cary, NC). Mixed model analyses of variance (PROC MIXED) were conducted to assess effects of the factors 'termite number' and 'time' had on CO_2 and CH_4 response. A repeated measures model with time as the repeated factor was used. Colony designation was considered a random effect. The overall simple effects of number of termites given time were assessed with a SLICE option in an LSMEANS statement. The linear relationship of CO_2 to the number of termites for each level of time were evaluated with planned linear contrasts. A randomized complete block model with repeated measures was utilized with replicate and colony as blocking factors, number of termites as the main unit factor, and time as the repeated measures factor. Simple effects of time given number of termites and number of termites given time were estimated and tested on least square means.

Results

Results of this experiment show that population and time have a significant effect on CO_2 (F=8.62; df = 27; P<0.0001) and CH_4 (F = 28.93; df = 27; P = <0.0001) emissions from *R. flavipes*. Table 2 shows the overall effect that the combined three termite populations and time has on termite CO_2 and CH_4 emissions and the effect population has at specific times, in this case-samples extracted at 0, 20, 40 and 60 min. Data show that small populations can produce detectable CO_2 and CH_4. Interestingly, at time zero as population within each group

increased, so did variation in CO_2 output (Table 1). As time increases there is a high degree of variability between different populations. Figure 1 shows the relationship of CO_2 emitted by different populations of termites within each sampling time. Overall, both Table 2 and Figure 1 show that larger populations of termites have the greatest statistical significance compared with smaller group sizes at different times.

Emissions of methane by the three combined termite colonies is somewhat more simple in respect to statistical significance where the effect of population over time is concerned. Table 1 shows that as time and population size increase, there is a higher degree of statistical significance between each population. Similarly, Figure 2 shows no significance between and populations at time zero. Also, each population at each sampling time shows greater significance.

Amounts of CO_2 produced by termites was variable, including at time zero. Only control replicates with no termites present across time were not significantly different from each other, giving a clear indication that CO_2 emission amounts are influence by population number. Similar to CO_2 results, CH_4 emissions from larger population numbers showed the highest significance.

Discussion

The variation in CO_2 emissions from termites in this study can be explained several ways. Termites, and arthropods in general, regulate CO_2, O_2 and H_2O through respiration by opening and closing of spiracles. Researchers have shown that amounts of CO_2 released are dependent on castes within subterranean termite colonies (Shelton and Appel 2001). At the time this experiment was initiated, only mature and workers were used, with the addition of five soldier termites, to aid in maintaining a comfortable foraging environment. However, after one week and just prior to gas sampling a number of nymphs had developed in several of the foraging arenas, primarily from Colony1. Colonies 2 and 3 did not develop nymphs. Shelton and Appel

(2001) demonstrated that CO_2 can diffuse through soft termite cuticle as well as through spiracles. Also, termite CO_2 emissions amounts have been shown to be species specific (Jamali et al. 2013). Shelton and Appel (2001) demonstrated the differences in CO_2 produced by *R. flavipes* and *C. formosanus*, where *R. flavipes* produced significantly more CO_2 than *C. formosanus*. Termite symbiotic microbe digestion of organic matter produces H_2, CO_2, acetate, and CH_4, with amounts of each depending on diet (Sanderson 1996). Erratic gas exchange is influenced by discontinuous gas exchange, which occurs in many terrestrial arthropods. The drywood termite *Zootermopsis nevadensis*, family Termopsidae, demonstrates this phenomenon to protect its oxygen-sensitive symbiotic bacteria by closing spiracles until it is necessary to release CO_2 to maintain low O_2 levels in the tracheal system (Lighton and Ottesen 2005). Since CO_2 comes from more than one source it is possible that this explains variability in CO_2 emissions from termites in this study.

Methane emissions from termites in this study are less erratic compared with CO_2 because CH_4 emanates from one internal source, the gut. However, similar to CO_2 production, CH_4 emission is also species dependent (Velu et al. 2011). However, this study indicates that *R. flavipes* in similar population numbers emits similar amounts of CH_4. This is shown by the similar volumes and tight standard errors in our data (Table. 4.2). Cao et al. (2010) demonstrated similar results while investigating H_2 and CH_4 emissions from *R. flavipes*, *R. virginicus*, and *Coptotermes formosanus*. The authors utilized 20 workers and sampled gases six times over 72 hours. *Reticulitermes virginicus* and *R. flavipes* showed increased CH_4 levels over time, with *C. formosanus* showing no detectable CH_4 during the 72 hours (Cao et al. 2010). There is little understanding of population dynamics of *R. flavipes* in relation to CH_4 emissions. Data presented here lays ground work for possible modelling of termite contributions to the atmospheric budget of CO_2 and CH_4.

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Table 4.1. Staggered sampling times for collecting gasses for each defined termite group.

Termite Number	*Time-0	*20 minutes	*40-minutes	*60-minutes
0	0	20	40	60
50	2	22	42	1:02
100	4	24	44	1:04
150	6	26	46	1:06
200	8	28	48	1:08
250	10	30	50	1:10
300	12	32	52	1:12

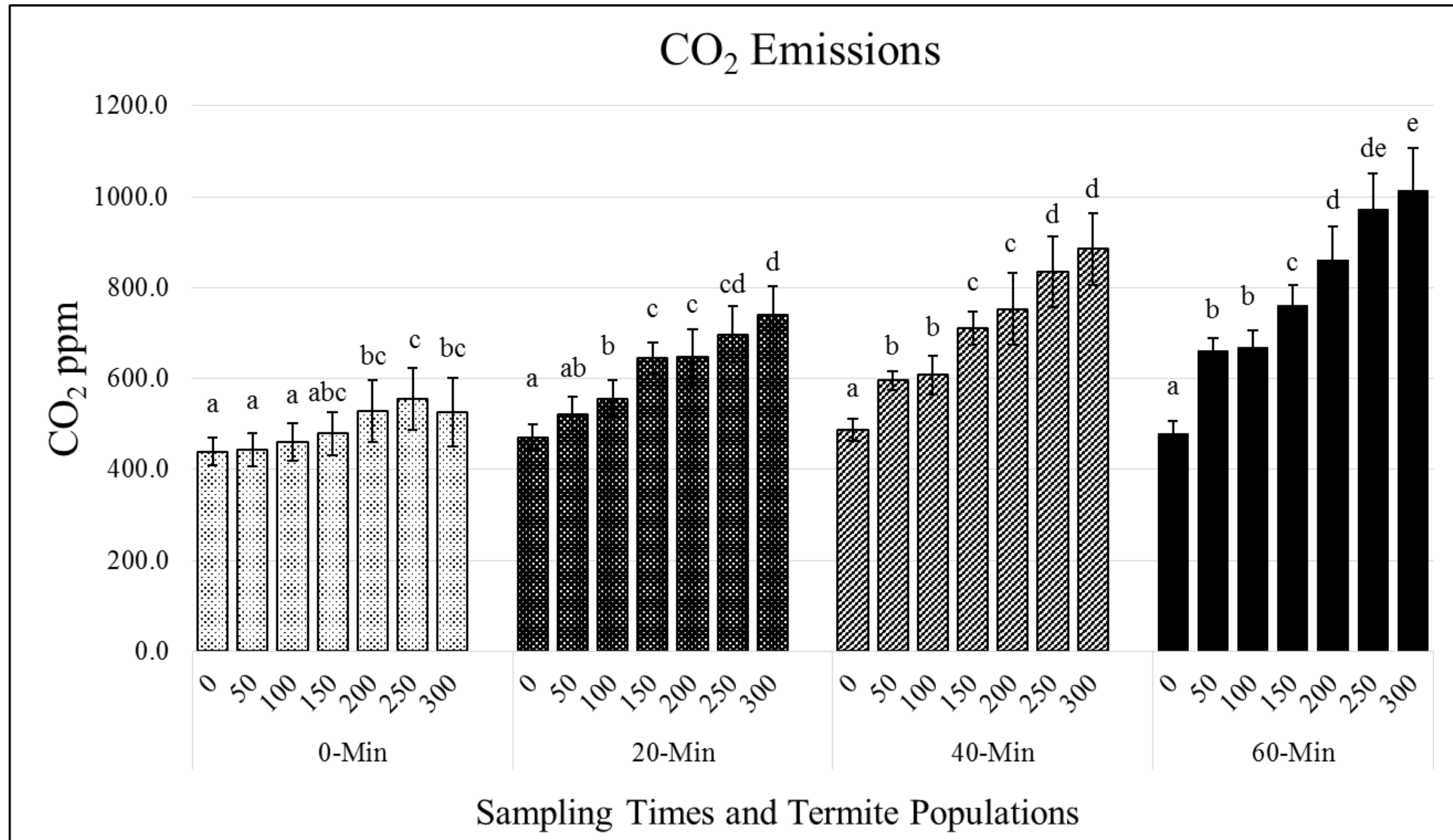
*Staggering sampling times for each group at two minute intervals ensured samples were taken at 20 minute intervals. The minutes for each termite number show the time at which each sample was taken after the initial sample.

Table 4.2. CO_2 and CH_4 ppm emissions over time from a defined number (population) of *R. flavipes*. Mean ± SEM.

Time Minutes	Population	CO_2*	CH_4*
T-0	0	439.4 ± 76.0 a	2.57 ± 0.29 a
	50	442.6 ± 67.9 ab	2.87 ± 0.29 a
	100	459.9 ± 41.1 ab	2.63 ± 0.32 a
	150	478.9 ± 47.7 abc	2.73 ± 0.30 a
	200	528.0 ± 68.2 abcd	2.65 ± 0.32 a
	250	556.2 ± 67.9 abcde	2.66 ± 0.33 a
	300	526.1 ± 76.0 abcd	2.61 ± 0.32 a
T-20	0	471.4 ± 28.8 abc	2.75 ± 0.27 a
	50	522.2 ± 37.7 abcd	3.40 ± 0.35 ab
	100	555.1 ± 41.2 abcde	4.03 ± 0.45 abc
	150	644.8 ± 33.9 defg	5.01 ± 0.45 bcd
	200	648.2 ± 61.2 defg	5.36 ± 0.59 bcde
	250	697.5 ± 62.9 efgh	6.21 ± 0.59 de
	300	739.9 ± 63.8 fghij	7.25 ± 0.59 ef
T-40	0	487.8 ± 24.6 abc	2.48 ± 0.30 a
	50	595.5 ± 20.7 bcdef	4.33 ± 0.37 abcd
	100	607.7 ± 41.2 cdefg	5.34 ± 0.62 bcde
	150	711.1 ± 36.8 fghi	7.04 ± 0.78 ef
	200	753.2 ± 78.4 ghij	8.32 ± 1.13 fg
	250	834.7 ± 77.9 hijk	9.88 ± 1.34 gh
	300	885.1 ± 79.0 jkl	12.46 ± 0.91 jk
T-60	0	479.1 ± 27.1 abc	2.87 ± 0.29 a
	50	660.9 ± 27.7 defg	5.87 ± 0.25 cde
	100	669.4 ± 35.5 defg	7.12 ± 0.36 ef
	150	760.0 ± 45.8 ghij	8.62 ± 1.09 fg
	200	860.0 ± 73.5 ijk	11.35 ± 1.06 hi
	250	972.0 ± 79.7 kl	14.15 ± 1.46 l
	300	1014.2 ± 93.2 k	16.48 ± 1.30 k

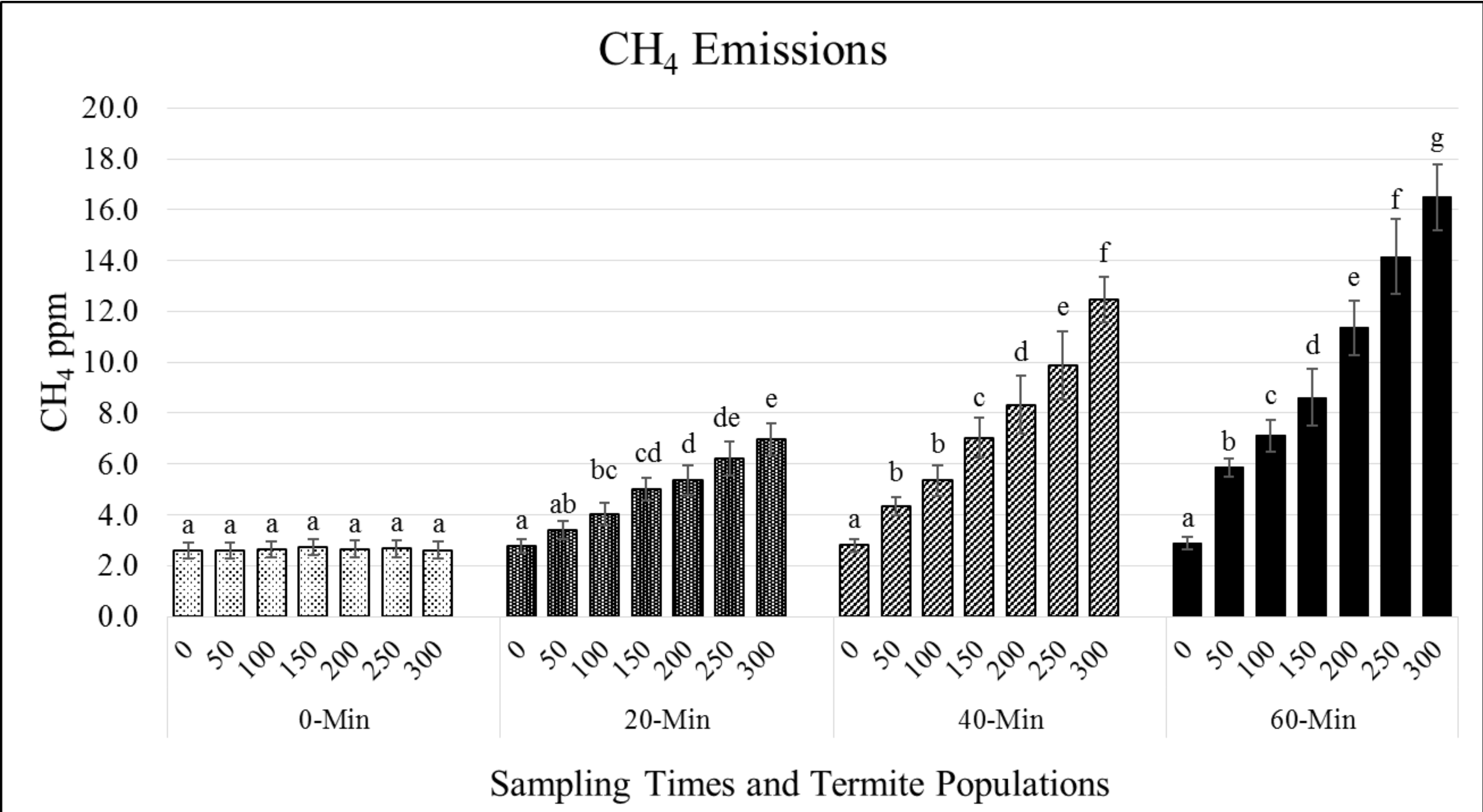
*CO_2 and CH_4 ppm means with the same letter within each column are not significantly different. P=0.05.

Figure 4.1. CO_2 emissions for each termite group at each sampling event. Means ± SEM.



Means for each group at each sampling event with the same letter are not significantly different. P=0.05.

Figure 4.2. CH_4 emissions for each termite group at each sampling event. Means ± SEM.



Means for each group at each sampling event with the same letter are not significantly different. *P*=0.05.

CHAPTER V

CO_2 AND CH_4 EMMISSIONS FROM TALLGRASS PRAIRIE SOIL AND FROM FORAGING SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)

Abstract Differences in subterranean termite metabolic gas emissions have been observed in laboratory experiments**.** The primary difficulty in measuring subterranean termite gases in natural ecosystems is non-homogeneous termite distribution in soil. This experiment was designed to aggregate foraging *Reticulitermes flavipes* Kollar in chambers and measure their gas emissions in a near natural field environment. Throughout 2014 and 2015, metabolic gas emissions from soil and termites were measured by using flux chambers randomly placed within a 30.5- x 30.5-m grids on The Joseph H. Williams Tallgrass Prairie Preserve. Flux chambers were replicated ten times for each of the four different treatments. Results show that aggregated termites produced significant amounts of CO_2 and CH_4 during spring and summer months of 2014 and 2015 compared with termite-free controls. Natural, non-disturbed soils emitted no CH_4, but emitted significant amounts of CO_2 during the same period compared with sterilized soil controls. There were no statistical differences among all treatments during cold winter months. Termites contribute significant amounts of these gases to overall baseline background gases also contributed to by soil fauna and flora. Results suggest that seasonal environmental factors such as soil moisture and soil temperature affect CO_2 and CH_4 emissions from termites as well as CO_2 from soils.

Key Words Carbon dioxide, gas flux, methane, *Reticulitermes flavipes*, tallgrass prairie**,**

The Joseph H. Williams Tallgrass Prairie Preserve (TGPP) encompasses 15,700 ha of natural prairie landscape located in Osage County, northeastern Oklahoma (36º50'N, 96º25'W). The TGPP is owned and managed by The Nature Conservancy. The historic 11,800-ha Barnard Ranch cornerstone property was purchased by The Nature Conservancy in late 1989, while additional properties were subsequently purchased to bring the preserve to its current size (Hamilton 2007). The TGPP consists of a variety of grassland, forested, cross-timbers, riparian wetland, and disturbed habitats. Known flora for these areas consists of 763 species in 411 genera and 109 families, with 12% of these being non-native to Oklahoma. Approximately 90% of the native flora consists of Big bluestem (*Andropogon gerardii*), Indian grass (*Sorghastrum nutans*), Tall dropseed (*Sporobolus compositus*), switchgrass (*Panicum virgatum*), and Little bluestem (*Schizachyrium scoparium*) (Palmer 2007). There are strong interactions between soil invertebrates and microbes, plant growth, and prairie soil respiration (Seastedt et al. 1988; Sims and Bradford 2001)

Globally, wood-feeding subterranean termites inhabit many ecosystems and are important recyclers of cellulosic material. During recycling they influence soil fertility, organic matter and moisture content, and soil classification. Similar to other soil-dwelling arthropods like ants, termites act as soil engineers, altering soil physical characteristics by tunneling and excavating soil and moving it from lower soil horizons to the surface (Jouquet et al. 2006). This process increases soil fertility, enhances the water percolation rate and soil water-holding capacity, and influences rhizosphere nutrient cycling and root dynamics (Whiles and Charlton 2006; Frouz and Jilkova 2008). Termite activities in soil contribute to heterogeneity of both aerobic and anaerobic soil microbes (Lavelle et al. 1992).

Pierce and Sjogersten (2009) estimated that ≈10% of atmospheric CO_2 comes from soil. This amounts to ten times greater output than CO_2 produced by fossil fuels (Raich and Potter 1997). Nutrient rich prairie soils in temperate regions of North American constitute one of the largest sources of CO_2, N_2O and CH_4 originating from aerobic and anaerobic bacteria deposited by soil-dwelling invertebrates such as earthworms and micro-and macro-arthropods, which includes termites (Panzer 2002; Smith et al. 2003; Hendrix et al. 2006). Microbial digestion of organic matter produces large quantities of CO_2 into the atmosphere by diffusing from the soil surface-atmosphere interface (Kern and Johnson 1993; Kuzyakov 2006). Plants directly affect soil respiration through root respiration, and indirectly by deposition of detritus within soil and on the soil surface that soil microbes feed upon (Raich and Tufekciogul 2000).

The TGPP is home to *Reticulitermes flavipes* and *R. hageni,* both found in the cross-timber areas adjacent to open prairie. Smith et al. (2012) estimated *R. flavipes* foraging populations were 103,193 (±7,081) to 422,780 (±19,297), with *R. hageni* foraging populations ranging from 44,170 (±4,879) to 207,141 (±9,190). Brown et al. (2008) estimated foraging populations from 10,000 to 180,000 foragers on the TGPP proper, with estimated foraging territories ranging from 9.0 to 92.3 m^2.

Cook (1932) was the first to postulate that a gas, which he hypothesized was methane, was produced by the degradation of cellulosic material by symbiotic prokaryotes in the termite hindgut. Methane is produced in hindgut of termites by the fermentation of glucose as cellulose is decomposed by gut microorganisms. The CO_2 and H_2 produced are further converted to CH_4 and acetate by anaerobic bacteria (Sugimoto et al. 1998). This methane arises from processes of the methanogenic *Archaea,* organisms that catalyze the reaction $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$. Other microbes are responsible for acetogenesis and catalyze the reaction $4H_2 + 2CO_2 \rightarrow$

$CH_3COOH + 2H_2O$, which also occurs in the termite hindgut. This demonstrates that competition occurs for the same H_2 molecules required for both reactions, and that feeding habits of termites determine within which pathway the H_2 molecule is utilized most (Leadbetter and Breznak 1996). Wood-feeding termites such as *R. flavipes* were found to produce more acetate than CH_4 during the fermentation processes in the hindgut (Brauman et al. 1992).

Early research that indicates termites are a potentially large source of greenhouse gases is a currently debated issue. The first large comprehensive study on termite-produced greenhouse gases (CH_4, CO_2, CO, H_2) suggested they are a significant source of the overall budget of these trace gases in the atmosphere (Zimmerman et al. 1982). From laboratory experiments it was estimated that the lower termite *Reticulitermes tibialis* Banks, and higher termite *Gnathamitermes perplexus*, had the potential to produce 0.111 and 0.310mg CO_2 per termite per day, respectively, and that *R. tibialis* produced 0.794mg CH_4 per termite per day, while *G. perplexus* produced 0.397mg CH_4 per termite per day. They also estimated that both species combined contributed 5×10^{16}g of CO_2 and 1.5×10^{14}g CH_4 per year to the global atmosphere. Rasmussen and Khalil (1983) estimated that methane produced by termites is at least 50 times higher than reported by Zimmerman et al. (1982). Fraser et al. (1986) estimated that termites produce less than 5% of the total global $CH_4 + CO_2$ combined budget, based on the high degree of variation in termite feeding preferences. Sieler et al. (1984) conducted CO_2 and CH_4 flux studies with six termite species, *Hodotermes*, *Macrotermes*, *Odontotermes*, *Trinervitermes*, *Cubitermes* and *Amitermes*, from a broad leaf savannah in South Africa, and found that flux rates varied according to genus. Martius et al. (1993) determined that CH_4 emitted from wood-feeding *Nasutitermes* spp. in Amazon forests was dependent on termite biomass, which varied from each experiment location, and estimated that termites produced only 5% of global methane.

An abundance of soil-dwelling organisms, including termites, collectively produce normal atmospheric background CO_2. The objective of the research presented here is to determine the contribution of termite-produced metabolic gases to total normal background gases emanating from soil on the TGPP.

Materials and Methods

Study Site. The TGPP is located between 36.37º and 39.90ºN latitude and 96.32º and 96.49ºW longitude in northeast Oklahoma, and encompasses 15,410 ha of land, 90% consisting of grasslands populated predominately by *Andropogon gerardii*, *Sorghastrum nutans*, *Panicum virgatum,* and *Schizachyrium scoparium*. A linear band of mixed hardwood forest cross-timbers, originating in Kansas and ending in Texas, consisting predominately of two oak species, *Quercus stellata* and *Quercus marilandica*, runs north-to-south across eastern TGPP. Riparian areas bordering several creeks contain a diverse assortment of xeric hardwoods (Palmer 2007). This research site is subject to yearly prescribed burning.

Experimental Design. This experiment utilized a completely randomized grid with 81 interior intersections, 3.0-m on center. The 'checkerboard' grid consisted of nine rows both horizontally and vertically, with each intersection representing one of 81 station location points, of which 40 were used. Each treatment's 10 flux chamber replicates were individually randomly assigned to one of the 81 intersections.

Treatments. There were four treatments. **(1)** Termite active stations (Treatment A); **(2)** gas monitoring of non-disturbed natural soil (Treatment B); **(3)** Sterilized soil controls (Treatment C); and **(4)** Termite excluded control (Treatment D).

Flux Chambers. Flux chambers were modified from Parkin and Venterae (2010), and constructed from 3.78-liter metal paint cans measuring 18.8cm tall x 16.0cm diameter, with an

epoxy coating on the interior surfaces to prevent corrosion. Chamber bottoms were removed to allow for influx of gases from the soil. Lids were modified to include a sampling septum as described by Mehra et al. (2013) and Dyksjtra et al. (2009). This allowed for interior atmosphere sampling and also accommodated a vent tube to prevent pressure build-up when the lid was in place, while also allowing for increase in interior CO_2 and CH_4 concentrations (Parkin and Venterae 2010). Each sampling septum was installed by punching a 1.97-cm diameter hole in the lid and inserting a 2.0-cm diameter rubber septum (Hutchinson and Livingston 2001, Butnor et al. 2005, Livingston et al. 2006). The vent tube was constructed using 0.95-cm outside diameter (0.63-cm inside diameter) flexible copper tubing inserted through the lid and secured using a 0.95-cm-diameter bulkhead-fitting rubber O-ring to seal the insertion hole (Fig. 5.1). Once cut to 15.2-cm length, the copper tubing was bent to form a double-curved 'C' shape in a manner to allow insertion into the bulkhead fitting and through the rubber O-ring, and also position the open ends of the tube approximately 5mm above and below the lid surfaces (Figs. 5.1, 5.2).

To minimize disturbing the soil, soil monitoring flux chambers were inserted into the soil by tracing the bottom edge of the cylindrical chamber onto the soil surface and vertically cutting into the soil along the traced edge line with a curved, serrated-edge trowel down to a depth of 7 cm. The chamber was then inserted into the cut soil by gently tapping on the chamber top edge with a rubber mallet until the chamber bottom edge reached 7-cm deep. Soil was then firmly packed against both the interior and exterior walls of the chamber at the soil surface-wall interface to create a tight seal.

Gas Collection. Methods for sampling and storage of gases collected were modified from Rochette and Bertrand (2003). Glass vials used for extracting gas samples each consisted of a

20ml crimp-top vial with a 20mm grey butyl septa and 20mm aluminum crimp ring seal (ThermoScientific, Rockwood, TN, part #60180-506, #60180-744, and #500-334). The 20ml vials were prepared as follows. The grey butyl septum was placed on each vial, followed by the aluminum crimp ring seal using a manual crimping tool (ThermoScientific, Rockwood, TN, part #C4020-100). The atmosphere inside the collection vials was evacuated for 0.30 minutes using a Welch Duo-Seal® vacuum pump (Sargent-Welch Scientific Co., Skokie, IL. Model #1405). Vials were used in conjunction with a two-way, dual-point Vacutainer Eclipse blood collection needle (Daigger Scientific, Vernon Hills, IL, Product #EF2392B) attached to a modified 50ml conical centrifuge tube that acted as a holder for the vial. The longer needle of the dual-point needle is inserted into the flux chamber's septum, the shorter needle is covered by a butyl rubber sheath. This sheath allows the user to insert this needle through the septum and into the flux chamber without venting the internal chamber gases, but separates as the needle penetrates the vial's rubber septum, allowing the vial to draw air from inside the flux chamber. Then needle reseals when the vial is removed from the holder.

Gas Flux Determination. The first group of gas samples was extracted one month after initial field placement of the flux chambers. Flux of gases from all plots were determined by extracting gas samples in sequence at 0-, 30-, and 60-minute timed intervals from each flux chamber. Time-0 sample was taken immediately after the lid was placed on the flux chamber, followed by time 30- and 60-minute samples (Parkin and Venterea 2010; Mosier et al. 2006). Crimp-top sampling vials were returned to the lab within 24- to 48-hours, where the air samples were analyzed using a gas chromatograph (GC). Gas samples in the collection vials can be stored for up to five days at 2°C prior to analysis without degradation of the sample (Rochette and Bertrand 2003).

Gas Chromatography. Gas samples were analyzed with a Varian®450 GC that incorporated a flame ionization detector (FID) for CH_4 analysis, and a thermal-conductivity detector (TCD) for CO_2. Gases were separated by an 80/100-mesh-packed column (Mosier et al. 2006; Parkin and Venterea 2010). The GC is interfaced with a Dell OptiPlex desktop computer with Windows XP® Office operating system. Varian's Galaxie® data collection software controls all GC functions including run time, and injector, oven, and detector temperatures. Galaxie software also integrates data and automatically calculates CO_2 and CH_4 ppm. During analyses, injector temperature was set at 135°C, FID at 300°C, and TCD at 120°C. Samples were analyzed using an isothermal oven temperature program at 50°C for 7.0-min followed by 1.0-min stabilizing time, totaling 8.0 minutes. A 5.0ml air sample from each vial was injected into the GC for analysis.

Flux Analyses. GC analysis provided the area under target peaks for CO_2. Dividing the area under the target peak by the area under the standard peak provides a percentage measurement of the target peak in ppm. Data are reported as ppm (mg kg^{-1}). Linear regression determined slope by ppm change over time (0 min; 30 min; 60 min).

Results

Figures 5.2 and 5.3 show CO_2 and CH_4 emissions for all treatments analyzed monthly. Termites produced significantly more CO_2 during May, June, August, and October of 2014 as well as during May and July of 2015 when compared across all months ($P<0.0001$). In July 2014, termite CO_2 was not significantly different between termites and soil, but both were significantly different compared with controls. Using test of effects slices to compare the four treatments for each month showed no significance for November 2014, January, March, and December of 2015. There was significance among treatments during May, June, July, August

September and October (Table 5.1). PROC CORR (SAS Institute, PC version 9.1) analyses established that air temperature, relative humidity, and soil moisture content influenced CO_2 emissions from termites ($P>0.0569$). Similarly, Shelton and Appel (2000) determined that air temperature influenced CO_2 emissions from *Cryptotermes cavifrons* Banks, *Incisitermes tabgae* Snyder, and *Incisitermes minor* Hagen in a linear response. However, in the current study, soil temperature did not directly correlate with CO_2 emission ($P=0.0659$; Table 5.1). Controls without termites showed no correlation between CO_2 produced and air temperature, soil moisture or relative humidity ($P\geq0.1689$). Soil moisture was the only environmental factor that demonstrated a significant correlation ($P=0.0392$). Likewise, soil controls showed no significant correlation with CO_2 emission ($P=0.0789$) for air temperature, soil moisture or relative humidity. Again, soil temperature was significant ($P=0.0306$). Figure 5.1 shows the relationship between CO_2, month, and treatment over 19 months. CO_2 emission from termite-active plots in May was significantly greater compared with all other treatments and months ($P<0.0001$). However, termite-active plots and total-soil plots produced significantly more CO_2 in warmer months compared with sterilized soil, and termite-free controls during the same months. Termite-active plots and total-soil plots produced statistically similar results to controls during the same months (Fig. 5.2). On average, soil total CO_2 emissions were less than plots with aggregated termites.

Figure 5.2 shows CH_4 emissions from all four treatments for the months gas samples were extracted. These data show termites-active plots demonstrated strong significance over total-soil plots, sterile-soil and termite-free plots during May, June, July, August, ($P<0.0001$) of 2014, as well as May and July of 2015. During colder months, there were no significance differences among the four treatments ($P\geq0.1761$; Fig. 5.2). However, when all treatments were compared across 19 months, *R. flavipes* active plots emitted significantly greater CH_4 during May (data for

May 2014 and 2015, as well as July 2014 and 2015, were averaged together) compared with June, July, and August. June, July, and August CH_4 emissions from termite-active plots were significantly greater than termite-free controls for the same months as well as for other treatments during cold months (Figure 5.2). This is reflected in the correlation analysis. Of the environmental conditions, relative humidity, showed no correlation ($P \geq 0.1135$) to methane emissions from termite-active plots. However, soil temperature, air temperature, and soil moisture showed significant correlation ($P < 0.0569$) to CH_4 emissions from termite-active plots. CH_4 emissions showed little correlation to air temperature, soil temperature, soil moisture, or relative humidity ($P \geq 0.0751$; Table 5.2).

Discussion

Emission data suggest that air temperature, soil temperature, and soil moisture significantly influence subterranean termite CO_2 and CH_4 emissions from tallgrass prairie soil. Air temperatures on the TGPP during relatively warm spring and summer months ranged from 16.0 to 27.9°C, and soil temperatures ranged from 16.6 to 22.3°C. Shelton and Appel (2000) demonstrated as air temperatures increased from 20-40°C, *I. minor*, *I. tabogae*, and *C. cavifrons* produced 1.92g CO_2 h^{-1}, 1.66g CO_2 h^{-1}, and 1.62g CO_2 h^{-1}, respectively. Their results demonstrated that CO_2 output from these three species increases linearly with temperature. Also, their results corroborate emission data of Jamali et al. (2011), who described similar results for CH_4 emissions from four mound-building termite species. Their results showed a correlation with gas emissions and diurnal and seasonal climate variations, including air temperature variations in the Northern Territory of Australia.

Termite and other soil-dwelling arthropod movement and foraging activity is partly dependent on soil moisture content (Mackay et al. 1986). Being soft-bodied insects, termites are

susceptible to desiccation and rely on moisture-rich environments for survival and proliferation (Suiter et al. 2009). This is true for *Microcerotermes nervosus*, where its biomass in soil or mounds is directly related to soil moisture during wet seasons on tropical savannas (Jamali et al. 2013). Similarly, during the current study it was observed that when soil moisture was above 14.6% on the TGPP, the average number of termites present in flux monitoring chambers was greater than when soil was dry. In general, as termite numbers increased so did overall CO_2 and CH_4 emissions. Jamali et al. (2013) observed similar comparisons by showing that as termite biomass increased so did CO_2 and CH_4 flux from termite mounds and surrounding savanna soil, with large variations between mounds. On the TGPP, rainfall is the primary contributor to soil water and is essential for microbial and root growth as well as termite survival (Suiter et al. 2009; Cook and Orchard 2008). During early spring 2014, most of Oklahoma was starting to emerge from one of the worst droughts in state history, with parts of northcentral Oklahoma experiencing extreme drought (National Weather Service, NOAA). The TGPP, being located in northcentral Oklahoma, was impacted by this drought. However, by May, June, and July 2014, the TGPP received 68.8, 105.4, and 101.9mm (2.71, 4.15, and 4.01 inches) of rainfall, respectively. During August 2014, only 19.6mm (0.77 inches) of rain fell on the TGPP. During October, November and December 2014, 122.7, 37.3, and 46.5mm (4.83, 1.47, and 1.83 inches) of rainfall, respectively, was reported (Oklahoma Climatology Survey 2014). These moisture variations are reflected in the data that show both soil and termites producing relatively large amounts of CO_2 during the wettest months. During the wettest months, termites also emitted the greatest amount of CH_4. Rainfall on the TGPP during May, June, July and August 2015 was recorded as 208.3, 62.2, 117.9, and 100.3mm (8.20, 2.45, 4.64, 3.95 inches), respectively, demonstrating monthly variation in total rainfall on the TGPP. During September and October

2015 little rainfall was recorded, whereas November and December 2015 rainfall was 35.3 and 48.3mm (1.39 and 1.90 inches), respectively. During November and December 2015 rainfall was similar to summer months with 100.6 and 72.1mm (3.96 and 2.84 inches), respectively (Oklahoma Climatology Survey 2015).

In stable, tropical warm temperatures and moisture-rich environments, CO_2 and CH_4 emissions from termites could be expected to level off. However, on the TGPP with its variable rainfall amounts this was not the case during either 2014 or 2015. Weather data showed that as soil and air temperature increased during 2014 and 2015 summer months, soil moisture decreased both years (Oklahoma Climatology Survey 2014-2015). Thus, gas emissions would be expected to vary with changing soil moisture.

Root respiration is influenced by diurnal temperature changes and soil moisture content depending on plant species (Bouma et al. 1997). Liu et al. (2002) determined that both soil temperature and moisture content regulate CO_2 flux from microbes as well as root systems on a tallgrass prairie in Texas. In addition, Yuste et al. (2007) suggested that the amount of CO_2 produced by soil microbes was influenced by moisture and temperature, which regulated microbe metabolism. Also, soil temperature accounted for 80% seasonal CO_2 flux variation in a hardwood forest (Davidson et al. 1998). In another study, gas flux estimates of a grass prairie showed that soil temperature accounted for only 46% of CO_2 flux, and soil water content accounted for 26%. Roots were estimated to contribute 52% of overall CO_2 flux (Mielnick and Dugas 2000). These comparisons with our data validate that soil temperature, air temperature, and soil moisture affect CO_2 emissions from TGPP soils and its soil-dwelling termites.

In summary, this study distinguished total soil CO_2 from termite-produced CO_2, and also demonstrated that environmental factors influence greenhouse gas emissions for both total-soil plots and plots containing active termites.

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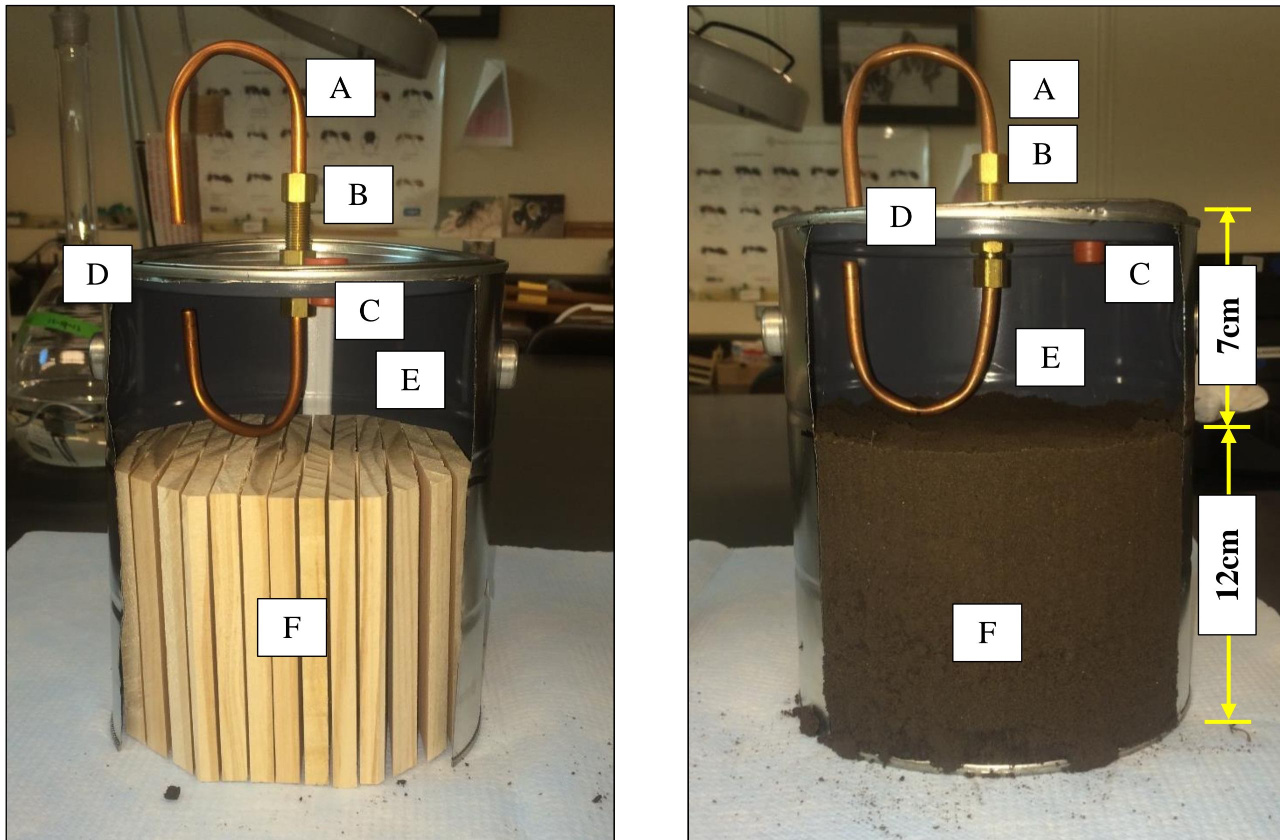
Table 5.1. Slice option results for each month

Month	F-Value	treatments *df*	ppm *df*	P-Value
January	1.10	3	410	0.3489
March	2.66	3	430	0.0476
May	401.96	3	267	<0.0001
June	40.98	3	422	<0.0001
July	56.71	3	267	<0.0001
August	19.50	3	430	<0.0001
September	5.33	3	410	0.0013
October	6.30	3	408	0.0003
November	0.65	3	408	0.5855
December	0.20	3	408	0.8941

Table 5.2. Correlation: effects of environmental conditions on CO_2 and CH_4 emissions. N=12.

	Environmental Conditions							
	Soil Temperature		Air Temperature		Soil Moisture		Relative Humidity	
Treatment	CO_2 *P*-value r	CH_4 *P*-value r	CO_2 *P*-value r	CH_4 *P*-value r	CO_2 *P*-value r	CH_4 *P*-value r	CO_2 *P*-value r	CH_4 *P*-value r
Termite gases	0.061 0.56	0.014 0.69	0.020 0.66	0.006 0.74	0.020 0.62	0.021 0.65	0.087 0.53	0.113 0.48
Soil	0.008 0.72	0.257 0.36	0.001 0.82	0.336 0.30	0.034 0.61	0.865 -0.05	0.080 0.53	0.075 0.12
Termite Control	0.040 0.60	0.358 0.29	0.079 0.54	0.313 0.32	0.012 0.97	0.382 0.22	0.0697 0.54	0.289 0.36
Soil Control	0.0306 0.62	0.3579 0.29	0.0789 0.53	0.3208 0.31	0.3511 0.30	0.2204 0.38	0.1919 0.40	0.3626 0.29

Figure 5.1. Cut-away of flux chambers containing wood billets or sterile soil.



A=Vent tube; B=Bulkhead fitting; C=septa; D=lid; E=Head space
F=In-ground portion (Wood billets, or Soil)

Figure 5.2. Stainless-steel-mesh-wrapped flux chamber (mesh-covered open bottom)

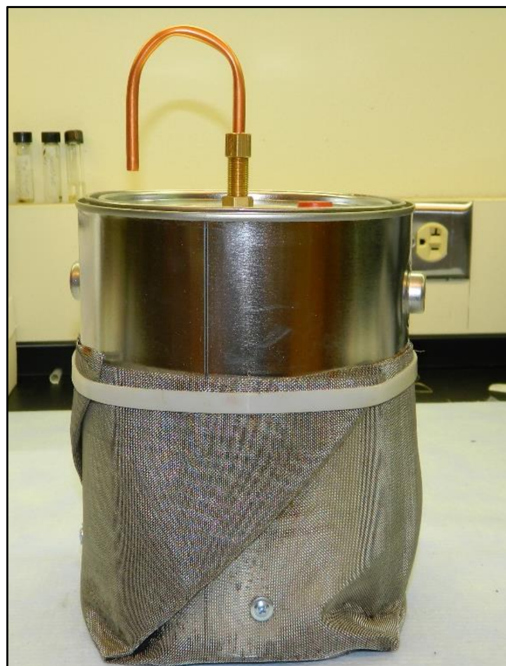
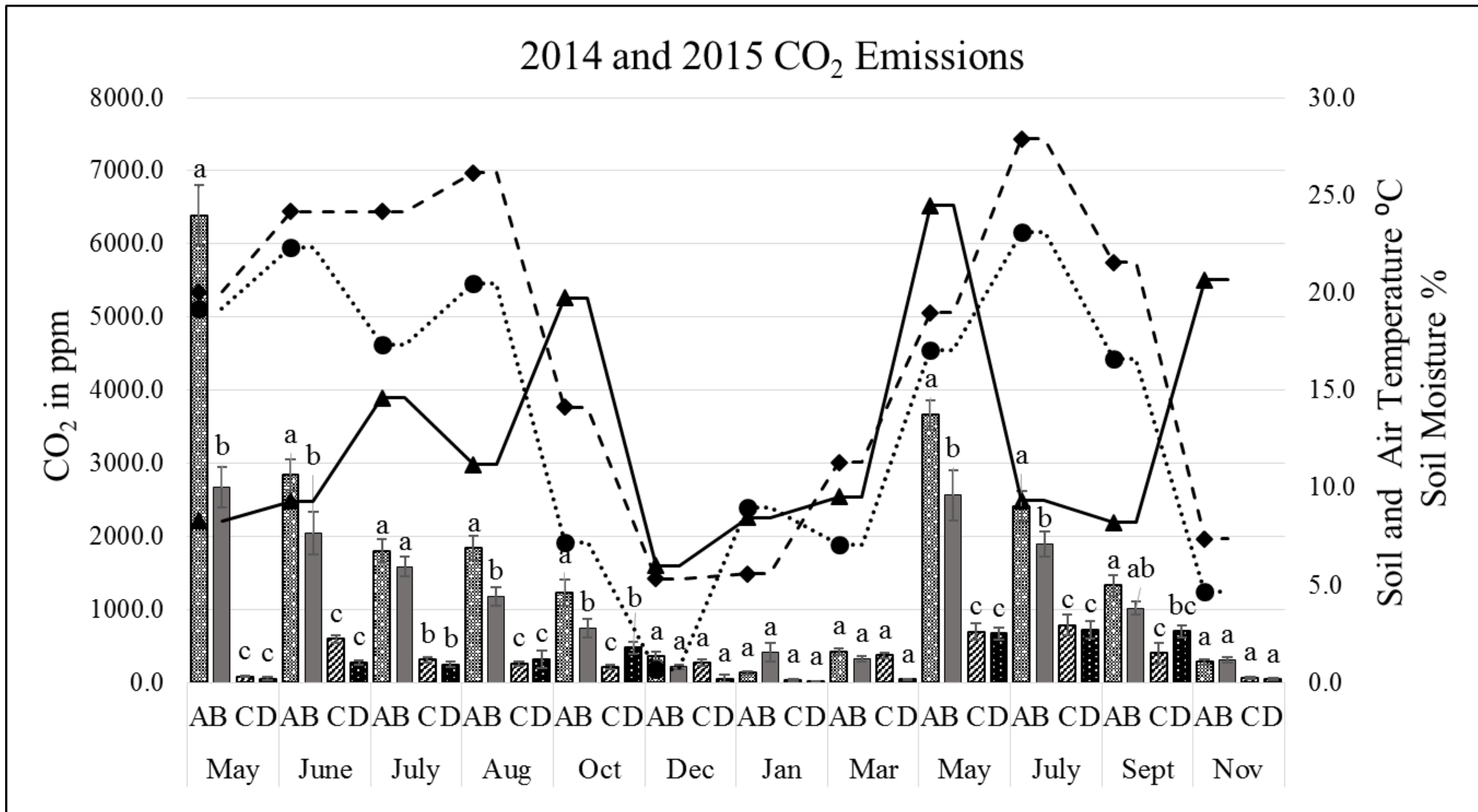


Figure 5.3. CO_2 emissions from termites and soil within months and environmental conditions. Means ± SEM

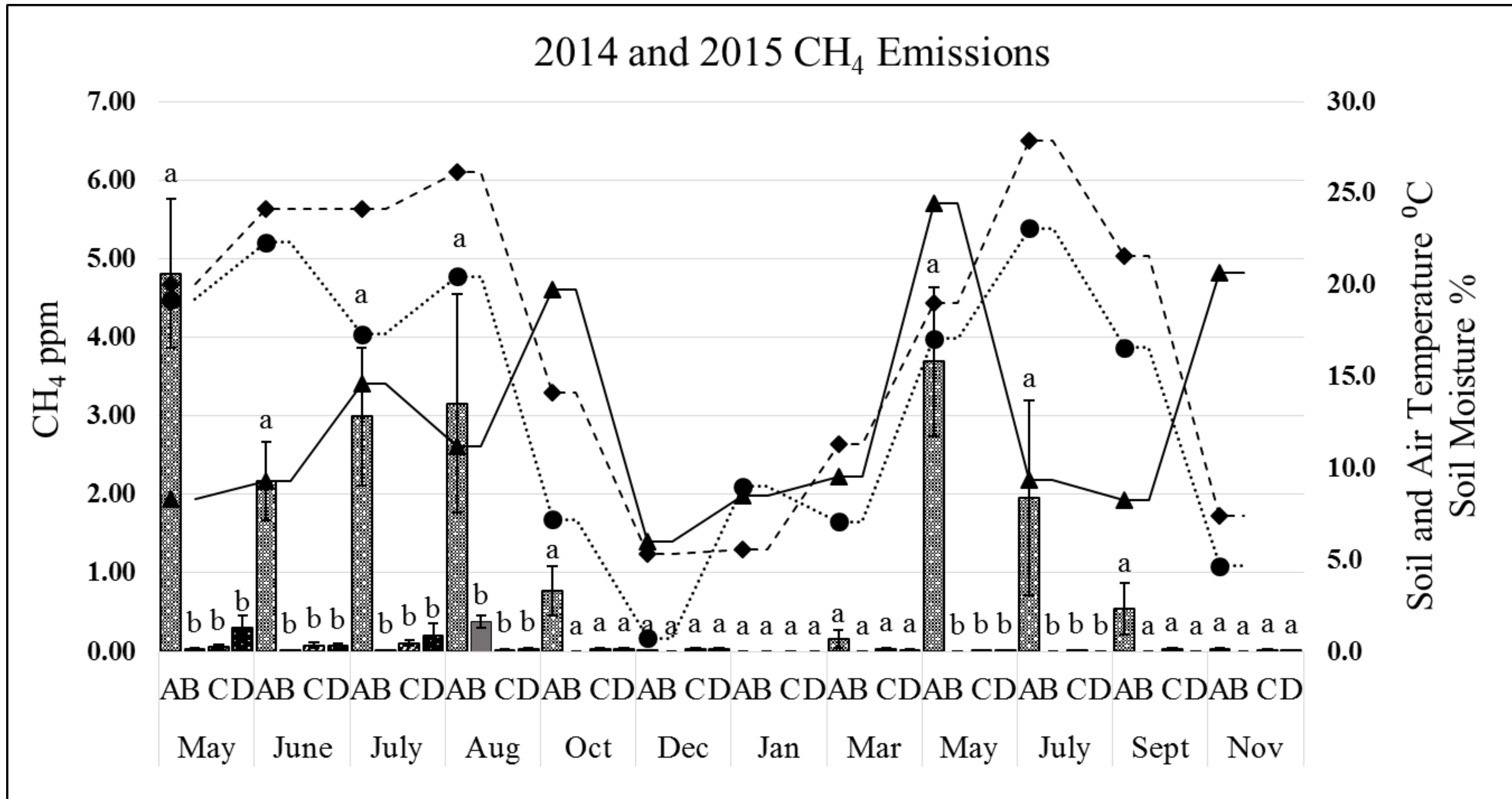


Means within each month with the same letter are not significantly different. *P*=0.05.

= Soil Temperature ºC — = Air Temperature ºC — = Soil Moisture%

= '**A**'Termite CO2 — = '**B**' Soil CH_4 — = '**C**' Termite Control — = '**D**' Soil Control

Figure 5.4. CH_4 emissions from termites and soil within months and environmental conditions. Means ± SEM

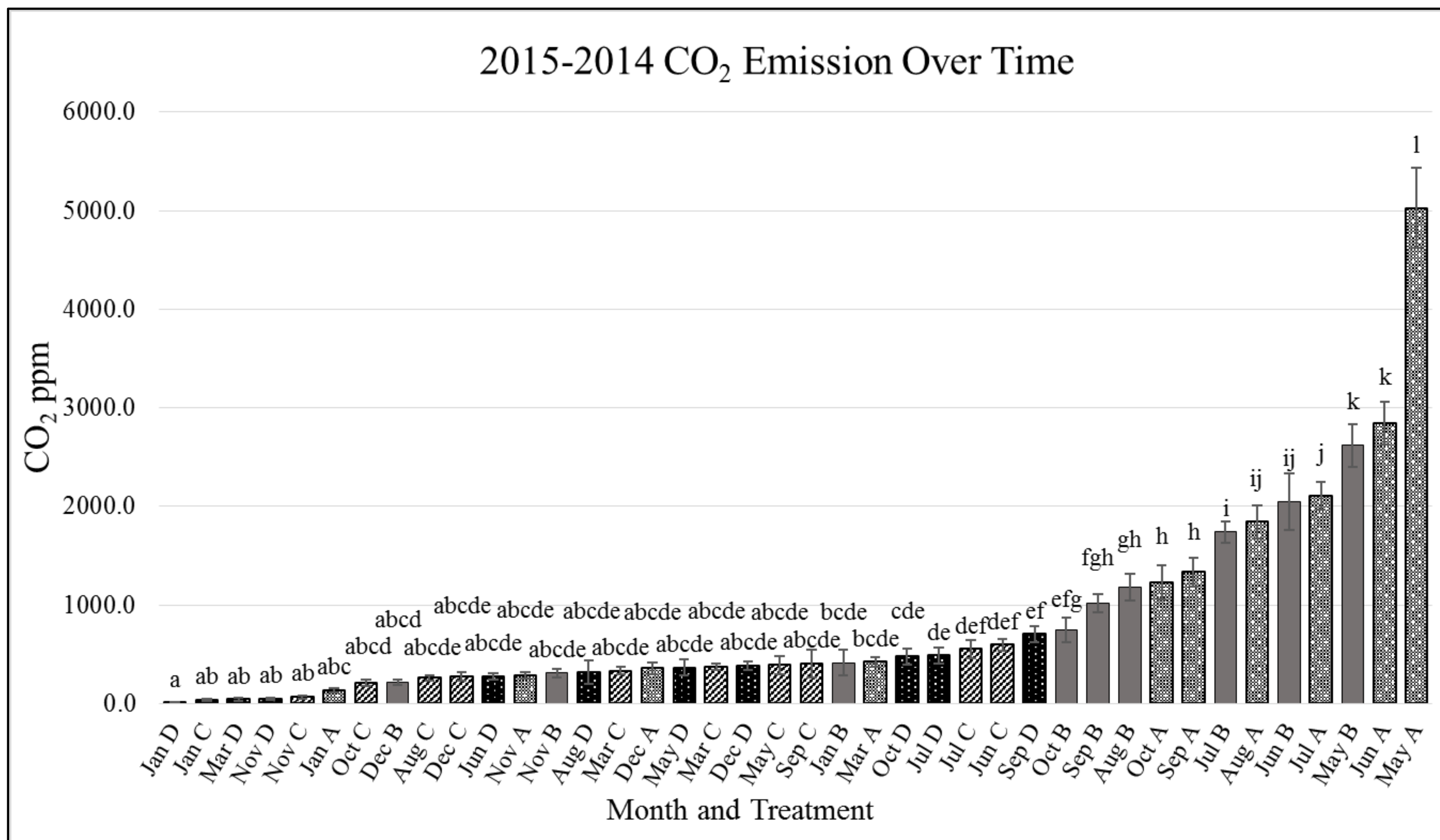


Means within each month with the same letter are not significantly different. *P*=0.05.

= Soil Temperature ºC = Air Temperature ºC = Soil Moisture%

= **'A'** Termite CH_4 = **'B'** Soil CH_4 = **'C'** Termite Control = **'D'** Soil Control

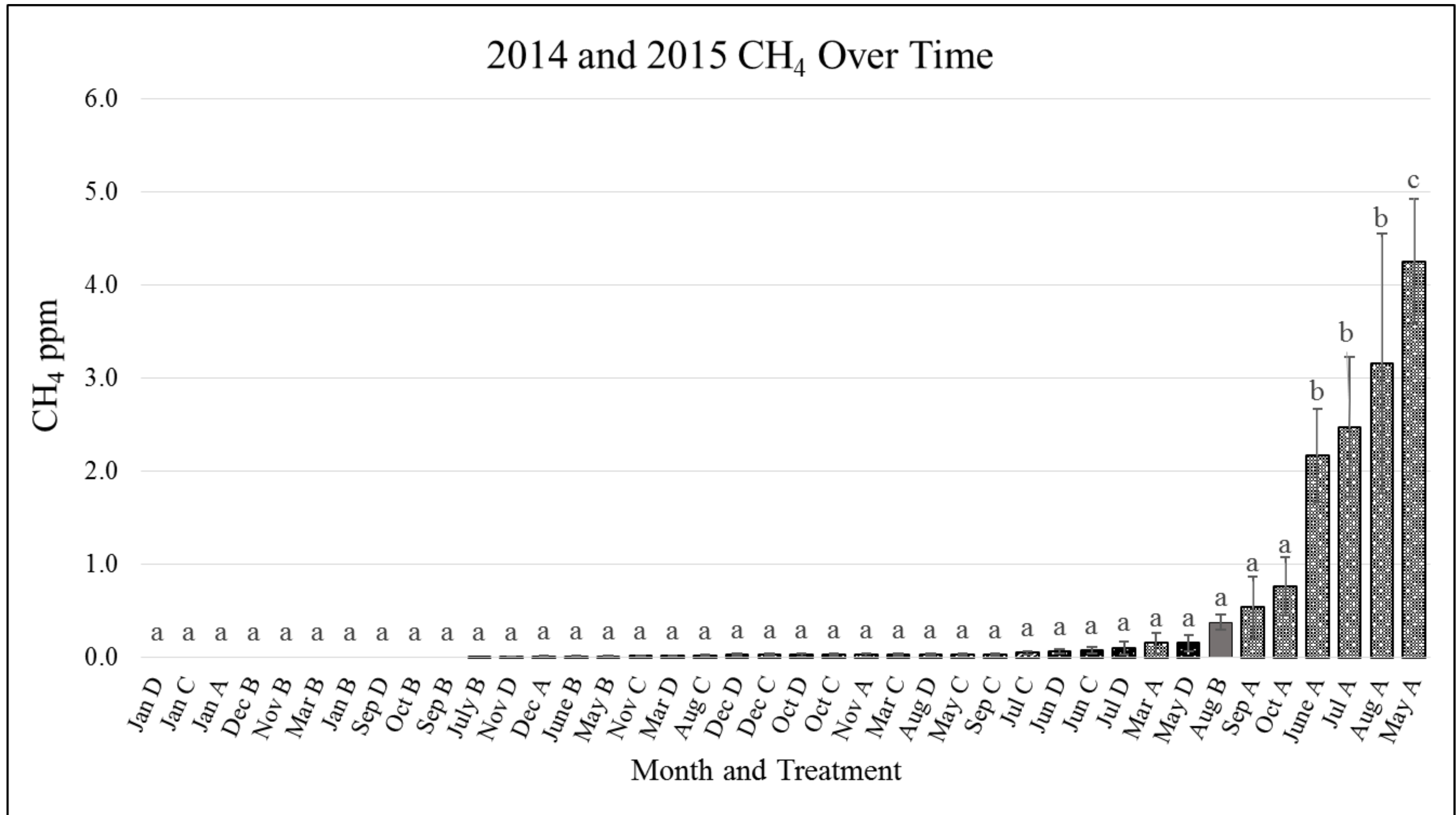
Figure 5.5. CO_2 emissions from all treatments over 19 months. Means ± SEM.



*Means with the same letter are not significantly different. *P*=0.05.

= **'A'** Termite CH_4 = **'B'** Soil CH_4 = **'C'** Termite Control = **'D'** Soil Control

Figure 5.6. CH_4 emissions from all treatments over 19 months. Mean ± SEM



*Means with the same letter are not significantly different. *P*=0.05.

= **'A'** Termite CH_4 = **'B'** Soil CH_4 = **'C'** Termite Control = **'D'** Soil Control

CHAPTER VI

SUMMARY

The production and types of metabolic gases from many species of mound-building subterranean termites have been well studied in Africa, Australia and South America (Darlington et al. 1996; Martius et al. 1996; Jamali et al. 2011). Contributions of these gases have not been well defined because subterranean termites are not evenly distributed in soil, and because of the amounts CO_2 coming from background sources such as soil microbes, plant roots and other soil-dwelling arthropods (Shelton and Appel 2001; Smith et al. 2003).

Field studies during 2013 demonstrated the complexity of discriminating between abiotic and biotic gasses generated by soil from gasses produced by termites. Results indicate that CO_2 flux during January, March, April, June, July, and December was similar among all three treatments. However, CO_2 flux in control plots during May was significantly greater compared with termite-active plots. Overall CO_2 flux from both termite-infested and termite-free plots was great enough to obscure CO_2 contributions from subterranean termites alone. Therefore, although total normal baseline CO_2 emissions from soil were measured, the specific amount of CO_2 that termites alone contributed to this flux could not be accurately determined.

When metabolic gases from laboratory-reared *R. flavipes* were studied, subtle but significant changes in CO_2 and CH_4 emissions where observed. Analyses indicated that as overall time increased so did significance of gas output between populations. For example, for gas concentrations analyzed within the 60-minute sampling period, 300 termites were significantly

different over 50 termites. However, when analyzed over time, CO_2 and CH_4 emissions from 300 termites at 60 minutes were significantly different compared with 300 termites at time zero. More importantly, CO_2 and CH_4 emissions from groups of 50 termites at 60 minutes were significant compared with 50 termites at Time-0, demonstrating that significant changes in CO_2 and CH_4 can be detected from small populations of termites. CO_2 emissions from populations within sampling times 20, 40, 60 minutes demonstrated strong significance with each having a *P* value of <0.0001. Time-0 populations showed significant differences with a *P* value of 0.0207. CH_4 emissions from populations within each group showed strong significance ($P < 0.0001$) for each sampling time. These data show that small changes in CO_2 and CH_4 from subterranean termites can be detected.

As suggested by Sugimotto et al. (2000), we have established a protocol by which direct measurements of subterranean termite metabolic gases can be made. Throughout 2014 and 2015, gas emissions emanating from termites and soils were measured on the TGPP. Foraging *R. flavipes* produced significant amounts of CO_2 and CH_4, depending on their population density. Soils emitted no CH_4 but emitted significant amounts of CO_2 during the same period compared with controls. Meteorological data indicate that termite activity is governed by environmental conditions in the soil as well as the atmosphere similar to that described by Jamali et al. (2011). Termite populations increased in flux chambers as soil temperature and moisture content rose. However, as soil moisture declined and temperatures continued to rise during hot summer months, termite populations declined. During cold months there were no termites present nor were there any metabolic gases detected. This seasonal effect on termites appears to have the same effect on soil respiration, which corroborates with that demonstrated by Yuste et al. (2007).

Increasing numbers of termites produce more CO_2 and CH_4 over time. More importantly, termites comprise just one group of contributors of a much larger community of soil organisms producing CO_2. Termites appear to be the main TGPP soil arthropod group producing detectable CH_4. It was determined that *R. flavipes* produced measurable concentrations of CO_2 and CH_4 on the TGPP. More sensitive detection equipment and improved soil gas monitoring methods are needed to better determine accurate amounts of metabolic gases that subterranean termites contribute to total normal baseline gas emissions on the TGPP.

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