Methane and carbon dioxide exchange in the tropical savannas of northern Australia: The role of termites

By

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Abstract

Termites are one of the most uncertain components of global CH_4 budget mainly because of the lack of long-term field based studies from different biogeographical regions. This thesis investigated the exchange of CH_4 and CO_2 between termites and atmosphere, and between soil and atmosphere in the tropical savannas of northern Australia.

Diurnal variations in CH_4 fluxes were measured from mounds of *Microcerotermes nervosus*, *Microcerotermes serratus* and *Tumulitermes pastinator* every four hours over a 24 hour period. There was large diurnal variation in mound CH_4 fluxes caused by diurnal temperature patterns. Mound CH_4 fluxes measured between 10:00 and 12:00 hours best represented the mean daily flux. Seasonal measurements of mound CH_4 fluxes were up to 25-fold greater in the wet season than the dry season and always greater in the wet season for all investigated species. Detailed studies in *M. nervosus* revealed that these differences were not associated with changes in environmental pattern but seasonal changes in termite mound population size. The magnitude of diurnal and seasonal variations in mound CH_4 fluxes measured in this study suggest that estimates of global CH_4 emissions from termites that do not account for such variations will contain larger errors and uncertainty.

The contribution of mound-building, hypogeal and wood-nesting termites to the CH_4 balance was estimated for a savanna woodland at Howard Springs near Darwin. Methane fluxes were measured from termite mounds and from the soil - from which CH_4 fluxes from hypogeal termites were estimated. Methane fluxes from wood-nesting termites were estimated based on known species abundance. Termites were an annual CH_4 source of +0.24 kg CH_4-C ha^{-1} y^{-1} and soils a CH_4 sink of -1.14 kg CH_4-C ha^{-1} y^{-1}. Thus, termites offset 21% of CH_4 consumed by soil methanotrophs, but overall the savanna ecosystem was a sink for CH_4 of -0.90 kg CH_4-C ha^{-1} y^{-1}.

Two indirect methods were tested to predict CH_4 and CO_2 fluxes from termite mounds. The first predicted mound CH_4 fluxes from 'easier-to-measure' mound CO_2 fluxes. The

second predicted CH_4 and CO_2 fluxes from termite mounds based on the relationship between internal mound concentrations and external mound flux. For both indirect methods the prediction errors were small when calculated separately for each species, whereas, a generic relationship or predictions between species resulted in large errors, probably associated with different mound structures for different species.

This study shows that CO_2 emissions from termite mounds are up to two orders of magnitude greater than CH_4 emissions, when expressed in CO_2-equivalents. There was large variation in both CH_4 and CO_2 fluxes from termite mounds and soil among different sites which suggests caution when scaling up fluxes from the plot or site scale to a regional or greater scale.

This study filled important knowledge gaps in the ecosystem ecology of termites and Australian savannas. This study establishes North Australian savannas as one of the few biogeographical regions where the contribution of termites to ecosystem CH_4 exchange has been investigated. The study highlights the difficulties associated with predicting CH_4 flux from termites on a biome scale, which are caused by the high temporal and species-specific variability in flux. Future studies will have to consider these issues in order to reduce the uncertainty of the role of termites in the global CH_4 budget.

Declaration

This is to certify that:

i. The thesis comprises only my original work.

ii. Due acknowledgement has been made in the text to all other material used.

iii. The thesis is less than 100,000 words in length, exclusive of tables, illustrations, bibliography, and appendices.

Hizbullah Jamali

Preface

This PhD thesis consists of six chapters three of which have been published. The major research work for these publications was carried out by me while the co-authors contributed in the form of overall supervision from experimental design to manuscript writing. The citations for the published chapters are as under:

Chapter 2

Jamali H., Livesley S. J., Dawes, T. Z., Cook, G. D., Hutley, L. B., and Arndt, S. K. (2011) Diurnal and seasonal variations in CH_4 flux from termite mounds in the tropical savannas of Northern Territory, Australia. *Journal of Agricultural and Forest Meteorology* 151 (11): 1471-1479.

Chapter 3

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Chapter 4

Jamali H., Livesley S. J., Dawes, T. Z., Hutley, L. B., and Arndt, S. K. (2011) Termite mound emissions of CH_4 and CO_2 are primarily determined by seasonal changes in termite biomass and behaviour. *Oecologia* 167 (2): 525-534.

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1 Introduction

There is unequivocal evidence that the earth is undergoing global climate change as a result of increased concentration of greenhouse gases (GHG) in the atmosphere (IPCC, 2007), although the extent and regional distribution of the effects of climate change is less certain (Dalal and Allen, 2008). Besides water vapor, greenhouse gases contribute significantly to global warming with carbon dioxide (CO_2) contributing 60%, methane (CH_4) 20%, ozone (O_3) 10% and nitrous oxide (N_2O) 6%; a minor contribution is made by chlorofluorocarbons (CFC) and volatile organic compounds (VOC) (Forster et al., 2007). Since the industrial revolution (approx. 1750 AD), the atmospheric concentration of CO_2 has increased from 280 ppm to 379 ppm, CH_4 from 715 ppb to 1774 ppb and N_2O from 270 ppb to 319 ppb in 2005 (Forster et al., 2007). Several of the major greenhouse gases occur naturally, but increases in their atmospheric concentrations over the last 250 years are due largely to human activities; other industrial greenhouse gases like CFCs are entirely the result of human activities (Solomon et al., 2007). Naturally occurring GHGs, such as CO_2, CH_4 and N_2O, are chemically stable and persist in the atmosphere for longer periods so that their emission has a long-term influence on climate (Solomon et al., 2007). On a 100 year time horizon, the Global Warming Potential (GWP) of CH_4 and N_2O is 25 and 298 times that of CO_2, respectively (Forster et al., 2007).

The major sources of CO_2 are biological respiration, fossil fuel use, biomass burning and industrial processes, and major sinks of CO_2 are biological CO_2 fixation (photosynthesis) by vegetation, phytoplankton and microorganisms and carbonate formation (Dalal and Allen, 2008). The major sources of CH_4 are submerged soils, freshwater and oceans, CH_4 hydrates, fossil fuel use, enteric fermentation, animal wastes, domestic sewage, landfills and biomass burning. The largest sink of CH_4 is consumption in the troposphere by OH-radicals (>85%) while oxidation in aerobic soils (<10%) is the only terrestrial sink of CH_4 (Dalal and Allen, 2008). The major N_2O sources are natural systems, agriculture (N fertilizers, legume N and soil mineral N), biomass burning, fossil fuel use and industrial processes. The major sinks of N_2O include its reduction to N_2 by O_3 in troposphere and

stratosphere, which also results in the depletion of O_3 layer in the stratosphere; additionally soils can act as a minor N_2O sink (Dalal and Allen, 2008).

Natural ecosystems play an important role both as a sink of GHG, including those from anthropogenic activities, as well as sources of GHG. Tropical forests have the greatest net primary production (NPP) followed by tropical savannas, whilst the other natural ecosystems have a NPP at least an order of a magnitude smaller than tropical forests and tropical savannas (Saugier et al., 2001). Almost 30% of CH_4 emissions are from natural sources, and N_2O emissions from natural ecosystems comprise 55% of the total global N_2O emissions (Denman and Brasseur, 2007; Forster et al., 2007). However, little is known about the biogeographical distribution of CH_4 and N_2O exchange at a global scale.

1.1 Savannas

Savannas are one of the world's most extensive biomes that occur in more than 20 countries, predominantly in seasonal tropics covering approximately 27.6 million km^2 or around one-sixth of the world's land surface (Hutley and Setterfield, 2008). Savannas have been estimated to account for approximately 30% of the net primary production (NPP) of all terrestrial ecosystems (Grace et al., 2006). Savannas are characterized by the coexistence of trees and grasses, from being near treeless grasslands to woody dominated open-forest/woodlands of up to 80% woody cover (Hutley and Setterfield, 2008). Savanna distribution is determined by the seasonality of climate, defined by strongly alternating wet and dry seasons with rainfall ranging from 300 to 2000 mm y^{-1} and a dry season lasting between 2 and 9 months (Hutley and Setterfield, 2008). Savanna structure and distribution is largely determined by four key environmental factors: 1) plant available moisture; 2) plant available nutrients; 3) fire regime; and 4) herbivory by both vertebrates and invertebrates (Hutley et al., 2000; Hutley and Setterfield, 2008; Williams et al., 1996). Because of their size and largely tropical distribution, savannas significantly influence global carbon dynamics (Beringer et al., 2007). The relatively high NPP of savannas (19.9Gt C y^{-1}) is comparable with that of tropical forests (21.99Gt C y^{-1}) (Grace et al., 2006).

In Australia, savannas form a wide continental arc spreading over the northern half of the Northern Territory and north Queensland, and northwest Western Australia (Mott et al., 1985). Australian tropical savannas cover an area of ~2 million km^2 or a quarter of the continental land surface and are the predominant vegetation type in the northern Australia where rainfall is above 600 mm y^{-1} (Fox et al., 2001; Hutley and Setterfield, 2008; Mott et al., 1985). There are large variations in soil and climatic characteristics which are reflected in a wide variety of vegetation types within Australian savannas (Mott et al., 1985). Tree cover is typically dominated by *Eucalyptus tetrodonta*, *E. dichromophloia*, *E. miniata*, *E. pruinosa*, *Melaleuca viridiflora*, and *M. nervosa*, with declines in canopy cover density as rainfall decreases with increasing distance from the northern coast (Fox et al., 2001; Hutley and Setterfield, 2008). The ground layer is dominated by annual and perennial grasses from the *Sarga*, *Heteropogan*, and *Schizachrium* genera with a variety of other tall grasses (>1m height) dominating the ground layer of the monsoonal savannas, which extend from Western Australia to the Cape York Peninsula in Queensland (Fox et al., 2001; Hutley and Setterfield, 2008).

In most Australian savannas, soil nutrient status is very low probably because these soils are weathered relics of earlier pedological processes (Holt and Coventry, 1990; Mott et al., 1985). In contrast with other tropical savannas, Australian savannas support only a small human population with only few areas completely cleared for intensive agriculture (Williams et al., 1996). Thus, Australia still has large sections of ecologically intact savannas which retain their original physiognomic and functional properties (Mott et al., 1985). Fire is one of the most significant drivers of savanna function in Australia. Changes in fire recurrence and frequency influences the tree and grass balance, which dramatically alters ecosystem structure and the carbon storage capacity of savannas as a larger tree-grass-ratio would result in a larger carbon sink and vice versa (Beringer et al., 2007). In contrast with African savannas, Australia supports few native herbivores which makes invertebrates, especially termites, critical to the functioning of ecosystems, especially in terms of nutrient cycling (Mott et al., 1985).

1.1.1 Greenhouse gas exchange in savannas

Because of the large area tropical savannas cover, these ecosystems have been identified as a major source of trace gas emissions, however seasonal variation and uncertainty in the magnitude of these emission is considerable (Bousquet et al., 2006; Grace et al., 2006; Potter et al., 1996). The exchange of CO_2, CH_4 and N_2O in natural savanna ecosystems is predominantly a function of (Livesley et al., 2011):

- gross primary productivity (CO_2)
- plant and soil respiration (CO_2)
- termite activity (CH_4, CO_2)
- periods of wetland inundation and water level (CH_4, N_2O)
- soil methane oxidation (CH_4)
- soil nitrification and denitrification (N_2O)
- fire events (CO_2, CH_4 and N_2O)

However, scope of this thesis is limited to CH_4 and CO_2 exchange in tropical savannas. Fires result in direct release of greenhouse gases to the atmosphere (Cook and Meyer, 2009), as well as may affect soil-atmosphere exchange of these gases by altering soil C inputs, nutrient inputs, surface microbial activity, surface moisture and temperature (Castaldi et al., 2010; Livesley et al., 2011). Swamps and ephemeral wetlands in savannas produce CH_4 as a result of methanogenic bacteria activity in the saturated soil conditions (Otter and Scholes, 2000). Large CH_4 emissions have been reported from the soils of an African savanna in the wet season (Brümmer et al., 2009a). Methane uptake (oxidation) by soil methanotrophic bacteria is the only terrestrial sink of atmospheric CH_4 and has been shown to occur consistently in aerobic savanna soils (Dalal et al., 2008; Potter et al., 1996). Termites occur throughout savanna ecosystems and are one of the more uncertain components of regional and global CH_4 and CO_2 budgets (Bignell et al., 1997; Sugimoto et al., 2000).

1.2 Termites

Termites are terrestrial insects (order: Isoptera) occurring between 45ºN and 45ºS, or approximately two-thirds of the Earth's land surface (Lee and Woods, 1971). Termites

are a large and diverse group with over 2,600 described species in 281 genera, 7 families and 14 subfamilies (Kambhampati and Eggleton, 2000). Highest species diversity of termites is found in tropical forests (Eggleton 2000), where they are the most important invertebrate decomposers (Bignell & Eggleton 2000). Termites are broadly divided into two groups: **1) Lower termites** which harbor in their alimentary tract a dense and diverse population of bacteria and cellulose digesting, flagellate protozoa; **2) Higher termites** harbor a dense and diverse array of gut bacteria, but typically lack protozoa, and although they are limited to only one family (i.e. Termitidae), they constitute more than 70% of the total known species and more than 85% of known genera (Kambhampati and Eggleton, 2000; Krishna, 1970). The distribution of lower and higher termite species in different biogeographical regions is shown below in Table 1.1.

Table 1.1: Summary of species number distribution in biogeographical regions; Source: (Kambhampati and Eggleton, 2000).

Geographic region	**Lower termites**	**Higher termites**	**Total**
Nearctic	26	12	38
West Palaearctic	11	7	18
East Palaearctic	18	4	22
Neotropical	132	349	481
Afrotropical	63	591	664
Malagasy	23	29	52
Oriental	379	651	1030
Papuan	45	45	90
Australia	56	197	253
Total	**753**	**1885**	**2648**

Termites are social insects that live together as a colony in a nest which can comprise of several dozen to hundreds of thousands of termites. Termite nests vary from a loose association of connected galleries within the soil to several meters high soil-built epigeal mounds.

1.2.1 Feeding groups

Although generally recognized for their ability to consume wood, the nutritional ecology of termites is quite diverse and not limited to xylophagy; the consumption of wood (Brauman et al., 1992). In the evolutionary process from lower termites to higher termites, termites have changed their diet from wood to grass, litter, soil, lichens etc. (Wood and Sands, 1978). Thus, all lower termites are wood-feeders, whereas higher termites include all trophic groups. However, because of a lack of research on the natural history and feeding habits of termites, it is not yet possible to assign termite species to functional feeding groups with complete certainty (Bignell et al., 1997). Termites can be divided into the following broadly overlapping trophic groups:

1) **Soil feeders:** These termites feed predominantly on the upper mineral soil horizons, presumably deriving nutrition from the humic compounds therein (Lee and Woods, 1971). This group is found only in the Apicotermitinae, Termitinae and Nasutitermitinae (Bignell and Eggleton, 2000).
2) **Wood-soil interface feeders:** This group feeds predominantly within soil under logs, within soil plastered on the surface of rotting logs or within highly decayed wood. This group overlaps with both adjacent categories and is only found in the Termitinae, Apicotermitinae and Nasutitermitinae (Bignell and Eggleton, 2000).
3) **Wood feeders:** This group feeds on wood and excavates galleries in larger items of woody litter. Most lower termites are wood-feeders, and there are wood-feeding species in all sub-families of the Termitinae except the Apicotermitinae (Bignell and Eggleton, 2000).
4) **Litter foragers:** Termites foraging on leaves and small woody items often take food material back to the nest and store temporarily. Litter foraging termites are usually more conspicuous than other feeding groups because of the numerous galleries or soil sheets constructed over, litter, wood and ground surface. This group occurs in the Macrotermitinae, Apicotermitinae, Termitinae and Nasutitermitinae (Bignell and Eggleton, 2000).
5) **Grass foragers:** These termites usually forage for dead dry standing grass, which they store in large quantities in their mounds. Grass-feeders are found in the

Hodotermitinae, Macrotermitinae, Termitinae and Nasutitermitinae (Bignell and Eggleton, 2000).

6) **Minor feeding groups:** The wood-, litter- and grass-feeders also include termites (Macrotermitinae) that feed on a fungus (*Termitomyces* spp.) that grows within termite nest and degrades the plant material collected by the workers (Sanderson, 1996). Fungus-growing termites do not occur in the Americas, Australia and New Guinea (Martius, 1994; Pearce and Waite, 1994). Some termites feed on algae and lichens on tree bark while others may feed opportunistically on dung and vertebrate corpses. Some termite species are known to feed obligatory on termite mounds built by other species (Bignell and Eggleton, 2000).

The above trophic groups have the major drawback that no distinction is made between living and dead plant tissues. Living trees are attacked by species of *Coptotermes*, and small roots and root hairs may be consumed by apparent soil-feeders (Bignell and Eggleton, 2000). It is hard to assign Macrotermitinae to any single trophic group as the workers of different ages feed on varying combinations of food sources (Bignell and Eggleton, 2000).

1.2.2 Termites in Australia

There are more than 348 termite species reported from Australia, more than 90 of which are undescribed (Watson and Gay, 1991) and with many more still yet to be recognized (Anderson et al., 2005). Higher termites (Termitidae) constitute more than 75% of total species reported from Australia (Table 1.2). Lower termites are richer in species on sites in Australia than in other regions (Gay and Calaby, 1970). In contrast with other continents where rainforests are extremely rich in termite fauna, there is a paucity of species in Australian rainforests (Watson and Gay, 1991). However, Australian savannas may be slightly richer in total species as compared to African savannas (Braithwaite et al., 1988). According to the classification by Grassé (1986), Australian termite species can be divided in five families of lower and higher termites (Table 1.2).

Table 1.2: Classification of Australian termites (Isoptera) according to Grassé (1986).

Family	Number of reported species
Lower termites	
▪ Mastotermitidae	1
▪ Kalotermitidae	46
▪ Termopsidae (incl. Hodotermitidae)	5
▪ Rhinotermitidae (incl. Serritermitidae)	30
Higher termites	
▪ Termitidae	266
Total	**348**

Australian termite fauna includes one endemic family, Mastotermitidae. This family is left with only one living species, *Mastotermes darwiniensis*, which was confined to tropical Australia, but is now established in New Guinea (Watson and Gay, 1991). *M. darwiniensis* occurs widely in the tropical areas of Queensland (Qld), Northern Territory (NT) and Western Australia (WA) and normally nest in the boles of trees, in logs or stumps, or underground (Watson and Abbey, 1993). The Kalotermitidae are similar in species richness in Africa and Australia, while Rhinotermitidae are richer in Australia than elsewhere (Braithwaite et al., 1988).

Termitidae (higher termites) is the largest family of Isoptera. However, two of the four subfamilies of Termitidae – the fungus-growing Macrotermitinae, prominent in Africa and Asia, and Apicotermitinae – do not occur in Australia (Braithwaite et al., 1988; Watson and Gay, 1991). *Amitermes* is the largest Australian genus with around 100 species reported so far, most abundantly found in northern, western and central Australia (Watson and Abbey, 1993). Most of the *Amitermes* either live subterranean or build mounds, the most commonly known being the magnetic mounds of *A. meridionalis* and *A. laurensis* (Watson and Gay, 1991). *Drepanotermes*, with more than 20 reported species, is an endemic genus from Australia that mostly build mounds but some species take over mounds built by other species (Watson and Gay, 1991). With the greatest concentration of species found in north-western Australia (Watson and Abbey, 1993),

Drepanotermes primarily feed on dry grasses and forbs, leaf litter and twigs which they usually cut in 1 cm small pieces and store in nests. *Microcerotermes* with 16 reported species, all wood-eaters and found all over Australia except south-eastern corner (Watson and Abbey, 1993), are mostly subterranean but some species build small conical or domed mounds (Watson and Gay, 1991). *Nasutitermes* genus has more than 30 species and is distributed throughout Australia particularly in the northern half of the continent (Braithwaite et al., 1988; Watson and Abbey, 1993). The genus includes both subterranean species as well mound-builders with mounds up to 7m high (*N. triodiae*) and colony sizes of 1–2 million individuals (Watson and Gay, 1991). Food material of *Nasutitermes* generally includes grass, vegetable debris, and wood. *Tumulitermes* is an endemic genus of higher termites in Australia which includes more than 50 species and is widely distributed throughout Australia except the south-eastern region (Watson and Abbey, 1993). All *Tumulitermes* are surface foragers and store grass in their nests which can be subterranean or epigeal mounds (Watson and Gay, 1991). Termitidae in Australia also includes many other genera including formerly *Termes*-group which are not described here.

1.2.3 Termites in savannas

Termites essentially act as primary consumers and play a dominant role in the ecological functioning of savanna ecosystems via nutrient cycling and maintaining soil structure (Dangerfield et al., 1998; Dawes, 2010; Holt and Coventry, 1990; Lavelle et al., 1997). With biomass densities comparable to large ungulates and megaherbivores (Moe et al., 2009), termites account for 40-60% of the total soil macrofauna biomass, and consume up to 55% of surface litter and about 20% of the grass standing crop (Wood and Sands, 1978). Termites constituted 61% of total macroinvertebrate abundance in northern Australian savannas (Dawes-Gromadzki, 2007). In the tropical savannas of Queensland, Australia, termites were responsible for up to 20% of organic matter decomposition (Holt et al., 1990).

In the process of nest construction, termites modify the physical, chemical and biochemical characteristics of the soil they use for construction, as well as the soil of

nearby areas (Lee and Wood, 1971). Termites use soil, together with their saliva and faeces, to construct their nests which may be epigeal (mounds), subterranean, or within or attached to trees (Wood, 1988). Therefore, organic compounds, particularly C and N, in general are more abundant in termite nests than in surrounding soils (Lee and Wood, 1971; Lee and Woods, 1971; López-Hernandez, 2001). Also CO_2 emissions per gram of soil has been found to be much greater from the mound material as compared to adjacent soils when incubated in laboratory (Holt, 1987; López-Hernandez, 2001) because of greater microbial biomass in mounds as compared to adjacent soil (Holt, 1998). It has been suggested that much of the northern Australia owes its present soil mantle to termite-transported material (Lee, 1983) as termites are estimated to form a 20 cm thick sandy A horizon of the soils from erosion and degradation of termite mounds over a period of 8000 years (Holt et al., 1980).

1.3 Greenhouse gas emissions from termites

The contribution of termites to the GHG emissions is by far of greatest significance among the invertebrates (Bignell et al., 1997). Termites produce a range of trace gases including CO_2, CH_4, N_2O, H_2 (hydrogen), CO (carbon monoxide), and $CHCl_3$ (chloroform) which can affect atmospheric chemistry (Sugimoto et al., 2000). However, only CO_2 and CH_4 are emitted in sufficient quantity to make termites a potentially significant source to global budgets (Khalil et al., 1990). While CO_2 emissions from termites are mainly the result of termite respiration, CH_4 production by termites is a complex process.

1.3.1 Methane production in termites

CH_4 production by termites was first reported by Cook (1932) who observed the evolution of a gas from *Zootermopsis navadensis* and described it as H_2 and/or CH_4. Breznak (1975), who reported the first quantitative figures for CH_4 emissions from termites, determined the CH_4 production rate on termite body weight basis from three lower termite species namely *Reticulitermes flavipes*, *Coptotermes formosanus* and *Crytotermes brevis*.

All termites have a dense and diverse symbiotic microbial community in their hindgut which aide in anaerobic metabolism resulting in CH_4 production (Breznak, 1975). The hindgut of a termite is analogous to an anaerobic fermentation chamber where the wood polysaccharides (cellulose + hemicelluloses), which constitute about 70% of the dry weight of wood, under go up to 99% degradation mainly driven by hindgut microbiota (Odelson and Breznak, 1983). Methane production rates are highly variable among different trophic groups of termites because of major differences in the hindgut microbiota. Termites tend to increase CH_4 emission rates during dietary evolution from wood-to-soil-feeding forms or from lower to higher termites (Sugimoto et al., 1998b). Cellulose degradation is mainly driven by hindgut protozoa in lower termites and by bacteria in higher termites (Sugimoto et al., 1998b). In wood-, litter- and grass-feeding termites, the fermentation of plant material is realized by an abundant homoacetogenic microflora which first hydrolyze cellulose and ferment each glucose ($C_6H_{12}O_6$) monomer to acetate (CH_3COOH), CO_2 and H_2 (Odelson and Breznak, 1983).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

Methane is produced by reduction of CO_2 by methanogenic bacteria (2):

$$4H_2 + 2CO_2 \rightarrow 2CH_4 + 2O_2 \quad (2)$$

Rates of CO_2 reduction to acetate by gut contents of xylophagous termites have been found to be greater than fungus-growing and soil-feeding termites, thus resulting in large acetate production but very low CH_4 emissions (Brauman et al., 1992; Breznak and Switzer, 1986; Rouland et al., 1993). This is because the digestive metabolism of xylophagous species is mainly characterized by an abundant, mainly acetogenic, anaerobic microflora (Rouland et al., 1993). In contrast, most of the soil-feeding and the fungus-growing termites show little or no acetogenesis from CO_2 + H_2 resulting in larger CH_4 emissions but little or no acetate production (Brauman et al., 1992; Rouland et al., 1993). In soil-feeding termites, although bacterial density was lower than xylophagous

species, it was characterized by a very abundant methanogenic microflora and the absence of acetogenic bacteria (Rouland et al., 1993). Among the castes, workers produce more CH_4 as compared to soldiers and nymphs (Sugimoto et al., 1998b).

These and other similar studies (Brauman et al., 1992; Fraser et al., 1986; Rouland et al., 1993; Sugimoto et al., 1998b; Wheeler et al., 1996) suggested that CH_4 production by termites was not a constant function of carbon consumed. These studies also concluded that soil-feeders were the greatest CH_4 producers on termite body mass basis, thus overturning the key assumption of Collins and Wood (1984) that soil-feeders were the smallest CH_4 producers of all trophic groups (Table 1.3).

Table 1.3: Magnitude of mean CH_4 flux rates from isolated workers of different termite species measured in laboratory as reported in literature; source: Bignell et al.(1997), Brauman et al.(1992) and Sugimoto et al.(1998b).

Feeding group	**No. of species**	**Mean CH_4 flux (μmol g-termite^{-1} h^{-1})**	**Standard deviation**
Soil	21	0.416	0.274
Wood-soil	3	0.406	0.452
Litter	9	0.354	0.292
Wood	34	0.176	0.234
Grass	3	0.140	0.080

Hackstein and Stumm (1994) studied different taxa of terrestrial arthropods and concluded that methanogenic bacteria occur in the hindguts of nearly all tropical representatives of millipedes (Diplopoda), cockroaches (Blattaria), scarab beetles (Scarabaeidae), termites (Isoptera), while such methanogens were absent from 66 other arthropod species investigated. They also suggested that CH_4 fluxes from termites are, on a weight-specific basis, not necessarily exceptional.

Large variations have also been reported in mound fluxes of CH_4 and CO_2 from different termite species measured in the field (Brümmer et al., 2009a; Delmas and Servant, 1992;

Eggleton et al., 1999; Khalil et al., 1990; MacDonald et al., 1998; MacDonald et al., 1999; Seiler et al., 1984). It has been suggested that a proportion of CH_4 produced by termites in mounds might be oxidized by methanotrophic bacteria in mound material before being released to the atmosphere (Bignell et al., 1997; Khalil et al., 1990). Sugimoto et al. (1998a) estimated the fraction of CH_4 oxidized during transport to the atmosphere using stable isotope ratios of carbon (C). They observed greater $^{13}CH_4$ in CH_4 released from mounds than the CH_4 emitted by isolated termites, indicating that a portion of CH_4 produced inside mound is oxidized before being released to the atmosphere. Assuming that isotopic fractionation occurs only in the process of oxidation, Sugimoto et al. (1998a) suggested that 53 to 83% of CH_4 produced in small-mounds is oxidized before being released to the atmosphere, and that this proportion is even greater for fungus-growing and large-mound-making species. Another reason for such species variation in mound fluxes can be the difference in CH_4 and CO_2 production rates per unit termite biomass. Ignoring such variations in flux rates and/or mound distribution of different termite species can result in large errors in scaling up to the landscape or regional levels.

1.3.2 Global emissions of CH_4 and CO_2 from termites

Termites were first highlighted as a potentially large global source of CH_4 by Zimmerman et al. (1982) who estimated global termite emissions at 75-310 Tg y^{-1}, which equates to 13-56% of all CH_4 sources in the biosphere (Table 1.3). They based this estimate on the ability of termites to convert biomass to CH_4, as measured in the laboratory, and used annual estimates of biomass consumed by termites to deduce this annual CH_4 release. This global estimate was revised downwards to 10-100 Tg y^{-1} by Rasmussen and Khalil (1983), based on laboratory measurements of CH_4 emissions per termite from five colonies of *Zootermopsis angusticollis* and estimates of the global termite population (Table 1.3). Collins and Wood (1984) suggested that Zimmerman et al.'s (1982) estimate of biomass consumed by termites per year (33×10^{15} g y^{-1}) was at least an order of magnitude too high, because the species of termites studied were not globally representative, and would likewise lead to an overestimate of global CH_4

emissions. Collins and Wood (1984) assumed that soil-feeders would produce less CH_4 per termite than wood-feeders.

Table 1.4: Global estimates of methane emissions from termites as reported in literature.

Reference	**CH_4 emissions (Tg/year)**	**Methods**
Zimmerman et al. (1982)	75-310	Two species measured in laboratory
Rasmussen and Khalil (1983)	10-100	One species measured in laboratory
Collins and Wood (1984)	10-30	Based on amount of wood consumed by termites
Seiler et al. (1984)	2-5	Mounds measured in field in Africa
Fraser et al. (1986)	6 - 42	Six species measured in laboratory
Khalil et al. (1990)	12 - 20	Mounds measured in field in Australia
Martius et al. (1993)	5 - 36	Five South American species (*Nasutitermes)* measured in laboratory
Hackstein and Stumm (1994)	6 - 51	Four wood-eating species measured in laboratory
Sanderson (1996)	18 - 22	Global database on termite distribution, biomass and fluxes
Bignell et al. (1997)	17 - 96	Suite of laboratory CH_4 flux measurements and review
Sugimoto et al. (1998a)	1 - 8	Laboratory and field measures of flux and CH_4 oxidation by mounds
Sugimoto et al. (2000)	10 - 20	A review
Brümmer et al. (2009a)	1	Mounds of one species measured in field in Africa

Fraser et al. (1986) estimated global CH_4 emissions of 15 Tg y^{-1} from termites ranging between 6 and 42 Tg CH_4 y^{-1} using laboratory experiments from a number of termite

species from tropical and temperate regions of the Northern and Southern hemispheres (Table 1.3). The first laboratory study of termite CH_4 fluxes in South America was conducted by Martius et al. (1993) using a wood-feeding termite species (*Nasutitermes* spp.). From global estimates of termite biomass Martius et al. (1993) then estimated global CH_4 emissions from termites at 26 Tg CH_4 y^{-1}, or less than 5% of the global CH_4 source budget (Table 1.3). On the basis of CH_4 flux measurements from four wood-feeding termite species and estimates of global termite biomass, Hackstein and Stumm (1994) concluded that the most termites could produce globally is 51 Tg CH_4 y^{-1} (Table 1.3).

Sanderson (1996) compiled a global database of regional estimates of termite biomass and fluxes of CH_4 and CO_2. They assigned termite biomasses to various ecosystems using published measurements from literature and a high resolution database of vegetation categories. The assigned termite biomasses were then combined with literature measurements of CH_4 and CO_2 fluxes from termites and extrapolated to give global emission estimates at19.7 ± 1.5 Tg CH_4 y^{-1} (Table 1.3) and 3500 ± 700 Tg CO_2 y^{-1}.

First field measurements of CH_4 emissions from termites were conducted by Seiler et al. (1984) in a South African savanna who measured CH_4 emissions from the mounds of five higher termite species and one lower termite species. Seiler et al. (1984) estimated the global CH_4 emissions from termites between 2 and 5 Tg CH_4 y^{-1} based on the conservative estimates of biomass consumption (Collins and Wood, 1984) and biomass conversion to CH_4 by termites. In another field study, Khalil et al. (1990) measured fluxes of CO_2, CH_4, N_2O, H_2, CO, and $CHCl_3$ from the mounds of *Coptotermes lacteus* and *Amitermes laurensis* in Australia. Mound fluxes of only CO_2, CH_4 and $CHCl_3$ were positive through all the seasons suggesting negligible termite emissions of other gases (Khalil et al., 1990). On the assumption that total biomass consumed by termites is 7.3 Pg y^{-1} (1 pg = 10^{15}), Khalil et al. (1990) estimated the global termite emissions of 12 - 20 Tg CH_4 y^{-1} (Table 1.3) and 4 - 8 Pg CO_2 y^{-1}.

One of the most comprehensive works on termite fluxes published to date is by Bignell et al. (1997) who reviewed the data reported in literature, and also carried out a suite of experiments in the laboratory and in field. They demonstrated that termite emissions of CH_4 and CO_2 were greater when incubated in the presence of mound material and soil than when incubated isolated. As such, the interaction between termites and their nest material will have serious implications on estimates based on measurements of isolated termites only. They concluded that insufficient data exist to show beyond doubt that termites have or have not a significant role in global carbon fluxes. Bignell et al. (1997) estimated the limits of the global termite emissions at 17 - 96 Tg y^{-1} for CH_4 and 735 - 3557 Tg y^{-1} for CO_2, or 3 - 19 % and 0.5 - 2% of the then global source budgets of CH_4 and CO_2, respectively. In a laboratory based study, Sugimoto et al. (1998a) calculated global CH_4 emissions from termites between 1.5 and 7.4 Tg y^{-1}, or 0.3 to 1.3% of the then global CH_4 source budget.

Sugimoto et al. (2000), a comprehensive review, assessed different methods and argued that direct measurements of net CH_4 and CO_2 emissions from termites in natural settings (in their nests or in soil) are the best data for scaling-up calculations. They noted that flux calculations are still hampered by uncertainties over termite biomass distribution and a general failure to consider local and landscape-level CH_4 oxidation by methanotrophic microorganisms in mounds and soil. After reviewing the available evidence, they concluded that termite emissions will contribute less than 4% and 2% to the global source budgets of CH_4 and CO_2, respectively.

1.3.3 Termite emissions as a component of ecosystem exchange

It is important to assess the termite emissions as a contributory component of the GHG exchange within the ecosystem they are found to truly gauge their importance. Delmas and Servant (1992) measured mound CH_4 fluxes from 10 termite species in the tropical forests of central Africa and concluded that termites were a minor component in the CH_4 source budget. CH_4 emissions from forest fires and flooded lowlands were the major components in CH_4 source budget although the ecosystem was a net CH_4 sink because of larger CH_4 uptake by soil (Delmas et al., 1991; Delmas and Servant, 1992).

MacDonald et al. (1998; 1999) measured CH_4 fluxes from termite mounds and soils in West Africa and Borneo, and observed CH_4 emissions from termite mounds as well as in from some soil because of termite activity in soil profiles. At a regional scale, however, CH_4 oxidation by soil exceeded CH_4 emissions from termites (MacDonald et al., 1998). In a similar study in Sabah, Malaysia (Eggleton et al., 1999), CH_4 emissions were observed from soil which were significantly correlated to the termite biomass in soil profiles. Although a significant gross CH_4 source, termite emissions of CH_4 were offset by CH_4 oxidation in soil at all sites studied (Eggleton et al., 1999). These results suggest that the oxidation capacity of soils would be greater in the absence of CH_4 emissions associated with termite activity within soil profiles. More recently, Brümmer et al. (2009a; 2009b) found that termite mounds of *Cubitermes fungifaber* were a significantly greater point source of CO_2, CH_4 and N_2O as compared to adjacent soil in the South-Sudanian savanna of Burkina Faso. From the measured mound flux rates in field and basal area covered by mounds, Brümmer et al. (2009a) calculated that termite mounds were a CH_4 source of 0.26 kg CH_4 ha^{-1} y^{-1} and CO_2 source of 0.07 t CO_2 ha^{-1} y^{-1} which contributed 8.8% and 0.4% to the total soil CH_4 and CO_2 emissions, respectively.

1.4 Key knowledge gaps in the context of this study

The uncertainty in termite flux estimates is mainly associated with the lack of ***long-term field studies*** from different biogeographical regions including ***the tropical savannas of northern Australia***. Studies conducted in laboratories cannot be the representative of field conditions (Bignell et al., 1997). Termite species composition is highly variable in different biogeographical regions as is the flux rate among different species. It is important to investigate and account for such ***variation in flux and distribution of different termite species***.

There are only a few studies that have measured the termite fluxes in natural conditions, most of which are limited to the emissions from termite mounds thus ignoring the potential contribution from non-mound-dwelling termite species. Quantification of the ***contribution of non-mound-building termites*** is critical for a realistic estimate on

greenhouse gas emissions from termites. Also important to investigate is the ***role of termites in CH_4 and CO_2 balance of an ecosystem*** they are found in. With a few exceptions, most of the studies have ignored this important aspect of termite flux research and rather tried to reach a hasty conclusion on global estimate.

Temperature can influence the magnitude of emissions from termites (Zimmerman and Greenberg, 1983), which can cause large variations in fluxes at a diurnal (Seiler et al., 1984) and seasonal (Khalil et al., 1990) scale. However, the potential ***diurnal and seasonal variations in fluxes*** from termite mounds because of the temperature variations have not been studied in detail. In the tropical savannas of northern Australia, there is limited variation in temperature at seasonal scale but large variations occur diurnally which can also influence the termite fluxes. Also, there is a distinct wet and dry season in these tropical savannas, the effects of which on termite fluxes are unknown. It is important to investigate the magnitude of variation in fluxes which can potentially result in large errors if not accounted for in scaling up. It is also important to understand processes and identify the ***drivers causing diurnal and seasonal variations*** which can be incorporated in modeling these fluxes.

The fewer field-based studies on CH_4 fluxes from termites are also probably due to the difficulty associated with making such measurements as compared to soil fluxes: e.g. chamber installation, remote sites, harsh climate, costs and labor time for analysis. In comparison to CO_2 flux that can be measured relatively easily using an Infrared Gas Analyzer (IRGA), CH_4 fluxes are most often measured through conventional gas sample collection and concentration analysis through gas chromatography back in the laboratory. Only in the last few years, have field-based CH_4 analyzer become available and they remain expensive in comparison to an IRGA. If developed, ***indirect methods for predicting GHG fluxes*** from intact termite mounds, such as used by Khalil et al. (1990), can be helpful if accurate to an acceptable level.

1.5 Objectives

This study was conducted in the tropical savannas of northern Australia. The objectives were to investigate the:

1) Diurnal variations in CH_4 flux from termite mounds and the environmental factors controlling these fluxes
2) Seasonal variations in CH_4 and CO_2 flux from termite mounds and the factors determining such variations
3) Variation in CH_4 and CO_2 flux from mounds of different termite species
4) Importance of termites to the CH_4 balance of a tropical savanna woodland at Howard Springs
5) Relative importance of CH_4 and CO_2 emissions from termite mounds at sites with variable mound density and species distribution;
6) Validity of indirect methods for predicting CH_4 and CO_2 fluxes from termite mounds

1.6 References

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2 Diurnal and seasonal variations in CH_4 flux from termite mounds in tropical savannas of the Northern Territory, Australia

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Abstract

Termites are estimated to contribute between <5 and 19% of the global methane (CH_4) emissions. These estimates have large uncertainties because of the limited number of field-based studies and species studied, as well as issues of diurnal and seasonal variations. We measured CH_4 fluxes from four common mound-building termite species (*Microcerotermes nervosus*, *M. serratus*, *Tumulitermes pastinator* and *Amitermes darwini*) diurnally and seasonally in tropical savannas in the Northern Territory, Australia. Our results showed that there were significant diel and seasonal variations of CH_4 emissions from termite mounds and we observed large species specific differences. On a diurnal basis, CH_4 fluxes were least at the coolest time of the day (~07.00 hours) and greatest at the warmest (~15.00 hours) for all species for both wet and dry seasons. We observed a strong and significant positive correlation between CH_4 flux and mound temperature for all species. A mound excavation experiment demonstrated that the positive temperature effect on CH_4 emissions was not related to termite movement in and out of a mound but probably a direct effect of temperature on methanogenesis in the termite gut. Fluxes in the wet season were 5 to 26 fold greater than those in the dry season. A multiple stepwise regression model including mound temperature and mound water content described 70-99% of the seasonal variations in CH_4 fluxes for different species. CH_4 fluxes from *M. nervosus*, which was the most abundant mound-building termite species at our sites, had significantly lower fluxes than the other three species

measured. Our data demonstrate that CH_4 flux estimates could result in large under- or over-estimation of CH_4 emissions from termites if the diurnal, seasonal and species specific variations are not accounted for, especially when flux data are extrapolated to landscape scales.

2.1 Introduction

Methane (CH_4) has a global warming potential 25 times that of CO_2 on a 100-years time horizon (Forster et al., 2007). More than 580 Tg of CH_4 are released to the atmosphere annually, with over 70% originating from biogenic sources (Denman and Brasseur, 2007) and with almost one-third of this global CH_4 emission arising from natural ecosystem sources (Dalal et al., 2008). However, the global CH_4 budget and the contribution of different sources and sinks is still highly uncertain (Denman and Brasseur, 2007). Better knowledge of the current global CH_4 budget will help reduce uncertainties in future projections of climate change. The regional patterns of surface emissions show that most of the global year-to-year variability lies in tropics (Bousquet et al., 2006).

Tropical and sub-tropical savannas cover around one-sixth of the world's land surface, account for 30% of total net primary production of all terrestrial ecosystems (Grace et al., 2006), and cover a quarter of the Australian continent with an area of ~2 million km^2 (Chen et al., 2003). Savannas mostly occur in the seasonal tropics and are characterized by relatively aseasonal temperature pattern but highly seasonal rainfall. Global climate models generally perform poorly in savanna ecosystems (Hutley et al., 2005), because of the intra- and inter-annual variability in these globally important regions (Brümmer et al., 2009). Therefore it is of importance to conduct long-term studies for a reliable quantification of biosphere-atmosphere trace gas exchange.

Termites are terrestrial insects occurring between 45° N and 45° S, or approximately two thirds of the earth's land surface (Lee and Wood, 1971). Most termites use soil, together with their saliva and faeces, to construct their nests which may be epigeal (mounds), subterranean, or within or attached to trees (Wood, 1988). Termites have a dense and diverse microbial community in their hindguts which produce CH_4 as part of a fermentation process during digestion of organic matter (Brauman et al., 1992). Termites are an integral component of nutrient cycling in tropical ecosystems. Despite their fundamental role in nutrient dynamics their role as a source of CH_4 in savanna ecosystems is uncertain. The initial estimates of 75-310 Tg of CH_4 per year, i.e. 13-56% of the global CH_4 emissions (Zimmerman et al., 1982), highlighted the role termites play as a significant source of CH_4. In later studies most of the assumptions made by

Zimmerman et al. (1982) were refined (Sugimoto et al., 2000) and the contribution of termites was estimated to be <5% (Khalil et al., 1990; Sanderson, 1996; Seiler et al., 1984) or at most between 3-19% of the global CH_4 budget (Bignell et al., 1997).

Errors and uncertainties associated with these estimates as to the contribution of termites to the CH_4 budget are largely due to: i) lack of measured emissions rates from representative field experiments, and ii) the scaling factors used (global abundance, biomass of termites, or the number of nests in the world) (Khalil et al., 1990). In this study we address uncertainties associated with the first parameter, i.e. measured emission rates in the field.

Diurnal and seasonal variation in CH_4 flux from termite mounds can potentially lead to a large under- or over-estimate of annual termite CH_4 source strength when based on short-term, field-based measurements. As demonstrated by several authors, an increase in temperature results in an increase in CH_4 fluxes from termites (Sanderson, 1996), but this effect varies considerably among termite species (Fraser et al., 1986; Khalil et al., 1990; Seiler et al., 1984; Zimmerman and Greenberg, 1983). There is generally a diurnal variation in temperature in all ecosystems where termites are found, which may potentially cause a diurnal variation in CH_4 flux as well. Seiler et al. (1984) observed diurnal variation in CH_4 flux from termite mounds in African savannas but since then no systematic study has been conducted to investigate these variations over a 24 hour cycle. Seasonal variations in CH_4 flux are also important. For example, Khalil et al., (1990) reported that a mound of *Coptotermes lacteus* in the subtropical (Southern) region of Australia produced more CH_4 in summer because of the higher temperatures than in the other three seasons combined. However the effect of seasonal moisture variations on CH_4 fluxes from termite mounds of different species is unknown. CH_4 Flux variations among different termite species (Brauman et al., 1992; Rouland et al., 1993; Wheeler et al., 1996) and distribution of termite population according to species are also important factors to account for when scaling the results up to an ecosystem scale.

The examples outlined above confirm that at present there are large uncertainties regarding the magnitude and temporal variability of CH_4 emissions from termite mounds. For example, are measurements at any point in time during a day representative of the mean CH_4 emission for that day? Are there differences among seasons or different species? These questions are important for the accurate calculation of annual CH_4 budgets for savanna termites and for the design of monitoring programs of CH_4 emissions from termites.

This is the first study to investigate the diurnal and seasonal variations of CH_4 fluxes from termite mounds using a fully replicated experimental design in the field. Objectives of this study were to quantify: 1) diurnal and seasonal variations of CH_4 fluxes from termite mounds, 2) environmental factors controlling these CH_4 fluxes, 3) species differences in CH_4 fluxes from termite mounds, and 4) site comparison of CH_4 fluxes from the mounds of the same termite species.

2.2 Materials and methods

2.2.1 Sites

This study was conducted at four savanna locations in the Northern Territory of Australia. The soils and climates of these four locations are broadly similar. The first site was CSIRO's Tropical Ecosystem Research Center (TERC), (12º 24'S, 130º 55'E) which is on the outskirts of Darwin city and comprises savanna woodland dominated by *Eucalyptus miniata* Cunn. Ex Schauter and *E. tetrodonta* F. Muell., over an understorey of annual / perennial C4 grasses and a thick litter layer (Dawes-Gromadzki and Spain, 2003). At the TERC site, one plot of 50 x 50 m was selected in an area that had been unburnt for >5 years (TERC A), and another 50 x 50 m plot was selected in an area that had been unburnt for >20 years (TERC B). Of the available mounds within these 50 x 50 m plots, five of *Microcerotermes nervosus* were selected at TERC A, and four of *M. serratus* were selected at TERC B. The second site was located within Charles Darwin National Park (CDNP; 12º 27'S, 130º 50'E) 5.5 km east of Darwin and featured similar savanna vegetation to that of the TERC site (Dawes-Gromadzki, 2008). Five mounds of

Tumulitermes pastinator were selected in a 50 x 50 m plot at the CDNP site in an area that was burnt almost every year.

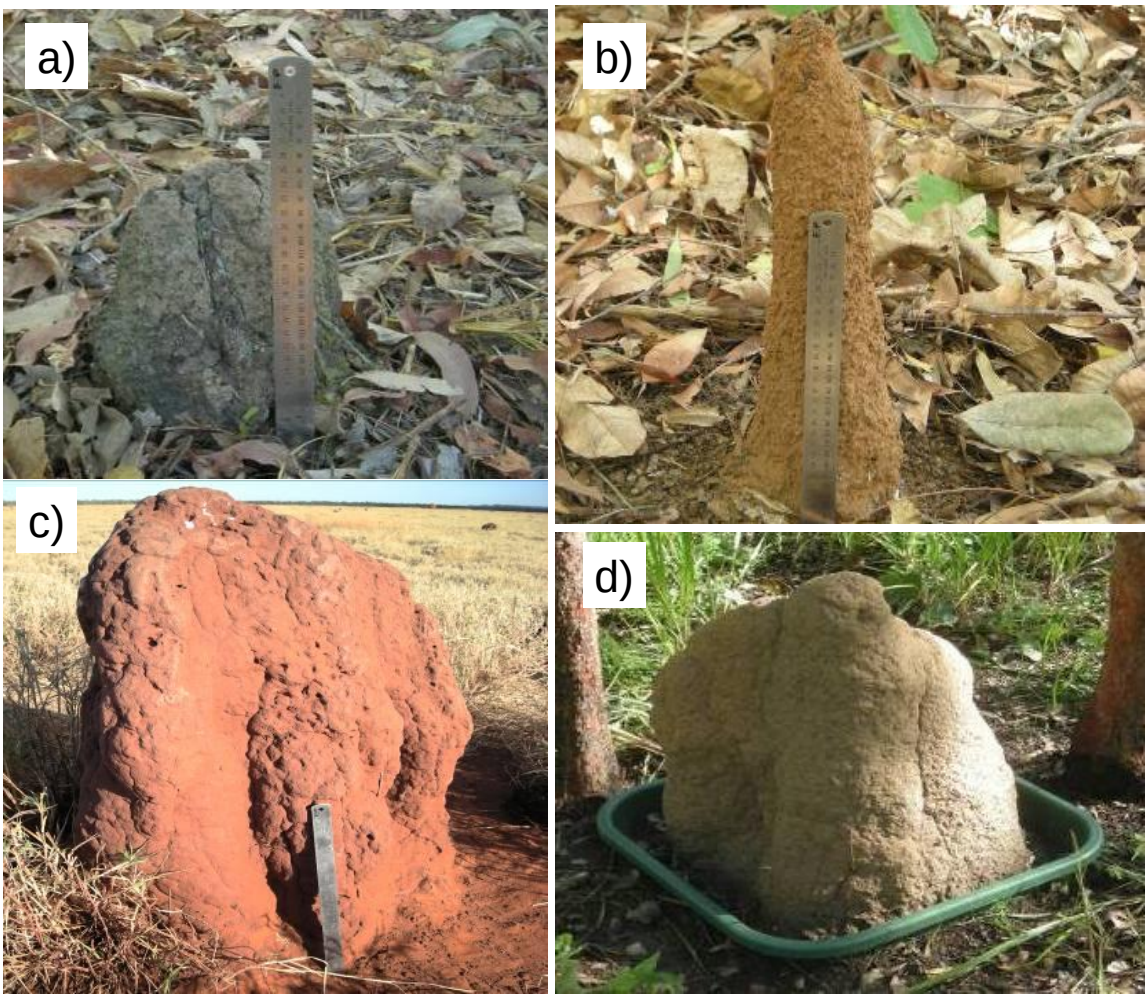


Fig. 2.1: Mounds of four termite species namely: a) *Microcerotermes serratus*, b) *Microcerotermes nervosus*, c) *Amitermes darwini*, and d) *Tumulitermes pastinator*, shown with a 30 cm ruler

The third site was located at Howard Springs, (Beringer et al., 2007) 35 km south-east of Darwin (12º 29'S, 130º 08'E) similarly dominated by *E. miniata* and *E. tetrodonta* species and a tall understorey of Sorghum C4 grass. At the Howard Springs site four mounds of *M. nervosus* were selected in each of the four randomly selected 50 x 50 m plots (total mounds = 16) in an area where fire occurred approximately every 2 to 3 years.

Finally, the fourth site was located ~190 km south of Darwin within the Douglas-Daly River catchments (DD; 14º 00'S, 131º 20'E). At the DD site, four mounds of *M. nervosus* were selected in a 50 x 50 m plot in an area of savanna woodland dominated by *E. miniata* and *E. tetrodonta* trees over a grassy understorey that experiences a low frequency of fire (1 in 5 years, L. Hutley pers. comm.), and four mounds of *Amitermes darwini* were selected in a cattle grazed pasture containing cultivated grass species *Heteropogan contortus*, *Eragrostis* species and *Eragrostii* species.

Mounds of *M. nervosus* were irregular cone-shaped, while the other three species were irregular dome-shaped (Fig. 2.1). To accommodate these irregular mound structures an approximate cylinder volume was estimated as:

$$V = pi \times R^2_{avg} \times H \quad (1)$$

where V is mound volume (m^3), pi is equal to 3.1416; R_{avg} is the average mound radius (m) measured at four to five points along the mound height H (m) using a diameter tape. Mound volumes ranged between 0.002 and 0.01 m^3 for *M. nervosus*, 0.004 and 0.01 m^3 for *M. serratus*, 0.03 and 0.08 m^3 for *T. pastinator*, and 0.02 and 0.09 m^3 for *A. darwini*. During the period of CH_4 flux measurement from termite mounds at these four site locations (November 2007- June 2008), mean minimum and maximum temperatures in Darwin at the height of the wet season (February) were 24.7 and 30.8ºC, respectively, and at the middle of the dry season (June) were 20.1 to 31.4ºC, respectively. The annual rainfall in 2008 was 1970 mm, with 1450 mm falling in the three months of January, February and March (wet season) (Bureau of Meteorology, 2008).

2.2.2 CH_4 flux measurements

CH_4 fluxes from termite mounds were measured in-situ using static manual chambers of five different volumes ranging between 0.02 and 0.2 m^3 constructed from polyvinylchloride. Net chamber headspace volume was calculated by subtracting the mound volume from the chamber volume. A collar was permanently installed around the base of each termite mound to a depth of < 3 cm in order to minimize disturbance to any sub-terranean termite galleries whilst enabling the rapid and gas-tight connection of a

headspace chamber to that collar. A chamber of equal circumference to the collar was carefully placed over the selected termite mound and connected to the collar using a ribbon of closed cell foam and several tension spring-clamps. Samples of chamber headspace gas (20 ml) were taken at 0, 10, 20 and 30 minutes after attaching the chamber, using a 25 ml Terumo™ syringe fitted with a two-way stop-cock and 23G needle inserted through a rubber septum. Each 20 ml gas sample was immediately transferred to a pre-evacuated 12 ml glass vial (Labco Exetainer) such that it was over-pressurized. These glass vials were transported by road to The University of Melbourne , Creswick laboratories for analysis of CH_4 concentration using an auto-injected gas chromatograph (GC, Shimadzu™, GC17a) fitted with a CTC auto-sampler, ten port Valco™ valve, a 1.8 m poropak Q separation column attached to a flame ionization detector (FID).

2.2.3 CH_4 flux calculation

Methane flux (µL CH_4 m^{-2} h^{-1}) was calculated using equation (2):

$$F_{\mu l} = \frac{V}{A} \times \frac{dC}{dt} \tag{2}$$

where V is volume of chamber headspace (L), A is basal area of mound (m^2), and dC/dt is the slope (µL L^{-1} h^{-1}) calculated by using a linear regression of four CH_4 concentrations at time 0, 10, 20 and 30 minutes.

This flux ($F_{\mu L}$) was then converted to µmol CH_4 m^{-2} h^{-1} ($F_{\mu mol}$) by accounting for temperature, pressure and volume based on the ideal gas law by using equation (3):

$$F_{\mu mol} = \frac{F_{\mu L} \times P}{R \times T} \tag{3}$$

where $F_{\mu mol}$ is the flux in µmol CH_4 m^{-2} h^{-1}, $F_{\mu L}$ is the flux in µL CH_4 m^{-2} h^{-1}, P is atmospheric pressure in kPa at the site according to altitude, R is 8.3144 (the ideal gas constant in L kPa mol^{-1} K^{-1}), and T is air temperature in K (273 + ºC). Fluxes in µmol CH_4 m^{-2} h^{-1} were then converted to µg CH_4-C m^{-2} h^{-1} based on molecular and elemental mass.

2.2.4 Diurnal flux measurements

Mounds of three termite species; *M. nervosus* (TERC A, n = 5), *M. serratus* (TERC B, n = 4), and *T. pastinator* (CDNP, n = 5), were measured every 4 hours over a 24 hour period to establish the diurnal variations in CH_4 flux. Mounds of all three species were measured over a continuous 24 hour period in February 2008 (wet season), April 2008 (wet-to-dry transition) and June 2008 (dry season). *M. nervosus* (TERC A) mounds were also measured in December 2007 (dry-to-wet transition).

The diurnal variation in CH_4 flux measurements allowed us to calculate the Q_{10} temperature coefficient which is a measure of the rate of change of a biological or chemical system, in this case, CH_4 flux, as a consequence of increasing the temperature by 10°C, and was calculated as follows:

$$Q_{10} = \left(\frac{F_2}{F_1} \right)^{\frac{10}{(T_2 - T_1)}} \tag{4}$$

where $F_{1,2}$ are CH_4 flux at two different temperatures, and T is corresponding mound temperature (°C). Q_{10} was calculated for a temperature range of 25 to 35 ºC.

Further experimentation was carried out to investigate whether temperature or termite behavioral activity, e.g. termite movement in and out of the mound, was the main causal factor for the diurnal CH_4 flux variations. Two mounds of *M. nervosus* were selected for this experiment which consisted of three sequential treatments: i) Diurnal CH_4 fluxes measured in-situ under normal diurnal temperature variation. ii) Diurnal CH_4 fluxes measured ex-situ after the same two mounds had been excavated using a spade to cut the base of the mound below the soil surface so that the mound remained intact and contained the majority of termite population. These ex-situ mounds were placed in plastic buckets to prevent termite movement out of the mound but experiencing normal diurnal temperature variations. iii) Diurnal CH_4 fluxes measured on the same two ex-situ mounds in the laboratory at four different temperatures ranging between 24 and 29 ºC, and by making the usually warmest time of the day (i.e. 14.00 hours) coolest and vice versa by using an air-conditioner.

2.2.5 Seasonal flux measurements

We measured CH_4 fluxes from replicate termite mounds of the four selected species at intervals through the wet and dry seasons. *M. nervosus* mounds were measured at TERC A (n = 5), Howard Springs (n = 16) and Douglas-Daly (n = 4). *M. serratus* mounds were measured at TERC B (n = 4), *T. pastinator* mounds were measured at CDNP (n = 4) and *A. darwini* mounds were measured at Douglas-Daly (n = 4). The exact dates and frequency of measurements differed for different species and sites, but in all cases CH_4 fluxes were measured in wet, dry and wet-to-dry-transition seasons, except for *A. Darwini* where fluxes were measured in wet and dry seasons only. For *M. nervosus*, fluxes were also measured in dry-to-wet-transition season at the Douglas-Daly site. Overall, these termite mound CH_4 flux measurements were representative of broad seasonal differences. All the seasonal measurements were made at ~1100 hours.

2.2.6 Auxiliary environmental measurements

Mound temperature (T_{mound}) was measured immediately after CH_4 flux using a hand held Cole-Palmer^R stainless steel temperature probe, with an accuracy of ±1ºC, inserted 6 cm into the mound. Mound water content (WC_{mound}) was measured for all species, except *M. serratus*, by collecting mound pieces from five nearby mounds of the same species and weighing these, oven drying at 105 ºC and reweighing. For *M. serratus*, the mound population was too small to enable repeated destructive sampling.

2.2.7 Data processing and analysis

SPSS 16.0 was used for the statistical analysis of data. One-way analyses of variances (ANOVA) were used to test for significance of differences in mean CH_4 fluxes and mean T_{mound} at diurnal (n = 282) and seasonal (n = 159) scales. Least Standardized Difference (LSD) Post Hoc tests were used to identify significantly different means among treatments ($p \leq 0.05$). Both seasonal and diurnal data were transformed using $\log_{10}$ ($\log_{10}$ flux + 1) for improving normality.

For the diurnal variations in CH_4 flux and mound temperature one-way ANOVA was used to investigate significant differences according to time of day for each termite mound species separately. This was repeated for each month's diurnal CH_4 data

separately. Linear regression analysis was used to evaluate the significance of the relationship between CH_4 flux and mound temperature. Analysis of covariance (ANCOVA) was repeated for each species to compare the slopes for different months in order to check if relationship between CH_4 flux and temperature was consistent across seasons within a species.

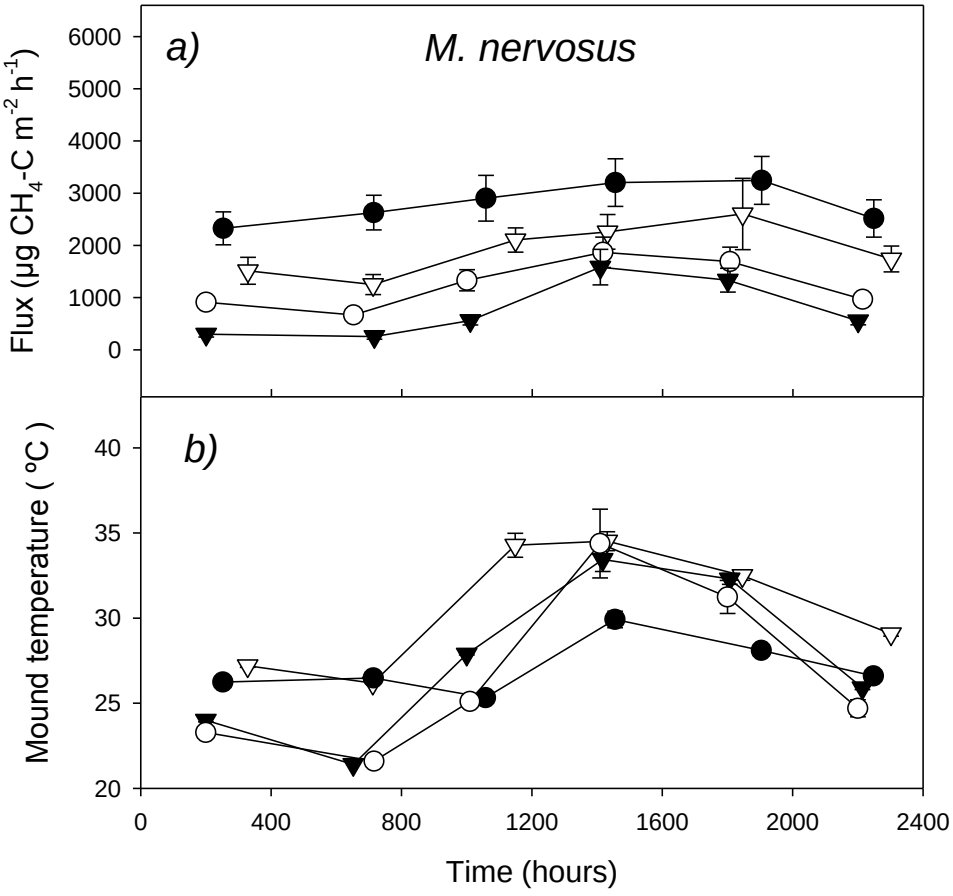
For the seasonal variations in CH_4 fluxes one-way ANOVA was repeated for each species on each site. Stepwise multiple-regression was used to analyze the significance of the relationship of CH_4 flux with mound water content (WC_{mound}) and mound temperature (T_{mound}). One-way ANOVA was used to investigate significant differences amongst species (n = 4) for wet season (March, n = 18) and dry season (May and June, n = 36) measurements separately. *M. nervosus* was the only species to be measured seasonally on more than one site (n = 3), as such, one-way ANOVA was used to investigate significant differences amongst different sites for the wet season measurements (March, n = 23), and the dry season measurements (May and June, n = 46) separately.

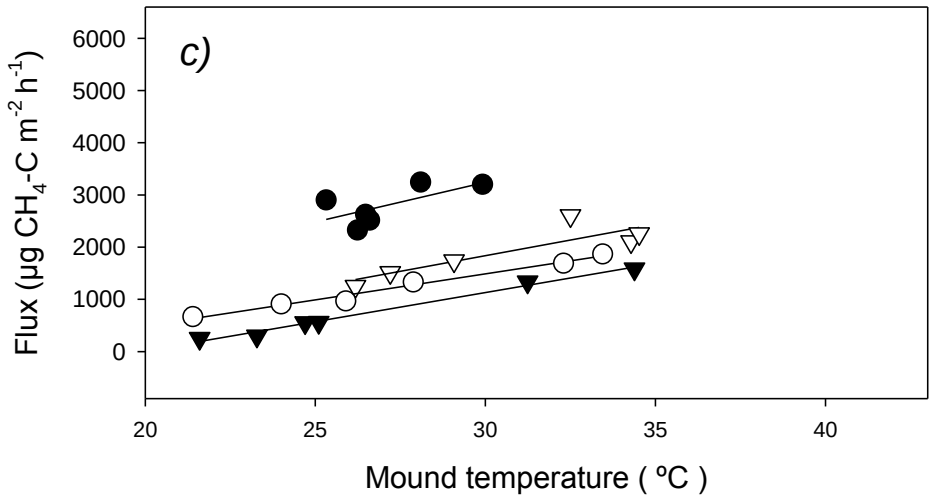
2.3 Results

2.3.1 Diurnal variation in CH_4 flux and time of day

For mounds of *M. nervosus, M. serratus and T. pastinator*, the greatest CH_4 flux within a diurnal cycle was measured between 15.00 and 19.00 hours and the smallest between 03.00 or 07.00 hours, regardless of the month or season of measurement (Figures 2.2, 2.3 and 2.4). CH_4 flux variations were significant in 9 of the 10 diurnal measurement events for the above three species. The variation in CH_4 flux closely followed the variation in mound temperature within a 24 hour diurnal cycle. Among the diurnal measurements, the greatest temperature range (23.3 – 40.3ºC) was measured in *T. pastinator* mounds in June and smallest (26.2 - 29.9ºC) in *M. nervosus* mounds in February.

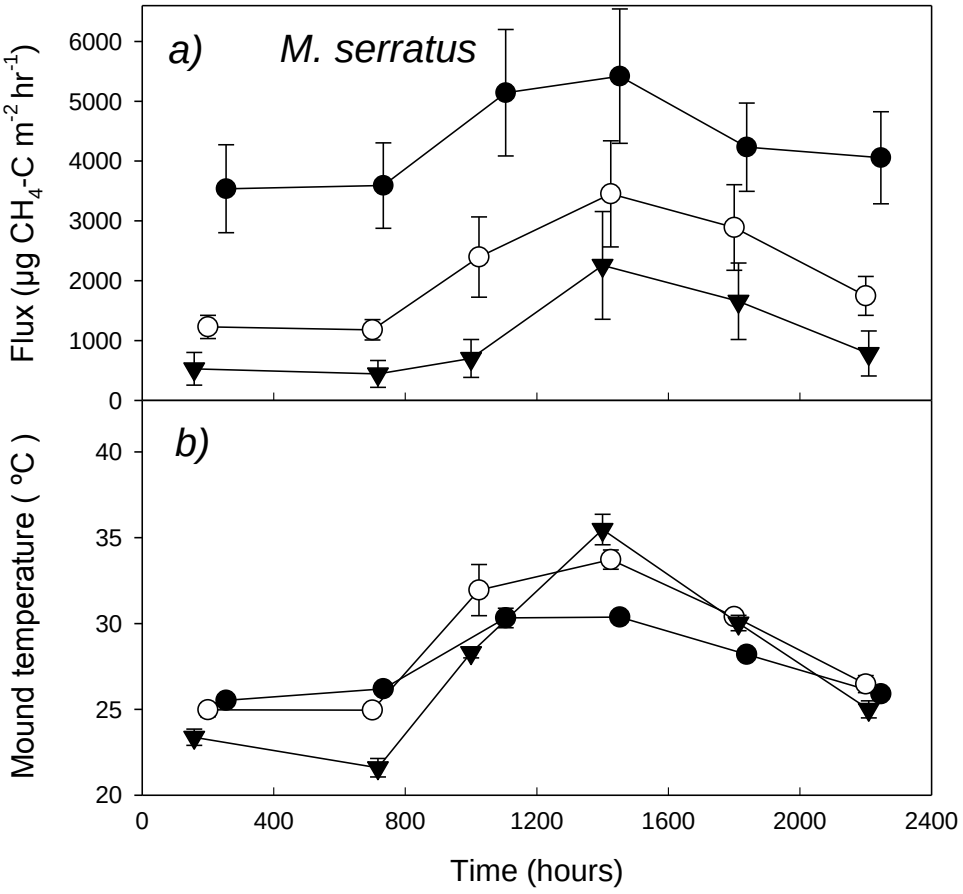
In daylight hours (07.00 – 18.00 hours) CH_4 fluxes measured at ~ 11.00 hours were closest to the mean diurnal CH_4 flux in 8 of the 10 diurnal measurement events. At night (19.00 – 0600 hours), CH_4 fluxes measured at 23.00 hours were closest to the mean diurnal CH_4 flux in 7 of the 10 diurnal measurement events. Mound temperatures measured at 15.00 hours had the greatest absolute difference from the mean diurnal mound temperature in 9 of the 10 diurnal measurement events.

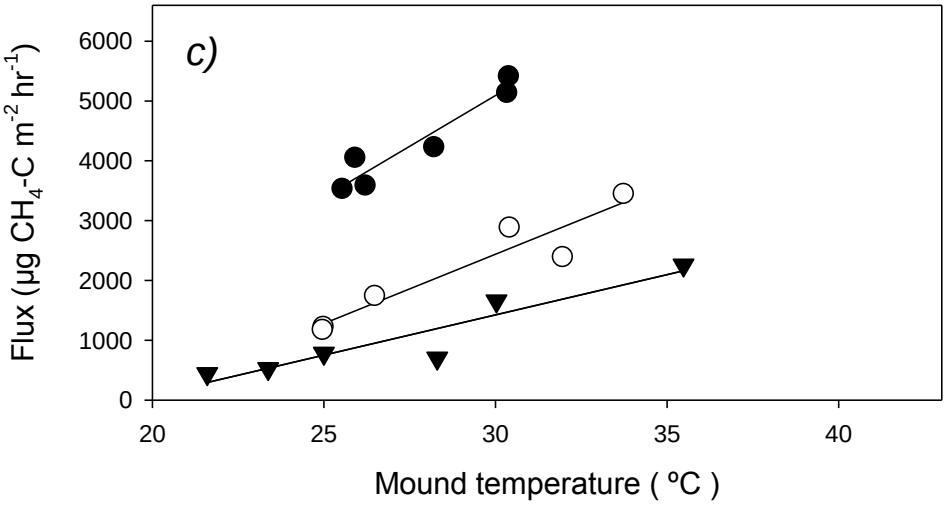




▽	Dec 2007	R^2 = 0.75	P = 0.02	Q10 = 1.97
●	Feb 2008	R^2 = 0.44	P = 0.15	Q10 = 1.61
○	Apr 2008	R^2 = 0.98	P < 0.01	Q10 = 2
▼	Jun 2007	R^2 = 0.98	P < 0.01	Q10 = 2.95

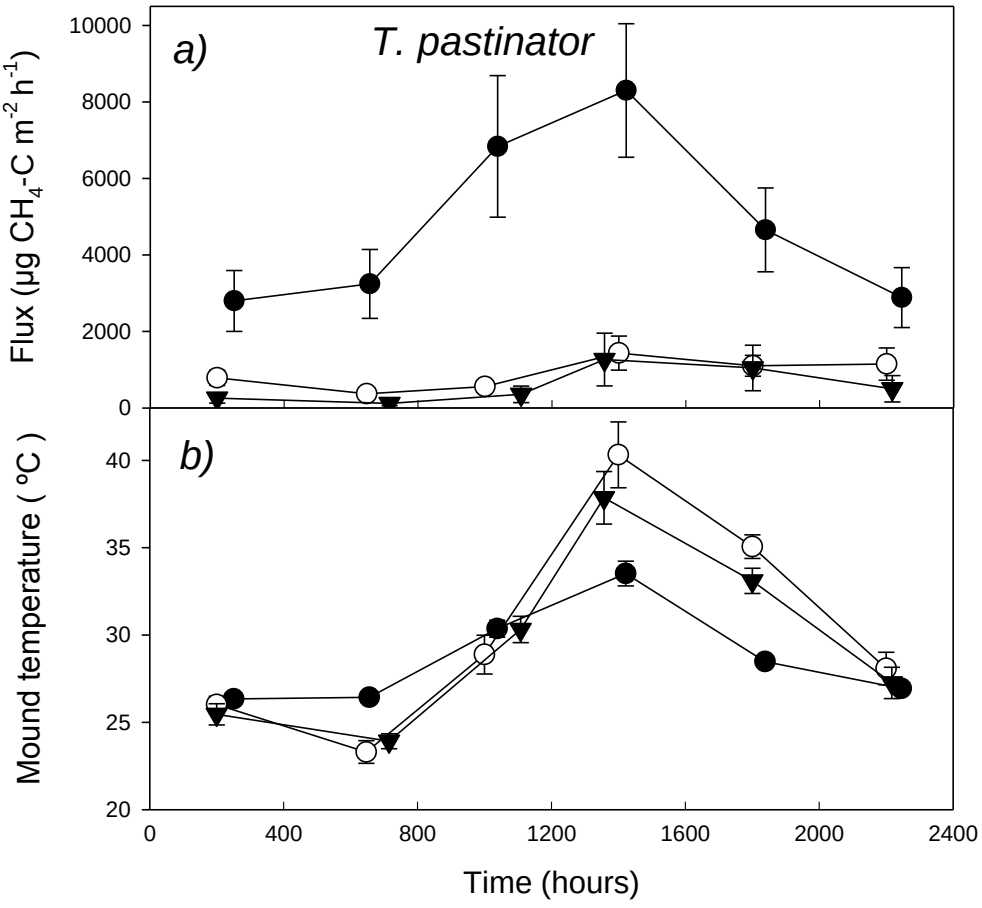
Figure 2.2 (**opposite**): Diurnal CH_4 and mound temperature variations in the *M. nervosus* mounds; error bars are standard errors of the mean; differences in slopes of regression line of mound CH_4 flux and mound temperature among different seasons/months are not significant.

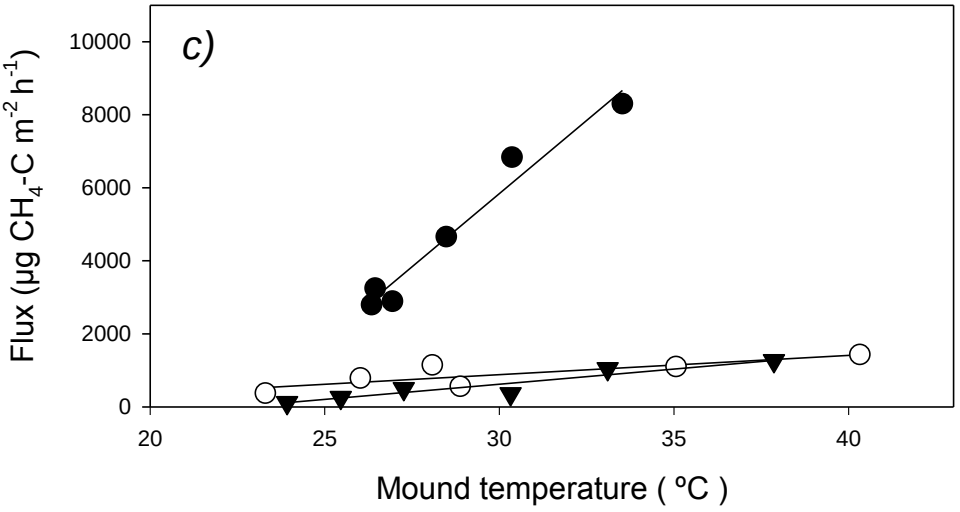




●	Feb 2008	R^2 = 0.91	P < 0.01	Q10 = 2
○	Apr 2008	R^2 = 0.90	P < 0.01	Q10 = 2.81
▼	Jun 2008	R^2 = 0.88	P < 0.01	Q10 = 2.79

Figure 2.3 (**opposite**): Diurnal CH_4 flux and mound temperature variations in M. serratus mounds; error bars are standard errors of the mean; the dry season (June) slope of the regression line of mound CH_4 flux and mound temperature is significantly different from the slopes in wet (February; $P < 0.01$) and wet-to-dry-transition (April; $P = 0.04$) seasons; differences among slopes for other months are not significant.





●	Feb 2008	R^2 = 0.96	P < 0.01	Q10 = 5.36
○	Apr 2008	R^2 = 0.69	P = 0.04	Q10 = 1.85
▼	Jun 2008	R^2 = 0.88	P < 0.01	Q10 = 5.05

Figure 2.4 (**opposite**): Diurnal CH_4 flux and mound temperature variations in T. pastinator mounds; error bars are standard errors of the mean; note the change of scale on y-axis; the wet season (February) slope of the regression line of mound CH_4 flux and mound temperature is significantly different from the slope in wet-to-dry-transition (April; P = 0.04) season; differences among slopes for other months are not significant.

2.3.1.1 Correlation between CH_4 flux and mound temperature

There was a significant correlation ($P \leq 0.05$) between CH_4 flux and mound temperature in 9 of the 10 diurnal measurement campaigns. The *M. nervosus* mound measurement event in February 2008 (wet season) showed no significant relationship and no significant CH_4 flux variations, probably because of the small diurnal temperature range. The R^2 between CH_4 flux and mound temperature ranged from 0.44 – 0.98 for *M. nervosus*, 0.88 – 0.91 for *M. serratus* and 0.69 – 0.96 for *T. pastinator* (Figures 2.2c, 2.3c and 2.4c).

The mounds of different termite species showed different responses to temperature among seasons as indicated by the slopes of regression lines for CH_4 flux vs. T_{mound} (Figures 2.2c, 2.3c and 2.4c). For *M. nervosus*, the slopes of different months were not significantly different, i.e. the response of flux to temperature was consistent across seasons (Figure 2.2c). For *M. serratus*, the slope of dry season (June) was significantly different from that of wet (February, $p < 0.01$) and wet-to-dry-transition (April, $p = 0.04$; Figure 2.3c). For *T. pastinator*, slope of wet season (February) significantly differed from dry (June, $p < 0.01$) and wet-to-dry-transition (April, $p < 0.01$) seasons (Figure 2.4c).
For *M. nervosus* (Fig. 2.2) and *M. serratus* (Fig. 2.3) Q_{10} values were greater in the dry season than the wet season. In comparison, Q_{10} values for *T. pastinator* mounds were very large in the dry (5.05) and wet seasons (5.36) but more similar (1.85) to those of other species in the wet-to-dry-transition in April.

The experiment in which CH_4 fluxes were measured from two *M. nervosus* mounds i) in-situ, ii) excavated (ex-situ) and kept under normal temperature variations and, iii) in a temperature controlled laboratory, showed a strong relationship between CH_4 flux and mound temperature in all three treatments with R^2 values of 0.99, 0.92 and 0.95,

respectively (Fig. 2.5). This experiment produced Q_{10} values of 2.1, 1.5 and 2.1 which were similar to those established from repeated *in-situ* diurnal measurement of *M. nervosus* mounds.

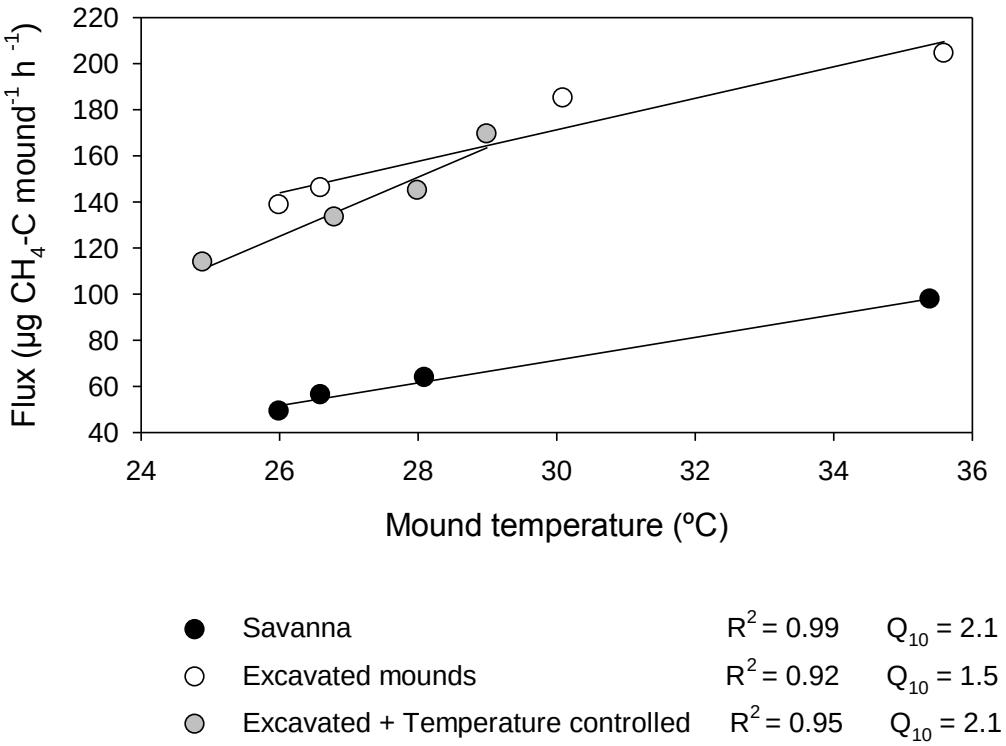


Figure 2.5: Diurnal CH_4 flux and mound temperature variations measured, i) under field conditions, ii) under field conditions with mounds excavated and termite movement restricted , and iii) excavated mounds measured in temperature controlled conditions in the laboratory where the natural diurnal temperature pattern was altered. A strong relationship was found in all three treatments. Q_{10} values here are also similar to the ones measured in regular diurnal field experiments.

2.3.2 Seasonal variations

2.3.2.1 Mound CH_4 flux

In all species, mound CH_4 fluxes were significantly greater in the wet season than in the dry season ($P < 0.01$). For *M. nervosus* mounds measured in both wet and dry seasons,

mean CH_4 fluxes ranged from 4444 (wet season) to 558 (dry season) µg CH_4-C m^{-2} h^{-1} at TERC (Fig. 2.6a; n = 5). A similar range, but with lower maximum flux rates, were observed for *M. nervosus* mounds at Howard Springs (Fig. 2.6b; n = 12) and the Douglas-Daly site (Figure 2.7a; n = 4). Regardless of site, there was an 8 to 9 fold difference in CH_4 fluxes between wet and dry season measurements for *M. nervosus* mounds.

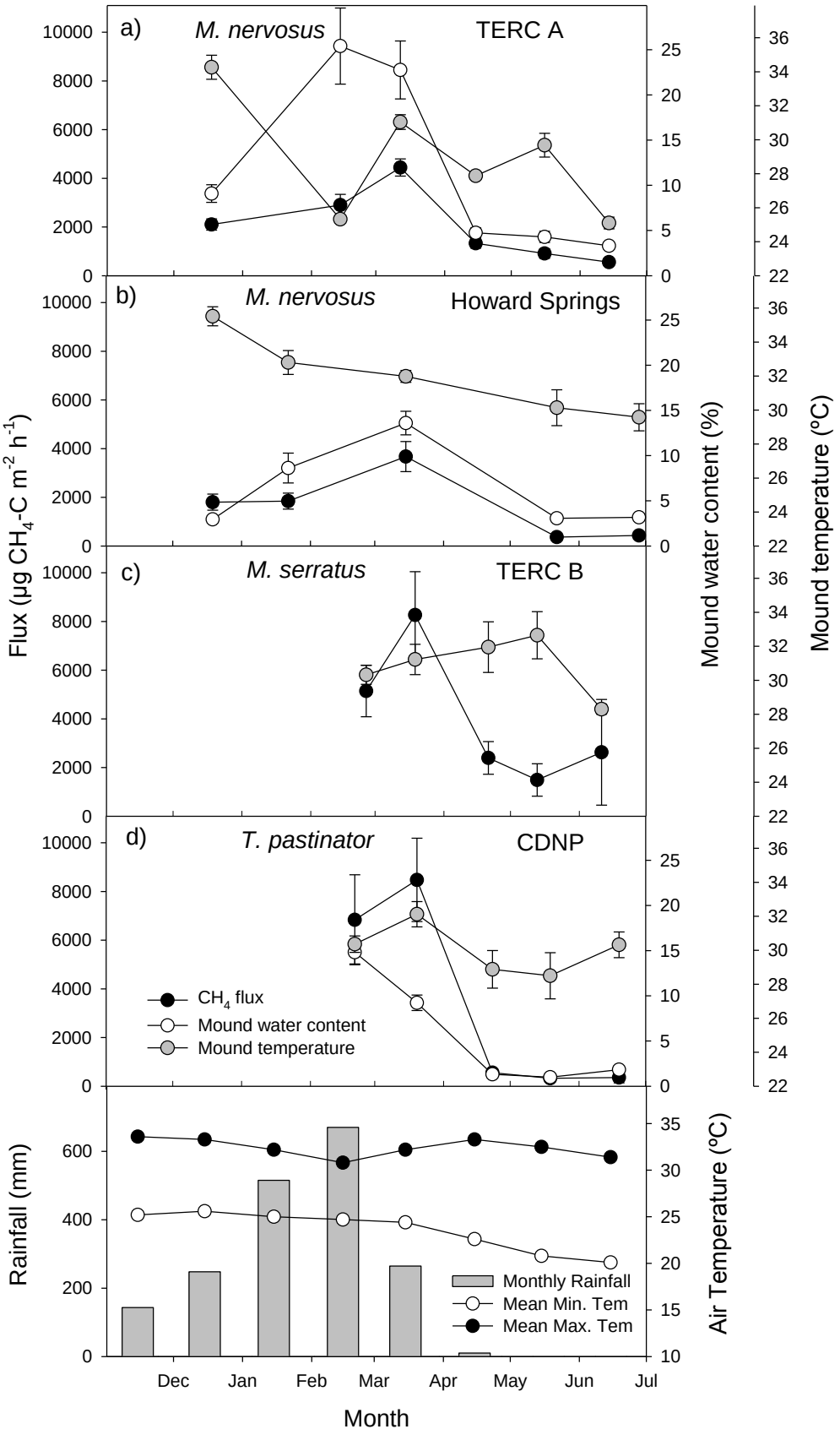

a)
M. nervosus
TERC A
b)
M. nervosus
Howard Springs
c)
M. serratus
TERC B
d)
T. pastinator
CDNP
CH4 flux
Mound water content
Mound temperature
Flux (µg CH4-C m-2 h-1)
Mound water content (%)
Mound temperature (ºC)
Rainfall (mm)
Air Temperature (ºC)
Monthly Rainfall
Mean Min. Tem
Mean Max. Tem
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Month

Figure 2.6 (**opposite**): Seasonal CH_4 fluxes from the mounds of *M. nervosus*, *M. serratus* and *T. pastinator;* error bars are standard errors of the mean; regression analysis of data is presented in Table 2.1. The bottom panel (e) shows climate data from Darwin Airport met station (Bureau of Meteorology, 2008), and is representative of all three sites in this figure.

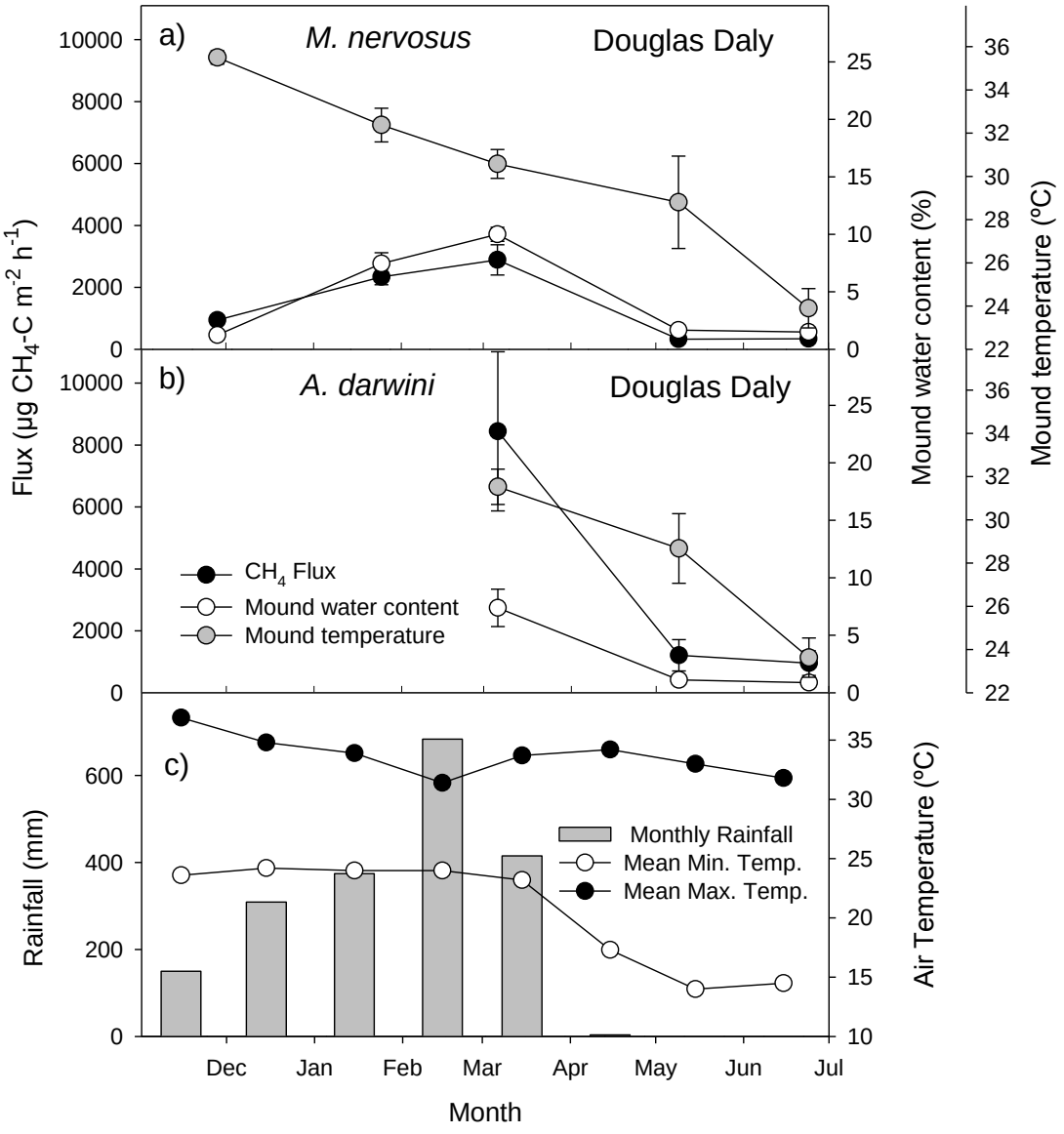


Figure 2.7: Seasonal CH_4 fluxes from the mounds of *M. nervosus* and *A. darwini* at Douglas-Daly; error bars are standard errors of the mean; regression analysis of data is

presented in Table 2.1. The bottom panel (c) climate data is from Douglas-Daly Research Farm meteorological station (Bureau of Meteorology, 2008).

Table 2.1: The relationship between environmental drivers and CH_4 fluxes from the mounds of termite species *M. nervosus, M. serratus, T. pastinator* and *A. darwini* at a seasonal scale, as determined by linear regression and stepwise multiple linear regression analysis of CH_4 flux, mound water content (WC_{mound}) and mound temperature (T_{mound}).

Species	**Site**	**Variable**	**P value**	**R^2**
M. nervosus	TERC	T_{mound}	ns	0.18
		WC_{mound}	<0.001	0.66
		Whole equation	<0.001	0.84
M. nervosus	Howard Springs	T_{mound}	<0.001	0.50
		WC_{mound}	<0.001	0.73
		Whole equation	<0.001	0.85
M. nervosus	Douglas Daly	T_{mound}	ns	0.30
		WC_{mound}	<0.001	0.69
		Whole equation	<0.001	0.85
M. serratus	TERC	T_{mound}	ns	0.30
		WC_{mound}	-	-
		Whole equation	-	-
T. pastinator	CDNP	T_{mound}	ns	0.30
		WC_{mound}	0.001	0.65
		Whole equation	0.001	0.70
A. darwini	Douglas Daly	T_{mound}	0.038	0.72
		WC_{mound}	<0.001	0.99
		Whole equation	<0.001	0.99

For *M. serratus* mounds, CH_4 fluxes ranged from 8267 in the wet season (March) to 700 μg CH_4-C m^{-2} h^{-1} in dry season (May), a 12 fold difference (Fig 2.6c; n = 4). For *T.*

pastinator mounds, CH_4 fluxes ranged from 8475 in the wet season (March) to 325 µg CH_4-C m^{-2} h^{-1} in the dry season (May), a 26 fold difference (Figure 2.6d; n = 5). For *A. darwini* mounds, CH_4 fluxes ranged from 8441 in wet season (March) to 956 µg CH_4-C m^{-2} h^{-1} in the dry season (May), a 9 fold difference (Figure 2.7b; n = 4).

2.3.2.2 Mound water content

The gravimetric water content of mound walls (WC_{mound}) also differed between the different seasons and ranged between 7.4 % (*A. darwini*) and 22.8 % (*M. nervosus*, TERC A) in the wet season and between 1.0 % (*A. darwini*) and 3.8 % (*M. nervosus*, TERC A) in the dry season. However, sporadic rain events of the wet season followed by drying made comparison of WC_{mound} amongst sites and species within the wet season problematic.

2.3.2.3 Mound temperature

In general, termite mound temperatures (T_{mound}) measured at ~11.00 hours, i.e. time of flux measurements, were lower in the dry season compared to the wet season. Temperatures ranged from 23.6 (*A. darwini*) to 32.7 ºC (*M. serratus*) in the dry season and from 30.6 (*M. nervosus*) to 32.1 ºC (*T. pastinator*) in the wet season (Figures 2.6 and 2.7). The seasonal range of T_{mound} measured at ~11.00 hours was much smaller than the diurnal temperature range (Figures 2.2, 2.3 and 2.4).

2.3.2.4 Seasonal CH_4 flux patterns as a function of mound water content and mound temperature

Linear regression between mound CH_4 flux and WC_{mound} was able to explain 65 to 99% of the seasonal variation, whereas regression between mound CH_4 flux and T_{mound} could explain less seasonal variation (18 to 72%) and was only significant for *M. nervosus* mounds ($P < 0.01$) at Howard Springs and *A. darwini* mounds ($P = 0.038$). A stepwise multiple linear regression (Table 2.1) with T_{mound} and WC_{mound} combined best defined the seasonal variations in mound CH_4 flux explaining 70 to 99% of the seasonal variation ($P < 0.01$).

2.3.3 Site comparison of *M. nervosus* CH_4 fluxes

The average seasonal CH_4 fluxes from *M. nervosus* mounds were in the order of TERC > Howard Springs > Douglas-Daly (Figures 2.6 and 2.7). Mound CH_4 fluxes at TERC were 1.2 fold greater than in Howard Springs and 1.5 fold greater than in Douglas-Daly in the wet season and 1.8 and 2.2 fold greater in the dry season, respectively. Mound CH_4 fluxes were only significantly different between *M. nervosus* in TERC and Douglas-Daly, in the dry season ($P = 0.02$).

2.3.4 Species comparison of CH_4 fluxes

In the wet season (March) when CH_4 flux was greatest, the average seasonal measurements of mound CH_4 fluxes were in the order of *T. pastinator* > *A. darwini* > *M. serratus* > *M. nervosus*, but differences among the first three species were small (<3%). CH_4 fluxes from *M. nervosus* mounds were almost half that from the other three species in the wet season (Figure 2.8). In the dry season, however, variation in CH_4 flux among termite mound species was much smaller in magnitude (Figure 2.8).

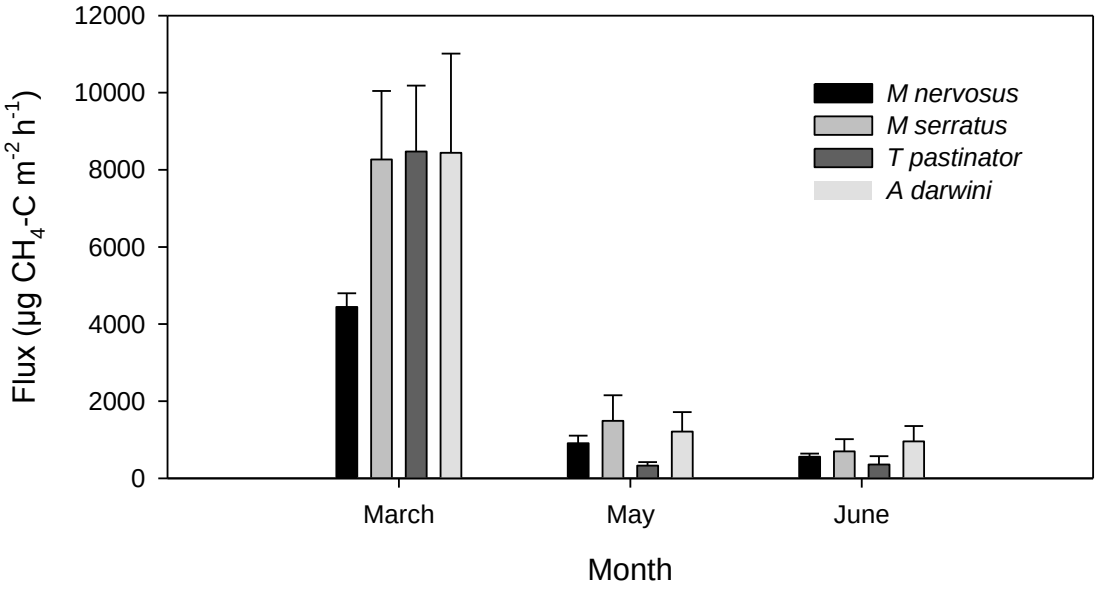


Figure 2.8: CH_4 flux variations among four termite species in wet (March) and dry (May and June) seasons.

2.4 Discussion

2.4.1 Diurnal variations in CH_4 flux

Our field and laboratory-based experiment results strongly suggest temperature as the main factor causing significant diurnal variation in mound CH_4 flux. This highlights the critical importance of understanding the diurnal variation in CH_4 flux and of measuring CH_4 fluxes continuously from termite mounds or at a time that best represents the daily mean, in our study between 10.00 and 12.00 hours. Measuring mound CH_4 flux in the afternoon can result in an overestimation of up to an order of magnitude greater than the actual mean daily flux.

Diurnal variation in CH_4 flux from termite mounds has not been presented before for a 24 hour period. The only other study (Seiler et al., 1984) that reported diurnal variations in CH_4 flux from termite mounds (*Trinvervitermes* spp.) was based on measurements made between 05.00 and 19.00 hours. Seiler et al. (1984) observed greatest CH_4 fluxes in the late afternoon and smallest in the early morning, which concurs with our findings. Diurnal variations in CO_2 fluxes from termite mounds have been reported by other studies (Peakin and Josens, 1978; Seiler et al., 1984).

Increased food consumption by termites at higher temperatures can possibly lead to higher rates of CH_4 production as a by-product of cellulose breakdown (Fraser et al., 1986). Doubled food consumption rates for an increase of 5 ºC in termite habitats have been reported for many species (Becker, 1970). Howick et al., (1975) found *Mastotermes darwiniensis* wood consumption rates increased from 25 to 35 % when their habitat temperature was raised from 26 to 32ºC. However, the possible influence of other factors e.g. availability of food, effect of temperature on respiration has also been suggested (Fraser et al., 1986). In another study (Lepage, 1981) termite abundance in the soil outside the mound was found to be greatest early in the morning in the dry season, and greatest in the afternoon in the wet season (frequently a time of cloud cover and rainfall); suggesting that high temperatures may limit foraging activities outside the mound (Ohiago and Woods, 1976). However, our ex-situ mound excavation experiment showed a strong relationship (R^2 = 0.92 and 0.95) between CH_4 flux and T_{mound} even when

termite movement in and out of the mound had been prevented. Thus, while rates of food consumption and foraging activities can possibly contribute to diurnal variations in mound CH_4 fluxes, temperature seems to be the major factor driving these variations in CH_4 fluxes from the termite mounds.

Different species showed a different diurnal temperature response in different seasons. The response of *M. nervosus* to diurnal temperature variation was consistent across the seasons, while *M. serratus* and *T. pastinator* showed significant differences in their response to diurnal temperature variation according to the season. The variation in mound CH_4 flux response to temperature across seasons can possibly be because of seasonal variations in moisture, food availability and/or mound population dynamics. Further research is needed to investigate the causes for this variable temperature response to CH_4 fluxes across seasons in termite species.

The Q_{10} calculated from the diurnal measurements ranged from 1.6 to 5.4, which is similar to the 2.6 – 5.2 range reported by Fraser et al. (1986) for four Australian termite species in a laboratory experiment. In another laboratory experiment, Zimmerman and Greenberg, (1983) reported a mean Q_{10} of 4.8 and 3.4 for *Zootermopsis augusticollis* using two different laboratory incubation methods. Khalil et al. (1990) reported almost an order of a magnitude increase in CH_4 fluxes as a result of a 10°C increase in temperature during seasonal measurements of *Coptotermes lacteus* mounds near Melbourne, Australia. These results suggest there could be an increase in termite-produced CH_4 as a result of increased temperatures because of climate change. However, climate change may also change rainfall regimes and carbon resource supply through NPP, but the impact of these factors upon termite activity and CH_4 emissions is unclear.

2.4.2 Seasonal variations in CH_4 flux

The magnitude of the increase in CH_4 fluxes from dry to wet season (5 to 26 fold) reveals the importance of detailed seasonal measurements in preparing annual CH_4 budget for a savanna landscape. Khalil et al. (1990) reported summer termite fluxes that were greater than the rest of the seasons combined, which is similar to our findings.

An interaction model of T_{mound} and WC_{mound} best described these seasonal flux variations. T_{mound} effect on CH_4 fluxes was less pronounced than WC_{mound} probably because of the aseasonal, relatively constant pattern of mean daily temperatures in tropical savannas (Khalil et al., 1990). While temperature has a direct effect on termite emissions, it is yet to be established if moisture has also any direct effect on termite emissions. An immediate response of CH_4 fluxes to both soil moisture and soil temperature in the mounds of *Cubitermes fungifaber* have been reported in African savannas (Brümmer et al., 2009). Higher termite activity in response to higher soil moisture and air humidity levels (Abensperg-Traun and De Boer, 1990; Buxton, 1981; Mackay et al., 1986; Wheeler et al., 1996), and higher food availability and/or digestibility can also be reasons for greater mound CH_4 flux in the wet season. We observed greater mound building activity in the wet and transition seasons as compared to the dry season. However, it is not clear if the greater activity in the wet season can also result in higher emissions per unit termite biomass. Changes in termite population across seasons can also result in seasonal CH_4 flux variations. More research is needed to discern if the seasonal variations in mound CH_4 flux are because of, i) variations in emissions per unit termite biomass, ii) seasonal variations in termite numbers within a mound, or iii) a combination of both.

2.4.3 Species and site variations

Variations in CH_4 fluxes from mounds of different termite species are not unexpected because of the variable biomass in mounds of different species and variable digestive microbiology across species and within colonies of the same species (Martius et al., 1993). Termites from different feeding groups produce different amounts of CH_4 per unit termite biomass because of differences in acetogenesis and methanogenesis activity by gut bacteria; for example soil-feeders are reported to produce more CH_4 per termite as compared to other feeding groups (Brauman et al., 1992; Rouland et al., 1993). Wheeler et al. (1996), found only seven out of thirteen species studied in the laboratory produced CH_4 and within a given species not all colonies produced CH_4. In our study, all termite mounds produced substantial amounts of CH_4 in both wet and dry seasons. The lowest emissions were from *M. nervosus*, which is also the most abundant mound-building termite species in this savanna ecosystem (Dawes-Gromadzki and Spain, 2003). It is

important to account for both species differences in CH_4 fluxes and mound density (number per hectare) when scaling up results to the landscape level.

Variations in CH_4 fluxes from mounds of the same species at different sites can simply be a function of differences in biomass (termite numbers) in the measured mounds at different sites. Differences in the environmental conditions such as vegetation and rainfall at these sites may drive the differences in termite populations.

2.5 Conclusions

Our results demonstrate that CH_4 flux from termite mounds vary significantly during a 24 hour period but also between seasons and between species. These results indicate that great care needs to be taken when designing experiments or monitoring programs to estimate annual CH_4 budgets for termite species. It will be very difficult to scale up measurements that were made at one point in time to an annual budget due to the large diurnal, seasonal and species specific variations. CH_4 fluxes from termite mounds need to be measured at different times of the year to account for seasonal variability and each species needs to be characterized separately. For an accurate estimate of CH_4 emissions from termite mounds it is critically important to measure fluxes either continuously or at a time that is most representative of the daily flux mean (i.e. in our case 10.00 and 12.00 hours).

Our results also showed that stepwise multiple regression models can be used with high confidence to scale up CH_4 fluxes if environmental drivers such as mound temperature and mound moisture are known. However, we were unable to identify some of the mechanisms that drive the observed changes in CH_4 fluxes during the season. A more detailed characterization of termite population dynamics and species specific responses to temperature and moisture will be necessary to get a better mechanistic understanding of the processes controlling emissions from termites in the field.

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3 The importance of termites to the CH_4 balance of a tropical savanna woodland of northern Australia

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Abstract

Termites produce methane (CH_4) as a by-product of microbial metabolism of food in their hindguts, and are one of the most uncertain components of the regional and global CH_4 exchange estimates. This study was conducted at Howard Springs near Darwin, and presents the first estimate of CH_4 emissions from termites based on replicated *in situ* seasonal flux measurements in Australian savannas. Using measured fluxes of CH_4 between termite mounds and the atmosphere, and between soil and the atmosphere across seasons we determined net CH_4 flux within a tropical savanna woodland of northern Australia. By accounting for both mound-building and subterranean termite colony types, and estimating the contribution from tree-dwelling colonies it was calculated that termites were a CH_4 source of +0.24 kg CH_4-C ha^{-1} y^{-1} and soils were a CH_4 sink of -1.14 kg CH_4-C ha^{-1} y^{-1}. Termites offset 21% of CH_4 consumed by soil resulting in net sink strength of -0.90 kg CH_4-C ha^{-1} y^{-1} for these savannas. For *Microcerotermes nervosus* (Hill), the most abundant mound-building termite species at this site, mound basal area explained 48% of the variation in mound CH_4 flux. CH_4 emissions from termites offset 0.1% of the net biome productivity (NBP) and CH_4 consumption by soil adds 0.5% to the NBP of these tropical savannas at Howard Springs.

3.1 Introduction

The atmospheric concentrations of greenhouse gases including carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) have increased substantially from pre-industrial levels in response to human activities, resulting in global climate change (IPCC, 2007). CH_4, with a global source budget of 580 Tg y^{-1} (Denman and Brasseur, 2007), has a global warming potential (GWP) 25 times that of CO_2 on a 100-year time horizon (Forster et al., 2007). Almost one-third of CH_4 emissions originate from natural sources including submerged soil, CH_4 hydrates, oceans, fresh water and termites. The remaining two-thirds originate from anthropogenic sources such as fossil fuel energy production and use, paddy rice cultivation, enteric fermentation in the guts of ruminant animals, biomass burning, landfill, animal waste and domestic sewage (Dalal et al., 2008).

The largest sink for CH_4 is hydroxyl (OH^-) oxidation in the upper atmosphere, which accounts for more than 90% of global sink mechanisms. A smaller (~6%), but important, CH_4 sink is provided through CH_4 oxidation by methanotrophic bacteria in aerobic soils (Dalal et al., 2008). Soils are the only terrestrial sink for atmospheric CH_4, with estimated sink strength of 22-100 Tg CH_4 y^{-1} (Potter et al., 1996; Castaldi et al., 2006; Dutaur and Verchot, 2007).

The global terrestrial CH_4 sink is estimated to be -4.79 ± 0.61 kg CH_4 ha^{-1} y^{-1} of which soils in tropical and temperate forests act as the largest sinks. Terrestrial wetlands are the largest CH_4 source of +168.85 ± 38.16 kg CH_4 ha^{-1} y^{-1} (Dalal and Allen, 2008). CH_4 sink capacity for tropical savannas is comparatively low at -0.80 ± 0.51 kg CH_4 ha^{-1} y^{-1} (Dalal and Allen, 2008). Savannas cover around one-sixth of the global terrestrial land surface and account for 30% of total net primary production of all terrestrial vegetation (Grace et al., 2006). The main natural sources of CH_4 in the tropical savannas are: i) biomass burning, ii) ephemeral wetlands, and iii) termites. Savanna soils have been reported to be either a net CH_4 sink (MacDonald et al., 1999; Castaldi et al., 2006) as well as a net CH_4 source (Hao et al., 1988; Poth et al., 1995; Otter and Scholes, 2000; Brümmer et al., 2009) in both African and South American studies. Savanna soils and soils of other seasonally dry ecosystems represent approximately 40% of the global terrestrial CH_4 sink

through soil methanotrophic oxidation activity, not because of high oxidation rates but because of the large area these ecosystems cover (Potter et al., 1996). Savanna soils become a net CH_4 source when methanotrophic activity is surpassed by methanogenesis under anoxic soil conditions and/or through subterranean termite activity (MacDonald et al., 1999).

Termites play a major role in the ecological functioning of savanna ecosystems via nutrient cycling and maintaining soil structure (Holt and Coventry, 1990; Lavelle et al., 1997; Dangerfield et al., 1998; Dawes, 2010). Termites constitute 61% of total macroinvertebrates abundance in savannas (Dawes-Gromadzki, 2007), and generate an important 0.2 to 2.0% of global terrestrial CO_2 emissions (Sugimoto et al., 2000). However termites have been highlighted more often as being one of the most uncertain components of global and regional CH_4 balances. Zimmerman et al. (1982) suggested that termites represent about 30% of global annual CH_4 emissions, but these initial estimates have been revised down to between 3% and 20% in later studies (Seiler et al., 1984; Fraser et al., 1986; Khalil et al., 1990; Martius et al., 1993; Sanderson, 1996; Bignell et al., 1997; Sugimoto et al., 1998). All estimates of global termite CH_4 emissions have very wide confidence limits due to the errors and uncertainties associated with: i) measured emission rates in the field or even the laboratory, and ii) the scaling factors used, such as termite mound density or estimates of global termite biomass.

Estimates of CH_4 emissions from termites that are based on in situ flux measurements are considered more representative than laboratory based studies (Seiler et al., 1984; Khalil et al., 1990; Sugimoto et al., 1998). However, most field-based studies have been limited to epigeal mounds, thus ignoring the important contribution of subterranean (MacDonald et al., 1999) and wood-nesting (tree or log) termites. Other important issues to consider when scaling up are diurnal (Seiler et al., 1984; Jamali et al., 2011), seasonal (Khalil et al., 1990; Jamali et al., 2011) and species specific (Brauman et al., 1992; Rouland et al., 1993; Jamali et al., 2011) variations in CH_4 fluxes from termites.

In Australia, savannas cover a quarter of the continent (1.9 million km^2) which represents about 12% of the world's savanna biome (Williams et al., 2005). Research into C exchange processes in the tropical savannas of northern Australia has been limited to CO_2 exchange using eddy covariance methods and C stock assessments through destructive harvests and sampling (Eamus et al., 2001; Beringer et al., 2003; Chen et al., 2003; Beringer et al., 2007). While there are several studies reporting CH_4 source and sink strength of savanna ecosystems in Africa and South America (Castaldi et al., 2006), data from Australia are almost completely lacking.

This study builds upon Jamali et al. (2011) and is the first field based replicated study in Australian savannas which estimates the contribution of termites to the total ecosystem CH_4 balance of a savanna woodland. The objectives of this study were to: 1) scale up measured mound CH_4 fluxes to the hectare level using the mound density and size surveys at, and around, Howard Springs; 2) investigate the relationship between mound basal area and mound CH_4 flux for the most common termite species at Howard Springs, i.e. *M. nervosus*; 3) scale up measured soil CH_4 fluxes and estimate the contribution of hypogeal (subterranean) termites based on the proportion of positive flux emissions; 4) estimate the contribution that all termites make (epigeal, hypogeal and arboreal) to Howard Springs CH_4 balance at the ecosystem scale.

3.2 Materials and Methods

3.2.1 Site description

The study was conducted in a savanna woodland site within the flux footprint of an eddy covariance tower at Howard Springs (Beringer et al., 2007), 35 km south-east of Darwin (12º 29′ 39″ S, 130º 08′ 09″ E). The dominant tree species include *Eucalyptus miniata* (Cunn. Ex Schauer) and *Eucalyptus tetrodonta* (F. Muell), with sub-dominants including *Erythrophyleum chlorostachys* (F. Muell), *Terminalia ferdinandiana* (F. Muell), *Corymbia porrecta* (K. D. Hill and L. A. S. Johnson) and *Corymbia bleeseri* (K. D. Hill and L. A. S. Johnson). The understorey consists of semi-deciduous and deciduous small trees and shrubs and a seasonally continuous cover of annual and perennial C4 grasses

(Beringer et al., 2007). Usual fire recurrence is approximately 2 in every 3 years, but since 2006 the site has been subjected to annual low severity fire events (Hutley pers. comm.).

The climate of the region is wet-dry tropical, with a wet season occurring from November to April, and a dry season from May to October (Cook and Heerdegen, 2001). Rainfall during the observation years of 2007, 2008 and 2009 was 2245 mm, 1668 mm and 1562 mm, respectively at the meteorological station 11 km from the site (Bureau of Meteorology, 2009).

3.2.2 Mound and tree basal areas

Four plots of 50 x 50 m (0.25 ha) were established at Howard Springs and termite mound basal area (m^2 ha^{-1}) was calculated from the circumference measurements at the base of each mound (Fig. 3.1). The termite species inhabiting each mound were identified by collecting termite soldiers and storing them in vials of 70% ethanol for later identification at the CSIRO laboratories in Darwin. Tree basal area (m^2 ha^{-1}) was calculated from the measurements of stem diameter at breast height (DBH) taken at 1.3 m height for all trees in each plot. Stem basal area was subtracted from the 0.25 ha for appropriate scaling up of CH_4 fluxes from the remaining soil area and termite mound basal area.

Figure 3.1: Measurement of mound dimensions during mound surveys at Howard Springs

3.2.3 Mound CH_4 flux measurements

CH_4 fluxes were measured once every four to six weeks between December 2007 and June 2008 from 16 mounds (4 per plot) of *Microcerotermes nervosus* (Hill), the most abundant termite species at Howard Springs. Fluxes from mound-building termite species other than *M. nervosus* (Table 3.1) could not be measured at Howard Springs to any degree of replication because of the sparse mound distribution and logistical issues of plot size. To account for CH_4 fluxes from termite mounds other than *M. nervosus* analogous sites to Howard Springs were established that had mounds of other termite species at greater densities. These mound-building termite species included *Microcerotermes serratus* (Forggatt) at CSIRO's Tropical Ecosystem Research Centre (TERC), Darwin (n = 4), *Tumulitermes pastinator* (Hill) at Charles Darwin National Park (n = 5), and *Amitermes darwini* (Hill) in the Douglas-Daly region (n = 4). Fluxes from these additional sites were measured once every four weeks between February and June

2008, from wet season into dry season. *T. pastinator* is recognized as a common mound-building species in these savanna-woodland *E. tetrodonta*/*E. miniata* ecosystems (Watson and Abbey, 1993).

Jamali et al. (2011) demonstrated through diurnal CH_4 flux measurements from replicated mounds of *M. nervosus*, *M. serratus* and *T. pastinator* that fluxes measured between 10:00 and 12:00 hours best represented mean daily mound CH_4 flux. As such, mound CH_4 fluxes at Howard Springs were always measured between 10:00 and 12:00 hours local time.

Mound fluxes were measured using static manual chambers of volumes between 0.02 and 0.20 m^3 constructed from polyvinylchloride (Fig. 3.2a). Mound volume was calculated by multiplying the mound height by average mound basal area calculated from the mound circumference measured at four points along the height. Mound volume was subtracted from the chamber volume to calculate the net chamber headspace volume. Mound volumes ranged from 0.01 to 0.06 m^3 for *M. nervosus*, and from 0.004 to 0.09 m^3 for the remaining three species. Samples of chamber headspace gas (20 ml) were taken at 0, 10, 20 and 30 minutes after attaching the chamber, using a 25 ml Terumo™ syringe fitted with a two-way stop-cock and needle inserted into the chamber headspace through a rubber septum. Each 20 ml gas sample was transferred to a pre-evacuated 12 ml gas tight vial (Labco Exetainer) so that each was over-pressurized. These gas samples were transported by road to the Creswick laboratories of The University of Melbourne for CH_4 concentration analysis using a gas chromatograph (GC, Shimadzu™, GC17a) fitted with a CTC auto-sampler, ten port Valco™ valve, a 1.8 m poropak Q separation column attached to a flame ionization detector (FID). Flux in µL CH_4 m^{-2} h^{-1} was calculated using the linear regression of the four CH_4 concentrations to obtain a slope with units of ppm (v) $hour^{-1}$, and then multiplied by the chamber volume (L) and divided by the mound basal area (m^2). This flux was then converted to µmol CH_4 m^{-2} h^{-1} thus correcting for the relationship between temperature, pressure and volume on the basis of ideal gas law. Fluxes in µmol CH_4 m^{-2} h^{-1} were then converted to µg CH_4-C m^{-2} h^{-1} and used in all the

statistical analyses. Further details on mound CH_4 flux calculation and mound volume calculation including equations can be found in Jamali et al. (2011).

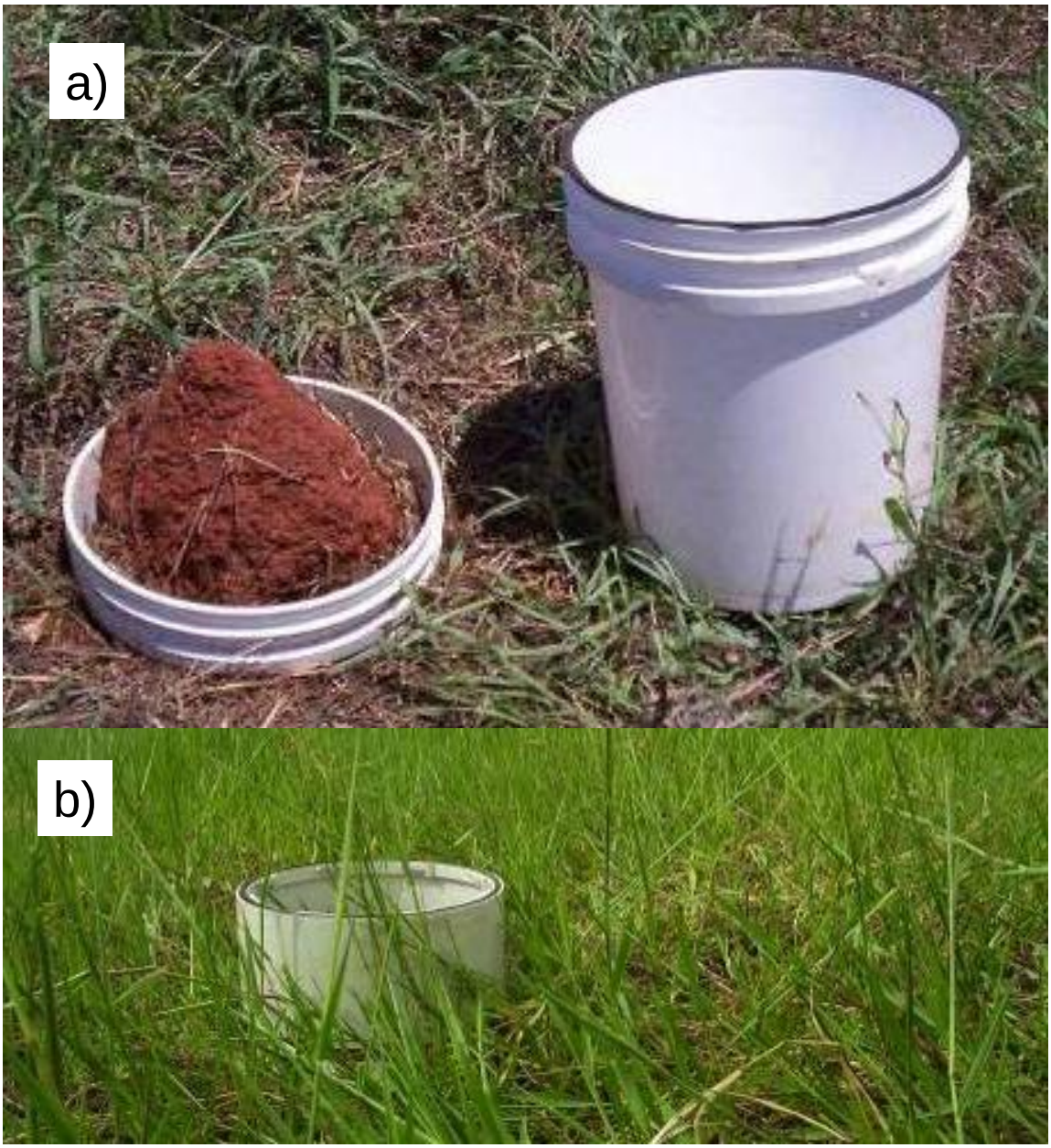

Figure 3.2: Manual chambers for measuring methane fluxes from **a)** termite mounds, and **b)** soil

3.2.4 Soil CH_4 flux measurements

Soil CH_4 fluxes were measured in three of the four 50 x 50 m plots every four to six weeks from October 2007 to January 2009. Five static manual chambers of 24 cm diameter and 20 cm height (Fig. 3.2b) were inserted 3 cm in the ground, and headspace gas samples (20 ml) were collected through a rubber septum using a 25 ml syringe fitted with a two-way stopcock and needle at 0, 20, 40 and 60 minutes. Gas samples were stored in pre-evacuated gas vials of 12 ml volume and analyzed as described above in section 'mound flux measurements'. Soil CH_4 fluxes were calculated in µg CH_4-C m^{-2} h^{-1} in the same way as for termite mounds.

3.2.5 Data analysis

3.2.5.1 Relationship between mound basal area and mound CH_4 flux

The mean mound CH_4 fluxes of 16 separate *M. nervosus* mounds measured twice in the wet and twice in the dry season (n = 64), were used to analyze the relationship between mound basal area and mound CH_4 flux. Simple linear regression and second-degree polynomial regression were applied.

3.2.5.2 Spatial and temporal scaling up

Measured CH_4 fluxes (µg CH_4-C m^{-2} h^{-1}) of soil and mounds were scaled up based on the assumption that fluxes measured at one point in time represented the mean daily flux for the rest of the days in the same month. Monthly flux (kg CH_4-C ha^{-1}) was calculated by multiplying the measured flux by the number of hours per day (24) and by the number of days in each month while taking in account the unit conversion. The CH_4 fluxes for months when measurements were not taken were estimated by using the mean measured flux of other months in the same season, or transition one month before and one month after the month being estimated. For mounds, the average of CH_4 flux measured in the dry season months of May and June was used to represent mean CH_4 flux in the other dry season months (July, August and September). Likewise, the average mound CH_4 flux measured in the wet season months of January and March was used for February. The average of mound CH_4 flux measured in March and May was used to estimate the flux in wet-to-dry transition month of April. To estimate the mound CH_4 flux in dry-to-wet

transition month of November the average of flux in September and December was used, likewise, October was estimated from the average of September and November. For soil, the average of CH_4 fluxes measured in January and February was used to represent the soil CH_4 flux in March, and the flux measured in the dry season month of July was used for the other dry season months of June and August. For September the average of soil CH_4 flux measured in July and October was used. Annual flux (kg CH_4-C ha^{-1} y^{-1}) was calculated by summing up the monthly fluxes.

The contribution of termites to the CH_4 balance at Howard Springs was calculated separately for the three main termite nesting groups: i) epigeal (mounds protruding above ground), ii) hypogeal (below ground or subterranean nests), and iii) wood and arboreal nesting (in dead wood or trees) termites (Eggleton et al., 2002; Dawes-Gromadzki, 2008). Mound (epigeal) CH_4 fluxes were scaled up using proportional basal area covered by the mounds of *M. nervosus* and other termite species (Table 3.1). Methane fluxes from the mounds of *M. serratus*, *T. pastinator* and *A. darwini* were similar and as such were grouped together and a mean flux used in scaling up to the ecosystem level. It was assumed that this mean mound flux represented the CH_4 flux from the mounds of termite species other than *M. nervosus* recorded at the Howard Springs site (Table 3.1).

For *M. nervosus*, flux was also calculated for each mound in the four 0.25 ha plots using the second degree polynomial regression equation (Fig. 3.5) between mound basal area (m^2) and flux (µg CH_4-C $mound^{-1}$ h^{-1}). Adding these individual mound fluxes in four 0.25 ha plots resulted in a flux in µg CH_4-C ha^{-1} h^{-1} which was then converted to an annual flux by multiplying with the number of hours in a day (24) and number of days per year (365).

Table 3.1: Termite species collected from epigeal mounds at Howard Springs.

Species	**Family**
Microcerotermes nervosus	Termitidae
Microcerotermes serratus	Termitidae
Microcerotermes spp.	Termitidae
Nasutitermes eucalypti	Termitidae
Nasutitermes longipennis	Termitidae
Nasutitermes graveolus	Termitidae
Lophotermes septentrionalis	Termitidae
Ephelotermes melachoma	Termitidae
Macrognathotermes sunteri	Termitidae
Amitermes darwini	Termitidae
Coptotermes acinaciformis	Rhinotermitidae

For the contribution of subterranean termites, the soil flux data of chambers showing net CH_4 emissions and the percent area covered by such chambers as a proportion of the total area covered by soil chambers were used. For a conservative approach and to avoid mixing soil methanogenesis (i.e. CH_4 production as a result of soil microbial activity in anaerobic soils during wet season) with termite emissions, the soil emission values of the wet season were replaced with a mean soil emission rate from rest of the seasons when soils were too dry for methanogenic activity.

The contribution of wood and arboreal nesting termites was estimated from their relative contribution to termite species diversity as reported in literature (Braithwaite et al., 1988; Dawes-Gromadzki, 2008) for these north Australian savannas. As there are no data on the

biomass of this nesting group, it was assumed that their contribution in species composition equals their contribution in total termite biomass, and that they produce similar CH_4 fluxes as mound-building (epigeal) termites. Therefore, there is a great deal of uncertainty in the estimates of CH_4 flux for wood and arboreal nesting termites.

For scaling up soil fluxes, the net soil area was calculated by subtracting the basal area of mounds and trees from each 0.25 ha plot. Mean soil CH_4 flux for individual months were then summed up to calculate the annual soil flux.

3.3 Results

3.3.1 Mound and tree basal areas

Mean mound basal area in the 4 plots was 6.9 ± 2.8 m^2 ha^{-1}, which was 0.07% of the total soil area. Mean mound density was 127 ± 15 mounds ha^{-1}, in which *M. nervosus* mounds represented 60-80% (Fig. 3.3 and Fig. 3.4). The remaining 20-40% of termite mounds consisted of ten other species (Table 3.1). The mean basal area covered by mounds was 2.10 ± 0.7 m^2 ha^{-1} for *M. nervosus* and 4.75 ± 2.2 m^2 ha^{-1} for other species. Thus, despite being the most abundant mound-building species, the total basal area of *M. nervosus* mounds represented only 31% of the total mound basal area because of their smaller mound size (Fig. 3.3 and Fig. 3.4). Tree basal area was 11.3 ± 0.3 m^2 ha^{-1} and was dominated by *Eucalyptus miniata* and *E. tetrodonta*.

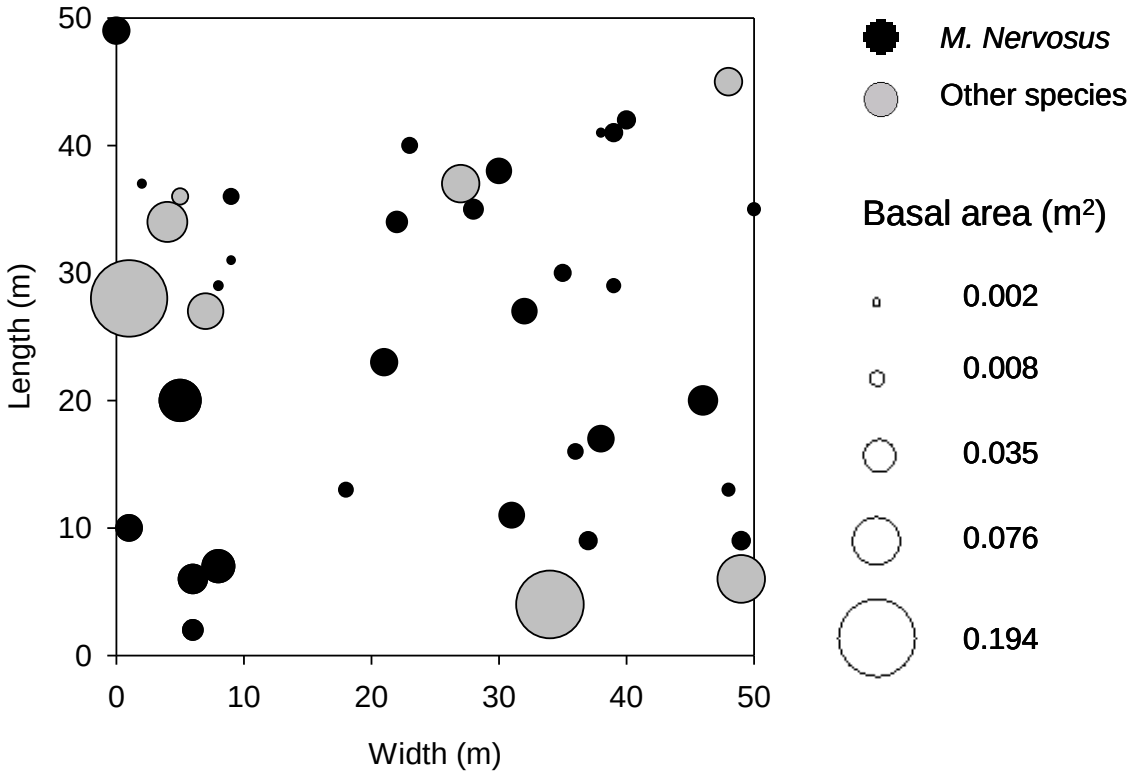

Figure 3.3: Mound distribution in one of the four plots at Howard Springs; note that size of circles representing mounds is proportionate to the basal area of mounds of different species but not proportionate to the plot dimensions.

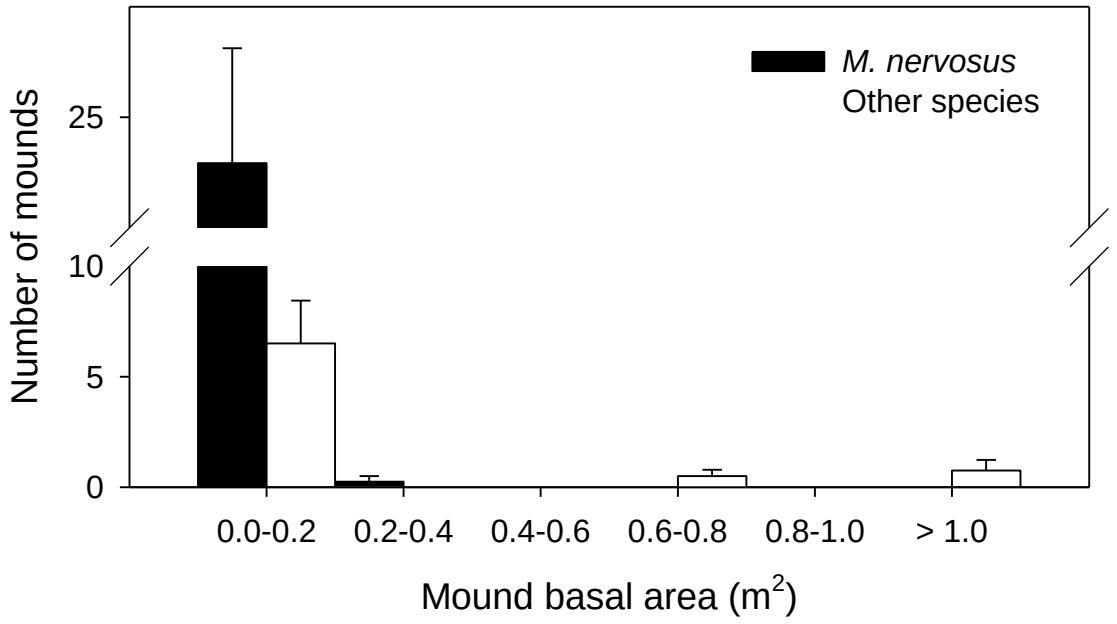

Figure 3.4: Mound distribution in the four 0.25 ha plots surveyed at Howard Springs; error bars are standard errors of the mean of four plots.

3.3.2 CH_4 fluxes from termite mounds

Mean CH_4 fluxes from the mounds of *M. nervosus* ranged from 468 µg CH_4-C m^{-2} h^{-1} (dry season) to 4074 µg CH_4-C m^{-2} h^{-1} (wet season), a 9-fold difference between dry and wet seasons (Table 3.2). Mean CH_4 fluxes from the mounds of *M. serratus* ranged between 700 and 8267 µg CH_4-C m^{-2} h^{-1}, *T. pastinator* between 325 and 8475 µg CH_4-C m^{-2} h^{-1}, and *A. darwini* between 956 and 8441 µg CH_4-C m^{-2} h^{-1}, with smaller fluxes measured in the dry season and greater fluxes in the wet season for all species (data not shown). Because of the similar magnitudes in measured CH_4 fluxes from *M. serratus*, *T. pastinator* and *A. darwini* mounds, these were combined to provide a mean non-*M. nervosus* mound flux, which ranged between 671 µg CH_4-C m^{-2} h^{-1} and 8394 µg CH_4-C m^{-2} h^{-1} according to the month, which was used in scaling up to an annual budget at the ecosystem level (Table 3.2).

Table 3.2: Mean CH_4 fluxes from termite mounds of *M. nervosus* (16 mounds), *M. serratus* (4 mounds), *Tumulitermes pastinator* (5 mounds) and *Amitermes darwini* (4 mounds); fluxes from *M. nervosus* and the other three species combined are shown in separate columns.

Month	**Flux (µg CH_4-C m^{-2} h^{-1})**	
	M. nervosus	**Other species combined**
January	2278[a]	3505[b]
February	3176[b]	6338[a]
March	4074[a]	8394[a]
April	2271[b]	1789[a]
May	468[a]	1009[a]
June	539[a]	671[a]
July	504[b]	840[b]
August	504[b]	840[b]
September	504[b]	840[b]
October	836[b]	1173[b]
November	1168[b]	1506[b]
December	1832[a]	2172[b]
Total area (m^2 ha^{-1})	2.1	4.8
Annual flux (kg CH_4-C ha^{-1} y^{-1})	0.03	0.10
Combined (kg CH_4-C ha^{-1} y^{-1})	0.13	

[a] Measured values

[b] Estimated values

3.3.3 Relationship between mound basal area and mound CH_4 flux

For *M. nervosus* (n = 16), the relationship between mound basal area and mound CH_4 flux was best described by a second-order polynomial regression model (R^2 = 0.48, $p \leq 0.01$), such that CH_4 flux was greatest from mounds with a basal area between 0.025 and 0.040 m^2 and decreased considerably for mounds >0.050 m^2 (Fig. 3.5). A simple linear regression was significant (R^2 = 0.28, $p \leq 0.05$) for mounds with basal area $\leq$ 0.04 m^2 (n = 14), and was even stronger (R^2 = 0.43, $p \leq 0.05$) for mounds with basal area $\leq$ 0.03 m^2 (n = 11) (data not shown). It should be noted that *M. nervosus* mounds with basal area $\leq$ 0.04 and $\leq$ 0.03 m^2 represented 90% and 80%, respectively, of the total 96 *M. nervosus* mounds measured at Howard Springs. This relationship was tested for *M. nervosus* only, as the mound replication for other termite species was small ($n \leq 5$).

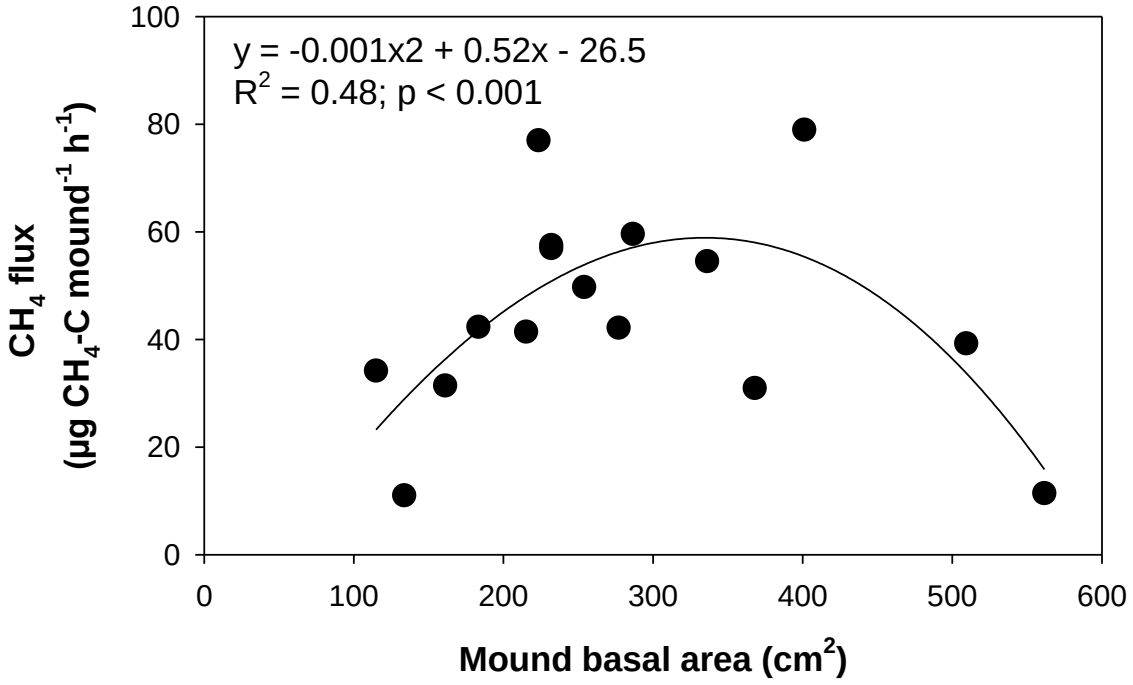

Figure 3.5: Relationship between mound basal area and mound CH_4 flux for *M. nervosus* (n = 16) measured at Howard Springs. The best fit for all the data was provided by the second-degree polynomial regression analysis shown in the plot.

3.3.4 CH_4 fluxes from soil

Although CH_4 uptake (negative flux) as well as CH_4 emissions (positive flux) were measured from individual soil-based chambers, the mean (n = 15) soil CH_4 flux was negative (i.e. uptake) in all months. Mean soil CH_4 fluxes ranged between -3.6 ± 2.6 and -16.3 ± 5.4 µg CH_4-C m^{-2} h^{-1} such that the mean flux rate for the whole year was -11.3 ± 1.3 µg CH_4-C m^{-2} h^{-1}, representing an annual flux of -1.05 kg CH_4-C ha^{-1} y^{-1}. The greatest single uptake and emission values were -36.7 and +40.4 µg CH_4-C m^{-2} h^{-1} as measured in the build-up to the wet season (November) and late-dry (October) seasons, respectively. Soil CH_4 emissions were measured in 7 to 33% of the 15 soil chambers according to the month of measurement, i.e. the remaining chambers measured soil CH_4 uptake.

Table 3.3: Mean soil CH_4 fluxes measured from three 50 x 50 m plots (n = 15). The % area of chambers showing emissions, mean CH_4 emissions, mean CH_4 uptake, and all chambers combined are shown separately. The annual sum of emissions and uptake are calculated based on the proportionate area covered by such chambers.

Month	**Area with emissions (% of total)**	**Mean soil fluxes (µg CH_4-C m^{-2} h^{-1})**		
		Emissions	**Uptake**	**Combined**
January [a]	26.7	+7.9	-7.8	-3.6
February [a]	33.3	+2.5	-10.2	-6.0
March [b]	30.0	+5.2	-9.0	-4.8
April [a]	6.7	+0.6	-13.2	-12.2
May [b]	13.3	+1.3	-18.3	-15.7
June [b]	13.3	+1.3	-18.3	-15.7
July [a]	13.3	+1.3	-18.3	-15.7
August [b]	13.3	+1.3	-18.3	-15.7
September [b]	15.0	+6.4	-17.3	-13.6
October [a]	16.7	+11.5	-16.2	-11.6
November [a]	26.7	+12.6	-26.8	-16.3
December [a]	13.3	+9.9	-17.0	-13.5
Total area (ha ha^{-1}) [c]				0.9982
Annual flux (kg CH_4-C ha^{-1} y^{-1})		+0.08	-1.14	-1.05

[a] Measured values

[b] Estimated values

[c] Soil area after subtracting mound and tree basal areas

3.3.5 Annual CH_4 budgets at the ecosystem scale

Epigeal (above ground mounds) nesting termites at Howard Springs emitted +0.13 ± 0.06 kg CH_4-C ha^{-1} y^{-1}, with +0.03 kg CH_4-C ha^{-1} y^{-1} being emitted from the mounds of *M. nervosus* and +0.10 kg CH_4-C ha^{-1} y^{-1} from the mounds of other species (Table 3.2). The contribution of *M. nervosus* mounds, as calculated using the second degree polynomial regression equation (Fig. 3.5) between mound basal area (m^2) and CH_4 flux was also +0.03 kg CH_4-C ha^{-1} y^{-1}, i. e. similar to that calculated by first method.

The estimated CH_4 emissions from hypogeal or soil-inhabiting termites were +0.08 kg CH_4-C ha^{-1} y^{-1} (Table 3.4). Wood and arboreal nesting termite species comprise 12% of the total number of termite species in these savannas (Braithwaite et al., 1988; Dawes-Gromadzki, 2008), and are estimated to emit +0.03 kg CH_4-C ha^{-1} y^{-1} (Table 3.4). Thus, at an ecosystem scale termites are estimated to emit +0.24 kg CH_4-C ha^{-1} y^{-1} at the Howard Springs savanna site (Table 3.4).

Table 3.4: Contribution of termite nesting groups to the total CH_4 fluxes from termites. The contribution of tree and log inhabiting termites was estimated based on surveys by Braithwaite et al. (1988).

Habitat group	**Actual contribution** (kg CH_4-C ha^{-1} y^{-1})	**Relative contribution** (% of total)
Mound-building	0.13	54
Soil-inhabiting (subterranean)	0.08	34
Tree and log inhabiting	0.03	12
Total	0.24	100

Net annual soil CH_4 flux, based on all soil flux measurements including uptake and emission chambers, was -1.05 kg CH_4-C ha^{-1} y^{-1}. However, if the soil flux measurements showing CH_4 emissions, which represent the contribution of soil-inhabiting/subterranean

termites, are excluded soil CH_4 uptake was -1.14 kg CH_4-C ha^{-1} y^{-1} (Table 3.3). At the ecosystem scale, net CH_4 exchange of the Howard Springs savanna woodland system, after accounting for +0.24 kg CH_4-C ha^{-1} y^{-1} emissions from termites was -0.90 kg CH_4-C ha^{-1} y^{-1}.

3.4 Discussion

3.4.1 CH_4 fluxes from termite mounds

Mean CH_4 fluxes from termite mounds ranged between +468 and +8394 µg CH_4-C m^{-2} h^{-1} in this study, which compare well to the mound based CH_4 fluxes reported in the literature broadly ranging between +38 and +35250 µg CH_4-C m^{-2} h^{-1} (Khalil et al., 1990; Martius et al., 1993; MacDonald et al., 1998; Brümmer et al., 2009). There are other studies that express CH_4 fluxes per unit termite biomass (Fraser et al., 1986; Brauman et al., 1992; Rouland et al., 1993) but it is not possible to compare these to our results because of the different methods and units used.

Annual CH_4 emissions from termite mounds at Howard Springs were estimated to be +0.13 ± 0.06 kg CH_4-C ha^{-1} y^{-1}. There is some uncertainty in these estimates as the fluxes from mounds of species other than *M. nervosus* were not measured at Howard Springs, although these sites were similar in dominant vegetation type. Our estimate of annual CH_4 emissions from termite mounds compares well to the chamber-based estimate of +0.20 kg CH_4-C ha^{-1} y^{-1} (Brümmer et al., 2009) from *Cubitermes fungifaber* mounds in a savanna in Burkina Faso, West Africa. MacDonald et al (1999) reported greater termite mound emissions of +0.4 and +0.9 kg CH_4-C ha^{-1} y^{-1} for near-primary and secondary forest in Cameroon, respectively, but similar emissions of <+0.2 kg CH_4-C ha^{-1} y^{-1} from termite mounds in Borneo forest ecosystems.

Though mounds of *M. nervosus* were the most abundant at Howard Springs, their basal area was only 31% of total mound basal area because of their small size compared to other species. Moreover, CH_4 fluxes from *M. nervosus* mounds were almost half the magnitude of fluxes from the other three mound termite species measured. Thus, it is

very important to account for species variation in CH_4 fluxes and mound distribution when scaling up to the ecosystem level.

3.4.2 Relationship between mound basal area and mound CH_4 flux

A significant second-order polynomial regression between mound basal area and CH_4 flux for *M. nervosus* mounds suggests that larger, and presumably older, mounds do not necessarily have a larger termite population than medium sized mounds. This assumes that for a given species, mound CH_4 fluxes are directly proportional to termite population size. For the vast majority of *M. nervosus* mounds (80%) with a basal area <0.04 m^2 a simple linear regression explained 43% of the variation in mound CH_4 flux. The only other study (Martius et al., 1993) to investigate the relationship between mound size and CH_4 flux did so in a laboratory using five mounds of *Nasutitermes ephratae* and found no significant correlation. The artificial laboratory conditions and small sample size may have influenced the findings of Martius et al. (1993), whereas we measured 16 mounds *in situ* through wet and dry season months.

3.4.3 CH_4 fluxes from soil

We measured a mean soil CH_4 uptake rate of -11.3 µg CH_4-C m^{-2} h^{-1} ranging between -40.4 and +36.7 µg CH_4-C m^{-2} h^{-1} at Howard Springs. In comparable studies of savanna ecosystems outside Australia, soil CH_4 fluxes of net uptake, neutral or emissions have been reported (Castaldi et al., 2006). It was estimated that subterranean or hypogeal nesting termites emitted 0.08 kg CH_4-C m^{-2} y^{-1}, thus offsetting 7% of -1.14 kg CH_4-C m^{-2} y^{-1} oxidized by savanna soil at Howard Springs, which is similar to the 7% offset reported for subterranean termites in a near-primary or the 16% reported for a secondary forest in Cameroon, Africa (MacDonald et al., 1999). In savannas, it is usually attempted to correlate soil CH_4 emissions with rainfall, soil moisture, soil temperature and other soil attributes, but not with termite biomass or activity (Hao et al., 1988; Castaldi et al., 2004; Castaldi et al., 2006; Sanhueza and Donoso, 2006; Brümmer et al., 2009). MacDonald et al. (1998; 1999) demonstrated that CH_4 emissions from tropical soils can be significantly correlated with termite biomass in the soil beneath those chambers. We also observed the presence of termites in some soil core samples collected from beneath chambers that emitted CH_4, but termite biomass was not quantified.

During the dry season soil CH_4 emissions are almost certainly due to the presence of termites. However, in the wet season both soil microbial methanogenesis and termite activity might have contributed to any measured soil CH_4 emissions. It appears that soil CH_4 emissions are mainly due to termite activity as these emissions were measured throughout the year (Table 3.3) regardless of soil moisture conditions. The complex interaction of CH_4 emissions from termite activity and CH_4 uptake (oxidation) from soil methanotroph activity is further complicated by the seasonal variations in soil moisture, termite biomass (MacDonald et al., 1998) and termite foraging activity (Lepage, 1982). A proportion of CH_4 originating from subterranean termites is potentially oxidized by soil methanotrophic activity as this CH_4 diffuses through the soil profile. Although this does not influence the net soil fluxes, the contribution of termites to the total CH_4 budget can therefore be underestimated. As such, the annual estimates of termite derived CH_4 is conservative and the percentage offset that termites provide to the Howard Springs CH_4 sink can be expected to be greater than that reported here. Conversely, termites can also enhance CH_4 oxidation rates by increasing the diffusivity of soils (Wood, 1988), and by supporting a larger and more active methanotroph population by increasing the CH_4 concentration in soil profile as CH_4 oxidation rates depend on CH_4 concentration (Bender and Conrad, 1995). Studies with a greater soil chamber replication and use of CH_4 isotopes (von Fischer and Hedin, 2002) can potentially improve the quantification and process-based understanding of methanotrophy, methanogenesis and termite-related CH_4 emissions within soil profile.

3.4.4 Termite CH_4 emissions and nesting groups

The contribution of termites from different nesting groups to the total termite CH_4 emissions at Howard Springs were estimated to be in order of epigeal mounds (54%) > soil-inhabiting (34%) > wood and arboreal nesting (12%) termites (Table 3.4). McDonald et al. (1999) reported that mound-building termites contributed between 51 and 64% to termite derived CH_4 emissions whilst the rest was produced by the subterranean termites. As such, the importance of termites to an ecosystem's CH_4 balance may be a considerable under-estimate when based on mound-building termites only. Although we included an estimate of the contribution that wood and arboreal nesting termites make to

ecosystem CH_4 emissions, this was not based on any direct flux measurements and represents a future research focus to measure these fluxes *in situ*.

3.4.5 Annual CH_4 budgets

The annual estimates expressed in kg CH_4-C ha^{-1} y^{-1} (Table 3.2, 3.3 and 3.4) were converted to CO_2-e by first converting CH_4-C to CH_4 (16/12 = 1.33) and then multiplying by global warming potential for CH_4 which is 25 (IPCC, 2007). On an annual basis, termites at the Howard Springs site emitted +8.0 kg CO_2-e ha^{-1} y^{-1} whereas the soil consumes -38.1 kg CO_2-e ha^{-1} y^{-1}. The Howard Springs savanna is dominated by *E. tetrodonta* and *E. miniata*, a widespread savanna-woodland type representing an area of 246,609 km^2 across the Northern Territory and Queensland in Australia (Fox et al., 2001).

Extrapolating CH_4 fluxes measured at Howard Springs across this vegetation type gives a regional estimate of termite CH_4 emissions of +0.20 Tg CO_2-e y^{-1} and a soil CH_4 sink of -0.94 Tg CO_2-e y^{-1}, such that termite derived emissions offset 21% of the CH_4 oxidized by soil methanotrophs. If applied to the 1.9 million km^2 of Australian savanna, termites may be a CH_4 source of approximately +1.5 Tg CO_2-e y^{-1} and soils a CH_4 sink of -7.2 Tg CO_2-e y^{-1}. There would be some uncertainty associated with this broader estimate given the differences in rainfall, vegetation, soil types and land use intensity across the entire savanna biome in Australia.

Brümmer et al (2009) reported that an African savanna ecosystem at Burkina Faso had a net CH_4 source of +76 kg CO_2-e ha^{-1} y^{-1} with 8.8% emitted from termite mounds and the rest from the soil. For a complete ecosystem perspective of savanna CH_4 exchange it would be important to also consider the emissions from fire events (Cook and Meyer, 2009; Russell-Smith et al., 2009; Cook et al., 2010) and ephemeral wetland inundations (Delmas et al., 1991; Otter and Scholes, 2000; Crutzen et al., 2006).

Termites are a major factor in savanna ecosystem processes, and have been estimated to account for 20% of total C mineralized (Holt, 1987), consume up to 55% of surface litter

and 20% of the standing crop (Wood and Sands, 1978). Beringer et al. (2007) measured CO_2 flux from the Howard Springs site using an eddy covariance tower and, after accounting for the loss of productivity and emissions due to fire, reported a net biome productivity (NBP) of -7333 kg CO_2-e ha^{-1} y^{-1} for these savannas. Given this NBP sink, at an ecosystem scale, CH_4 emissions from termites offset only 0.1% of the NBP while CH_4 uptake by soils add 0.5% to the NBP sink for this mesic savanna site.

Assuming a similar contribution of termites in the savannas and tropical rain forests worldwide (33.8 x 10^6 km^2), termites can globally produce 27 Tg CO_2-e y^{-1}, which is 0.2% of the global CH_4 source budget of 14500 Tg CO_2-e y^{-1} (Denman and Brasseur, 2007). This estimation is similar to a global estimate of 23 Tg CO_2-e y^{-1} by Brümmer et al. (2009), based on fluxes measured from mound-building termites in an African savanna. Global estimates of CH_4 emissions from termites based on global estimates of termite biomass range between 300 and 650 Tg CO_2-e y^{-1} (Fraser et al., 1986; Khalil et al., 1990; Martius et al., 1993; Sanderson, 1996), an order of magnitude greater than our estimates based on in situ mound flux measurements and termite nest abundance. It is important to conduct such field-based studies of termite fluxes in other geographic regions to further refine these global estimates.

3.5 Conclusions

By accounting for three termite nesting groups: i) epigeal, ii) subterranean and iii) tree dwelling termites it is apparent that termites can play an important role in the CH_4 balance of savannas at Howard Springs by emitting to the atmosphere +8.0 kg CO_2-e ha^{-1} y^{-1}, which offsets 21% of the -38.1 kg CO_2-e ha^{-1} y^{-1} taken up by soil through methanotroph bacteria. Termites may be a methane source of +1.5 Tg CO_2-e y^{-1} across Australian savannas and +27 Tg CO_2-e y^{-1} globally. This estimate is conservative as a sizable proportion of CH_4 produced by soil-inhabiting termites is oxidized within the soil profile. Regardless, our survey based estimates suggest that previous global estimates of CH_4 emissions from termites may be a considerable overestimate. However, it is important to refine the contribution of subterranean termites by using novel techniques such as isotopes.

3.6 References

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4 Termite mound emissions of CH_4 and CO_2 are primarily determined by seasonal changes in termite biomass and behaviour

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Abstract

Termites are a highly uncertain component in the global source budgets of CH_4 and CO_2. Large seasonal variations in termite mound fluxes of CH_4 and CO_2 have been reported in tropical savannas but the reason for this is largely unknown. This paper investigated the processes that govern these seasonal variations in CH_4 and CO_2 fluxes from the mounds of *Microcerotermes nervosus* Hill (Termitidae), a common termite species in Australian tropical savannas. Fluxes of CH_4 and CO_2 of termite mounds were 3.5 fold greater in the wet season as compared to the dry season and were a direct function of termite biomass. Termite biomass in mound samples was 10 fold greater in the wet season compared to the dry season. When expressed per unit termite biomass, termite fluxes were only 1.2 (CH_4) and 1.4 (CO_2) fold greater in the wet season as compared to the dry season and could not explain the large seasonal variations in mound fluxes of CH_4 and CO_2. Seasonal variation in both gas diffusivity through mound walls and CH_4 oxidation by mound material was negligible. These results highlight for the first time that seasonal termite population dynamics are the main driver for the observed seasonal differences in mound fluxes of CH_4 and CO_2. These findings highlight the need to combine measurements of gas fluxes from termite mounds with detailed studies of termite population dynamics to reduce the uncertainty in quantifying seasonal variations in termite mound fluxes of CH_4 and CO_2.

4.1 Introduction

Tropical savanna ecosystems have been identified as a major source of greenhouse gas emissions, however, uncertainty in the magnitude of these emissions is considerable (Bousquet et al. 2006; Grace et al. 2006). The exchange of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) in natural savanna ecosystems is predominantly a function of: a) gross primary production (CO_2), b) plant and soil respiration (CO_2), c) termite activity (CH_4), c) periods of wetland inundation and water level (CH_4, N_2O), d) soil CH_4 oxidation (CH_4), e) soil nitrification and denitrification (N_2O), and f) fire events (CO_2, CH_4, N_2O) (Livesley et al. 2011). Termites are one of the most uncertain contributors to the global budgets of CH_4 and CO_2 (Bignell et al. 1997; Brümmer et al. 2009; Khalil et al. 1990). Termite mounds have also been reported as a significant point source of N_2O; however, such N_2O fluxes were negligible from termite mounds in this study (unpublished data) and similarly by Khalil et al. (1990). The uncertainty in termite related fluxes is mainly associated with scaling up factors, such as global estimates of termite biomass, the number of nests (Khalil et al. 1990), and the lack of process-based understanding of CH_4 and CO_2 exchange between termites and the atmosphere. Most of previous studies used laboratory based termite fluxes in scaling up which ignore the fact that termite behaviour can be affected in artificial conditions (Bignell et al. 1997) and that not all CH_4 produced by termites is emitted to the atmosphere because of mound wall diffusivity and CH_4 oxidation by methanotrophs in the mound material (Sugimoto et al. 1998). The laboratory based studies scale up the emission rates using regional and global termite biomass estimates which are still lacking for many regions (Bignell et al. 1997; Sanderson 1996). Field based studies, though more realistic, mostly ignore the contribution of termites that do not construct mounds. Also, such field based studies are few and do not cover all biogeographical regions where termites occur.

An important factor for consideration in scaling is the large seasonal variations in termite mound fluxes of CH_4 and CO_2. Termite densities tend to be highest in savanna ecosystems that occur in highly seasonal climate zones (Hutley and Setterfield 2008). CH_4 fluxes from a mound of *Coptotermes lacteus* (Rhinotermitidae) in summer were greater than rest of the three seasons combined in the sub-tropical Australia, with large

seasonal variations in mound CO_2 fluxes observed (Khalil et al. 1990). In the tropical savannas of northern Australia, CH_4 fluxes from the mounds of four termite species were 5 to 26 fold greater in the wet season as compared to the dry season (Jamali et al. 2011). In an Australian tropical semi-arid woodland, Holt (1987) reported almost 4 fold greater CO_2 fluxes in the wet season as compared to the dry season from the mounds of *Amitermes laurensis* (Termitidae). The seasonal variations in mound gas fluxes have mainly been correlated with temperature in the sub-tropics (Khalil et al. 1990), and with moisture in the tropical savannas as there is limited seasonal variation in temperature (Holt 1987; Jamali et al. 2011). While the effect of temperature on termite fluxes of CH_4 and CO_2 have been reported (Jamali et al. 2011; Shelton and Appel 2000; Zimmerman and Greenberg 1983), the effect of moisture is still largely unknown. To our knowledge no process based study has been conducted which assesses the factors causing these seasonal variations in mound fluxes of CH_4 and CO_2 in the tropical savannas. Given the savanna biome occupies 15% of the global land surface, understanding these processes is required for accurate predictions of global greenhouse gas budgets.

The observed seasonality in mound fluxes of CH_4 and CO_2 can be caused by a number of different factors:

First, the observed seasonal variation in mound fluxes could be caused by a seasonal change in *emissions per unit termite biomass*. Environmental factors, such as temperature, moisture, or food quantity and quality can change the rates of metabolism and respiration in termites. Second seasonal variation in fluxes can be caused by *a change in the number of termites inside mound*. For example, certain termite species can lose up to 50% of their colony biomass as a result of swarming (Wood and Sands 1978). Also termite population in mounds can increase as part of reproductive life cycle. Third, seasonal variation in *termite activity*, such as foraging outside mounds, can result in seasonal variation in mound-based flux measurements. This can impact termite flux estimates based on termite mounds alone, as only a fraction of the termites in the colony will be present in the mound whilst the remainder of the termites will be emitting CH_4 and CO_2 elsewhere in the ecosystem. Fourth, termite mound walls are mainly composed of soil and can oxidize a fraction of CH_4 produced by termites inside mounds as a result

of methanotrophic activity (Sugimoto et al. 1998). Variable mound water contents across seasons can cause *variable CH_4 oxidation rates* (and thus variable mound CH_4 fluxes) as oxidation rates can be influenced by moisture. Seasonal CO_2 variation can also be partly due to the effect of moisture and temperature on the respiration of microbial biomass in the mound walls (Holt 1987). Fifth, seasonal variation in *gas diffusivity through mound wall* due to changing mound water content can also cause seasonal variation in net mound fluxes.

This study was aimed to investigate for the first time the factors causing seasonal variations in termite mound fluxes of CH_4 and CO_2 in Australian tropical savannas. All the experiments were conducted on *Microcerotermes nervosus*, one of the most common mound-building termite species in northern Australia (Watson and Abbey 1993). The objectives were to investigate the seasonality in: 1) CH_4 and CO_2 emissions per unit termite biomass, 2) termite biomass dynamics in mounds; 3) gas diffusivity of mound material and fluxes of mound material as a result of microbial activity; and 4) short term effect of temperature and moisture on CH_4 and CO_2 fluxes from termites (not mounds).

4.2 Methods

4.2.1 Site

Field work was conducted in a savanna woodland at the CSIRO Tropical Ecosystems Research Center (TERC 12º 24´ S, 130 º 55´ E), near Darwin in northern Australia. The vegetation is dominated by *Eucalyptus tetrodonta* F. Muell and *E. miniata* Cunn. ex Schauer over a ground layer of annual and perennial C4 grasses, and a thick litter layer (Dawes-Gromadzki and Spain 2003). This is widespread savanna type across north Australia at rainfall above 900 mm annually (Fox et al. 2001).

4.2.2 Field-based flux measurements

Five mounds of *Microcerotermes nervosus* were repeat-measured for CH_4 and CO_2 fluxes between February and December 2009 at intervals of four to six weeks. Mounds with easy access for measurements were selected. Fluxes were measured using static manual chambers of volume 0.02 m^3, constructed from polyvinylchloride (Fig. 4.1).

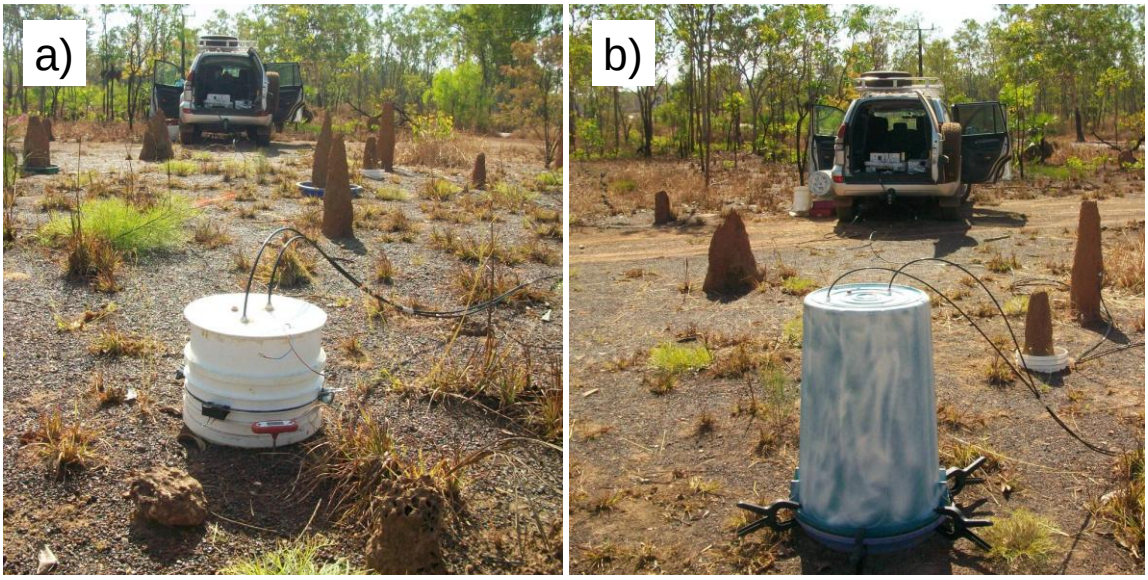

Figure 4.1: Methane and carbon dioxide flux measurements from **a)** soil, and **b)** termite mounds, using 'Fast Greenhouse Gas Analyzer (FGGA)'.

A collar was permanently installed around the mounds to a soil depth of 3 cm. A chamber of equal circumference to the collar was placed over the mound and connected to the collar using a ribbon of closed cell foam and several tension spring-clamps (Fig. 4.1). This chamber was then connected to a Los Gatos Research (LGR^{TM}) Fast Greenhouse Gas Analyzer (FGGA) through a pair of gas tubes and Swagelok^{TM} push-fittings (Fig. 4.1). LCD screen was attached to the FGGA which displayed the CH_4 and CO_2 concentrations measured at a frequency of 1Hz (i.e. one sample per second) for a period of five minutes per chamber. The operation of the FGGA is based on an off-axis integrated cavity output spectroscopy combined with a highly specific narrow band laser for the detection of CH_4 and CO_2 and strongly reflective mirrors to obtain a laser path length of 2–20×103 m. Further technical details on FGGA operation can be found in Hendriks et al. (2008). Flux was calculated from the linear change in concentration of CH_4 and CO_2 in the chamber headspace by multiplying the slope (ppm_v $hour^{-1}$) with the chamber volume (L) and dividing by the mound basal area (m^2). Flux was then corrected for temperature and pressure based on the ideal gas law.

4.2.3 Auxiliary environmental measurements

Mound temperature (T_{mound}) was measured immediately after the mound flux measurement by horizontally inserting a hand held Cole-Palmer® stainless steel temperature probe 6 cm into the mound. Mound water content was not directly measured to avoid destruction of the mounds required for repeat measurements. Instead, soil water content (%) was measured gravimetrically by collecting five soil core samples from the top 6 cm next to each mound using a brass soil sampling ring. These were weighed, oven dried at 105 ºC and reweighed. Monthly rainfall (mm) data were obtained from the Darwin Airport meteorological station of the Bureau of Meteorology, Australia which is located less than 2 km from the TERC site.

4.2.4 Flux and termite biomass measurements in the laboratory

Samples (n = 22) from *M. nervosus* mounds were collected in 3 L glass jars and equilibrated at 25ºC for five hours in a temperature controlled room at Charles Darwin University, Darwin, prior to measurement of CH_4 and CO_2 fluxes. Fluxes were measured by connecting the glass jars to the FGGA and observing a linear change in the headspace concentration of CH_4 and CO_2 at a frequency of 1 Hz for a period of 10 minutes (Fig. 4.2). Fresh biomass of termites was determined immediately afterwards by breaking down the mound samples and collecting individual termites (Fig. 4.3) using forceps. Workers and soldiers were weighed separately to an accuracy of 10^{-4} g. The mean biomass of an individual termite within a caste (i.e. workers, soldiers and alates) was determined by weighing 10 individuals from each caste from most of the mound samples. The volume of mound samples was measured, before breaking and removing the termites, by cling-wrapping the sample in a thin plastic sheet and placing it in a partially water filled calibrated container. The volume of displaced water was subtracted from the chamber volume to calculate the net headspace volume. A maximum of only two mound samples were collected and measured each day. This experiment was conducted in both the wet (n = 22) and the dry (n = 22) season and was completed within a two week period for both seasons.

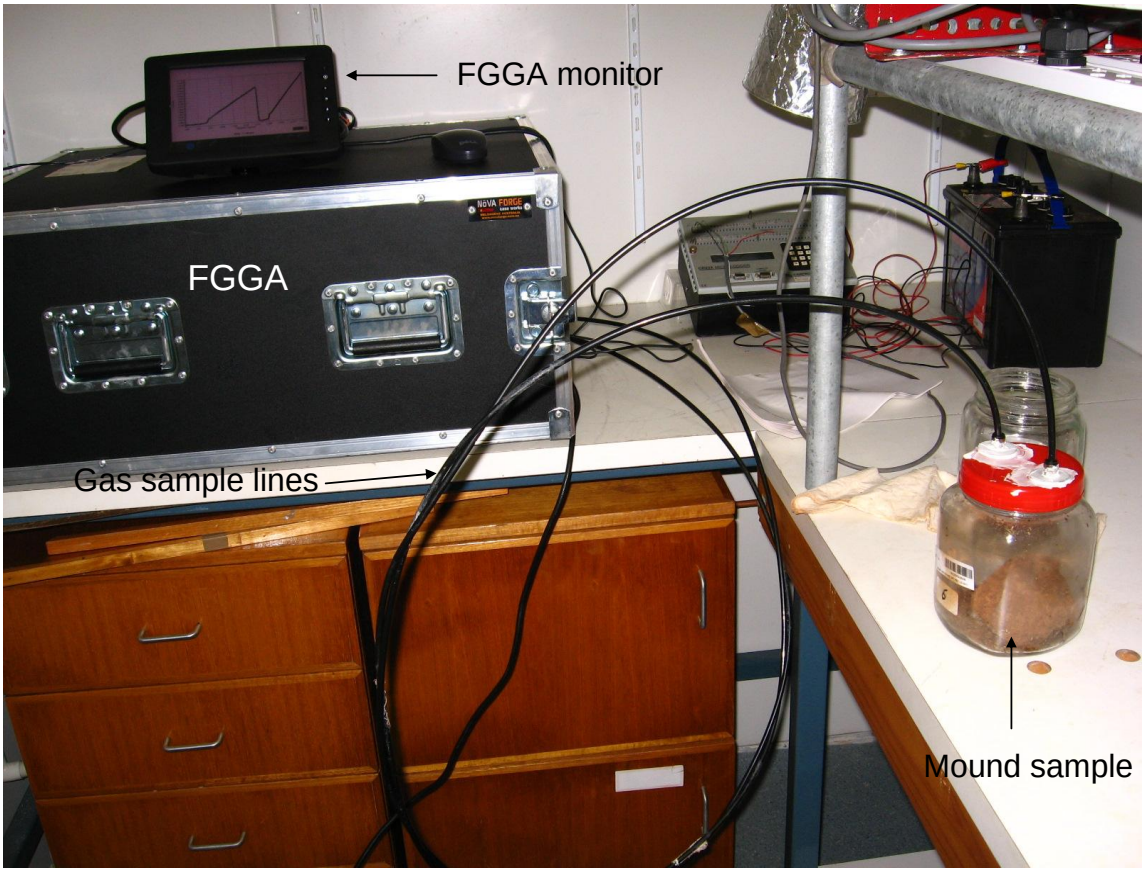

Figure 4.2: Methane and carbon dioxide flux measurements from mound samples in a temperature control room, using FGGA, for studying the effect of termite biomass, temperature and moisture on fluxes

Fluxes were also measured from the mound material following termite removal from mound samples. Mound material samples were incubated for 30 minutes using the same protocols described above for incubating mound samples containing termites but using 1L glass jars. Seasonal difference in fluxes from mound material would explain the contribution of CH_4 oxidation and microbial respiration to seasonal variations in mound fluxes of CH_4 and CO_2. These mound material fluxes were subtracted from the gross fluxes of mound samples, measured before removing the termites, for calculating the net CH_4 and CO_2 from termites only.

Figure 4.3: Termites collected from mound samples after flux measurement in the laboratory

4.2.5 Gas diffusivity measurements of mounds

Seasonal difference in gas diffusivity of mound walls was measured using two methods:

Indirect method: Seasonal difference in mound diffusivity was estimated indirectly by using the ratio of internal mound CH_4 concentration and mound CH_4 flux. Eleven (11) mounds of *M. nervosus* were repeat-measured for mound CH_4 flux and internal mound CH_4 concentration in the wet and the dry seasons. Internal mound CH_4 concentration was measured immediately after the mound flux measurements by collecting 20 ml gas samples from mounds using a syringe and tube. These gas samples were immediately

transferred to pre-evacuated glass vials (Labco Exetainer) which were then analyzed for CH_4 concentration (ppm) using an auto-injected gas chromatograph (GC, Shimadzu^{TM}, GC17a) at the Creswick laboratories of The University of Melbourne. Seasonal variation in the ratio of CH_4 flux to internal mound CH_4 concentration, and the consistency of relationship between mound CH_4 flux and internal mound CH_4 concentration across seasons would help explain if there is a seasonal difference in mound wall diffusivity.

Direct method: Gas diffusivity through mound wall was repeat measured in the wet and the dry seasons directly according to von Fischer et al. (2009) from the decrease in concentration of Sulphur hexa fluoride (SF_6) that was injected into the chamber headspace through a rubber septum using a syringe and needle. In this experiment, the *M. nervosus* mounds (n = 5) and chambers used were the same that were also being repeat measured for seasonal fluxes of CH_4 and CO_2 as described in section 2.2 above. A small fan was fitted inside each chamber to maintain good mixing of SF_6 within the chamber headspace. After chamber closure, twenty ml gas samples were collected from the chamber headspace at 2, 12, 22, 32 minutes using a syringe and a one-way stopcock. These gas samples were immediately transferred to pre-evacuated exetainers and analyzed for SF_6 and CH_4 concentration using a GC. Mound water content was measured by collecting samples from adjacent *M. nervosus* mounds (n = 5) and weighing at 105ºC for 48 hours. These mound samples were collected in a way that they largely included a portion of mound wall. Bulk density (g cm^{-3}) of these mound samples was calculated by measuring their volume as described under section 2.3 above (data not shown). Diffusion coefficients (cm^2 min^{-1}) for CH_4 were determined from that of SF_6 using their respective molecular weights as explained in von Fischer et al. (2009).

4.2.6 Effect of temperature and moisture on laboratory termite fluxes

The short term effect of temperature on termite fluxes was measured in the laboratory, using mound samples (n = 5) of *M. nervosus* that contained termites. These were kept in 3 L glass jars and housed in a temperature controlled room at the Charles Darwin University, Darwin, Australia. Fluxes were measured at three temperatures, 25ºC, 35ºC and 15ºC, after equilibrating for 6 hours at each temperature.

The effect of moisture on termite fluxes was investigated by measuring fluxes before and after placing wet calico cloth pieces in the jars at a constant temperature of 25ºC using the same protocols described for the temperature effect. Fluxes from the wet calico were also measured and subtracted from the total fluxes.

4.2.7 Statistical analysis

SPSS (Ver.16.0, SPSS Inc., Chicago, IL) was used for the statistical analyses of data. Statistical significance was defined at $p \leq 0.05$, unless otherwise stated. Note that original data was used in all the figures and transformed data was used for statistical tests where necessary as stated.

A simple linear regression (n = 30) was used for testing the relationship of mean mound fluxes (CH_4 and CO_2), measured in field, with mean mound temperature and mean soil water content.

A simple linear regression (n = 22) was used for testing the relationship between mound CH_4 flux (μg CH_4-C m^{-2} h^{-1}) and internal mound CH_4 concentration (ppm). A paired student T-test (n = 11) was used to compare the mound CH_4 flux to internal mound CH_4 concentration (ppm) ratio between the wet and the dry seasons. Paired student T-test was also used to compare the water content and gas diffusivity coefficients (log_{10} transformed) of mounds between the wet and the dry seasons.

For the fluxes measured from mound samples in the laboratory, a simple linear regression analysis was used for testing the relationship between termite biomass and flux (CH_4 and CO_2) separately for the wet (n = 22) and the dry (n = 22) seasons. An independent sample student T-test (n = 22) was used for analyzing the significance of difference in flux per unit termite biomass between the wet and the dry seasons; data was transformed using ln(flux). An independent sample student T-test (n = 22) was also used for testing the significance of difference in termite biomass per unit mound sample mass between the wet and the dry seasons; data was transformed using the log_{10}(termite biomass).

For analyzing the effect of temperature on fluxes a Q_{10} temperature coefficient, which is a measure of the rate of change of a biological or chemical system (in this case CH_4 and CO_2 flux) as a consequence of increasing the temperature by 10ºC; was calculated as follows:

$$Q_{10} = \left(\frac{F_2}{F_1} \right)^{\frac{10}{(T_2 - T_1)}} \quad (1)$$

where $F_{1,2}$ are fluxes at two different temperatures, and T is corresponding room temperature (°C). Q_{10} was calculated for a temperature range of 15 to 25 ºC and 25 to 35 ºC.

Paired sample student T-test was used for analyzing the effect of moisture on CH_4 and CO_2 fluxes from the mound samples (n = 5); data was transformed using $\log_{10}$(flux).

4.3 Results

4.3.1 Seasonal fluxes measured in field

CH_4

Mound CH_4 fluxes measured in field were 3.5 fold greater in the wet season (1465 ± 293 µg CH_4-C m^{-2} h^{-1}) compared to the dry season (417 ± 74 µg CH_4-C m^{-2} h^{-1}; Fig. 4.4). There was a significant relationship (R^2 = 0.69, $p \leq 0.05$) between soil water content and mound CH_4 flux, but no significant relationship between mound temperature and mound CH_4 flux (data not shown).

CO_2

Mean mound CO_2 flux was 3.5 fold greater in the wet season (601 ± 98 mg CO_2-C m^{-2} h^{-1}) than in the dry season (173 ± 34 mg CO_2-C m^{-2} h^{-1}; Fig. 4.4). The relationship of mound CO_2 flux was significant with soil water content (R^2 = 0.69, $p \leq 0.05$) but not significant with mound temperature (data not shown).

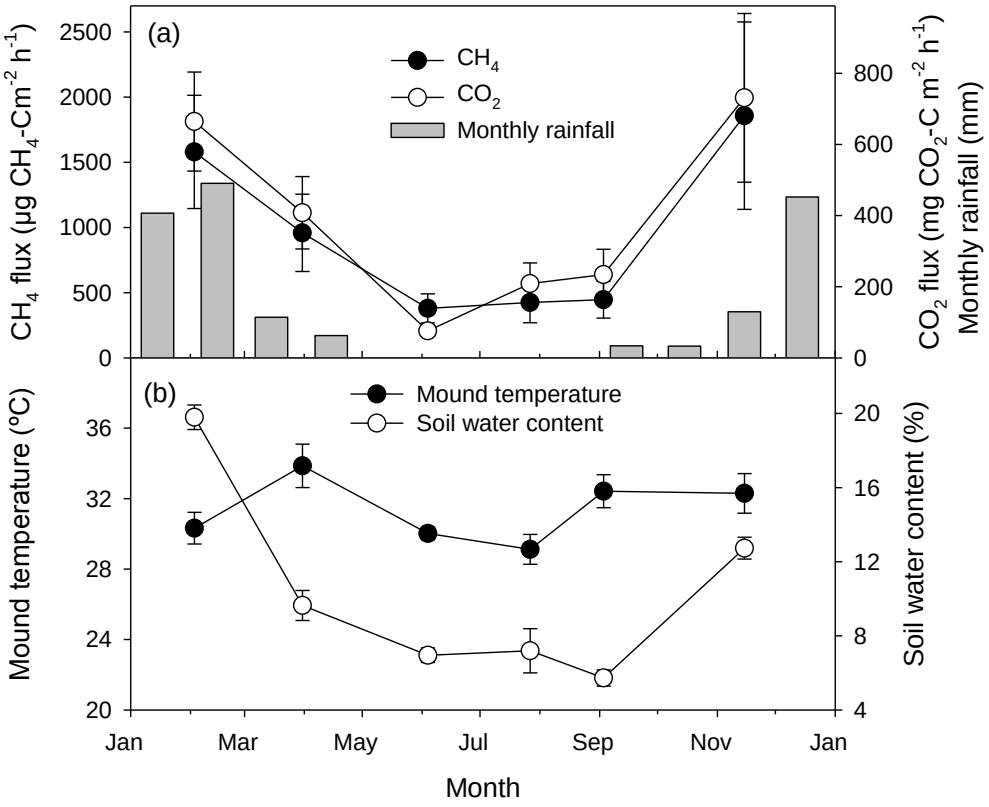

Figure 4.4: (a) Seasonal CH_4 and CO_2 fluxes from five termite mounds of *Microcerotermes nervosus* measured at TERC site and monthly rainfall from the meteorological station at Darwin Airport, and (b) seasonal mound temperature and gravimetric soil water content (%).

The 2009 dry season broke in the Darwin region with a 5.4 mm rainfall event in September (Bureau of Meteorology 2009). Mound fluxes were measured a few days before and within a few hours after this rainfall event. There was a 10 to 50% increase in mound CH_4 flux and a 10 to 80% increase in mound CO_2 flux as a result of this rain (Fig. 4.5). A paired student T-test showed a significant difference ($p \leq 0.05$) in mound fluxes (CH_4 and CO_2) measured before and after the rain event (Fig. 4.5); data was transformed using $\log_{10}$(flux). Mean mound temperature and mean gravimetric soil water content was 32.4 ºC and 5.7% respectively before this rain event and 33.4 ºC and 10.0 % after.

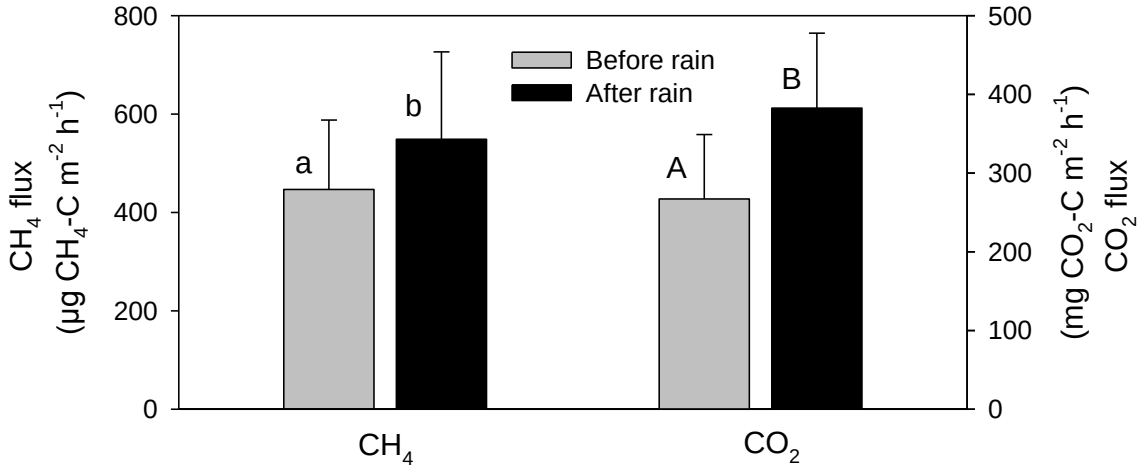

Figure 4.5: Mean mound fluxes of CH_4 and CO_2 (n = 5) measured from mounds of *M. nervosus* before and after the 'break of rains' (5.4 mm) in late dry season 2009 measured at TERC; error bars are standard error of the mean; case-wise letters show the significance of difference ($p \leq 0.05$) using transformed (log_{10}) flux values.

4.3.2 Gas diffusivity through mounds

Indirect method: There was a significant relationship between mound CH_4 flux and internal mound CH_4 concentration (R^2 = 0.89; $p \leq 0.001$) regardless of season (Fig. 4.6a). The difference in the ratio of mound CH_4 flux to internal mound CH_4 concentration between the wet season and the dry season was not significant (Fig. 4.6b).

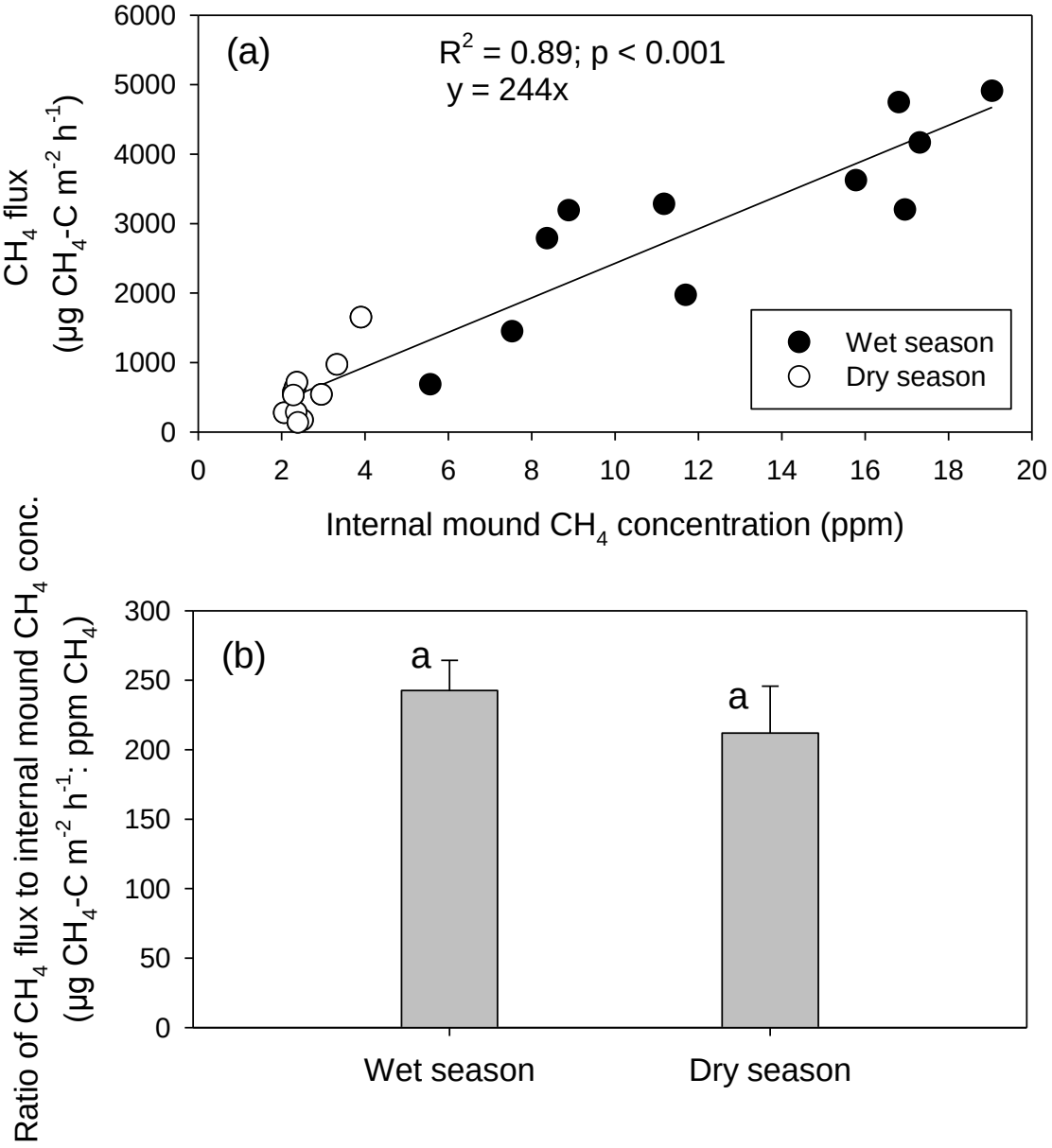

Figure 4.6: **(a)** Simple linear regression between mound CH_4 flux and internal mound CH_4 concentration (ppm); **(b)** seasonal variation in the ratio of mound CH_4 flux (µg CH_4-C m^{-2} h^{-1}) to internal mound CH_4 concentration (ppm), repeat-measured in the wet and the dry seasons; letters on top of bars show the significance ($p \leq 0.05$) of difference between the wet and the dry seasons.

Direct method: There was a significant difference ($p \leq 0.01$) in the gravimetric water content (%) of mound samples between the wet (14.2 ± 1.1%) and the dry (3.7 ± 1.0%) seasons (Fig. 4.7a). The difference in CH_4 diffusion coefficients of mounds between the wet and the dry seasons, however, was not significant (Fig. 4.7b).

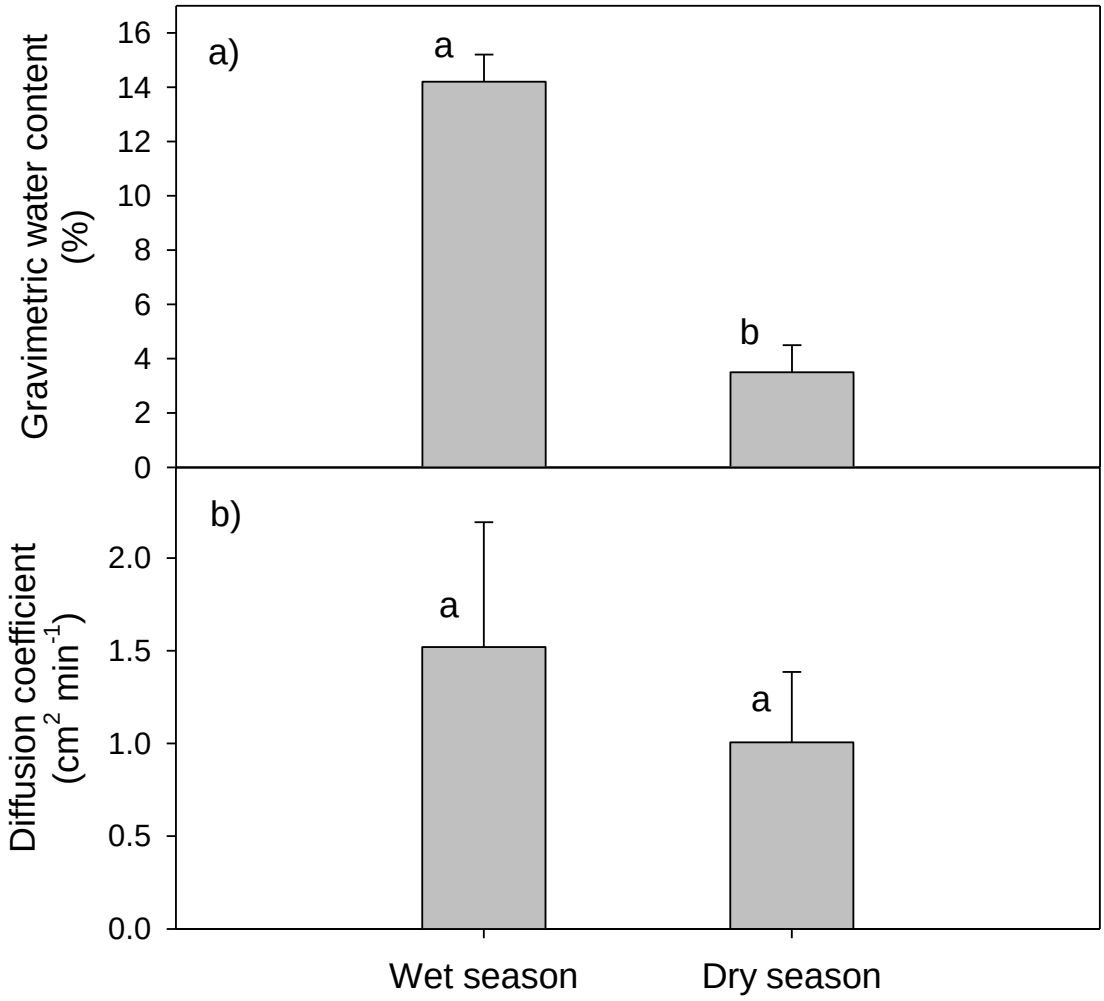

Figure 4.7: Seasonal change in mound water content (a) and gas diffusivity through mounds using SF_6 (b); error bars are standard errors of the mean; letters on top of bars show the significance ($p \leq 0.05$) of difference.

4.3.3 Fluxes measured in laboratory from mound samples

CH_4

There was a significant positive linear relationship between termite biomass and CH_4 flux both in the wet ($R^2 = 0.81$, $p \leq 0.001$) and the dry ($R^2 = 0.86$, $p \leq 0.001$) season (Fig. 4.8a). Mean CH_4 flux per unit termite biomass was significantly greater ($p \leq 0.01$) in the wet season (9.9 ± 0.8 μg CH_4-C g termite^{-1} d^{-1}) than in the dry season (8.1 ± 0.6 μg CH_4-C g termite^{-1} d^{-1}; Table 4.1); difference being 1.2 fold. CH_4 fluxes from the mound material after the termites had been removed were negligible both in the wet and the dry season (data not shown). We did not observe a linear change in CH_4 concentration in jars during incubation of mound material which indicates very low methanotrophic or methanogenic activity in the mound material regardless of season.

Table 4.1: Seasonal dynamics in CH_4 and CO_2 flux (per unit termite biomass) and termite biomass in mound samples of *M. nervosus* as measured in the laboratory.

	Wet season	**Dry season**	**Difference (p)**
Termite flux			
CH_4 (μg CH_4-C g-termite^{-1} d^{-1})	9.9 ± 0.8	8.1 ± 0.6	≤0.01
CO_2 (mg CO_2-C g-termite^{-1} d^{-1})	3.7 ± 0.8	2.7 ± 0.2	≤0.01
Biomass			
Mean biomass (g-termite kg-mound^{-1})	35.0 ± 3.8	3.6 ± 0.9	≤0.01
Mean mass of a worker (mg)	1.34 ± 0.04	1.41 ± 0.07	
Mean mass of a soldier (mg)	1.87 ± 0.02	1.91 ± 0.11	
Mean mass of an alate (mg)	-	2.8 ± 0.05	
Soldiers (% total biomass)	6	5	
Non-soldiers (% total biomass)	94	95	

CO_2

There was a significant positive linear relationship between termite biomass and CO_2 flux both in the wet ($R^2 = 0.85$, $p \leq 0.001$) and the dry ($R^2 = 0.91$, $p \leq 0.001$) season (Fig. 4.8b). Mean CO_2 flux per unit termite biomass in the wet season (3.7 ± 0.8 mg CO_2-C g-

termite^{-1} d^{-1}) was significantly greater ($p \leq 0.01$) than the flux in the dry season (2.7 ± 0.2 mg CO_2-C g-termite^{-1} d^{-1}; Table 4.1); difference being 1.4 fold. CO_2 fluxes from the mound material (microbial respiration), after removing the termites, were 5 fold greater in the wet season compared to the dry season (data not shown).

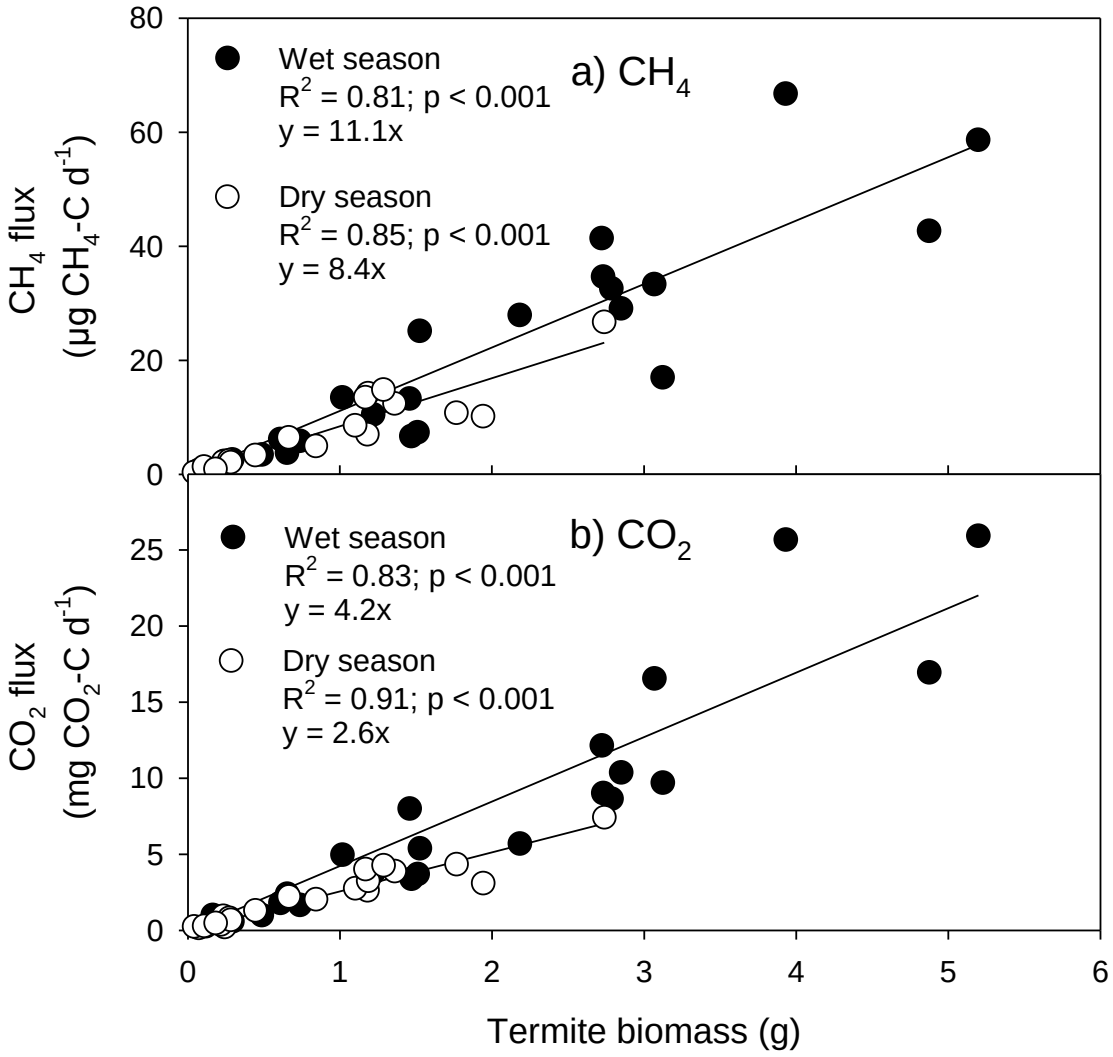

Figure 4.8: Simple linear regression analyses of: a) fresh termite biomass of *M. nervosus* and CH_4 flux, and b) fresh termite biomass of *M. nervosus* and CO_2 flux in the wet and the dry seasons of 2009 as measured from fresh mound samples in the laboratory.

4.3.4 Seasonal variation in termite biomass in mounds

As determined from mound samples, there was a significant relationship between termite biomass and mound mass (R^2 = 0.46, $p \leq 0.001$) in the wet season but not significant in the dry season (Fig. 4.9). Termite biomass was 10 fold greater in the wet season (35.0 ± 3.8 g-termite kg-mound^{-1}) compared to the dry season (3.6 ± 0.9 g-termite kg-mound^{-1}; Table 4.1). In the wet season, smaller mound samples were collected than in the dry season because of the greater termite biomass density and therefore the time required for separation and removal.

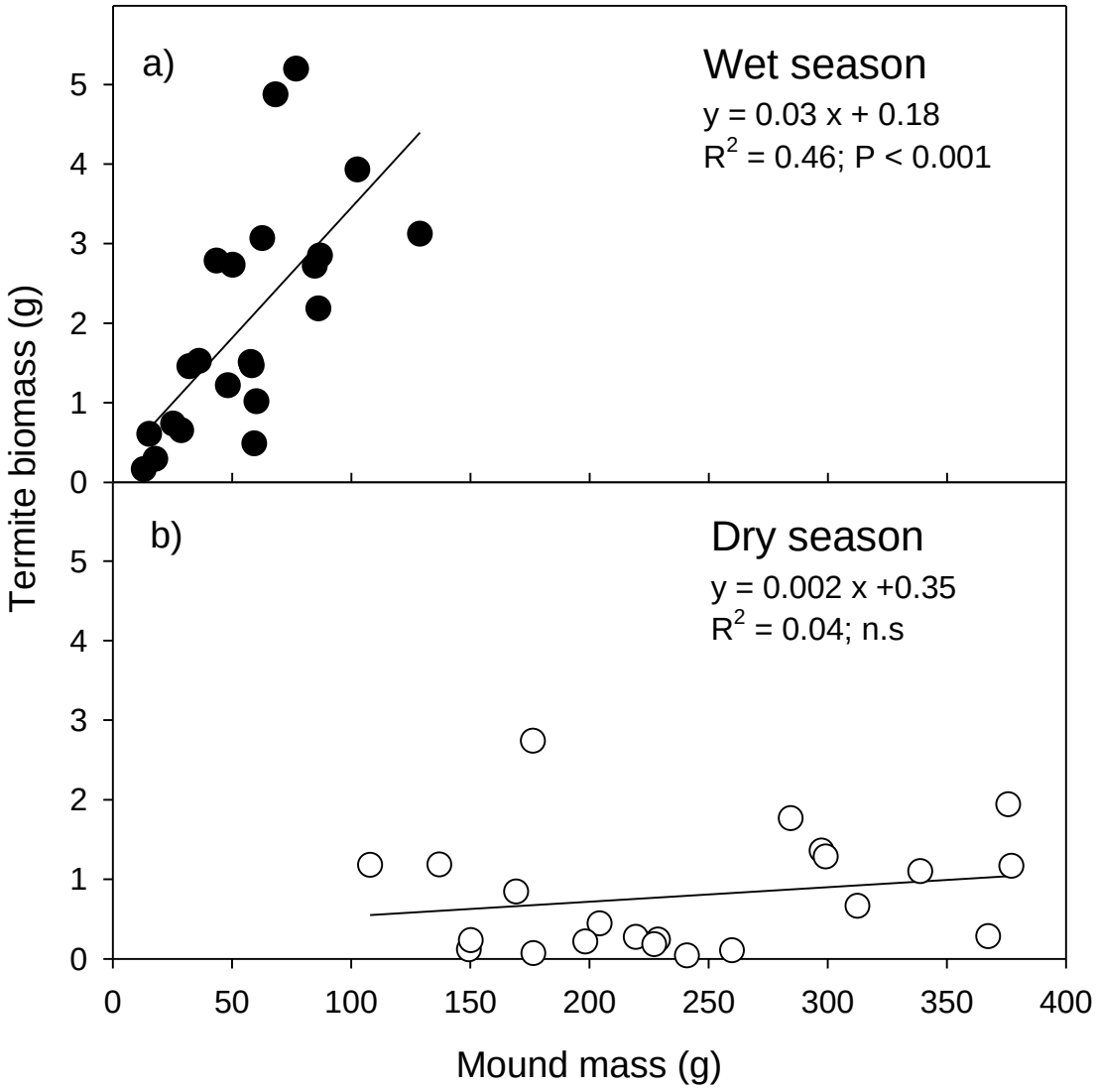

Figure 4.9: Simple linear regression analysis of mound mass (samples) and termite biomass in the: **a)** wet season, and **b)** dry season of 2009, for *M. nervosus*.

Soldiers comprised only 5 to 6% of the total termite biomass in a mound, with workers and alates comprising the rest (Table 4.1). Mean mass of an individual worker was similar in the wet (1.34 ± 0.04 mg) and the dry (1.41 ± 0.07 mg) season, as was the mean mass of an individual soldier in the wet (1.87 ± 0.02 mg) and the dry (1.91 ± 0.11 mg) seasons (Table 4.1). Mean mass of an alate could only be measured in the dry season (2.8 ± 0.05 mg) as winged alates leave the mounds early in the wet season (Table 4.1). Thus mass per termite was in the order of alate > soldier > worker.

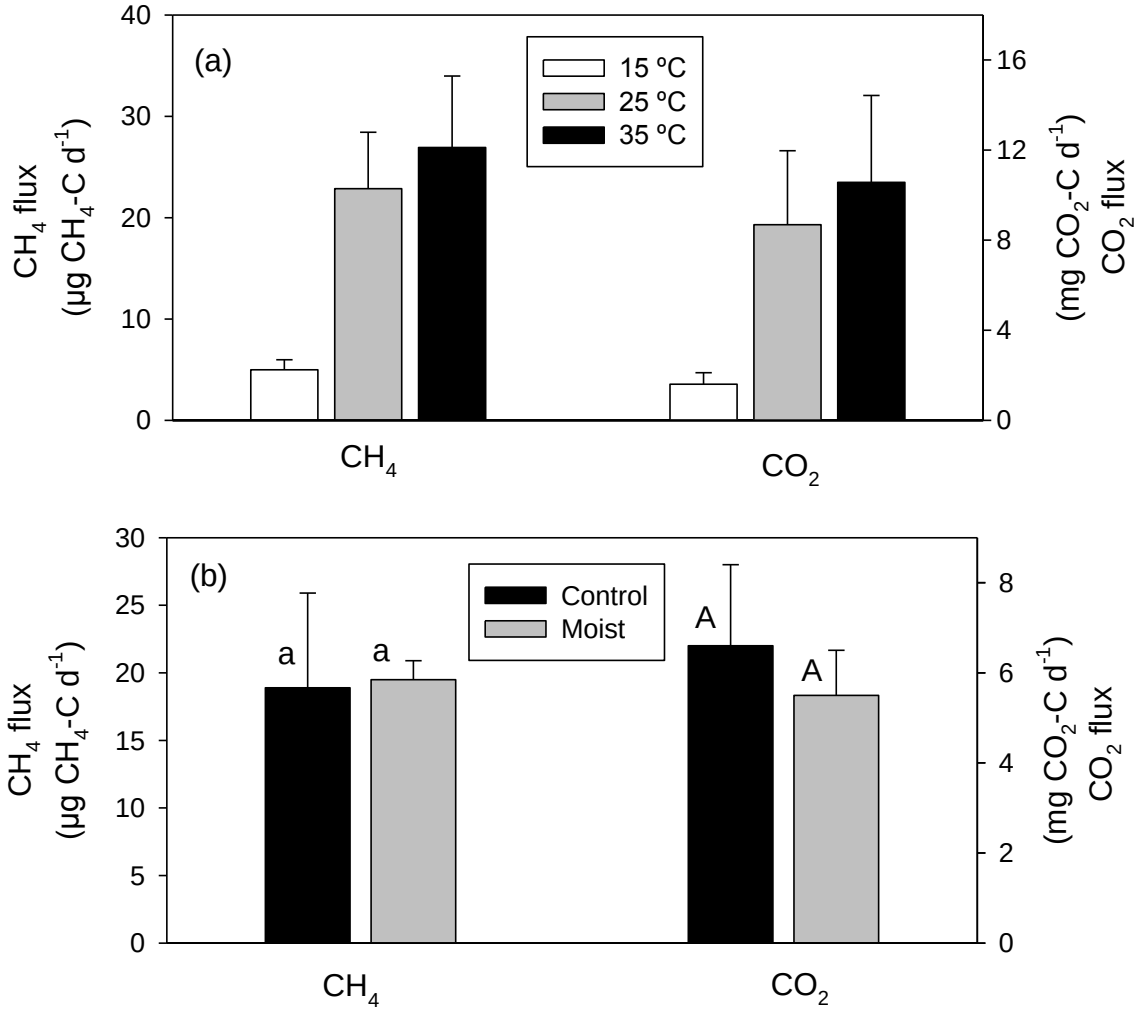

Figure 4.10: **(a)** Mean CH_4 and CO_2 fluxes measured from mound samples (n = 5) containing termites incubated at 15 ºC, 25 ºC and 35 ºC; error bars are standard errors of the mean; case-wise letters on top of the bars show the significance of differences in

fluxes measured at three different temperatures; for CH_4, Q_{10} values were 4.6 and 1.2 between 15 and 25 ºC and between 25 and 35 ºC, respectively; for CO_2, Q_{10} values were 5.4 and 1.4 between 15 and 25 ºC and between 25 and 35 ºC, respectively; **(b)** Mean fluxes of CH_4 and CO_2 measured at 25°C from five mound samples containing termites; before and after adding moist calico material pieces; case-wise letters on top of the bars show the significance of variations.

4.3.5 Effect of temperature and moisture on flux

For CH_4, the Q_{10} was 4.6 between 15 and 25 ºC and 1.2 between 25 and 35 ºC (Fig. 4.10a). For CO_2, the Q_{10} was 5.4 between 15 and 25 ºC and 1.4 between 25 and 35 ºC (Fig. 4.10a). The difference in CH_4 and CO_2 fluxes measured before and after adding moisture to the jars was not significant (Fig. 4.10b), although there was an increase in termite activity and gallery construction.

4.4. Discussion

4.4.1 Seasonal dynamics in termite mound biomass

This study demonstrates for the first time that seasonal variations in fluxes of CH_4 and CO_2 from termite mounds in tropical savannas are primarily derived from seasonal variation of termite biomass in those mounds. We found a 10 fold increase in termite biomass in mound samples in the wet season compared to the dry season (Table 4.1), with biomass closely correlated with flux (Fig. 4.8). We suggest that this was the main factor causing the seasonal variations in mound gas fluxes measured in the field, which were 3.5 fold in this study (Fig. 4.5) and 8-9 fold in Jamali et al. (2011).

The observed changes of termite biomass inside mounds can be driven by either the reproductive cycle or foraging activity of termites from inside to outside the mound. Our observation of greater biomass of termites inside mounds in the wet season is in contrast to the general understanding of population dynamics of Termitidae. It is believed that populations of tropical Termitidae peak in the dry season (Noirot 1969), with swarming occurring with the onset of rains between October and December (Hill 1942). Colony biomass can be reduced by up to 50% when alates leave the colony permanently during

swarming (Wood and Sands 1978), but this varies among different termite species (Lepage and Darlington 2000; Nutting 1969). Generally, swarming is immediately followed by egg production, which peaks during the wet season (Matsuura et al. 2007). However, this suggested lifecycle pattern does not concur with our termite population data and the observation that the greatest CH_4 and CO_2 fluxes from mounds occur in the wet season.

It is possible that termites were not present inside the mounds due to foraging activity. Termite foraging activity is governed by the energy and protein needs of the colony (Buxton 1981). In tropical areas this should be greatest in the dry season during nymphal (alate) maturation (Lepage and Darlington 2000) when the developing alates are fed by workers. Hence, it is probable that a large parts of the colony population were foraging outside the mounds in the dry season in order to meet the energy demands of the colony. This has been observed for harvester termites of *Macrotermes* species (Bodot 1967; Lepage 1982; Wood et al. 1977) and *Trinervitermes geminatus* (Ohiago 1979a; Ohiago 1979b) of family Termitidae studied in African savannas. Conversely, for *M. nervosus* Dawes-Gromadzki and Spain (2003) observed much greater foraging activity in the wet and transitional seasons as compared to the dry season at our field site in Darwin. However, these authors used surface baits to assess termite foraging activity and it is possible that as *M. nervosus* is a wood-eating species it can forage for food resources deeper within soil (e.g. dead roots) in the dry season where moisture conditions are more favorable (Abensperg-Traun 1991). *M. nervosus* is very sensitive to desiccation and termite specimen collected during the dry season in the field died within minutes when left exposed to the low humidity ambient atmosphere. It is therefore likely that seasonal changes in termite population in mounds may only be apparent because of changes in seasonal termite foraging activity. However, the life cycle of *M. nervosus* has not been investigated in detail and it is also possible that this species differs in its population dynamics to other Termitidae.

4.4.2 Seasonality in fluxes per unit termite biomass

Termites emitted slightly greater CH_4 and CO_2 per unit termite biomass in the wet season compared to the dry season. However, the magnitude of this seasonal variation in flux per

unit termite biomass was much smaller than the magnitude of observed seasonal variation in mound fluxes measured in the field which was 3.5 fold (CH_4 and CO_2) in this study and 8-9 fold (CH_4 only) in Jamali et al. (2011). Consequently, the large seasonal variations of CH_4 and CO_2 fluxes from termite mounds were not caused by changes in the flux of CH_4 and CO_2 by unit termite biomass. This seasonal variation in flux per unit termite biomass may be attributed to insect adaptation to xeric conditions, as metabolism and respiration processes can be an important source of water loss (Bartholomew et al. 1985; Edney 1977; Lighton 1990).

4.4.3 Seasonality in gas diffusivity and fluxes from mound material

Indirect estimates as well as the direct measurements of gas diffusivity through mound wall did not show any significant difference between the wet and the dry seasons. These results suggest that seasonal variation in gas diffusivity through mound wall was not a driving mechanism in the seasonal variation of mound fluxes. CH_4 fluxes of the termite mound material were negligible both in the wet and the dry season. We cannot rule out the possibility of CH_4 oxidation by mound material which could be better quantified using long term incubations and isotopic techniques (Sugimoto et al. 1998). However, the absence of measurable CH_4 oxidation in mound material, also reported elsewhere (Bignell et al. 1997), means that this process is unlikely to cause a significant variation in seasonal mound CH_4 fluxes. CO_2 fluxes as a result of microbial respiration from mound material partly contributed towards causing seasonal variations in mound fluxes of CO_2; however, we did not quantify their exact contribution.

4.4.4 Effect of temperature and moisture on flux

There was a positive correlation between temperature and termite fluxes of CH_4 and CO_2 in laboratory incubation experiments using mound samples (Fig. 4.10a). However, temperature fluctuations in tropical savannas are mainly observed on a diurnal basis (day/night) whereas the seasonal differences (wet/dry season) of mean temperatures are rather small (Fig. 4.7b). Hence, temperature is not likely to have been a major driver for the observed seasonal changes in CH_4 and CO_2 fluxes. The short-term effect of moisture on CH_4 and CO_2 fluxes from termites was not significant despite greater termite activity after the addition of a source of moisture (Fig. 4.10b). This further supports the argument

that seasonal variations in mound fluxes of CH_4 and CO_2 are principally driven by seasonal dynamics in termite population rather than the seasonal change in flux per unit termite biomass. It also suggests that an immediate response of mound fluxes to rainfall (Fig. 4.5) is because of termites being restricted to mounds and not because of any effect on their gut biology, metabolism or physiology.

4.5 Conclusions

This study is the first to investigate the processes responsible for large seasonal variations in CH_4 and CO_2 from termite mounds. The key finding of this paper is that these seasonal variations in mound fluxes of CH_4 and CO_2 in the tropical savannas of Australia are primarily derived from seasonal variation of termite biomass in mounds. Termites emit slightly greater CH_4 and CO_2 per unit termite biomass in the wet season as compared to the dry season but this does not account for the large seasonal differences observed in mound fluxes of CH_4 and CO_2. Our results highlight the need to integrate future studies of termite gas fluxes with detailed studies of termite population dynamics and behaviour for realistic estimates of greenhouse gas emissions from termites.

4.6 References

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5 Predicting CH_4 fluxes from termite mounds using indirect methods in tropical savannas of northern Australia

Abstract

Emissions of CH_4 from termites are usually highlighted more than CO_2; however, the general assumption that CH_4 flux from termite mounds is the most important factor in the overall greenhouse gas balance may not be realistic. Flux studies from termites are usually hampered by the difficulty in measuring such fluxes. The main objectives of this study were: 1) to investigate indirect methods for predicting fluxes from termite mounds based on (a) the relationship between mound CH_4 and mound CO_2 flux, and (b) the relationship between mound flux (CH_4 and CO_2) and the concentration of the respective gas inside mound, and 2) to investigate the relative importance of CH_4 and CO_2 fluxes from soil and termite mounds at TERC, Charles Darwin National Park, Howard Springs-savanna and Howard Springs-wetland sites in the tropical savannas of northern Australia. The magnitude of error in predicted mound CH_4 flux was small when predicted using the mound CO_2 flux of the same species. However, mound CH_4 flux of a species that has been predicted using mound CO_2 flux of another species resulted in large errors. Similarly, the error in predicted fluxes was smaller when predicted by using the internal mound concentration of these gases from the same species. Thus, these two methods can be used to predict fluxes with relatively smaller errors if used within a species; however, the relationship between flux and the predicting factor has to be established separately for each species. The annual greenhouse gas flux from termite mounds as well as soil was dominated by CO_2 with large variations among sites. Annual soil CH_4 flux was a net sink at TERC and Howard Springs-savanna site and a net CH_4 source at Charles Darwin National Park and Howard Springs-wetland site. It is important to account for such variations in annual flux among sites when scaling up from local to regional scales.

5.1 Introduction

Savannas occur in over 20 countries, largely in seasonal tropics, cover 20% of global land surface and produce almost 30% of global net primary production (Grace et al., 2006; Hutley and Setterfield, 2008). Savannas are characterized by a grass understorey with a tree/shrub over-storey in regions with distinct wet and dry seasons and frequent low-intensity fires through natural or human induced actions (Grace et al., 2006; Hutley and Beringer, 2010). Because of the extent of area these ecosystems cover, tropical savannas play an important role in the global carbon cycle. However, uncertainty in the magnitude of carbon stocks and greenhouse gas emissions is considerable (Bousquet et al., 2006; Grace et al., 2006). An important component of the carbon and greenhouse gas balance of savanna ecosystems is the exchange of the greenhouse gas methane. Methane exchange in tropical savannas is predominantly a function of: 1) soil CH_4 oxidation (uptake), 2) CH_4 emissions from seasonally inundated soils or ephemeral wetlands, 3) CH_4 emissions from fire events, and 4) CH_4 emissions from termites. Many of these processes are poorly quantified, which lead to large uncertainties regarding the overall methane budget of savannas.

There is a general consensus that termite mounds may be a large point source of CH_4 and CO_2 as compared to adjacent soils (Brümmer et al., 2009; Jamali et al., 2011a; Khalil et al., 1990; MacDonald et al., 1998; Seiler et al., 1984) but their contribution at the ecosystem scale is highly uncertain because of variable mound density, species differences among sites and difficulties in obtaining reliable and extensive field measurement. There are limited studies that have investigated CH_4 fluxes from termites in the field because of the difficulty associated with making such measurements (chamber installation, remote sites, harsh climate, costs and labor time for analysis). Fluxes of CO_2 can be measured cheaply and relatively easily using an Infrared Gas Analyzer (IRGA), whereas, CH_4 fluxes are most often measured through conventional gas sample collection and concentration analysis through gas chromatography back in the laboratory. Only in the last few years, have field-based CH_4 analyzer, such as one used in this study, become available and they remain expensive in comparison to an IRGA.

Khalil et al. (1990) used an indirect method to estimate CH_4 and CO_2 fluxes from intact termite mounds based on the relationship between mound flux, gas concentration inside mounds and gas concentration at ambient level as below:

$$F = \lambda\,(Cm - C_0) \qquad (1)$$

Where F is the flux from the mound (g CH_4 or CO_2 mound^{-1} h^{-1}), C_m and C_0 are gas (CH_4 or CO_2) concentrations (ppm) inside mound and in ambient air outside the mound, respectively, and λ is a constant derived from this equation. Khalil et al. (1990) calculated a mean λ value based on field measurements of F, C_m and C_0 from the mounds of *Coptotermes lacteus*. Khalil et al. (1990) validated this method and λ value using mounds of *Amitermes laurensis*, for which F, C_m and C_0 had been measured, and found good agreement between measured and predicted fluxes. As such, Khalil et al. (1990) assumed that this single λ value was similar for mounds of all termite species and indirectly calculated mound CH_4 and CO_2 fluxes of four other termite species using this method. If valid, the advantage of this method is that it takes in account the proportion of CH_4 produced inside mound by termites that is not emitted to the atmosphere because of gas diffusion barriers through the mound wall and CH_4 oxidation by methanotrophs in mound wall material (Khalil et al., 1990).

Another indirect method for estimating CH_4 fluxes from intact termite mounds could be by based on the potential relationship between mound CH_4 flux and mound CO_2 flux. Emissions of CH_4 and CO_2 from termite mounds are mainly a function of termite biomass, metabolic activity and respiration (CO_2 only). Jamali et al. (2011b) found a strong relationship between CH_4 and CO_2 fluxes ($R^2 > 0.8$) measured from termites (not mounds) of *Microcerotermes nervosus* in the laboratory. If this relationship between CH_4 and CO_2 flux also proves to be true for mound fluxes, it might be possible to use 'easier-to-measure' CO_2 fluxes for predicting mound CH_4 fluxes.

Although scope of most of the termite flux studies reported in literature is limited to CH_4 (Bignell et al., 1997; Fraser et al., 1986; MacDonald et al., 1998; Sanderson, 1996),

termites can also be an important source of CO_2. Emissions of CH_4 from termites are usually highlighted more than CO_2 because of their larger contribution in the CH_4 balance of an ecosystem as compared to CO_2 (Brümmer et al., 2009). However, the general assumption that CH_4 flux from termite mounds is the most important factor in the overall greenhouse gas balance may not be realistic. It is important to investigate the relative importance of CH_4 and CO_2 emission from termites to the total greenhouse gas balance of an ecosystem.

Objectives of this study were:

1. to investigate if the internal mound concentration of CH_4 and/or CO_2 can be used to predict the flux of the respective gas from the mound;
2. to investigate if CO_2 flux can be used to predict CH_4 flux of termite mounds; and
3. to study the relative importance of CH_4 and CO_2 emissions from termite mounds and soils at sites with variable mound density and termite species distribution.

5.2 Materials and methods

5.2.1 Site

Table 5.1: Site characteristics including location, tree basal area, litter mass, soil bulk density and mound bulk density; standard errors of the mean in parentheses.

Site	Location	Tree basal area (m^2 ha^{-1})	Litter mass (kg m^{-2})	Soil bulk density (g cm^{-3})	Mound bulk density (g cm^{-3})
TERC	12º24´S, 130º55´E	16.8	0.84 (0.06)	1.27 (0.02)	0.55 (0.05)[a]
CDNP	12°27′S, 130°50′E	10.9	0.92 (0.04)	1.57 (0.03)	1.00 (0.08)[b]
HS savanna	12°29′S, 131°00′E	4.2	0.12 (0.05)	1.79 (0.02)	0.51 (0.09)[c]
HS wetland	12°31′S, 131°07′E	1.5	0.00	1.55 (0.02)	0.67 (0.06)[d]

[a] = *Microcerotermes nervosus*; [b] = *Tumulitermes pastinator*; [c] = *Tumulitermes hastilis*; [d] = *Amitermes meridionalis*

This study was conducted at four 50 x 50 m plots, each in four different savanna locations near Darwin in the Northern Territory, Australia:

1) CSIRO's Tropical Ecosystems Research Center (TERC) is on the outskirts of Darwin city and is dominated by *Eucalyptus miniata* Cunn. Ex Schauter and *E. tetrodonta* F. Muell., over an understorey of annual / perennial C4 grasses with a thick litter layer (Table 5.1). TERC, with a tree basal area of 16.8 m^2 ha^{-1} (Table 5.1), has been protected from fires for > 20 years (Eager pers. comm.). Total termite mound basal area at this site was 18.4 m^2 ha^{-1}, with 21% of this basal area contributed from *Microcerotermes nervosus* mounds and the remainder from nine other termite species (Table 5.2). Five mounds of *M. nervosus* were selected for repeat measurement across the seasons. Mound walls of *M. nervosus* are soft with an internal honeycomb-like structure and the average mound size was 0.01 m^3.

2) Charles Darwin National Park (CDNP) is located 5.5 km east of Darwin and is dominated by *Eucalyptus miniata* Cunn. Ex Schauter and *E. tetrodonta* F. Muell., with an understorey of annual / perennial C4 grasses with a thick litter layer (Table 5.1). Tree basal area was 10.9 m^2 ha^{-1} (Table 5.1) at CDNP site. The area studied has not been burnt for > 10 years (Paul pers. comm.). Total termite mound basal area was 8.5 m^2 ha^{-1} with major contributions of 11% and 10% from the mounds of *Tumulitermes pastinator* and *M. nervosus*, respectively. The remaining mound basal area is made up from eight other termite species (Table 5.2). Five mounds of *T. pastinator* were selected for repeat measurements. Mounds of *T. pastinator* have a very hard outer wall with a strong internal honeycomb-like structure surrounding a large central gallery. Average mound size of *T. pastinator* was 0.02 m^3.

3) Located 21 km south-east of Darwin at Howard Springs (HS-savanna) was a similar savanna woodland to sites 1 and 2, but with a smaller tree basal area of 4.2 m^2 ha^{-1} and negligible litter (Table 5.1). Site 3 was burnt almost every year in early dry season (May). Total termite mound basal area at this site was 18.1 m^2 ha^{-1} more than 50% of which was covered by the mounds of *Tumulitermes hastilis* (Table 5.2). The remaining mounds were of *M. nervosus* and other species, which could not be identified as four samples were lost (Table 5.2). Fluxes were repeat-measured from five mounds of *T. hastilis* at this site.

Mound wall of *T. hastilis* was softest of all with honey comb-like mound structure. Average mound size for *T. hastilis* at this site was 0.01 m^3.

4) The fourth site was an ephemeral wetland (HS-wetland) with a few acacia trees (tree basal area 1.5 m^2 ha^{-1}) located 30 km south-east of Darwin in the Howard Springs locality (Table 5.1). Site 4 was inundated under water during the wet season between December and April. The C4 grass understorey appeared to be burnt frequently. All termite mounds at this site were of *Amitermes meridionalis* covering a total mound basal area of 6.2 m^2 ha^{-1} (Table 5.2). Always occurring in seasonally flooded alluvial plains, mounds of *A. meridionalis* are uniquely constructed like enormous tombstones, often more than two meters in height (Anderson et al., 2005). These mounds are commonly known as magnetic mounds because of their unique orientation along a north-south axis for temperature regulation inside mound (Anderson et al., 2005). Fluxes were measured from seven mounds of *A. meridionalis* at this site. Mound wall was softer for the smaller mounds and harder for the larger. Average mound size for *A. meridionalis* was 0.12 m^3 at this site.

Table 5.2: List of mound-building termite species collected from the 50 x 50m plots at four sites.

Site	Family/species
TERC	**Termitidae**
	1. *Amitermes darwini*
	2. *Ephelotermes melachoma*
	3. *Ephelotermes taylori*
	4. *Macrognathotermes errator*
	5. *Macrognathotermes sunteri*
	6. *Microcerotermes nervosus*
	7. *Nasutitermes eucalypti*
	8. *Nasutitermes longipennis*
	Rhinotermitidae
	9. *Coptotermes acinaciformis*
	10. *Schedorhinotermes actuosus*
CDNP	**Termitidae**
	1. *Amitermes germanus*
	2. *Drepanotermes septentrionalis*
	3. *Ephelotermes melachoma*
	4. *Macrognathotermes sunteri*
	5. *Microcerotermes nervosus*
	6. *Microcerotermes serratus*
	7. *Nasutitermes eucalypti*
	8. *Nasutitermes longipennis*
	9. *Tumulitermes pastinator*
	Rhinotermitidae
	10. *Schedorhinotermes actuosus*
HS - savanna	**Termitidae**
	1. *Microcerotermes nervosus*
	2. *Tumulitermes hastilis*
	3. Unidentified species
HS - wetland	**Termitidae**
	1. *Amitermes meridionalis*

5.2.2 CH_4 and CO_2 flux measurements from mounds and soil

Methane and carbon dioxide fluxes were measured from termite mounds and soil using manual chambers in situ, every four to six weeks between February and November 2009, which covers the wet and dry seasons and the transition months between these seasons. Chamber bases were permanently fixed around selected mounds throughout the measurement campaign and were connected to chamber tops of the same circumference

using spring clamps and closed cell foam during the flux measurement. Flux of CH_4 and CO_2 was measured in a closed dynamic set up (non-steady state) by connecting each chambers in turn to a Fast Greenhouse Gas Analyzer (Los Gatos Research, Mountain View, CA, USA) using an inlet and outlet gas line with Swagelok^{TM} push-fittings . Flux measures took 5 to 10 minutes to perform. See chapter 3 for further details. Fluxes from termite mounds were always measured between 10:00 and 12:00 hours local time as fluxes measured at this time best represent the mean daily flux (Jamali et al., 2011a).
Soil fluxes of CH_4 and CO_2 were measured on the same day, but between 09:00 and 11:00 hours, using the same protocols as for mound flux measurements. Chamber bases were permanently fixed in the soil at all sites throughout 2009.

5.2.3 Internal mound CH_4 and CO_2 concentration

The internal mound CH_4 and CO_2 concentrations were measured once in the wet and the dry seasons from the same mounds of four termite species that were also repeat-measured for fluxes of CH_4 and CO_2. Gas tubes were permanently installed 5 cm into the mound wall at a mid level height of the mound. The outer end of the gas tube was connected to a two-way stopcock which was opened only at the time of gas sample collection. Gas samples of 20 ml were collected from inside the mounds by connecting a syringe to the stopcock immediately after measuring mound fluxes. These gas samples and CH_4 and CO_2 calibration standards of increasing concentrations were injected into the Fast Greenhouse Gas Analyzer. The concentration of CH_4 and CO_2 in the collected gas samples were then determined by using the simple linear regression equation developed from the standard gas samples of CH_4 and CO_2 separately.

5.2.4 Relationship between fluxes and environmental variables

Mound temperature was measured immediately after flux measurements by inserting a hand held Cole-Palmer® stainless steel temperature probe inserted 6 cm into the mound at half point along the height. Soil temperature was measured at 3 cm soil depth. Soil water content was measured by collecting soil cores (diameter 72cm) from the top 6 cm oven dried at 105ºC. Mound water content was not measured to avoid destructive sampling. Previous measurements (data not shown) had showed a strong relationship between water content of *M. nervosus* mounds and soil water content (R^2 = 0.88) at TERC. Stepwise

multiple linear regressions were used to analyze the relationship of CH_4 and CO_2 flux from termite mounds with mound temperature and soil water content, and of CH_4 and CO_2 flux from soil with soil temperature and soil water content. The R^2 indicated the amount of variation in flux that can be explained by these environmental variables.

5.2.5 Indirect methods for estimating fluxes from termite mounds

Method 1: Using CO_2 flux from termite mounds to predict CH_4 flux

We tested the validity of this method on mounds of *Microcerotermes nervosus* (n = 5), *Tumulitermes pastinator* (n = 5), *Tumulitermes hastilis* (n = 5) and *Amitermes meridionalis* (n = 7), four common mound-building termite species of northern Australia (Watson and Abbey, 1993). Simple linear regression was used to analyze the relationship of mound CH_4 flux with mound CO_2 flux separately for each of the four termite species. The regression equation and the measured CO_2 flux from the mounds of a species were used to estimate mound CH_4 fluxes for the same species as well as other three species.

Method 2: Using internal mound CH_4 and CO_2 concentration to predict CH_4 and CO_2 fluxes from termite mounds

The validity of this method was tested using the mounds of same four species as in method 1 above. The flux (F), concentration inside mound (C_m) and concentration in ambient air (C_0) were measured for CH_4 and CO_2 both in the wet and the dry seasons from the mounds of above four termite species. A simple linear regression was used to analyze the relationship between the gas (CH_4 and CO_2) concentration inside mound and mound flux of the respective gases measured from same mounds. A mean value of λ was calculated using the data of each species, and this was then used to predict mound fluxes for the other three species based on their C_m and C_0 data.

For investigating the accuracy of predicted fluxes using above two methods, the mean absolute error (MAE) and relative mean absolute error (MAE %) were calculated as follows (Miehle et al., 2006):

$$MAE = \frac{\sum_{i=1}^{n} |Oi - Pi|}{n} \qquad (1)$$

$$MAE\% = 100\frac{MAE}{\hat{O}} \qquad (2)$$

Where *Oi* and *Pi* are observed and predicted fluxes, respectively, for the same mounds and $\bar{O}$ is the mean of observed fluxes from the same mounds of a species. MAE% expresses the error in predicted fluxes as a percentage of the mean of observed fluxes ($\bar{O}$) for the same species and does not allow for compensation of positive and negative prediction errors.

5.2.6 Annual CH_4 and CO_2 flux calculation

Annual flux was calculated for each termite species based on field measurements of CH_4 and CO_2 from mounds, thus accounting for seasonal variations in flux. For months with a direct flux measurement, the mean daily flux was simply flux m^{-2} h^{-1} scaled up to day^{-1}. For months without direct flux measurement, the mean daily flux for that month was estimated as being an average of the nearest 'measured' month preceding and nearest month antecedent.

Annual flux of CH_4 and CO_2 (kg CO_2-e ha^{-1} y^{-1}) from termite mounds was then calculated for each site by taking in account the basal area (m^2 ha^{-1}) covered by mounds of all termite species surveyed at these sites. The basal area of each mound was calculated from the measurements of mound circumference at ground level in each 50 x 50 m plot. Termite soldiers were then collected from each mound and the termite species identified at CSIRO, Darwin. For mounds built by termite species for which flux had not been measured, an average flux rate from the four species that had been measured was used.

Annual soil flux of CH_4 and CO_2 was calculated in kg CO_2-e ha^{-1} y^{-1} from the field measurements of soil flux as described for mounds using a global warming potential of 25 for CH_4. Tree stem basal area, calculated from the measurements of tree diameter at 1.3 m height for all trees in each 50 x 50 m plot, and total mound basal was subtracted to calculate the net soil area for scaling up soil fluxes to the hectare level.

5.3 Results

5.3.1 CH_4 and CO_2 fluxes from termite mounds

Table 5.3: Significant linear relationships of mound CH_4 and CO_2 fluxes with mound temperature (T_{mound}) and soil water content (W_{soil}) as determined by step-wise linear regression for individual termite species.

Mound CH_4 flux (log_{10})	**Coefficients**	**R^2**	**p value**	**n**
M. nervosus (TERC)	W_{soil} (0.061), T_{mound} (0.083)	0.60	≤ 0.001	30
T. pastinator (CDNP)	W_{soil} (0.149)	0.55	≤ 0.001	30
T. hastilis (HS-savanna)	-	-	n.s	30
A. meridionalis (HS-wetland)	W_{soil} (0.017)	0.13	≤ 0.05	35
Mound CO_2 flux (log_{10})				
M. nervosus (TERC)	W_{soil} (0.063), T_{mound} (0.079)	0.68	≤ 0.001	30
T. pastinator (CDNP)	W_{soil} (0.096)	0.60	≤ 0.001	30
T. hastilis (HS-savanna)	W_{soil} (0.027)	0.24	≤ 0.01	30
A. meridionalis (HS-wetland)	-	-	n.s	35

CH_4

Mound CH_4 fluxes were greater in the wet season as compared to the dry season for all species except *T. hastilis* which did not show an obvious seasonal pattern in flux (Fig. 5.1). Mean CH_4 flux (± standard error) were lowest from the mounds of *M. nervosus* ranging between 379 ± 111 (dry season) and 1857 ± 718 μg CH_4-C m^{-2} h^{-1} (wet season) while fluxes from the mounds of other three species were in a similar range and almost three fold greater than *M. nervosus* (Fig 5.1). Stepwise multiple linear regression indicated that mound CH_4 flux of *M. nervosus* was significantly positively correlated ($p \leq 0.001$) with soil water content and mound temperature, while that of *T. pastinator* with soil water content ($p \leq 0.001$) only (Table 5.3).

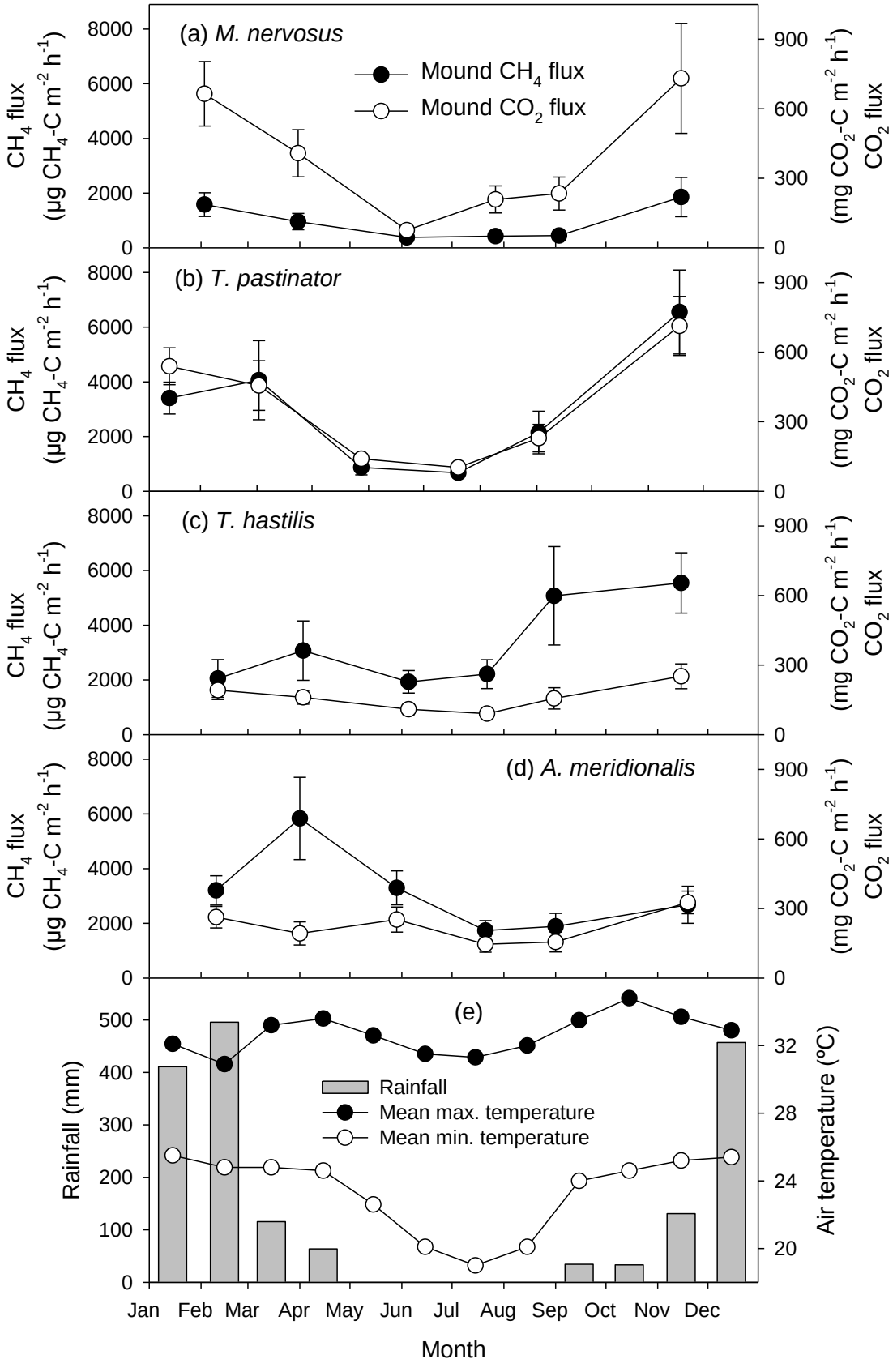
(a) M. nervosus
Mound CH4 flux
Mound CO2 flux
(b) T. pastinator
(c) T. hastilis
(d) A. meridionalis
(e)
Rainfall
Mean max. temperature
Mean min. temperature
CH4 flux (µg CH4-C m-2 h-1)
CO2 flux (mg CO2-C m-2 h-1)
Rainfall (mm)
Air temperature (ºC)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month

Figure 5.1 (**opposite**): Mean fluxes of CH_4 and CO_2 repeat-measured from mounds (n = 5-7) of four termite species at four different savanna sites; error bars are standard error of the mean; panel (e) shows 2009 monthly climate data for Darwin Airport (Bureau of Meteorology, Australia).

Methane fluxes from the mounds of *A. meridionalis* (measured at the ephemeral wetland site) showed a significant but weak relationship ($R^2 = 0.13$; $p \leq 0.05$) with soil water content (Table 5.3), however this relationship was stronger if measurements made in February season, when the soil was saturated, were excluded ($R^2 = 0.94$; $p \leq 0.001$). Methane fluxes from *T. hastilis* did not show any significant relationships with soil water content or mound temperature (Table 5.3).

CO_2

Mound CO_2 fluxes of all species showed a distinct seasonal pattern with greater fluxes in the wet season as compared to the dry season (Fig. 5.1). Mean mound CO_2 flux was similar for *M. nervosus* and *T. pastinator* ranging between 76 ± 2 (dry season) and 731 ± 237 mg CO_2-C m^{-2} h^{-1} (wet season) and was more than two fold greater as compared to *T. hastilis* and *A. meridionalis* (Fig. 5.1). Mound CO_2 flux of *M. nervosus* was significantly ($p \leq 0.001$) positively related to soil water content and mound temperature (Table 5.3). Fluxes from *T. pastinator* and *T. hastilis* were significantly ($p \leq 0.01$) and positively correlated with soil water content (Table 5.3). Mound CO_2 fluxes of *A. meridionalis* (measured at the ephemeral wetland site) did not show any significant relationships (Table 5.3).

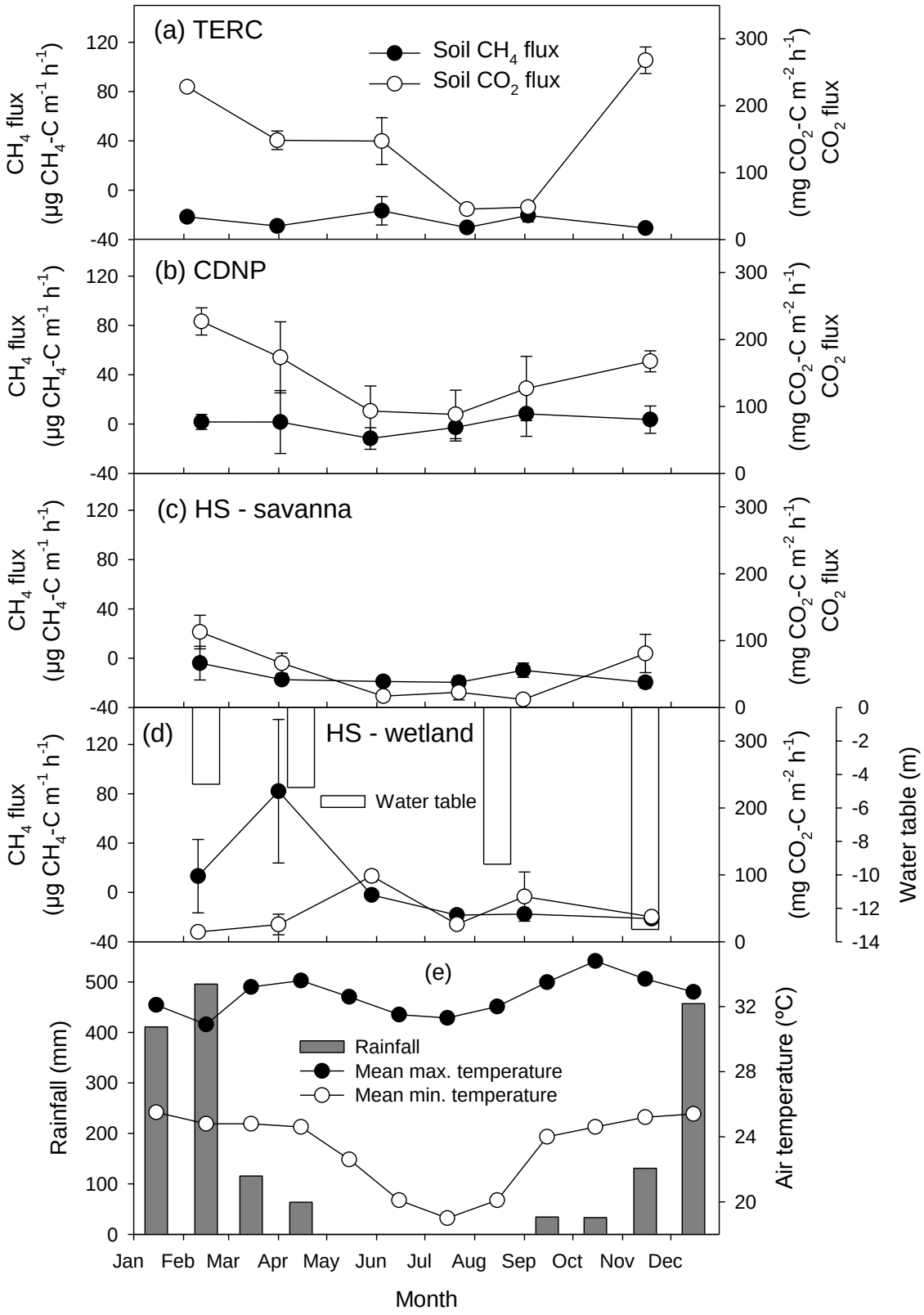
(a) TERC
Soil CH_4 flux
Soil CO_2 flux
(b) CDNP
(c) HS - savanna
(d) HS - wetland
Water table
(e)
Rainfall
Mean max. temperature
Mean min. temperature
CH_4 flux (µg CH_4-C m^{-1} h^{-1})
CO_2 flux (mg CO_2-C m^{-2} h^{-1})
Water table (m)
Rainfall (mm)
Air temperature (ºC)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month

Figure 5.2 (**opposite**): Mean soil fluxes (n = 5) of CH_4 and CO_2 measured at four different sites; error bars are standard error of the mean; panel (e) shows 2009 climate data from Darwin Airport meteorological station, Bureau of Meteorology, Australia.

5.3.2 CH_4 and CO_2 fluxes from soil

Table 5.4: Significant linear relationships of soil CH_4 and CO_2 fluxes with soil temperature (T_{soil}) at 5 cm and soil water content (W_{soil}) as determined by step-wise linear regression at four savanna sites.

Soil CH_4 flux (log_{10})	**Coefficients**	**R^2**	**p value (≤)**	**n**
TERC	-	-	n.s	25
CDNP	-	-	n.s	25
HS-savanna	-	-	n.s	25
HS-wetland	W_{soil} (0.009)	0.32	0.001	30
Soil CO_2 flux (log_{10})				
TERC	W_{soil} (0.104)	0.52	0.001	25
CDNP	W_{soil} (0.057)	0.35	0.01	25
HS-savanna	W_{soil} (0.096)	0.67	0.001	25
HS-wetland	-	-	n.s	30

CH_4

No distinct seasonal pattern was observed in soil CH_4 flux at TERC, CDNP and HS-savanna sites. Although some individual chambers showed CH_4 emissions, mean soil CH_4 flux (n = 5) was negative (i.e. soil CH_4 uptake) on all six measurement occasions at TERC and HS-savanna sites (Fig. 5.3). Whereas, mean CH_4 flux at CDNP site switched between uptake and emission in different seasons, ranging between +8.3 ± 18.2 and -11.7 ± 8.8 μg CH_4-C m^{-2} h^{-1} (Fig. 5.3). The relationship between soil CH_4 flux and soil water content and soil temperature was not significant at TERC, CDNP and HS-savanna sites (Table 5.4). At the HS-wetland site (ephemeral wetland), an obvious seasonal pattern in flux was observed with mean CH_4 fluxes (n = 5) being positive (i.e. soil CH_4 emissions) during the middle of the wet season, and negative (i.e. soil CH_4 uptake) in the drier months (Fig. 5.3d). Mean CH_4 fluxes (n = 5) at this site ranged between -18.4 ± 4.4 and

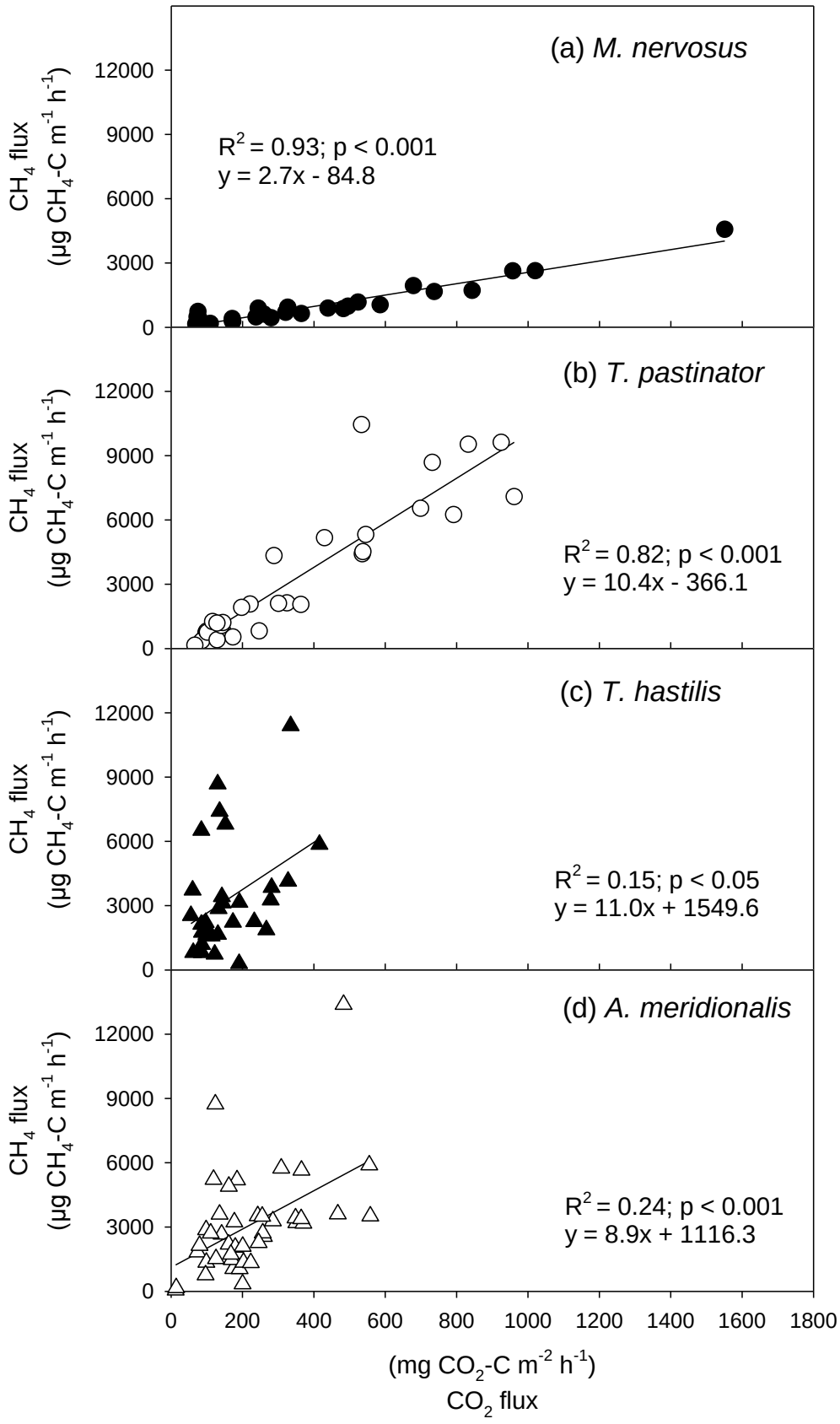
(a) M. nervosus
R2 = 0.93; p < 0.001
y = 2.7x - 84.8
(b) T. pastinator
R2 = 0.82; p < 0.001
y = 10.4x - 366.1
(c) T. hastilis
R2 = 0.15; p < 0.05
y = 11.0x + 1549.6
(d) A. meridionalis
R2 = 0.24; p < 0.001
y = 8.9x + 1116.3
CH4 flux
(µg CH4-C m-1 h-1)
0
3000
6000
9000
12000
0
200
400
600
800
1000
1200
1400
1600
1800
(mg CO2-C m-2 h-1)
CO2 flux

Figure 5.3 (**opposite**): Simple linear regression analysis between CH_4 and CO_2 fluxes from the mounds of four termite species.

+82.1 ± 130.3 μg CH_4-C m^{-2} h^{-1} in different months, with emissions occurring when the water table was within 5 meters of the soil surface in the wet season (Fig. 5.3d). There was also standing water up to 3 cm in depth in two of the chambers in the wet season (February and April). There was a significant ($p \leq 0.001$) and positive relationship between soil CH_4 flux and soil water content (Table 5.4).

CO_2

Soil CO_2 flux showed a distinct seasonal pattern at TERC, CDNP and HS-savanna sites with greater fluxes measured in the wet season and smaller in the dry season, and a significant ($p \leq 0.01$) relationship with soil water content at all three sites (Fig. 5.3). Seasonal flux pattern at HS-wetland site was different from the other three sites as greatest flux were measured in the early dry season and smallest in the wet season when the soils were saturated and water table within 5 meters of the surface (Fig. 5.3d). In the early dry season (May), the soil water content at this ephemeral wetland site was still similar or greater than that of other sites in the middle of the wet season (data not shown). The relationship between CO_2 fluxes and soil water content was only significant for <20% gravimetric soil water contents (data not shown), i.e. excluding wet season, saturated soil measurements. Soil CO_2 flux at TERC was greatest and similar to that at CDNP, ranging between 45 ± 6 and 268 ± 20 mg CO_2-C m^{-2} h^{-1}, and smallest at HS-wetland site ranging between 15 ± 5 and 98 ± 8 mg CO_2-C m^{-2} h^{-1} (Fig. 5.3).

5.3.3 Indirect methods for estimating mound fluxes

Method 1: Using CO_2 flux from termite mounds to predict CH_4 flux

In general, there was a similar pattern in the mound fluxes of CH_4 and CO_2 from all termite species (Fig. 5.1). The linear relationships between fluxes of CH_4 and CO_2 from termite mounds were stronger for *M. nervosus* ($R^2 = 0.93$; $p \leq 0.001$) and *T. pastinator*

(R^2 = 0.82; $p \leq 0.001$) as compared to *T. hastilis* (R^2 = 0.15; $p \leq 0.05$) and *A. meridionalis* (R^2 = 0.24; $p \leq 0.001$) mounds (Fig. 5.3). From the linear regression functions (Fig. 5.3), it is evident that there are similar relationships between CO_2 and CH_4 flux rates among the mounds of *T. pastinator*, *T. hastilis* and *A. meridionalis*; for every 1 mg of CO_2-C flux there is approximately 9 to 11 µg of CH_4-C emitted. Whereas, for mounds of *M. nervosus* <3 µg of CH_4-C is emitted for every 1 mg of CO_2-C flux.

In general, the predicted fluxes were more accurate (i.e. smaller MAE %), when mound CH_4 fluxes were predicted using the mound CO_2 fluxes of the same species (Table 5.5). Mound CH_4 fluxes were predicted more accurately for *M. nervosus* and *T. pastinator* with MAE% of 20 and 24, respectively, using the CO_2 fluxes from the same species (Table 5.5). When mound CH_4 fluxes predicted using the CO_2 flux of other species, the MAE% of the predicted fluxes ranged between 43 and 90, except *M. nervosus* which showed MAE% up to 520, i.e. least accurate of all (Table 5.5).

Table 5.5: The relative mean absolute error (MAE %) of mound CH_4 fluxes predicted using the concurrent mound CO_2 flux from mounds of the same species as well as mounds of other species.

Species [a]	**MAE% in predicted mound CH_4 fluxes** [a]			
	M. nervosus	*T. pastinator*	*T. hastilis*	*A. meridionalis*
M. nervosus	20	74	90	84
T. pastinator	291	24	65	47
T. hastilis	520	70	51	59
A. meridionalis	387	43	47	45

[a] mound CO_2 flux of these species is used for predicting the fluxes from the same and other species

[b] MAE % is the relative error in predicted flux as a percentage of mean observed flux of that species

Method 2: Using internal mound CH_4 and CO_2 concentration to predict CH_4 and CO_2 fluxes from termite mounds

CH_4

There was a significant relationship between mound CH_4 flux and CH_4 concentration inside mounds for all species (Fig. 5.4). This relationship was stronger for *M. nervosus*, *T. pastinator* and *A. meridionalis* species ($R^2 > 0.80$) as compared to *T. hastilis* ($R^2 = 0.58$) (Fig. 5.4). The value of λ was calculated for each species using equation 1. Methane fluxes were then predicted for the mounds of three species using the λ of fourth species and equation 1.

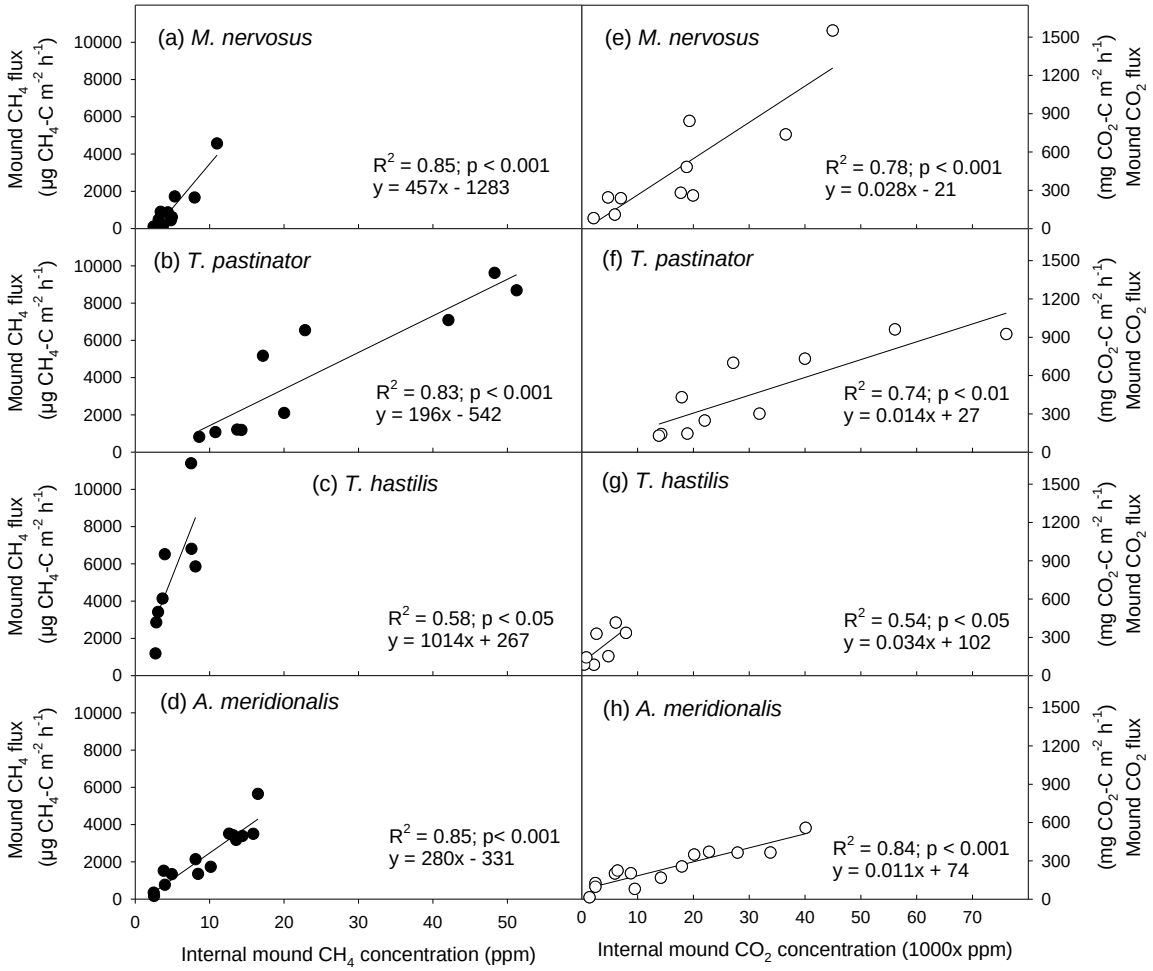

Figure 5.4: Relationship between CH_4 and CO_2 internal mound concentrations and respective CH_4 and CO_2 mound fluxes

CO_2

The relationship between mound CO_2 flux and CO_2 concentration inside mound was significant for all species, with $R^2 > 0.70$ for *M. nervosus*, *T. pastinator* and *A. meridionalis* and an R^2 of 0.54 for *T. hastilis* (Fig. 5.4). In general, the error (MAE%) in the predicted fluxes was smaller when predicted using the λ of the same species except *A. meridionalis* (Table 5.6). When using the λ of other species, the MAE% in the predicted mound CO_2 fluxes using λ calculated from *M. nervosus, T. pastinator* and *A. meridionalis* were similar (i.e. ≤ 109%), whereas, MAE% using λ from *T. hastilis* mounds were an order of magnitude greater (Table 5.4).

Table 5.6: The mean absolute error (MAE %) in fluxes of CH_4 and CO_2 predicted by using the Lambda (λ) relationship between the gas concentration difference inside and outside (ambient) the mounds and the fluxes of gas from those mounds.

Species [a]	**λ** [b] **(SE)**	**MAE% of predicted fluxes** [c]			
		M. nervosus	***T. pastinator***	***T. hastilis***	***A. meridionalis***
CH_4					
M. nervosus	297 (51)	39	62	82	19
T. pastinator	175 (27)	54	25	89	42
T. hastilis	1915 (264)	466	921	38	538
A. meridionalis	332 (36)	39	77	80	23
CO_2					
M. nervosus	31 (5)	36	109	58	102
T. pastinator	15 (2)	48	31	80	26
T. hastilis	389 (185)	1298	2494	437	2305
A. meridionalis	23 (4)	35	59	69	61

[a] λ calculated from these species is used for predicting the fluxes for species shown in columns

[b] μg CH_4-C m^{-2} h^{-1} ppm^{-1} for CH_4 and μg CO_2-C m^{-2} h^{-1} ppm^{-1} for CO_2

[c] MAE % is the relative error in predicted flux as a percentage of observed flux

The error (MAE%) in predicted fluxes using the λ of the same species was smaller as compared to when fluxes predicted using the λ of other species except *M. nervosus* (Table 5.4). When using the λ of other species, the MAE% in the predicted mound CH_4 fluxes using λ from *M. nervosus, T. pastinator* and *A. meridionalis* were similar (i.e. <100%), whereas, MAE% using λ from *T. hastilis* mounds were an order of magnitude greater (Table 5.6).

5.3.4 Annual fluxes

5.3.4.1 Termite mounds

Termite species with greatest mound CH_4 emissions and termite species with greatest mound CO_2 emissions were not the same (Table 5.7). Annual CH_4 flux estimates per m^2, on a CO_2-e basis, from the mounds of *M. nervosus* were 3- to 4-folds smaller than those from the other three termite species (Table 5.7). Whereas, the annual CO_2 flux estimates per m^2 from *M. nervosus* and *T. pastinator* were approximately two fold greater than those from *T. hastilis* and *A. meridionalis* (Table 5.7). On a CO_2-e basis, annual CH_4 flux estimates were between 5- (*T. hastilis*) and 46-fold (*M. nervosus*) smaller than the concurrent annual CO_2 flux estimates.

Table 5.7: Annual fluxes of CH_4 and CO_2 from termite mounds of four species in CO_2-e.

Species	**Site**	**Annual flux from termite mounds (kg CO_2-e m^{-2} y^{-1})**	
		CH_4	**CO_2**
M. nervosus	TERC	0.3	13.9
T. pastinator	CDNP	1.1	13.0
T. hastilis	HS-savanna	1.0	5.5
A. meridionalis	HS-wetland	0.9	7.4

Total mound basal area was 2- to 3-folds greater at TERC and HS-savanna sites as compared to CDNP and HS-wetland sites (Table 5.8). After accounting for the basal area of all mounds at each site, annual emissions from termite mounds were highly variable among sites (Table 5.8). Annual CH_4 fluxes from the termite mounds, on a CO_2-e basis,

were similar at TERC and HS-savanna sites, and almost 2-fold greater compared to the CDNP and HS-wetland sites (Table 5.8). Annual CO_2 fluxes from termite mounds, on a CO_2-e basis, were an order of magnitude greater than CH_4 flux at the same sites (Table 5.8). CDNP had the greatest annual flux of CO_2 from termite mounds (+166.6 kg CO_2-e ha^{-1} y^{-1}) even though it had very low annual CH_4 flux from mounds. The HS-wetland site had the lowest annual fluxes of CO_2 from termite mounds and the lowest flux for CH_4 (Table 5.8).

Table 5.8: Annual mean fluxes of CH_4 and CO_2 in kg CO_2-e ha^{-1} y^{-1} from termite mounds and soil at each of the four sites.

Site	Mound basal area (m^2 ha^{-1})	Termite mounds [e]		Soil [e]	
		CH_4	CO_2	CH_4	CO_2
TERC[a]	18.4	+ 13.4	+ 155.6	- 73.0	+ 51117
CDNP[b]	8.5	+ 7.0	+ 166.6	+ 2.9	+ 49523
HS-savanna[c]	18.1	+ 16.6	+ 140.4	- 41.7	+ 18654
HS-wetland[d]	6.2	+ 5.6	+ 45.5	+ 18.8	+ 13463

[a] TERC termite mounds were 21% *M. nervosus*, 79% other species

[b] CDNP termite mounds were 11% *T. pastinator*, 10% *M. nervosus*, 79% other species

[c] HS-savanna termite mounds were 51% *T. hastilis*, 2% *M. nervosus*, 47% others

[d] HS-wetland termite mounds were 100% *A. meridionalis*

[e] Fluxes expressed in kg CO_2-e ha^{-1} y^{-1}

5.3.4.2 Soil

The soil was a net CH_4 sink at two sites and a net CH_4 source at the other two, with TERC being the greatest CH_4 sink at -73.0 kg CO_2-e ha^{-1} y^{-1} and HS-wetland the greatest source at +18.8 kg CO_2-e ha^{-1} y^{-1} (Table 5.6).

Annual soil CO_2 fluxes were almost three orders of magnitude greater as compared to soil CH_4 fluxes at the same respective sites, when expressed on a CO_2-e basis. Annual soil CO_2 fluxes at TERC and CDNP sites were 2- to 4-folds greater than those at HS - savanna and HS-wetland site (Table 5.6).

5.4 Discussion

5.4.1 Indirect methods for estimation of flux from termite mounds

Method 1: Using CO_2 flux from termite mounds to predict CH_4 flux

The linear regression analysis (Fig. 5.3) and the magnitude of error in predicted fluxes (Table 5.5) suggest that CO_2 fluxes from termite mounds may be used to predict CH_4 fluxes for the same species. Even within a species, the error in predicted flux was large for some species (> 45 MAE: *T. hastilis* and *A. meridionalis*) and smaller in others (< 25 MAE: *M. nervosus* and *T. pastinator*) depending on the strength of relationship between mound CH_4 and mound CO_2 fluxes. However, this relationship is not consistent across species and using the regression function of one species to predict CH_4 fluxes for the mounds of other species can result in large errors. This inconsistency arises because the termite species that produced greatest CH_4 per unit mound were not the same that produced the greatest CO_2 (Table 5.7). For example, mounds of *M. nervosus* had smallest CH_4 fluxes but greatest CO_2 fluxes compared to other species. A similar observation was made by Khalil et al. (1990) who reported that CH_4 emissions from the mounds of *Amitermes laurensis* were 10-fold greater than *Coptotermes lacteus* but CO_2 emissions were 30-fold smaller.

In the laboratory, we found a strong relationship between CH_4 and CO_2 flux from termites of *M. nervosus* with $R^2 \geq 0.88$ (Jamali et al., 2011b). However, it seems there are intermediate processes between gas (CH_4 and CO_2) production by termites inside mounds and its release to the atmosphere.

First, a considerable portion of CH_4 produced inside mound can be oxidized by methanotrophic bacteria in mound material or the soil beneath the mound before being emitted to the atmosphere. Using CH_4 isotopes, Sugimoto et al. (1998) found that, for the mounds of different species from family Termitidae, 53% to 83% of total CH_4 produced inside mounds was oxidized before being emitted to the atmosphere. For the thick-walled and therefore less porous mounds of *Macrotermes annandalei*, almost all the CH_4 produced by termites inside mounds was oxidized resulting in near-zero CH_4 emissions from mounds to the atmosphere (Sugimoto et al., 1998). In our study, mounds of *T.*

pastinator had the thickest and hardest mound walls with a bulk density almost double that as compared to other species (Table 5.1). Mean CH_4 concentration inside the mounds of *T. pastinator* was almost 3 to 5 fold greater as compared to *T. hastilis* and *A. meridionalis,* despite similar mound CH_4 fluxes. These results suggest that CH_4 oxidation may be greater for the thick-walled mounds of *T. pastinator* as compared to other species. This variation in CH_4 oxidation within mounds of different species because of variable mound wall properties may contribute to the observed variation in the relationship between CH_4 and CO_2 fluxes from the mounds of different species.

Second, CO_2 emissions from termite mounds are not only due to termite respiration, as microbial respiration also occurs in the mound wall. Holt (1998) reported highly variable microbial population among the mounds of five Australian termite species (including *T. pastinator*) and concluded that the mound microenvironment in some termite species can be more conducive for microorganisms as compared to others because of mound properties such as mound bulk density and wall thickness. Thus, the proportional contribution of microbial respiration to the total CO_2 emissions from mound (termite respiration + microbial respiration) will vary among termite species. It can be hypothesized that species with a greater proportion of microbial respiration in total CO_2 fluxes from mounds and/or variable microbial respiration rates across seasons would tend to show weaker relationship between CH_4 and CO_2 fluxes from same mounds. A simple laboratory experiment (data not shown) indicated that microbial respiration in the mound material represented approximately 5% of total CO_2 emissions from a *M. nervosus* termite mound. We did not conduct such experiments for any of the other three species.

Based on the strength of relationship and smaller magnitude of error in predicted fluxes, our regression models for *M. nervosus* and *T. pastinator* can be used to predict CH_4 fluxes from the mounds of these species by only measuring CO_2 fluxes in field. However, we do not recommend using CO_2 flux as a predictor of CH_4 flux for other species without first developing species-specific models from field-based measurements. Similarly, we do not recommend using this approach when the relationship between CO_2 and CH_4 flux is weak (e.g. $R^2 < 0.5$).

Method 2: Using internal mound CH_4 and CO_2 concentration to predict respective fluxes from the same mounds

The linear regression analysis between mound flux and internal mound concentration suggests that this method may be used to predict mound fluxes for the same species. However, using this relationship (λ) based on one species to predict mound fluxes from other termite species, as used by Khalil et al. (1990), resulted in errors of up to 9 fold for CH_4 and up to 25 fold for CO_2 when compared to direct measured fluxes from the same mounds. Although there is a strong relationship between gas (CH_4 and CO_2) concentration inside a mound and emissions of the respective gases from the same mound for all species, this relationship is not consistent across species (Fig. 5.4). This is because of the variation in mound structure. Thick-walled mounds tend to accumulate gas inside the mound because of greater diffusive barriers resulting in greater internal gas concentrations but low mound fluxes and vice versa. For example, *T. hastilis* mounds were one of the greatest emitters despite having smaller internal gas concentrations because of softer mound wall resulting in a much higher λ value as compared to other species. We have demonstrated that using the λ value of *T. hastilis* for predicting mound fluxes from the thick-walled mounds of *T. pastinator* resulted in errors of 9-fold for CH_4 and 25-fold for CO_2 (Table 5.6). Therefore, predicting mound fluxes for some species using the λ value of other species may result in large and unacceptable errors.

In both the above methods, fluxes were generally predicted more accurately when using the predicting factor calculated from the mounds of the same species. The large errors in fluxes when predicted using the predicting factor of a different species are mainly caused by the variation in mound structure. Thus, both of the above methods can be used for estimating fluxes if predicting factors are developed for each or a group of species.

5.4.2 CH_4 and CO_2 fluxes from termite mounds

The seasonal pattern in fluxes of CH_4 and CO_2 for all species, except *T. hastilis*, concurs with previous findings (Brümmer et al., 2009; Holt, 1987; Jamali et al., 2011a) and is derived primarily from the seasonal population dynamics in termite mounds (Jamali et al., 2011b). The aseasonal pattern observed in CH_4 fluxes from the mounds of *T. hastilis*

(Fig. 5.1c) suggests that population dynamics for this species may differ from that reported previously for other species.

At the hectare scale, annual fluxes (CO_2-e) from termite mounds were dominated by CO_2 emissions, as mound CH_4 emissions contributed only 4 to 11% to the overall flux. The combined annual CO_2-e emissions of CH_4 and CO_2 from termite mounds were greatest at TERC and CDNP, which probably indicates the greater vegetation cover at these sites thus supporting larger termite populations. The smallest annual CO_2-e emissions from termite mounds were at HS-wetland site probably because the seasonally wet conditions only suit *A. meridionalis* (Anderson et al., 2005) as well as the smaller contribution of microbial respiration because of saturated conditions inhibiting microbial activity, litter accumulation and vegetation development. At a hectare scale, the annual emissions from termite mounds at these four sites are comparable to the 80 kg CO_2-e ha^{-1} y^{-1} from the mounds of *Cubitermes fungifaber* in the savannas of Burkina Faso, Africa (Brümmer et al., 2009).

5.4.3 CH_4 and CO_2 fluxes from soil

Soil CH_4 fluxes measured at TERC, CDNP and HS-savanna sites did not show an obvious seasonal pattern which concurs with the findings of Livesley et al. (2011) who measured soil CH_4 fluxes at Howard Springs. Flux rates measured in this study were similar in magnitude to those observed in other savanna ecosystems in Africa (Castaldi et al., 2006; Otter and Scholes, 2000; Prieme and Christensen, 1999) and South America (Anderson and Poth, 1998). Mean CH_4 fluxes at TERC and HS savanna sites showed soil CH_4 uptake in all months while fluxes at CDNP kept switching between soil CH_4 uptake and emissions in different months, but without showing any significant relationship with soil water content or soil temperature. This is probably because soil CH_4 emissions occurred when there was enough population of termites in soil under chamber to reverse the direction of CH_4 flux from uptake to emissions (MacDonald et al., 1999), as also observed elsewhere (Anderson and Poth, 1998; Livesley et al., 2011). Conversely, CH_4 fluxes at the ephemeral wetland site showed an obvious seasonal pattern with CH_4 emissions in the wet season and uptake in dry and transitional months (Fig. 5.2d). This

switch from CH_4 uptake to emissions at greater soil water contents has also been observed by Brümmer et al. (2009) in African savannas.

Soil CO_2 fluxes measured at TERC and CDNP sites ranged between 45 mg CO_2-C m^{-2} h^{-1} (dry season) and 268 mg CO_2-C m^{-2} h^{-1} (wet season), which is similar to the range of 65 – 219 mg CO_2-C m^{-2} h^{-1} reported by Chen et al. (2002) and, more recently, of 131 – 238 mg CO_2-C m^{-2} h^{-1} by Livesley et al. (2011), both measured at Howard Springs. Greater soil CO_2 fluxes in the wet season are mainly derived from increased fine-root biomass and growth (autotrophic respiration), and greater soil microbial activity (heterotrophic respiration) as a result of increased soil and litter moisture conditions (Castaldi et al., 2010; Chen et al., 2002; Holt et al., 1990; Keith et al., 1997; Raich and Tufekcioglu, 2000). Carbon dioxide fluxes from the HS savanna site showed a similar seasonal pattern but lower in magnitude as compared to TERC and CDNP. This probably can be explained by smaller tree cover and therefore root biomass, as well as smaller amount of litter at this site which could have resulted in reduced autotrophic and heterotrophic soil respiration rates directly (Maggs and Hewett, 1990; Raich and Tufekcioglu, 2000; Tewary et al., 1982), and indirectly by reducing the soil moisture as observed here (data not shown). At the ephemeral wetland site, very low CO_2 fluxes in the wet season could be because of saturated soil conditions (Davidson et al., 2000), as also observed in African savannas (Brümmer et al., 2009).

Annual soil fluxes were dominated by CO_2 at all sites with soil CH_4 fluxes contributing $\leq$0.2% to the combined soil flux of CH_4 and CO_2. Soil CH_4 fluxes at TERC and HS-savanna were a net CH_4 uptake, while fluxes at CDNP and HS-wetland sites showed net soil CH_4 emissions. Soil CH_4 emissions from HS-wetland site are not unexpected as this was an ephemeral wetland where anaerobic methanogenic activity in saturated soils led to wet season emissions (Brümmer et al., 2009). At CDNP, annual soil CH_4 flux was a net emission (+2.9 kg CO_2-e ha^{-1} y^{-1}) as some individual chambers indicated considerable subterranean termite activity (Livesley et al., 2011) that was able to switch the direction of annual soil CH_4 flux from uptake to emission. Such high variability in soil CH_4 flux

among sites suggests that scaling up will be problematic in tropical savanna landscapes of northern Australia or elsewhere.

Annual soil CH_4 flux at HS-wetland site was +18.8 kg CO_2-e ha^{-1} y^{-1} which highlights the importance of ephemeral wetlands as a potential CH_4 source in these ecosystems. Ephemeral wetlands cover almost 25% of the 1.9 million km^2 that Australian savannas cover, and are estimated to emit between 5 and 15 Tg CO_2-e y^{-1} (Deutscher et al., 2010). Our seasonal flux measurements from the ephemeral wetland site also highlight the fact that CH_4 uptake in the dry season months can offset a large portion, and potentially all, of CH_4 emitted from same location in the wet season, which is important to account for.

Annual soil CO_2 fluxes were much greater at TERC and CDNP sites, and were in close agreement with other annual estimates of soil CO_2 fluxes in this region (Chen et al., 2002; Livesley et al., 2011). Annual soil CO_2 flux was much smaller at HS-savanna (19 t CO_2-e ha^{-1} y^{-1}) and HS-wetland (13 t CO_2-e ha^{-1} y^{-1}) sites. An important fact here is that ephemeral wetland site showed smallest annual CO_2 flux from soil, and this reduction in CO_2 flux, because of saturated soil conditions, seems to offset all CH_4 emissions from this site when expressed in CO_2-e. Thus, CH_4 emissions from ephemeral wetlands might play an important role in the CH_4 budget; their contribution to the overall greenhouse gas balance of savanna ecosystems may be negligible.

5.5 Conclusions

Mound CH_4 flux of *M. nervosus* and *T. pastinator* can be predicted using the linear relationship between CH_4 and CO_2 flux for the same species. However, the relationship between mound CH_4 and CO_2 flux is not consistent across species and using the regression function of one species to predict CH_4 fluxes for the mounds of other species can result in large errors. Second method included predicting CH_4 and CO_2 flux from termite mounds based on the relationship of the concentrations of these gases inside mounds with the mound flux of the respected gases. If used within a species, this method can be applied to predict fluxes with relatively smaller errors. However, this relationship

was also not consistent across species because of the variable mound structure. Annual fluxes from termite mounds and soil were by far dominated by CO_2 at all sites. The large variability in annual flux from termite mounds and soil suggest caution in scaling up the local fluxes to a regional scale.

5.6 References

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6 Synthesis

The uncertainty in the global estimates of CH_4 emissions from termites is mainly associated with the lack of long-term field based studies from different biogeographical regions where termites occur. The variation in termite fluxes among different ecosystems is mainly derived from the diversity of species composition and the variable rates of fluxes among termite species. Tropical savannas are relatively rich in termite abundance and diversity. Savannas cover a quarter of the Australian continent but the contribution of termites to the CH_4 and CO_2 balance of these ecosystems is unknown. This thesis investigated the exchange of CH_4 and CO_2 between termites and atmosphere, and between soil and atmosphere in the tropical savannas of northern Australia.

The diurnal and seasonal variations from the mounds of different termite species as well as the processes responsible for such variations were investigated. Field as well as laboratory studies were used for the process-based understanding of diurnal and seasonal variation in flux. Variation in fluxes from the mounds of different termite species and the distribution and density of mounds for those species was quantified. This mound flux data and soil flux measurements were then used to estimate the contribution of termites to the CH_4 balance of a savanna woodland (Howard Springs near Darwin) in the tropical savannas of northern Australia. Two indirect methods for estimating mound CH_4 (and CO_2) fluxes were also tested. The relative importance of CH_4 and CO_2 to the total greenhouse gas emissions from termite mounds was investigated for four different sites in the tropical savannas of northern Australia.

Fluxes from termite mounds are often measured only at any one point in time in a day, thus not considering the potential **diurnal variations (Chapter 2)** in mound fluxes. In the tropical savannas of northern Australia, there is little variation in air temperature among different seasons but there can be a large difference between the daily minimum and maximum, typically increasing from 15 ºC to 35 ºC diurnally. This is the first study that quantified the diurnal variations in mound CH_4 flux in a 24 hour cycle. Methane fluxes were measured from the mounds of three termite species (*Microcerotermes*

nervosus, *M. serratus* and *Tumulitermes pastinator*) every four hours in a cycle of 24 hours directly in the field. Methane flux was greatest at the warmest time of the day, i.e. in the afternoon, and lowest at the coolest time of the day, i.e. early in the morning, for the mounds of all termite species. Further experiments revealed that the diurnal pattern in mound CH_4 flux was caused by the diurnal temperature pattern and not by the possible diurnal movement of termites in and out of the mound.

I demonstrated that mound CH_4 fluxes measured between 10:00 and 12:00 hours were representative of the mean daily flux derived from multiple measurements of a 24 hour period. Mound fluxes measured at ~14:00 hours (warmest) and ~06:00 hours (coolest) were up to 11-fold greater and 5-fold smaller, respectively, than the daily mean flux. These results suggest that mound fluxes should either be measured continuously or at least at a time determined as best representing the mean daily flux. Most of the global estimates published so far are based on either laboratory measurements at a constant temperature, or on a single measurement of mound flux at any time of the day. Flux rates derived from laboratory measurements or single field measurements have to be calibrated using annual temperature data before these could be used for scaling up. To my knowledge this was not considered in any of the previous global estimates. As such, published regional and global estimates can potentially contain large errors depending upon the magnitude of diurnal temperature variation in that region.

Another potential source of error in calculating annual fluxes from termites is the **seasonal variation (Chapters 2 and 5)** in CH_4 flux. The tropical savannas of northern Australia are characterized by a distinct wet and dry season. I quantified the seasonal variation in CH_4 flux from the mounds of six termite species commonly found in the tropical savannas of northern Australia and, for the first time, identified the likely **causes of seasonal variation (Chapter 4)** in mound CH_4 fluxes. Mound CH_4 fluxes were up to 25-fold greater in the wet season as compared to the dry season. Laboratory experiments showed that moisture content of mound and soil did not influence the CH_4 and CO_2 emissions rates of *M. nervosus*; instead, it was the seasonal fluctuation in termite population inside the mounds that caused the seasonal variation in mound fluxes from *M.*

nervosus. In sub-tropical and temperate regions, with large seasonal temperature variation, seasonal variation in CH_4 flux can be caused by termite population dynamics as well as temperature. Thus, estimates that do not consider seasonal variation in mound fluxes can have large errors – many published studies have not considered seasonal variation.

The magnitude of diurnal and seasonal variations in mound fluxes in my study highlight that the published global estimates, based on studies that did not account for such variations, will likely have large errors. The magnitude of such errors can be highly variable among different biogeographical regions and needs investigation. My results emphasize that future studies need to consider both diurnal and seasonal variation in methane flux from termites in order to account for the potentially large differences in flux during different times of the day or in different seasons.

Important natural sources of CH_4 in the tropical savannas include fire, swamps, ephemeral wetlands, and termites. Methane uptake (oxidation) by soil methanotrophic bacteria is the only terrestrial sink for atmospheric CH_4. The contribution of termites to the **overall CH_4 balance (Chapter 3)** of Australian tropical savannas is unknown. I quantified the contribution of termites to the CH_4 balance of a savanna woodland at Howard Springs near Darwin by measuring CH_4 fluxes from termite mounds as well as from soil. The contribution of hypogeal-nesting termites, that have their nests underground with no visible mound structure, was derived from soil fluxes and the contribution of wood-nesting or arboreal-nesting termites was estimated from species abundance measures. On an annual basis termites were a CH_4 source of +0.24 kg CH_4-C ha^{-1} y^{-1} and soils were a CH_4 sink of -1.14 kg CH_4-C ha^{-1} y^{-1}. Thus, termites offset 21% of CH_4 consumed by soil resulting in net sink strength of -0.90 kg CH_4-C ha^{-1} y^{-1} for the tropical savanna woodland at Howard Springs. Mound-building termites were estimated to emit +0.13 kg CH_4-C ha^{-1} y^{-1} and hypogeal termites +0.08 kg CH_4-C ha^{-1} y^{-1}. The hypogeal-nesting termites offset 7% of CH_4 oxidized by soil, which was estimated from the proportional area covered by soil chambers that showed CH_4 emissions rather than CH_4 uptake as a result of termite activity. A soil chamber shows CH_4 emissions when

termite activity and associated CH_4 production in the soil under that chamber exceeds the rate of CH_4 uptake by methanotroph bacteria in that same soil volume. When termite activity and CH_4 production under a soil chamber does not equal, or exceed, CH_4 uptake by methanotrophs, it will still offset a fraction of it. Unfortunately, this simple chamber method can not account for the partial offset of soil CH_4 uptake rates by hypogeal termite CH_4 production. Thus, the contribution of hypogeal termites may be greater than reported here, and more advanced methods making use of termicide or differences in ^{13}C-CH_4 isotope signatures are required. Although I included an estimate of the contribution that wood and arboreal nesting termites may make to ecosystem CH_4 emissions, this was not based on direct flux measurements and this represents a future research opportunity.

I investigated the validity of **two indirect methods (Chapter 5)** for predicting fluxes of CH_4 (and CO_2) from termite mounds. The first method involved predicting CH_4 fluxes from termite mounds from 'easier-to-measure' mound CO_2 fluxes. My results indicated that CO_2 fluxes from termite mounds may be used to predict CH_4 fluxes **from mounds of the same species**. However, the relationship between mound CH_4 and CO_2 flux is not consistent across species and using the regression function of one species to predict CH_4 fluxes for the mounds of other species can result in large errors. This is related to intermediate and complementary processes between gas (CH_4 and CO_2) production by termites inside mounds and its release to the atmosphere. These processes include i) CO_2 emissions as a result of microbial respiration from mound wall, ii) CH_4 oxidation by mound material, and iii) gas diffusivity through mound walls which seem to be variable among species. The second indirect method included predicting CH_4 and CO_2 flux from termite mounds based on the relationship between the concentrations of these gases inside a mound with the mound flux of the respected gases. If used within a species, this method can predict CH_4 and CO_2fluxes with relatively smaller errors. Although there was a strong relationship between gas (CH_4 and CO_2) concentration inside a mound and flux of the respective gases from the same mound for each individual species, this relationship was not consistent across species. These differences in the relationship were mainly associated with variable mound structure. For example, thick-walled mounds tended to accumulate gas inside the mound because of greater diffusive barriers resulting in greater

internal gas concentrations but low mound fluxes and vice versa. Thus, in both of the above methods, the relationship between flux and the indirect predicting factor has to be established separately for each species as a generic relationship between flux and the predicting factor resulted in unacceptable errors.

Although termites have been recognised for their CH_4 emissions, my results show that **CO_2 emissions (Chapter 5)** from termite mounds are up to two orders of magnitude greater than CH_4 emissions from the same mounds, when expressed in CO_2-equivalents (CO_2-e). There was large variation in CH_4 and CO_2 fluxes from termite mounds, and from soil, among different sites. The variation in mound fluxes among sites was mainly associated with the differences in mound abundance and species diversity as mound fluxes were highly variable among species. The variations in soil CH_4 flux among sites were mainly derived from the soil hydrological conditions and termite activity within the soil profile. These variations in fluxes among sites suggest caution in scaling up the fluxes from the plot or site scale to a regional or greater scale.

My study filled important knowledge gaps in the ecosystem ecology of termites and their role in the global CH_4 budget. North Australian savannas are now among the few biogeographical regions where the contribution of termites to the CH_4 balance of the ecosystem has been investigated. My study demonstrated that CH_4 flux from termites is significant but variable in time and among species. My case studies showed that CH_4 emissions from termites in Australia are of a similar order of magnitude as compared to termites in Africa or the Americas. The tropical savannas in this study were still a CH_4 sink – the strong continuous uptake of CH_4 by soil outweighed the CH_4 emissions from termites. This highlights that CH_4 flux studies should consider the ecosystem scale and not look at CH_4 flux within individual components, such as termites, in isolation. Similarly, CO_2 fluxes from termite mounds have thus far not been studied intensively even though CO_2 emissions from termite mounds are several orders of magnitude greater than concurrent CH_4 fluxes. The variability in CH_4 flux meant that it was very difficult to identify variables that allow an easier estimation of annual CH_4 flux. Methane flux varied during the day, between seasons and between species. The main drivers for CH_4 flux

variability were i) temperature, ii) changes in termite mound population density and iii) termite mound properties, and these drivers need to be considered when scaling up these fluxes. The large differences in the magnitude of CH_4 fluxes from the six termite species investigated in this study suggests that it will be difficult to accurately estimate the CH_4 budget of biomes, especially if there is high termite species diversity. There are more than 300 termite species in Australia and a representative estimate of the CH_4 flux from these species will be difficult without further studies that investigate the CH_4 flux *in situ* while considering diurnal and seasonal variation. As emissions of CH_4 and CO_2 were a function of termite biomass, all future termite flux studies should also integrate estimates of termite biomass to provide a better process-based understanding. More studies like this are needed from other biogeographical regions of Australia and overseas so as to refine the current estimates of CH_4 emissions from termites and savannas.