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

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Abstract

Termites are an uncertain component in the global source budgets of CH_4 and CO_2. Large seasonal variations in termite mound fluxes have been reported in tropical savannas which should be accounted for when scaling up to annual budgets. The factors driving these seasonal variations in termite mound fluxes are unknown. This paper aims to explain the processes responsible for these seasonal variations in CH_4 and CO_2 fluxes from the mounds of *Microcerotermes nervosus* (Hill) in Australian tropical savannas. Fluxes of CH_4 and CO_2 measured from termite mound sub-samples in the laboratory were a direct function of termite biomass in those mound sub-samples. Termite biomass in mound sub-samples was 10 fold greater in the wet season as compared to the dry season, and was the main factor responsible for the observed seasonal variations in mound fluxes. When expressed per unit termite biomass, termite fluxes were 1.2 (CH_4) and 1.4 (CO_2) fold greater in the wet season compared to the dry season. However, the slightly greater flux emissions per unit termite biomass in the wet season can only explain a small part of the large seasonal variations in mound fluxes. The role of mound diffusivity, measured indirectly, and seasonal variation in CH_4 oxidation by mound material was negligible in driving the seasonal variations in mound CH_4 fluxes. The short term effect of temperature on flux was significant while that of moisture was not. These results emphasize that seasonal termite population dynamics are likely the main driver for the observed seasonal differences in mound fluxes of CH_4 and CO_2. These findings highlight the need to combine future studies of termite fluxes with detailed studies of termite population dynamics.

1. Introduction

Termites are one of the most uncertain components in the global budgets of CH_4 and CO_2 (Bignell et al., 1997; Brümmer et al., 2009; Khalil et al., 1990). This uncertainty is mainly associated with scaling up factors, such as global estimates of termite biomass or 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. An important factor for consideration in the scaling up of fluxes from termites is the large seasonal variations in termite mound fluxes of CH_4 and CO_2. CH_4 fluxes from a mound of *Coptotermes lacteus* 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 as well (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., 2010). In an Australian tropical semi-arid woodland, Holt (1987) reported large seasonal variation in CO_2 fluxes from the mounds of *Amitermes laurensis*. These seasonal variations in mound 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 only a small variation in temperature in the tropical savannas on seasonal scale (Jamali et al., 2010). While the effect of temperature on termite fluxes of CH_4 and CO_2 have been reported (Jamali et al., 2010; Shelton and Appel, 2000; Zimmerman and Greenberg, 1983), the effect of moisture is still unknown. However, to our knowledge no process

based study has been conducted which could confirm the factors causing these seasonal variations in mound fluxes of CH_4 and CO_2 in the tropical savannas.

The observed seasonality in mound fluxes of CH_4 and CO_2 can be caused by a number of different factors, such as emissions per time, termite biomass, termite activity, gas diffusivity of termite mound material and CH_4 uptake by mound material:

First, these seasonal variations in mound fluxes could be caused by a seasonal change in *emissions per unit termite biomass*. Environmental factors, such as temperature, moisture, or food quality can change the rates of metabolism and respiration in termites.

Second, the seasonal dynamics in mound fluxes of CH_4 and CO_2 could also be caused by *changes in the number of termites per mound*. For example, certain termite species can lose up to 50% of their colony biomass as a result of swarming (Wood and Sands, 1978).

Third, seasonal variation in *termite activity* such as foraging outside mounds can result in seasonal variation in mound-based flux measurements. This can have implications on 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 variations in mound fluxes of CO_2 can also be partly because of the effect of moisture and temperature on the respiration of microbial biomass in the mound walls (Holt, 1987).

Fifth, seasonal variation in *mound diffusivity* as a result of changing mound water content can also cause seasonal variation in mound fluxes.

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

2. Methods

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).

2.2 Field-based flux measurements

Five mounds of *Microcerotermes nervosus* (Hill) were repeat-measured for CH_4 and CO_2 fluxes between February and December 2009, at intervals of four to six weeks. Mound

115 selection was not random; rather mounds in locations that permitted easy access for
116 measurements were selected. Fluxes were measured using static manual chambers of
117 volume 0.02 m^3, constructed from polyvinylchloride. A collar was permanently installed
118 around the mounds to a soil depth of 3 cm. A chamber of equal circumference to the
119 collar was carefully placed over the mound and connected to the collar using a ribbon of
120 closed cell foam and several tension spring-clamps. This chamber was then connected on
121 the other end to a Los Gatos Research (LGR^{TM}) Fast Greenhouse Gas Analyzer (FGGA)
122 through a pair of gas tubes and Swagelok^{TM} push-fittings. A LCD screen was attached to
123 the FGGA which displayed the CH_4 and CO_2 concentrations measured at a frequency of
124 1Hz (i.e. one sample per second) for a period of five minutes per chamber. The operation
125 of the FGGA is based on an off-axis integrated cavity output spectroscopy combined with
126 a highly specific narrow band laser for the detection of CH_4 and CO_2 strongly reflective
127 mirrors to obtain a laser path length of 2–20×103 m. Further technical details on FGGA
128 operation can be found in Hendriks et al. (2008). Flux was calculated from the linear
129 change in the concentration of CH_4 and CO_2 in the chamber headspace by multiplying the
130 slope (ppm_v $hour^{-1}$) by the chamber volume (L) and dividing by the mound basal area
131 (m^2). Flux was then corrected for temperature and pressure based on the ideal gas law.
132

133 ### 2.2.1 Auxiliary environmental measurements

134 Mound temperature (T_{mound}) was measured immediately after the mound flux
135 measurement by horizontally inserting a hand held Cole-Palmer® stainless steel
136 temperature probe 6 cm into the mound. Mound water content was not directly measured
137 to avoid destruction of the mounds required for repeat measurement of CH_4 and CO_2 flux
138 across the seasons. 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 was obtained from the Darwin Airport meteorological station of the Bureau of Meteorology, Australia; located less than 2 km from the TERC site.

2.3 Flux and termite biomass measurements in the laboratory

Sub-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, 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. Termite biomass was determined immediately afterwards by breaking down the mound sub-samples and collecting individual termites using forceps. The fresh biomass of 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 sub-samples. The volume of mound sub-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. As hand sorting is a time consuming process, a maximum of only two mound sub-samples were collected and measured each day. This experiment was carried out in both the wet (n = 22) and the dry (n = 22) season and was completed within a two week period for both seasons.

Fluxes were also measured from the mound material that was left over after removing the termites from mound sub-samples. These mound material samples were incubated for 20 minutes using the same set up as described above for incubating mound-samples containing termites but using 1L glass jars. Seasonal difference in fluxes from mound material would explain the role of CH_4 oxidation and microbial respiration in causing seasonal variations in mound fluxes of CH_4 and CO_2. These fluxes from mound material were subtracted from the gross fluxes of mound sub-samples, measured before removing the termites, for calculating the net CH_4 and CO_2 from termites only.

2.4 Gas diffusivity measurements of mounds

Seasonal difference in gas diffusivity of mound wall was measured indirectly by using the ratio of internal mound CH_4 concentration and mound CH_4 flux. In this experiment, 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 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 CH_4 flux to internal mound CH_4 concentration ratio, 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.

2.5 Effect of temperature and moisture on laboratory termite fluxes

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

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

195 ## 2.6 Data analysis and presentation

196 SPSS^{TM} 16.0 was used for the statistical analyses of data. Statistical significance was
197 defined at $p \leq 0.05$, unless otherwise stated. Note that original data was used in all the
198 figures and transformed data was used for statistical tests where necessary as stated.
199 A simple linear regression (n = 30) was used for testing the relationship of mean mound
200 fluxes (CH_4 and CO_2), measured in field, with mean mound temperature and mean soil
201 water content.
202 A simple linear regression (n = 22) was used for testing the relationship between mound
203 CH_4 flux (µg CH_4-C m^{-2} h^{-1}) and internal mound CH_4 concentration (ppm). A paired T-
204 test (n = 11) was used for analyzing the significance of difference in the mound CH_4 flux
205 to mound internal CH_4 concentration (ppm) ratio between the wet and the dry seasons.
206 For the fluxes measured from mound sub-samples in the laboratory, a simple linear
207 regression analysis was used for testing the relationship between termite biomass and flux
208 (CH_4 and CO_2) separately for the wet (n = 22) and the dry (n = 22) seasons. An

209 independent sample T-test (n = 22) was used for analyzing the significance of difference
210 in flux per unit termite biomass between the wet and the dry seasons; data was
211 transformed using ln(flux). An independent sample T-test (n = 22) was also used for
212 testing the significance of difference in termite biomass per unit mound sub-sample mass
213 between the wet and the dry seasons; data was transformed using the $\log_{10}$(termite
214 biomass).
215 For analyzing the effect of temperature on fluxes a Q_{10} temperature coefficient, which is a
216 measure of the rate of change of a biological or chemical system (in this case CH_4 and
217 CO_2 flux) as a consequence of increasing the temperature by 10ºC; was calculated as
218 follows:

219 $$Q_{10} = \left(\frac{F_2}{F_1}\right)^{\frac{10}{(T_2 - T_1)}} \quad (1)$$

220 where $F_{1,2}$ are fluxes at two different temperatures, and T is corresponding room
221 temperature (°C). Q_{10} was calculated for a temperature range of 15 to 25 ºC and 25 to 35
222 ºC.
223 Paired sample T-test was used for analyzing the effect of moisture on CH_4 and CO_2
224 fluxes from the mound sub-samples (n = 5); data was transformed using $\log_{10}$(flux).
225

226 # 3. Results

227 ## 3. 1. Seasonal fluxes measured in field

228 ### CH_4

229 Mound CH_4 fluxes measured in field from *M. nervosus* were 3.5 fold greater in the wet
230 season compared to the dry season. Mean mound CH_4 flux was 1465 ± 293 µg CH_4-C m^{-2}

h^{-1} in the wet season and 417 ± 74 µg CH_4-C m^{-2} h^{-1} in the dry season (Fig. 1). 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 (Table 1).

CO_2

Mean mound CO_2 flux was 601 ± 98 mg CO_2-C m^{-2} h^{-1} in the wet season and 173 ± 34 mg CO_2-C m^{-2} h^{-1} in the dry season, i.e. a 3.5 fold difference (Fig. 1). There was a significant relationship (R^2 = 0.69, $p \leq 0.05$) between soil water content and mound CO_2 flux, but no significant relationship between mound temperature and mound CO_2 flux (Table 1).

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. 2). A paired 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. 2); data was transformed using $\log_{10}$(flux). Mean mound temperature and mean gravimetric soil water content was 32.4 ºC and 5.7% before this rain event and 33.4 ºC and 10.0 % after the rain, respectively (data not shown).

3.1.1 Measurements for mound diffusivity

There was a significant relationship between mound CH_4 flux and internal mound CH_4 concentration (R^2 = 0.85; $p \leq 0.01$) regardless of season (Fig. 3a). The difference in 'mound CH_4 flux to internal mound CH_4 concentration ratio' between the wet and the dry season was not significant (Fig. 3b).

255

256 ## 3.2 Fluxes measured in laboratory from mound sub-samples

257 ### CH_4

258 There was a strong and significant positive linear relationship between termite biomass
259 and CH_4 flux both in the wet ($R^2 = 0.81$, $p \leq 0.001$) and the dry ($R^2 = 0.86$, $p \leq 0.001$)
260 season (Fig. 4a). Mean CH_4 flux was 9.9 ± 0.8 µg CH_4-C g termite^{-1} d^{-1} in the wet season
261 which was significantly greater ($p \leq 0.01$) than the 8.1 ± 0.6 µg CH_4-C g termite^{-1} d^{-1} in
262 the dry season (Table 2). Thus, mean CH_4 flux expressed per unit biomass was 1.2 fold
263 greater in the wet season than the dry season. CH_4 fluxes from the mound material after
264 the termites had been removed were negligible both in the wet and the dry season (data
265 not shown). We did not observe a linear change in CH_4 concentration in jars during
266 incubation of mound material which indicates very low methanotrophic or methanogenic
267 activity in the mound material regardless of season.

268

269 ### CO_2

270 There was a significant positive linear relationship between termite biomass and CO_2 flux
271 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. 4b).
272 Mean CO_2 flux was 3.7 ± 0.8 mg CO_2-C g-termite^{-1} d^{-1} in the wet season which was
273 significantly greater ($p \leq 0.01$) than the 2.7 ± 0.2 mg CO_2-C g-termite^{-1} d^{-1} in the dry
274 season (Table 2), i.e. 1.4 fold greater on a per unit biomass basis. CO_2 fluxes from the
275 mound material (microbial respiration), after removing the termites, were greater in the
276 wet season as compared to the dry season (data not shown).

277

278 ## 3.3 Seasonal variation in termite biomass in mounds

279 As determined from mound sub-samples, there was a significant relationship between
280 termite biomass and mound mass (R^2 = 0.46, $p \leq 0.001$) in the wet season but not
281 significant in the dry season (Fig. 5). Termite biomass was 35.0 ± 3.8 g-termite kg-
282 mound^{-1} in the wet season and 3.6 ± 0.9 g-termite kg-mound^{-1} in the dry season (Table 2).
283 Thus, mean termite biomass in mound sub-samples was 10 fold greater in the wet season
284 as compared to the dry season. In the wet season, smaller mound sub-samples were
285 collected than in the dry season because of the greater termite biomass density and
286 therefore the time required for separation and removal.

287

288 Soldiers comprised only 5 to 6% of the total termite biomass in a mound, with workers
289 and alates comprising the rest (Table 2). The proportional contribution of workers and
290 alates was not determined because of their similar physical appearance. Mean mass of an
291 individual worker was similar in the wet (1.34 ± 0.04 mg) and the dry (1.41 ± 0.07 mg)
292 seasons, as was the mean mass of an individual soldier in the wet (1.87 ± 0.02 mg) and
293 the dry (1.91 ± 0.11 mg) seasons (Table 2). Mean mass of an alate could only be
294 measured in the dry season (2.8 ± 0.05 mg) as winged alates leave the mounds early in
295 the wet season (Table 2). Thus mass per termite was in the order of alate > soldier >
296 worker.

297

298 ## 3.4 Effect of temperature and moisture on flux

299 For CH_4, the Q_{10} was 4.6 between 15 and 25 ºC and 1.2 between 25 and 35 ºC (Fig. 6).
300 For CO_2, the Q_{10} was 5.4 between 15 and 25 ºC and 1.4 between 25 and 35 ºC (Fig. 6a).

The difference in CH_4 and CO_2 fluxes measured before and after adding moisture to the jars was not significant (Fig. 6b), although there was an increase in termite activity and gallery construction.

4. Discussion

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 mainly caused by the seasonal variation in termite biomass in those mounds. We found a 10 fold increase in termite biomass in mound sub-samples in the wet season as compared to the dry season (Table 2). We suggest that this was the main factor causing the seasonal variations in mound fluxes measured in the field which were 3.5 fold in this study (Fig. 1) and 8-9 fold in Jamali et al. (2010), as fluxes of CH_4 and CO_2 from mound sub-samples were a function of termite biomass in those mound sub-samples (Fig. 4).

There are three probable explanations for the observed seasonal dynamics of *M. nervosus* termite biomass in these mounds:

i) Seasonal dynamics in termite mound population as part of the reproductive cycle

The literature on the life cycle of Australian termites is scarce. For *M. nervosus*, like most tropical species of family Termitidae, swarming usually occurs with the onset of rains between October and December (Hill, 1942); during which time winged alates establish new colonies (Nutting, 1969). Termite colony biomass can be reduced by up to 50% as a result of swarming (Wood and Sands, 1978), but this varies for 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).

Mature termite populations peak in the dry season, followed by swarming with the onset of rains (Noirot, 1969). However, this suggested lifecycle pattern does not concur with our observations that the greatest CH_4 and CO_2 fluxes and greatest termite biomass in *M. nervosus* mounds occur in the wet season (Jamali et al., 2010).

ii) Seasonal pattern in termite foraging activity

One possible explanation may be that there are a large number of termites foraging outside the mound in the dry season compared to the wet season, which could explain apparent lower termite numbers in the mound. This is supported by suggestion that termite foraging activity is governed by the energy and protein needs of the colony (Buxton, 1981) which, in tropical areas is greatest in the dry season during nymphal (alate) maturation (Lepage and Darlington, 2000). For the *Macrotermes* species (Bodot, 1967; Lepage, 1982; Wood et al., 1977) and *Trinervitermes geminatus* (Ohiago, 1979) studied in African savannas, the peak of foraging activity always occurred in the dry season. This has been suggested as the main reason for smaller termite population in the dry season in the mounds of *Trinervitermes ebenerianus* in the Nigerian savannas (Sands, 1965). In a humid tropical forest of Cameroon, Dibog et al. (1998) observed greater termite abundance and species richness in the soil (not mounds) in the dry season as compared to the wet season. Furthermore, the abundance and species richness of these termites was significantly and negatively correlated to the amount of rainfall 48 hours preceding termite sampling. In our study, we observed an increase in CH_4 and CO_2 flux from termite mounds following the 'break of rains' after the 2009 dry season (Fig. 3). This response of mound flux to rainfall may be associated with termites being restricted

to the mounds due to the wet conditions outside mounds not being suitable for foraging (Dawes-Gromadzki and Spain, 2003).

iii) Seasonal vertical movement of termites

Another theoretical explanation could be vertical movements of termites within the mound. Water requirements for termites are generally very high as most species are poorly protected against dehydration (Collins, 1969). Termites usually maintain high humidity levels within the mound (Noirot, 1970). However, in the dry season when the desired humidity level cannot be sustained, the upper part of the mound is often left empty (Noirot, 1970). Thus, in the dry season the majority of some termite populations resides in the lower and/or underground sections of the mound, where conditions are more moist and humid (Noirot, 1970; Noirot and Darlington, 2000). If this was the case for *M. nervosus* it could have resulted in the smaller termite biomass observed in our mound sub-samples during the dry season compared to the wet season as we have only sampled the aboveground portion of mounds. However, we know of no evidence that there are significant diffusive barriers within the mound which could affect CH_4 and CO_2 fluxes because of termite presence in a particular section of mound.

Further experimentation involving sampling from both aboveground and belowground mound portions will confirm whether the observed seasonal change in termite population is real or only apparent because of termite activity and termite movement within the mound and to subterranean chambers. The latter case will have significant implications on the estimates based on mound fluxes alone as termites will be emitting CH_4 and CO_2 elsewhere in the ecosystem.

4.2 Seasonality in fluxes per unit termite biomass

372 Seasonal variation in flux per unit termite biomass played only a small role in causing the
373 seasonal variations in mound fluxes of CH_4 and CO_2. The magnitude of the seasonal
374 variation in flux per unit termite biomass was much smaller than the magnitude of
375 observed seasonal variation in mound fluxes measured in the field which was 3.5 fold
376 (CH_4 and CO_2) in this study and 8-9 fold (CH_4 only) in a recent study (Jamali et al.,
377 2010). This seasonal variation in flux per unit termite biomass may be attributed to insect
378 adaptation to xeric conditions, as metabolism and respiration processes can be an
379 important source of water loss (Bartholomew et al., 1985; Edney, 1977; Lighton, 1990).
380

381 ## 4.3 Seasonality in mound diffusivity and fluxes from mound material

382 Mound CH_4 fluxes measured in the field were strongly related to CH_4 concentration
383 inside mounds (Fig. 5a) regardless of season. The difference in the ratio of 'mound CH_4
384 flux to internal mound CH_4 concentration' between wet and dry seasons was not
385 significant (Fig. 5b), thus ruling out the role of changing mound wall diffusivity as a
386 driving mechanism in the seasonal variations of mound fluxes. If at all, mound diffusivity
387 is likely to be reduced in the wet season as a result of surface moisture restricting pore
388 continuity in the outer mound wall and therefore cannot explain the greater mound fluxes
389 in the wet season.
390 CH_4 fluxes from the termite mound material were negligible both in the wet and the dry
391 season. We cannot rule out the possibility of CH_4 oxidation by mound material which can
392 be better quantified using long term incubations and isotopic techniques (Sugimoto et al.,
393 1998). However, the absence of measurable CH_4 oxidation in mound material, also
394 reported elsewhere (Bignell et al., 1997), means that this process is unlikely to cause a
395 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 directly quantify their exact contribution.

4.2 Effect of temperature and moisture on flux

There was a positive correlation between temperature and termite fluxes of CH_4 and CO_2. 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. Hence, temperature would not be a major driver for the observed seasonal changes in CH_4 and CO_2 fluxes.

The short-term effect of moisture on termite fluxes was not significant despite greater termite activity after the addition of a source of moisture (Fig. 6b). This further supports the argument that seasonal variations in mound fluxes are principally driven by seasonal dynamics in termite population rather than the change in flux per unit termite biomass. It also suggests that an immediate response of mound fluxes to rainfall (Fig. 4) is because of termites being restricted to mounds and not because of any effect on their gut biology, metabolism or physiology.

5. Conclusions

Large seasonal variations in mound fluxes of CH_4 and CO_2 are mainly caused by the seasonal dynamics in 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 for mound fluxes of CH_4 and CO_2. Mound diffusivity and CH_4 uptake by methanotrophic bacteria in the mound

material play a negligible role and do not influence seasonal variations in mound fluxes. These results emphasize that termite population dynamics are the main driver for the observed seasonal differences in CH_4 and CO_2 fluxes from termite mounds. Although our results could not confirm which processes determined termite population size inside the mound they highlight the need to integrate future studies of termite fluxes with detailed studies of termite population dynamics.

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Tables

Table 1: Relationship of mound fluxes measured in the field with mound temperature and soil water content as determined by a simple linear relationship

Variable	**CH_4**		**CO_2**	
	R^2	p	R^2	p
Mound temperature	0.07	n.s	0.10	n.s
Soil water content	0.69	≤ 0.05	0.69	≤ 0.05

Table 2: Seasonal dynamics in flux (per unit termite biomass) and termite biomass in mound sub-samples of *M. nervosus* as measured in the laboratory

	Wet season	**Dry season**	**Difference (p)**
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	

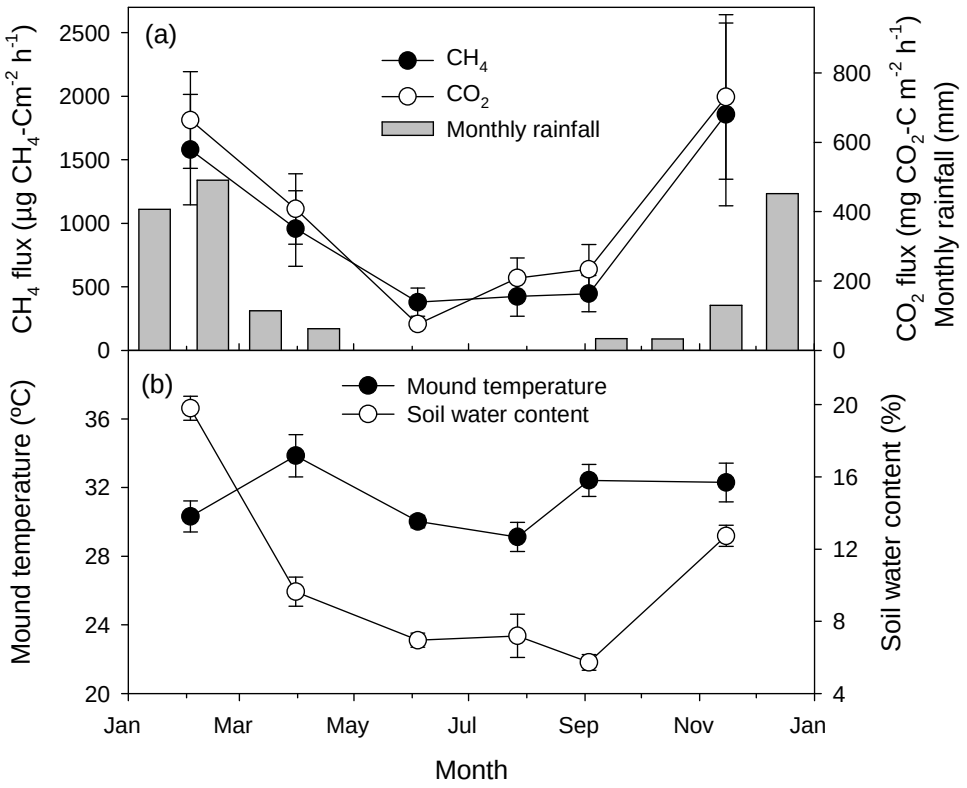
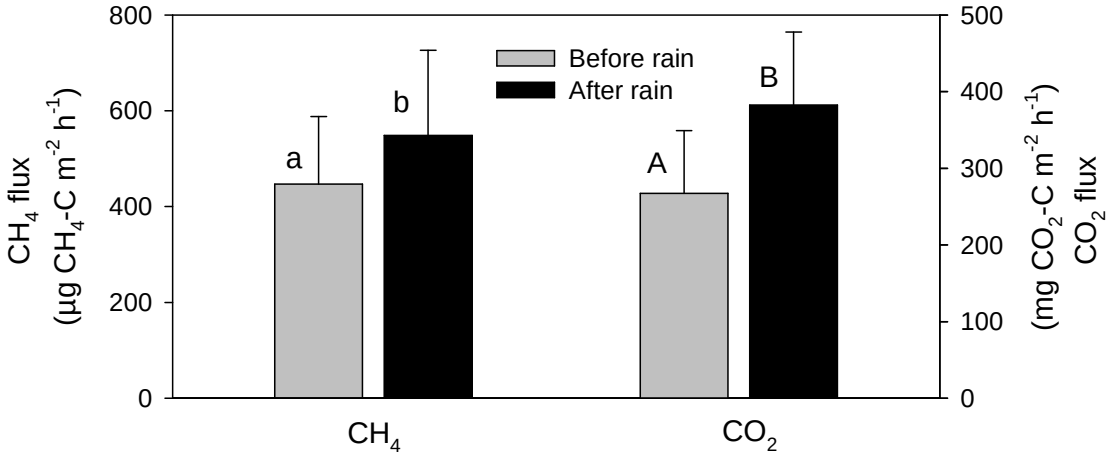
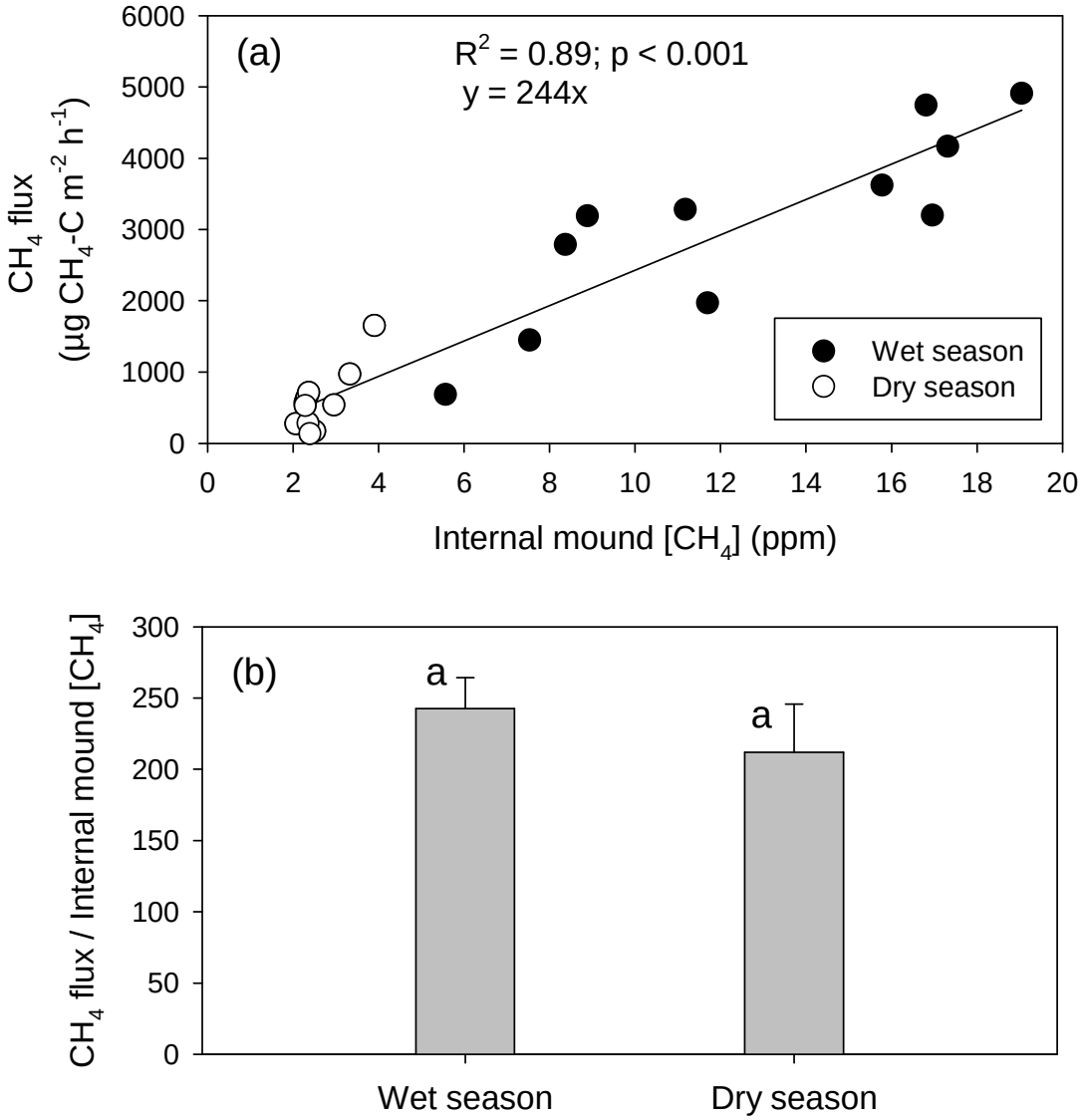


Figure 1: a) Seasonal CH_4 and CO_2 fluxes from five termite mounds of *Microcerotermes nervosus* measured at TERC site and monthly rainfall from the meteorological station at Darwin Airport, and b) seasonal mound temperature and gravimetric soil water content (%)



557

558 Figure 2: Mean mound fluxes of CH_4 and CO_2 (n = 5) measured from mounds of *M.*
559 *nervosus* before and after the 'break of rains' (5.4 mm) in late dry season 2009 measured
560 at TERC; error bars are standard error of the mean; case-wise letters show the
561 significance of difference ($p \leq 0.05$)



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563 Figure 3: (a) Simple linear regression between mound CH_4 flux and internal mound CH_4
564 concentration (ppm); (b) seasonal variation in 'mound CH_4 flux (µg CH_4-C m^{-2} h^{-1}) to
565 internal mound CH_4 concentration (ppm) ratio' repeat-measured in the wet and the dry
566 seasons; letters on top of bars show the significance ($p \leq 0.05$) of difference between the
567 wet and the dry seasons

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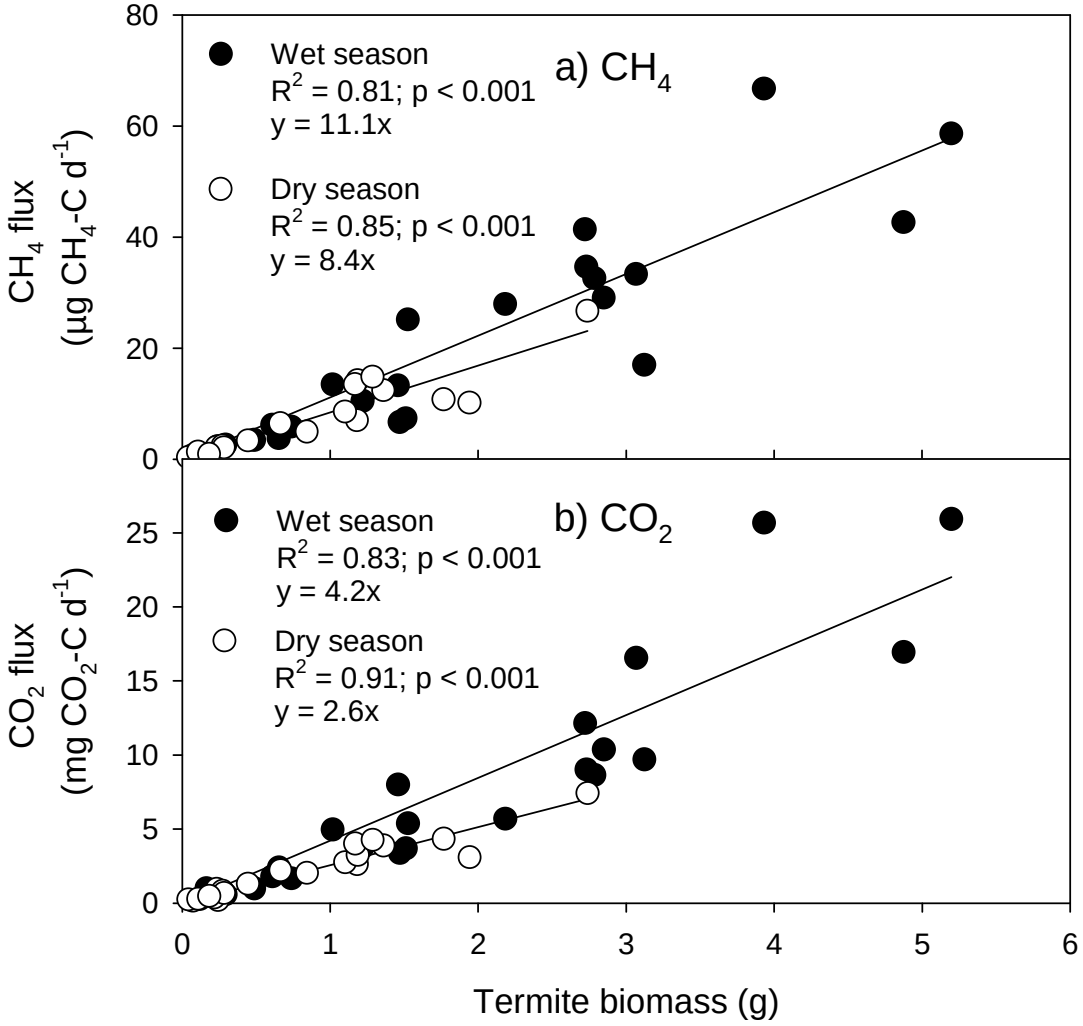
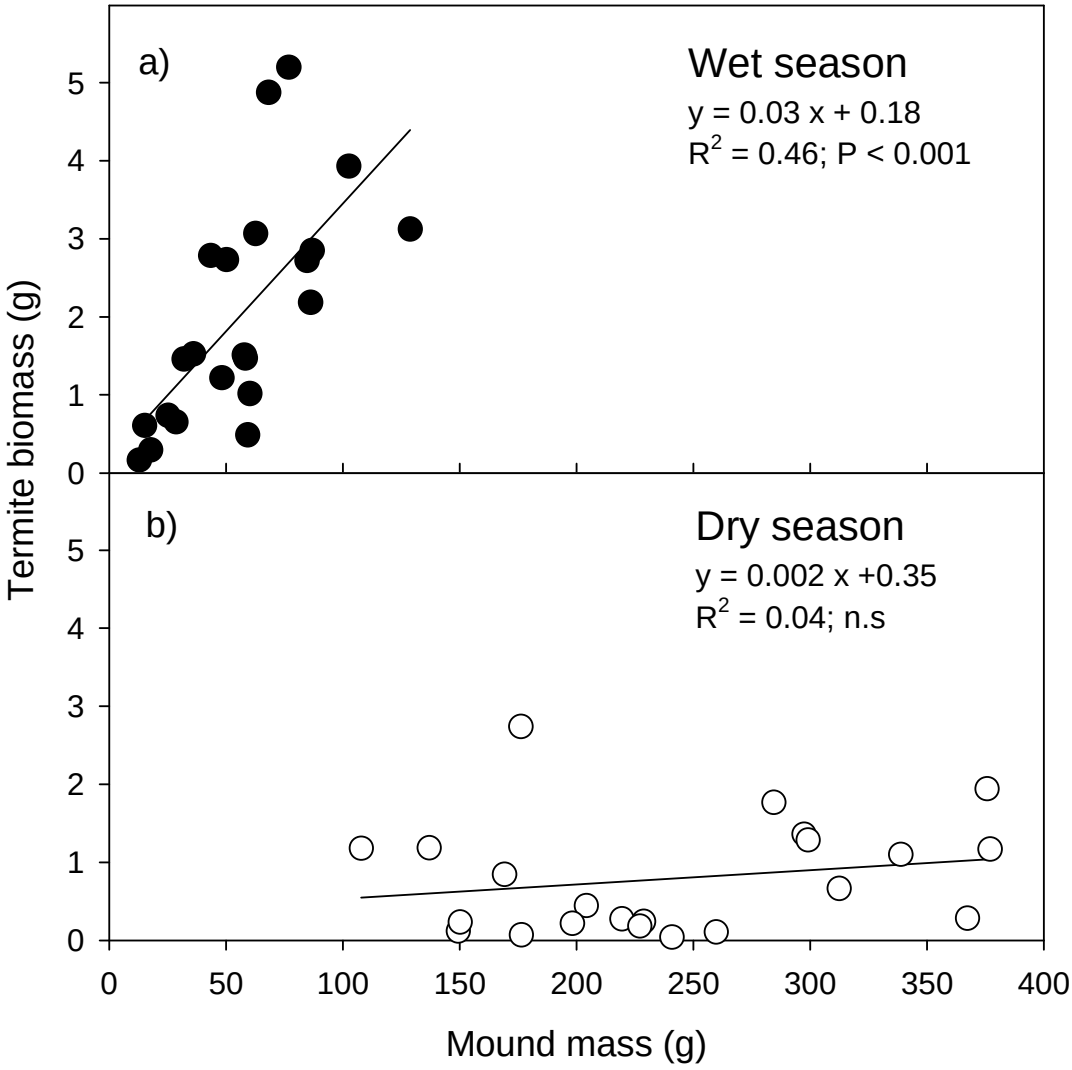


Figure 4: Simple linear regression analyses of: a) fresh termite biomass of *M. nervosus* and CH_4 flux, and b) fresh termite biomass of *M. nervosus* and CO_2 flux in the wet and the dry seasons of 2009 as measured from fresh mound sub-samples in the laboratory.



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582 Figure 5: Simple linear regression analysis of mound mass (sub-samples) and termite

583 biomass in the: a) wet season, and b) dry season of 2009, for *M. nervosus*.

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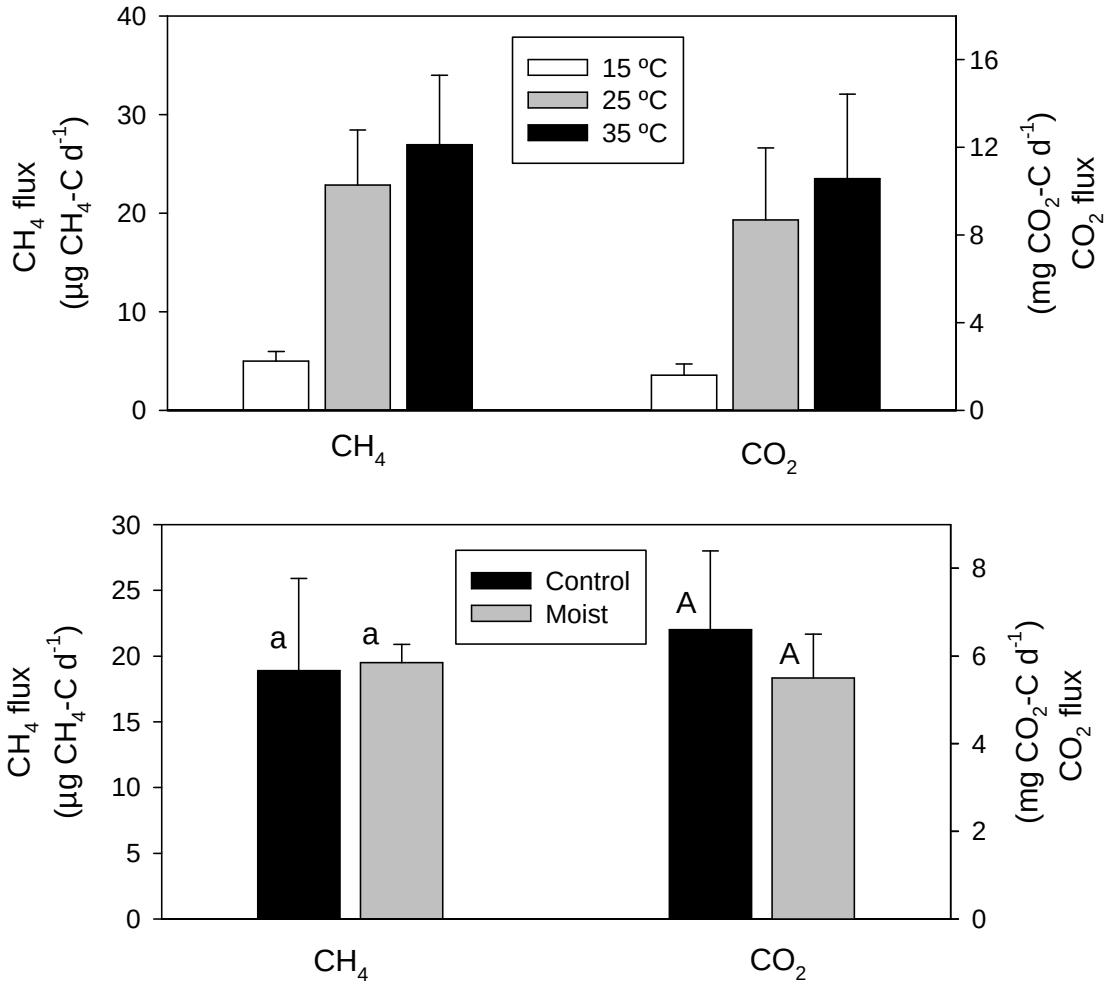

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586 Figure 6: (a) Mean CH_4 and CO_2 fluxes measured from mound sub-samples (n = 5)
587 containing termites incubated at 15 ºC, 25 ºC and 35 ºC; error bars are standard errors of
588 the mean; case-wise letters on top of the bars show the significance of differences in
589 fluxes measured at three different temperatures; for CH_4, Q_{10} values were 4.6 and 1.2
590 between 15 and 25 ºC and between 25 and 35 ºC, respectively; for CO_2, Q_{10} values were
591 5.4 and 1.4 between 15 and 25 ºC and between 25 and 35 ºC, respectively.
592 (b) Mean fluxes of CH_4 and CO_2 measured at 25°C from five mound sub-samples
593 containing termites; before and after adding moist calico material pieces; case-wise letters
594 on top of the bars show the significance of variations.

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