Review of the paper: The relationship between termite mound CH_4/CO_2 emissions and internal concentration ratios are species specific

Reviewer comments in **bold** and response in normal font

Response to the comments of anonymous Referee #2

The paper presents data on annual soil plus termites CO_2 and CH_4 fluxes for 4 savanna areas of Australia and analyses in detail the relationship between internal mound concentration and observed mound flux for both gases, over different termite species.
The paper presents several interesting and useful observations which are useful in terms of interpretation of field data for this quite complicated issue represented by GHG from termites, for which a relatively small number of studies is available. However a revision of the paper is needed. Several paragraphs need to be better explained, clarified or revised. The methodological part also needs a much better description relatively to flux and conc. determination and some considerations on how the proposed measurements done by Los Gatos instrument and dynamic chambers compare with all the other published values made by GC and static chambers. Also the part relative to the uncertainty associated to the observed relationship between variable is missing. This point is quite relevant given that the reported relationships are proposed as alternative methods for CH_4 flux determination and are given the limitation of being specie specific. In both cases it would be important to know which uncertainty we introduce.

We appreciate the detailed comments by the reviewer and have revised the manuscript in the light of specific comments as shown below.

Introduction
Page 17315 lines 25-27. However, the general assumption that CH4 is the largest emitted greenhouse gas from termites may not be realistic. I would change this sentence because: a) to say "general assumption" either refer to something widely accepted or when it is related to science not widely investigated, as in this case, then we need to mention who has made this assumption; b) I don't really think this is the general

knowledge about termite fluxes, the global emission of these gases from Sanderson literature review and extrapolations gives 19.7_1.5 Mt CH_4 yr^{-1} (413.7 Mt COequ) and 3500 _ 700 Mt CO_2 yr^{-1}, exactly the ration in the order of magnitude found by Brummer et al. 2009 you are citing. So even if considered in COeq CH_4 is significantly less than CO_2. And this is a quite established result, given that Sanderson arrives to this total starting from review from all over the world. For this reason I would reconsider the entire paragraph from 17315 line 25 to 17316 line 8.

As suggested, the sentence at Page 17315 lines 25-27 (However, the general assumption that…) has been deleted and the remaining paragraph revised which now reads as:

"For example, in an African savanna, mound CH_4 emissions measured from one termite species contributed 8.8% to the total (soil + mounds) CH_4 emissions of that landscape, whereas termite CO_2 emissions contributed 0.4% to the total (soil + mounds) CO_2 emissions (Brümmer et al., 2009). In this study, termite mound emissions of CH_4 were an order of magnitude smaller than termite mound emissions of CO_2 (Brümmer et al., 2009). As such, it is important to investigate whether the relative contribution of CH_4 and CO_2 emissions is consistent among termite species across savanna landscape."

Page 17316 lines 22-26: It is not clear if in this study you measured fluxes from termites directly or termites mounds and related it to termite biomass, please specify. All the paragraph is not clear for a reader who did not read your article, there are several assumptions which are not obvious. Please rephrase.

As suggested, the mentioned paragraph has been rephrased and reads as below:

"In a laboratory experiment, Jamali et al. (2011b) demonstrated that CH_4 and CO_2 emissions from termites (not mounds) of *M. nervosus* species were a strong function of termite biomass which also suggests a correlation between CH_4 and CO_2 emissions from termites and termite mounds. If true, such a relationship will make it possible to use 'easier-to-measure' CO_2 fluxes for predicting mound CH_4 fluxes."

"Another indirect method for estimating mound CH_4 flux could be based on the relationship between mound CH_4 flux and CH_4 concentration inside that mound (Khalil

et al., 1990). If valid, the advantage of this method is that it takes into account the proportion of CH_4 produced inside a mound by termites that is not emitted to the atmosphere due to both the gas diffusion barrier imposed by mound wall and CH_4 oxidation by methanotrophs in mound wall material (Sugimoto et al., 1998)."

Again this paragraph is not clear to me. You say that you found a correlation between internal CH_4 conc and mound flux. This would mean that knowing the internal CH_4 conc you can predict the methane flux outside the mound. Then you add that the advantage of the method is that it takes into account CH_4 oxidation and diffusion limits to internal CH_4. Here again we miss some piece of information because the reader does not know if this relationship is valid for the same species always, or depends on other factors (mound size, mound age, mound primary mineral material, mound level of wetness, etc). In the latter case we could agree, in the second no. So you need to define all of this better if you want to mention it.

This paragraph has been revised in the light of above comments from reviewer 2. Regarding validity of this relationship across species, this is one of the objectives (4) of this study and is discussed in detail in results and discussion. After the revision, the paragraph at 17316 line 26 to 17317 line 9 reads as:

"Another indirect method for estimating mound CH_4 flux could be based on the relationship between mound CH_4 flux and CH_4 concentration inside that mound, first used by Khalil et al. (1990), as below:

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

Where F is mound CH_4 flux, Cm and C_0 are CH_4 concentrations inside mound and in ambient air outside the mound, respectively, and λ is a constant derived from this equation. The constant λ calculated using equation 1 is then used to estimate mound CH_4 flux from termite mounds for which only Cm and C_0 are measured in field. Khalil et al. (1990) calculated a λ value using field measurements of one termite species and used it to estimate mound CH_4 fluxes from different termite species, thus assuming that the relationship between mound CH_4 flux and CH_4 concentration inside a mound is consistent among different species. This assumption may not be true as the mound structure can be variable for different termite species. The same approach (equation 1) may also be used to predict mound CO_2 fluxes but with same uncertainty for the mounds of different termite species. Additionally, given the possible correlation between mound CH_4 flux and mound CO_2 flux, we also hypothesize a correlation between mound CH_4 flux and CO_2 concentration inside mounds which should enable the prediction of mound CH_4 flux by only measuring CO_2 concentration inside a mound. It is important to investigate the relationships of gas concentration and mound flux, and the validity of these relationships across the mounds of different termite species."

Page 17317 lines 5-9: Taking into consideration what just said above, your assumptions which introduce the objectives of the work need to be better defined in terms of what is certain and what is not. We just said that it is not clear if your CH_4 flux vs. CH_4 conc is always true, the same could apply to CO_2 flux vs. CO_2 conc, so that the extrapolation of CH_4 fluxes from CO_2/CH_4 ratio would become highly uncertain. Please in the premise constraint the uncertainty.

This issue was addressed by the re-wording of the paragraph 17316 line 26 to 17317 line 9 as outlined above.

Objectives:

Your 5 objectives are fine if constraints to the uncertainty in the extrapolation have been already dealt with in some your previous work, otherwise a 6th objective should be to see if changing conditions can change the relationship between internal conc and external fluxes and CO_2/CH_4 ratio.

We believe the objectives cover the scope of this study and therefore may not need revision.

Methods

Paragraph 2.2 Please give details on your chambers, shape, material, size, eventual equilibrators of pressure, connection to gas analyser, etc. Given that the experimental set up is relevant it would be good that the experimental set up with los gatos analyzer is described with some details here rather than refer to a published paper. Please specify the amount of gas volume that los gatos apparatus need to flash the cell and the system and its flux rate (l/min) so that the reader has an idea of the dimension of air circulation inside the chamber per unit of time.

As suggested, more details have been added to the paragraph which now reads as:

"Methane and CO_2 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. Fluxes from termite mounds were always measured between 10:00 and 12:00 hours as this time best represents the daily average flux (Jamali et al. 2011a). Chamber bases were permanently fixed around selected mounds throughout the measurement campaign and were connected to chamber tops of the same circumference. Chambers were constructed from polyvinylchloride with their sizes ranging from 0.02 and 0.20 m^3 for termite mounds and 0.009 m^3 for soil. Flux of CH_4 and CO_2 was measured in a closed dynamic set up (non-steady state and non-vented) by connecting each chamber in turn to the Fast Greenhouse Gas Analyser (Los Gatos Research, Mountain View, CA, USA) - hereon referred to as FGGA - using an inlet and outlet gas line with Swagelok™ push-fittings (Jamali et al. 2011b). Chamber closure time was five minutes for measuring mound fluxes and ten minutes for soil fluxes, during which the FGGA collected and analysed gas samples at 1Hz frequency. The measuring cell of the FGGA is 0.0004 m^3 and the internal pump rate is 0.0033 m^3 min^{-1} – this translates into a complete flush of the measuring cell every 7.4 seconds."

Paragraph 2.3 Again here define what is an FFGA writing it's extended name, and define the necessary infos to understand the experimental procedure, direct injection? Which volume? Which conditions… etc. in the field, in the lab, calibration…etc. etc. Another important point could be to know if a GC measurement of CO2 and CH4 is equal to the one by FFGA. The reason I say this is because the most common approach to measure CH4 is GC and few groups use Los Gatos for this kind of experiments. Very frequently measurements by static chambers-GC tend to underestimate fluxes measured by IR or different kinds of lasers. Also for some species like CH4 many laser setups tend to have problems in resolving the bands for CH_4 and water vapour. So the question here is if the two techniques are comparable and you have scientific evidence of this or instead we could surely apply your results to determinations by FFGA but extrapolate to GC with a certain caution or a defined uncertainty.

We acknowledge the fact that there have been reports of disagreement between the results of different instruments (e.g. GC vs. Los Gatos FGGA) and different techniques (close chamber vs. micrometeorological methods), and therefore our study should not be treated as an exception. Our tests showed a strong correlation of $R^2 > 0.8$ between the fluxes measured by Los Gatos FGGA and GC and we did not decte any bias. However, given the main objectives of this study were to investigate relative contribution of CH_4 and CO_2 fluxes, and the relationships between internal gas concentrations and fluxes, rather than a comparison with other studies, we think this agreement between GC and Los Gatos FGGA results was sufficient to not influence the findings of this study.

As suggested, a brief experimental procedure of sample injection and extended name of FGGA has now been added to the mentioned paragraph which now reads as:

"The internal mound CH_4 and CO_2 concentrations were measured once each 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. Nylon tubes were permanently installed 5 cm into the mound wall at a mid-level height of the mound with the outer end of the tube 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, and injected into the Fast Greenhouse Gas Analyser (hereon referred to as FGGA) in field. The concentrations of CH_4 and CO_2 in the gas samples were determined by using a calibration equation developed in the laboratory by injecting 20ml of known concentrations of CH_4 and CO_2 in the same way as in the field."

Results

Page 17322 line 7 – Looking at Fig. 1 A. meridionalis seems equally not well defined as *T. hastilis* although the lack of response to rain occurs in different periods for the two sampled species. Isn't it?

The reviewer has correctly pointed out that the seasonality of fluxes from *A. meridionalis* is not as clearly defined as *M. nervosus* and *T. pastinator* which is further obscured by the scale of Fig. 1. However, the seasonal pattern of fluxes from *A. meridionalis* were more clearly defined than *T. hastilis* as confirmed by the statistically significant relationship between flux and water content for *A. meridionalis*, which was not significant for *T. hastilis* (Table 3).

Page 17323 line 10 No distinct seasonal patterns were observed in soil CH_4 flux at TERC, CDNP and HS savanna sites This is quite an unexpected result. Soils from seasonally dry ecosystems usually are characterized by a defined distinction in dry and

wet CH_4 soil fluxes (see review by Castaldi et al. 2006). In these soils CH_4 oxidation is surely an important contribution to the net CH_4 exchange, and during the wet season reduced gas diffusion should decrease the sink and increase occurrence of CH_4 production hotspots, plus probably also stimulate termites activity. In any case I would expect, as found often in literature to see a good sink during the dry season and a small sink or little source in the wet.

The reviewer has correctly pointed out that usually it is expected to observe a seasonal pattern in soil CH_4 fluxes in seasonally dry ecosystems as wet conditions theoretically result in reduced methanotrophic activity and increased methanogenic activity. However, we did not observe such a seasonal pattern in fluxes on three of our sites which also concur with the findings of Livesley et al. (2011) who studied soil CH_4 fluxes in these savannas in more detail. The soils in the study region are very sandy and are not prone to water logging. In effect, rainfall drains very quickly in these soils and consequently soil based methanogenic activity was negligent in the wet season. What is more surprising is the large CH_4 uptake capacity on the dry season in soils that is very dry. However, recent (and yet unpublished) results from Benedikt Fest indicate that methanotrophs in some Australian ecosystems seem to have a greater tolerance to dry soil conditions and show activity even in very dry conditions. Another explanation is the presence of subterranean termite activity as we did not separate the soil chambers with termite presence from the ones without termites which would have required digging soil under chambers. Hence, yes, our results do not conform to general trends observed elsewhere, but there are explanations for this observation.

Regarding seasonal termite activity, it is not clear if wet conditions would stimulate or hamper termite activity in soil as we had observed increase termite presence in mounds in the wet season as compared to the dry season in a previous study (Jamali et al. 2011b) which suggest reduced termite activity outside mound or in soil in the wet season.

Page 17324 line 18. It is true that the 4 regressions are all significant, however, the first two show a clear distribution of point along the regression slope, whereas the 3rd and 4th appear like a quite scattered group of points driven by fewer points. I expect that the error associated to the 4 regression should be very different, small in the former cases and much bigger in the latter, thus making the relationship robust only for the first 2 cases. This should be mentioned and described in the results.

It is correct this correlation is stronger for first two species as compared to the last two, which has been described at 17324 lines 19-22 as below:

"The correlation between fluxes of CH_4 and CO_2 from termite mounds was 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* ($R^2 = 0.24$; $p \leq 0.001$) (Fig. 3)."

This is now further discussed in section 4.3 through additional sentences which read as:

"The linear regression analysis (Fig. 3) suggests that mound CO_2 fluxes can be used to predict mound CH_4 fluxes but with variable accuracy across species. For example, this relationship was stronger ($R^2 > 0.8$) for *M. nervosus* and *T. pastinator* but weaker ($R^2 < 0.3$) for *T. hastilis* and *A. meridionalis* which suggests this method may not be used for the latter two species. An important question here is: can we use the regression equation of one species to predict fluxes from other species? Answer is 'No'. This is because regression slopes are highly variable for different species (Fig. 3). For example, slope of *M. nervosus* is approximately three (3) times smaller than other three species, and therefore will result in a 3-fold error if the regression equation of *M. nervosus* is used to predict fluxes of other three species and vice versa."

Discussion

Page 17326 lines 17-20. There are two mistakes in this sentence: first the total COeq flux in HS wetland is higher than in HS savanna, second in Table 1 we cannot find mound basal areas reported.

The reviewer has correctly pointed out the errors in the sentence which arose mainly because of the reference to incorrect table. Please note Table 5 shows annual emissions from the mounds of each termite species while Table 6 shows the annual mound emissions per site after accounting for mound basal area covered by different mounds at that site. The scaling up method has been explained in Section 2.6. As such, Table 6 shows that HS savanna site (157 kg CO_2-e ha^{-1} y^{-1}) has higher emissions than HS-wetland (51 kg CO_2-e ha^{-1} y^{-1}). The revised sentence now reads as:

“The annual termite mound emissions (CH_4 + CO_2) in CO_2-e, after accounting for mound basal area on each site, were greater at TERC, CDNP and HS-savanna sites than HS-wetland site which corresponds to the mound basal area (Table 6) and thus termite biomass at these sites.”

Page 17326 lines 24-26. Again it is mentioned that HS wetland CO2eq fluxes are the lowest. However looking at table 5 I calculate the sum of CH_4 plus CO_2 equal to 6.5 and 8.3 for HS savanna and HS wetland, respectively. If you make wrong calculations also the discussion should be revised where you try to explain the observed differences. However, I think that error should be reported together with annual estimates in Table 5 so to have clearly the evidence that maybe 6.5 and 8.3 are not so different to justify too many speculations.

Again, this ambiguity has arisen because of not clearly referring to the correct table which is Table 6 and not Table 5. However, in the light of comments by reviewer 1 this sentence has been deleted.

Page 17327 lines 9-11: are you sure that on the base of the few measurements done, we can drive such conclusion? What about *A. meridionalis*

We agree that there was not enough evidence to make this conclusion. Therefore, this sentence has been deleted and replaced by the sentence below:

“It is not clear why the seasonal pattern of CH_4 fluxes from the mounds *T. hastilis* was different from the mounds of other species (Fig. 1).”

Page 17327 lines 15 net “annual uptake”: add “annual

This has been done, as shown below:

“Soil CH_4 fluxes at TERC and HS-savanna resulted in a net annual CH_4 uptake, while soil fluxes at CDNP and HS-wetland sites produced net annual CH_4 emissions.”

Page 17327 lines 21 the shift refers to a passage from something to something else, where is the shift here? Do you want to say that on annual base the site is a net source differently from the other sites?

This sentence has been revised and reads as:

"At CDNP, annual soil CH_4 flux was net emission of +2.9 kg CO_2-e ha^{-1} y^{-1} mainly because CH_4 emissions from a small number of soil chambers, resulting from subterranean termite activity, offset the CH_4 uptake by rest of the chambers, as shown elsewhere (MacDonald et al., 1999)."

Page 17327 lines 21: do you expect this only to be a particular feature of Northern Australia? I don't think so.

We agree that patchy termite distribution can also be experienced in other areas. Therefore, this sentence has been revised and reads as:

"Such high spatial variability in soil CH_4 flux among sites suggests that scaling up to regional level will be problematic in tropical savanna landscapes of northern Australia and other ecosystems with patchy distribution of subterranean termite activity."

Page 17328 lines 1-2 this accuracy or uncertainty should be mentioned and discussed.

As suggested, the said paragraph has been extended to include more details and reads as:

"The linear regression analysis (Fig. 3) suggests that mound CO_2 fluxes can be used to predict mound CH_4 fluxes but with variable accuracy across species. For example, this relationship was stronger ($R^2 > 0.8$) for *M. nervosus* and *T. pastinator* but weaker ($R^2 < 0.3$) for *T. hastilis* and *A. meridionalis* which suggests this method may not be used for the latter two species. An important question here is: can we use the regression equation of one species to predict fluxes from other species? Answer is 'No'. This is because regression slopes are highly variable for different species (Fig. 3). For example, slope of *M. nervosus* is approximately three times smaller than other three species, and therefore will result in a 3-fold error if the regression equation of *M. nervosus* is used to predict fluxes of other three species and vice versa."

Page 17328 lines 3-4. I don't understand how this sentence justify the previous. Why different accuracy should be related to different CO_2/CH_4 ratios for a given species? If you don't clarify this the logical passage for the next long paragraph is missing.

This sentence has been revised and reads as:

"The slopes of mound CH_4 flux vs. mound CO_2 flux vary among termite species because of the variable CH_4 per unit CO_2 production rates for different species. For example, mounds of

M. nervosus had the smallest CH_4 fluxes but greatest CO_2 fluxes compared to other species (Table 5)."

Page 17329 lines 20-21: here again could be interesting to compare the error associated to the extrapolation of CH_4 fluxes starting from CH_4 concentrations, for one species, with the error associated to the extrapolation from one specie to another. I think that the key point of the paper is to propose a method to improve uncertainty in extrapolations using other approaches, so uncertainty must be discussed.

We agree with the reviewer that it is important to highlight the error associated with the extrapolation using the equation developed for one species and applied on other species. However, we believe the message intended here is the conclusion that error (5-fold for CH_4 and 3-fold for CO_2 and so on) associated with such extrapolation is too big to ignore. This error might be small for some species as compared to others but that will remain uncertain as it will not be practical to develop such a relationship for each species given there are more than 150 termite species in the study area. The explanation below should be sufficient to discuss this aspect.

"The linear regression analysis between mound flux and internal mound gas concentration (CH_4 and CO_2) suggests that this method may be used to predict mound fluxes for a given species. However, using the equation developed for one species to predict mound fluxes from another termite species, as suggested by Khalil et al. (1990), could have resulted in errors of more than 5-fold for CH_4 and 3-fold for CO_2 in our study. Similarly, CO_2 concentration inside mound may be used to predict mound CH_4 flux from the same mound using our regression models. However again, using a generic relationship of CO_2 concentration inside a mound to predict mound CH_4 flux may result in 13-fold errors in predicted fluxes."

Page 17330 lines 5-9: It doesn't seem from Sanderson 1997 estimates of termite biomass density that semiarid savannas present overall higher density of termite biomass than humid areas, it would be worth to check your statement.

It is correct this sentence does not refer to Sanderson 1996 rather a personal communication by a senior ecologist. This reference has been added accordingly as below:

"Lower rainfall savanna areas can be populated by far higher mound densities than observed in this study (Russel-Smith pers. comm.)."

Conclusions

Conclusions need to be revised. They should just summarize the main findings you have and you can be sure about and we can mention in future works or use as starting points for future investigation

Conclusions have been revised in the light of comments from the reviewer and read as:

"This study confirmed that termite mounds are a greater source of CO_2 as compared to CH_4 on an annual CO_2-e basis. However, CH_4 and CO_2 emissions from termite mounds contributed less than 1% to the total CH_4 and CO_2 emissions from mounds and soil combined in CO_2-e. Our results indicate that there is no easy way to measure, or indirectly determine, the CH_4 flux for a variety of termite species. There were significant relationships between CH_4 concentration and CH_4 flux and also significant relationships between mound CH_4 and CO_2 flux. However, all these relationships had different slopes for different species and were therefore species specific. Using the regression function of one species to predict CH_4 fluxes for the mounds of other species would result in large errors. These species-specific relationships may be linked to the different processes that determine mound CO_2 or CH_4 concentration and mound CO_2 and CH_4 flux, and need further investigation. Our results clearly indicate that the large variability among different termite species results in different relationships between internal mound concentration and fluxes, and that generic equations cannot and should not be applied, as they would result in large errors."

Page 17330 lines 20-21. I think this is an already known concept. Maybe you can say that this study confirms or supports previous observations that…

This sentence has been revised and reads as:

"This study confirmed that termite mounds are a greater source of CO_2 as compared to CH_4 on an annual CO_2-e basis."

Page 17330 lines 24-25: This can be more a suggestion in the discussion than a conclusion because you really did not test this in a focused experiment, for example using the same relationship to predict measurement for the same specie taken in the same site the next year or in a different sites.

As suggested by the reviewer, this sentence has been revised and reads as:

“There were significant relationships between CH_4 concentration and CH_4 flux and also significant relationships between mound CH_4 and CO_2 flux.”

Page 17331 lines 3-6: again this is speculation and not a conclusion as you only know wall thickness among the mentioned variables.

We agree with reviewer’s comment. Lines 3-6 have been deleted while lines 1-3 have been revised and read as:
“These species-specific relationships may be linked to the different factors and processes that determine mound CO_2 or CH_4 concentration and mound CO_2 and CH_4 flux (such as mound wall thickness), and need further investigation.”

Table 4 – correct sOIL CH4 flux
This has been done.