Anonymous Referee #2

Comments on the article '**Assessing CO2 and CH4 fluxes from mounds of African fungus-growing termites'** from Matti Räsänen and co-authors

The article is well written, and contains interesting field data about a topic which is still relatively unknown, and of which relatively little data exists. The amount of mounds on which this story is based is small, but this is understandable considering the field conditions, and the difficulty to find suitable mounds. Also, the experiment is well designed in the sense that many environmental variables were measured. It is nice how some entomology studies are used to interpret the data.

There are a few general comments which should be addressed by the authors, followed by many detailed suggestions.

General comments

Dependency between species, nest type and location

The authors explain the difference between open and closed mounds in the beginning, and introduce the 2 termite species. They briefly indicate that 1 species makes closed mounds, and the other species open mounds. So, these variables (open/closed mound and species 1/species 2) are not independent. Nevertheless, this is not superclear through the paper. Looking at Table 1, termite species and mound type are listed as independent variables (separate columns), but they are not independent. Additionally to this, the authors try to compare the 2 different species in 2 different environments. But, looking at Table 1, the M. michaelseni only appears once in the bushland (out of 6 mounds), and the M. subhyalinus appears only 2 in the grassland (out of 6 mounds). While it is likely out of practical considerations that the authors decide to not look for equal sampling at each side, this should be discussed better.

So, I would advise the authors to better discuss and evaluate the fact that mound type and species are not independent and to better discuss whether observed differences between grassland and bushland are a consequence of the environment, or the fact that they were basically measuring different termite species (with different nest properties).

We thank the referee for all the helpful comments and suggestions.

In Table 1, we have merged the termite species and mound type columns to better indicate that these are not independent variables. We have added discussion point regarding the species, nest type and location:

"Our sampling design, with one species dominating the bushland and the other the grassland site, does not allow us to compare the two species reliably but their more or less similar gas fluxes (Figures 4 and 6) suggest that the two species (or mound types) do not have major differences in their CO_2 or CH_4 emissions. This results was expected as the two termite

species are closely related (Bagine et al., 1994; Vesala et al., 2017), have very similar population sizes and compositions (Darlington, 1984), and also share their dietary niches (Vesala et al., 2022b). The highest fluxes of both gases were registered from closed mounds of M. michaelseni but this may reflect the fact that the M. michaelseni mounds measured here most likely housed larger termite populations with larger fungus gardens than the studied M. subhyalinus mounds."

Discussion of diurnal variation

The part about the diurnal variation is interesting, but it is difficult to judge how valid/representative the measurements are. Please add on how many days the measurements were made, and how representative 4 or 5 time points are to determine a daily variation. Also, the authors mention that the largest CO_2 flux coincides with the highest wind speed. But, to what extent can wind speed be a factor of influence on the CO_2 flux if the mound is sheltered from wind speed during the measurement? Can the authors discuss this?

We have now specified the number of nighttime measurements in the method section. The S1 mound was measured on 15 November 2016 and S2 on 7 November 2016 from evening until next day morning. During each measurement the mounds were sampled by three repeated measurements from which the standard error was calculated and presented in the Fig. 5.

The diurnal sampling time points were focused on the afternoon to morning period when there is expected transition in the convective flow of the mound and because there is a lack of measurements from these periods. The sampling was less dense during the daytime when it is likely that there is a steady increase up to early afternoon based on earlier studies. A previous study measured during 6 time points, and there is no indication of fast temporal variation in the mound flux (Jamali et al., 2011). In addition, our diurnal flux measurements trend is in agreement with the high-frequency internal CO2 concentration measurements of *M. michaelseni* mounds (Ocko et al., 2017).

We observe that the wind speed maximum coincides with the largest diurnal flux value. Despite of that, the wind speed is not expected to drive the mound fluxes which originate from the deep mound structures (termite nest and fungus gardens) not affected by the ambient conditions. Wind can, however, disrupt the flux measurements, especially in case of larger mounds that do not fit completely into the chamber. Figure S2 shows how the concentration measurement looks like when the mound is not fully covered by the chamber and wind affects the measurements.

Discussion of differences between seasons

The differences between dry and wet season are interesting, but please discuss it further. For example, on page 17, line 15, the decrease in CH_4/CO_2 ratio is mentioned, seen from dry to wet season, and possibly linked to the activity of methanotrophic bacteria. So, the authors mention that during the wet season, mounds have higher water content, so these bacteria

become more active. But, you also mention (page 19, line 5) that the mounds represent a stable humidity and temperature. Is there any data or literature which actually shows that the mound humidity changes with season? Or is this 'just' an hypothesis to fit your observation? Please elaborate.

Although air humidity within the nest cavities remains constantly at a high, almost saturated, level (Luscher 1961; Agarwal 1980), the key variable for methanotrophic bacteria, inhabiting soil structures within and around the mounds, is the soil moisture which does vary between dry and wet seasons – also in termite mounds where moisture of soil wall structures seems to be largely controlled by precipitation (Jamali et al., 2011; Chen et al. 2019). We have now added the daily mean soil moisture measurements registered from the weather station to the figure 3a that shows that surface soil moisture was clearly higher during the second than during the first campaign. The first measurements were done after long dry period and the precipitation between the campaigns was 153 mm. Although soil moisture was not measured in the studied mounds, we assume that the water content of mound walls correlate with the soil moisture values.

Agarwal, V. B.: Temperature and relative humidity inside the mound of Odontotermes obesus (Rambur) (Isoptera: Termitidae). Proc. Indian Acad. Sci. (Anim. Sci.) 89, 91–99, https://doi.org/10.1007/BF03179148, 1980.

Chen, C., Wu, J., Zhu, X., Jiang, X., Liu, W., Zeng, H., and Meng, F.-R.: Hydrological characteristics and functions of termite mounds in areas with clear dry and rainy seasons, Agriculture, Ecosystems & Environment, 277, 25–35, https://doi.org/10.1016/j.agee.2019.03.001, 2019.

Lüscher, M.: Air-Conditioned Termite Nests, Sci. Am., 205(1), 138–145, doi:10.1038/scientificamerican0761-138, 1961.

Also, it is observed by the authors that the soil CH_4 flux is positive in the dry season, and uptake is seen in the wet season ('The dry season mean soil CH4 flux was positive at the grassland and bushland site, whereas the mean wet season flux was nearly zero with most fluxes being negative (Fig. 7, S5 and S6')).

This is unexpected, since usually higher soil moisture (wet season) leads to CH_4 emission, while uptake occurs when the soil is drier. Maybe I have overlooked it, but did the authors measure soil moisture during both season, and how was it indeed drier/wetter in the wet/dry season? The graphs in the suppl material, are they for wet or dry season? There the authors seem to find a weak relation between soil moisture and CH_4 flux. So, why do the authors observe more soil CH_4 flux in the dry season? Discuss this.

The main aim of the soil CH4 flux measurements was to measure the possible influence of the termite mound to the soil flux around the mound. The soil CH4 measurements were made at 2m, 4m and 6m distances from the mound. The soil fluxes at 2m distance from the mound are higher compared to the further distances. The soil CH4 flux values at further distances are in similar magnitude to previous measurements that were made in this area far from termite mounds (Wachiye et al., 2020). This means that a small proportion of the gases leave the nest from via the adjacent soil seen here at the 2m distance.

Our wet season soil CH4 fluxes around the mounds were lower than dry season fluxes. A previous study found the soil CH4 flux far from termite mounds to be near zero or negative throughout the year in this area (Wachiye et al., 2020), and thus we suspect that termites are the main source of the positive CH4 fluxes measured here. Changes in the abundance of termites in foraging networks surrounding the mounds could potentially vary seasonally. Especially during rains the foraging activity of *M. michaelseni* is low (Lepage, 1981), which could temporarily decrease the CH4 fluxes in surrounding soils. However, this should concurrently increase CH4 fluxes of the mounds, where the foragers need to move to. As also the mound CH4 fluxes were systematically lower during rainy season than during dry season, we interpret the observed seasonal differences in CH4 fluxes to be caused mainly by changes in soil methanotrophic activity. As already discussed above, we believe that increase in soil water content enhances microbial activity, including methane oxidation, compared to low moisture conditions in the end of the long dry season. We have added discussion points about this topic to the section 4.3.

Sample quantity/representativeness

This overlaps with the previous points, but just a general comment. The authors have only studied 12 mounds, only measured during 2 moments in the year, and only once diurnal variation was studied (during x days). Again, field conditions are hard, and it is understandable that the dataset is not larger. But I would encourage the authors to consider and evaluate this in their interpretation (can a comparison be made between dry and wet season based on just 2 moments? Can a conclusion on daily variation be made based on just a few measurements?).

It is true that our seasonal sampling does not capture the all the major periods of vegetation and soil moisture changes in the area. We have discussed the results of the seasonal variation in the relation to seasonal changes in biomass of fungus combs and in the nutritional changes that are driven by seasonal changes in vegetation. We now point out in discussion the limitation in our seasonal sampling of the mound fluxes.

The Macrotermes mounds are large compared to some mounds of other termite species. In field measurements, the challenge is to find mounds that can actually be measured and the actual measurements are difficult to perform on large mounds and especially during night in remote locations.

As mentioned above our diurnal measurement periods were focused on the day to night and night to day transition periods which are most interesting from the gas transfer point of view. Our result shows similar trend with the high-frequency internal CO2 concentration measurements of *M. michaelseni* mounds (Ocko et al., 2017). Given this agreement in the mound CO2 flux and internal CO2 concentration our work suggests that a longer-term continuous CO2 concentration measurement with occasional mound CO2 flux measurement might be a feasible approach to estimate the continuous termite flux from the mound.

One curiosity, are there no local Kenyan researchers involved in (writing up) this research?

We agree that so-called helicopter science is problematic, and local scientists should be involved in research projects. This has indeed been a case in many of projects and publications in Africa by our group (see e.g. Räsänen et al., 2017; Wachiye et al., 2020; 2022). However, there are not always local scientists interested in specific research topics and in such cases including a local name just as token would not be ethical. Thus in this paper we happen not to have any local scientists, unlike many of our other papers.

Räsänen, M., Aurela, M., Vakkari, V., Beukes, J. P., Tuovinen, J.-P., Van Zyl, P. G., Josipovic, M., Venter, A. D., Jaars, K., Siebert, S. J., Laurila, T., Rinne, J., and Laakso, L.: Carbon balance of a grazed savanna grassland ecosystem in South Africa, Biogeosciences, 14, 1039–1054, https://doi.org/10.5194/bg-14-1039-2017, 2017.

Wachiye, S., Merbold, L., Vesala, T., Rinne, J., Räsänen, M., Leitner, S., and Pellikka, P.: Soil greenhouse gas emissions under different land-use types in savanna ecosystems of Kenya, Biogeosciences, 17, 2149–2167, https://doi.org/10.5194/bg-17-2149-2020, 2020.

Wachiye, S., Pellikka, P., Rinne, J., Heiskanen, J., Abwanda, S., and Merbold, L.: Effects of livestock and wildlife grazing intensity on soil carbon dioxide flux in the savanna grassland of Kenya, Agriculture, Ecosystems & Environment, 325, 107713, https://doi.org/10.1016/j.agee.2021.107713, 2022.

Detailed suggestions

-Page 1, Line 19: is there a white space between ‘CO2’ and ‘and’?

The space was in subscript. This is now corrected.

-Page 4, line 12: to be result from→ to be a result of

Corrected

-Page 4, line 17: and thus may cause different fluxes-→ which may result in different fluxes

We have removed this sentence.

-Page 7: line 10-11: diurnal variation measurements were made: on how many days?

The mound S1 and S2 were measured from evening until next day morning (S1 on 15 November 2016 and S2 on 7 November 2016).

-Fig 2: add on the x-axes which method this number belongs to (Calculated Cone volume/)

Thank you. Corrected the x-axes text and specified the cone volume equation number in the caption text.

-Page 8, line 5: was→ were

Corrected

-Page 9, sentence on line 8 and 9: sentence is incorrect, check if comma or verb is missing?

Thank you. We rephrased the sentence to read "The relationship of the environmental variables to the fluxes and to the ratio of CH_4 to CO_2 flux was tested using all the measurements (n = 18)."

- Page 10: line 11-12: why are S1 and MR4 compared? They have a similar volume, but have a different species and environment, so is this comparison useful/valid?

This comparison may not be appropriate. We have removed the sentence.

- Figure 6: the legend of the triangles and circles is only given in the first figure (6a). Maybe add a sentence in the caption as well

We have now indicated this in the caption.

- Fig 6f: you plot here the standard deviation of the CO2 flux. Please add to your material and methods how you obtain the standard deviation. Is this the standard error of the linear regression slope?

We made always three repeated measurements with 5 min break in between. The standard deviation was calculated from the three repeated measurements. We have now explained this in the method section and in the Fig. 6 caption.

- Table 2: is there a white space missing between CH4 and flux?

The space was in subscript. This is now corrected.

- Page 16, line 12: mound outer dimensions correlate positively with the size….. This you take from literature, right? Although clear from the paper (you didn't count the termites), maybe clarify this to the reader (sentence below just a suggestion, feel free to ignore or improve)

As found by previous studies, the mound outer dimensions of the Macrotermes species (of which both our species belong to) correlate positively…..

Thank you. We rephrased the sentence and it now reads "As found by previous studies, the mound outer dimensions of both Macrotermes species correlate positively with …."

- Page 16, line 32: elaborate maybe 1 sentence what you mean with sterile, and why that leads to constant activity over the year

The paragraph was edited to clarify the idea that although the number of termites remain relatively constant, biomass changes in seasonally produced alates and fungus gardens could potentially cause some intra-annual variation in gas production.

-Page 17, line 5 and 8. CO2 not written in subscript

Corrected