

## ***Interactive comment on “The role of termite CH<sub>4</sub> emissions on ecosystem scale: a case study in the Amazon rain forest” by Hella van Asperen et al.***

**Hella van Asperen et al.**

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Received and published: 3 February 2021

### **Response to Referee 1**

*Interactive comment on “The role of termite CH<sub>4</sub> emissions on ecosystem scale: a case study in the Amazon rain forest” by Hella van Asperen et al, Received and published: 5 December 2020*

Van Asperen and co-authors studied methane and CO<sub>2</sub> emissions by a termite species at an upland site in the amazon basin. They report individual and mound-based emission factors comparable to previous studies, and suggest that methane emissions can

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be employed as as rapid and non-invasive method to estimate mount populations.

**Strength:** The manuscript addresses a timely and important research question (methane emissions by termites) and provides a much needed data point in a previously understudied areas (termites in the neotropics). The authors followed state of the art measurements at a surely logistically challenging field location. As a bonus, the authors present both a comprehensive literature review and some very rare data on emissions of other trace gases (N<sub>2</sub>O, CO) in the appendix. The manuscript is generally well written and surely of great interest for the Biogeosciences readership.

**Limitations:** Some of the measurements were poorly replicated: Only one control collar was placed at distance from termite mounts, and for termite weight estimates, only one measurement is presented.

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**Dear Lukas Kohl,**

**Thank you for your kind words and the time you spent on reviewing our manuscript. We are grateful for your suggestions, which we have used to improve the manuscript. Below you will find a point to point response to each of your raised concerns and, if applicable, the corrected and improved manuscript text.**

In addition, we would like to point out that:

- we have uploaded a revised text of §4.1 (First paragraph of discussion), which is shown at the end of this review;

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- we have uploaded a revised Figure 2, which is shown at the end of this review (Previous Figure 4 is Figure 2 in revised manuscript);
- we have uploaded the revised Table 2, which is shown at the end of this review (Previous Table 2 and 3 are now merged into Table 2);
- we have uploaded one additional figure, belonging to a discussion point in this review ('Review-Figure 1'), which is shown at the end of this review;
- the given values in the text might have changed due to an improved termite weight determination.

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**The choice for only one blank measurement was due to practical limitations, often a leading factor in these logistically challenging field conditions. To improve this part of the manuscript, we will substitute our blank measurement by additionally measured valley soil fluxes, performed as part of sub study.**

**Below we will:**

- **report the values of the additionally measured soil valley fluxes;**
- **argue why these values are more suitable than the original blank control value;**
- **provide the revised manuscript text and the new Figure 4 wherein the soil valley measurements are shown.**

**Additional measurements:** Additional flux measurements were done in the same week (March 2020) and performed in the same valley at approx. 500 m distance from the termite mounds. The chamber set up was as described in §2.5, with 10 soil collars

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and 3 repetitions. We observed soil CH<sub>4</sub> fluxes ranging between -0.12 to 2.89 nmol m<sup>-2</sup> s<sup>-1</sup>, (median= -0.02, average=0.15, sd=0.55).

Fluxes from the *original* blank collar ranged between 3.9-5.4 nmol m<sup>-2</sup> s<sup>-1</sup> and were thereby higher than the additionally measured soil valley fluxes (-0.12 to 2.89 nmol m<sup>-2</sup> s<sup>-1</sup>). The original blank collar fluxes were however quite similar to the mound adjacent fluxes (0.3-8.9 nmol m<sup>-2</sup> s<sup>-1</sup>, 16 locations). While the blank collar was not closely located to a mound (~5 m of mound nr. 15), comparison to these 2 sets of measurements points at the presence of a local CH<sub>4</sub> hotspot (Subke et al. 2018), thereby not being representative as a control collar. For the revised manuscript we will use the additional soil valley flux measurements as our 'blank collar'.

The aim of the blank 'control' measurement was to show the large difference between a 'normal' valley CH<sub>4</sub> emission (per area), and an emission (per area) when a termite mound is present. Considering the average mound emission of 25.2 nmol mound<sup>-1</sup> s<sup>-1</sup>, and the average valley soil emission of 0.03 nmol collar<sup>-1</sup> s<sup>-1</sup> (0.15 nmol m<sup>-2</sup> s<sup>-1</sup>), an average collar area emits a factor 630 more CH<sub>4</sub> when a termite mound is present. Including these complementary measurements will strengthen our message that termite mounds are hotspots in comparison to their surroundings.

We have included these additional measurements for comparison, by adapting Figure 4, and by adapting the manuscript text. Manuscript parts with major changes have been copied here below. In the revised manuscript, the original Figure 4 is now Figure 2.

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**In Methods, §2.5: Valley and mound adjacent soil fluxes**

Every mound adjacent soil flux measurement was 4 minutes, and the set of 4 collar measurements

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was performed once per mound, with exception of mound nr. 19. For mound nr. 13 and nr. 14, the measurements were performed on the 2<sup>nd</sup> measurement day, for mound nr. 15 and nr. 16, the measurements were done on the 3<sup>rd</sup> measurement day. Mound adjacent soil fluxes will be expressed per collar area (0.25 m<sup>2</sup>), to be better comparable to mound emissions. The same chamber set up was used in a sub study at a close by transect (~ 500 m from termite mounds) where, among others, valley soil fluxes were measured (10 collars, 3 repetitions). Measured soil fluxes from the valley will be shown for comparison.

### **In Results, §3.1: Mound CH<sub>4</sub> and CO<sub>2</sub> emissions**

Headspace concentrations increased strongly during chamber closure, and chamber concentrations reached up to 5750 nmol CH<sub>4</sub> mol<sup>-1</sup> and 1950 μmol CO<sub>2</sub> mol<sup>-1</sup>. Mound CH<sub>4</sub> emissions ranged between 17.0 and 34.8 nmol mound<sup>-1</sup> s<sup>-1</sup> (Fig. 1), with an average emission of 25.2 nmol mound<sup>-1</sup> s<sup>-1</sup>. Additional valley measurements showed heterogeneous soil CH<sub>4</sub> fluxes with small uptake and emission taking place alongside, ranging between -0.1 and 2.9 nmol m<sup>-2</sup> s<sup>-1</sup> (med=-0.02, avg=0.15, sd=0.54). Mound adjacent CH<sub>4</sub> soil fluxes, measured at 20 and 45 cm from the mound, ranged between 0.4 and 8.9 nmol CH<sub>4</sub> m<sup>-2</sup> s<sup>-1</sup> (avg=2.14, sd=2.00), and were on average enhanced in comparison to valley soils (Fig. 2). Soil valley CO<sub>2</sub> fluxes were found to range between 0.9 and 3.7 μmol m<sup>-2</sup> s<sup>-1</sup> (avg=2.14, sd=0.74) (Fig. 2). Mound adjacent soil CO<sub>2</sub> fluxes showed an average emission of 4.84 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (range=2.0-10.1, sd=2.01), thereby being enhanced with respect to the surrounding soils (Fig. 2). Mound CO<sub>2</sub> emissions, corrected for the average valley soil respiration, were ranging between 1.1 and 13.0 μmol mound<sup>-1</sup> s<sup>-1</sup>, with an average emission of 8.14 μmol mound<sup>-1</sup> s<sup>-1</sup> (Fig 1).

### **In Discussion, §4.3:**

Valley soil CH<sub>4</sub> and CO<sub>2</sub> fluxes were similar to what was found by earlier studies (Souza (2005), Moura (2012), Chambers et al. (2004), Zanchi et al. (2012)). On average, mound adjacent soil CH<sub>4</sub> and CO<sub>2</sub> fluxes were enhanced with respect to valley soils, although differences were small, and no clear emission pattern with 'distance to mound' was observed. While mound adjacent soil fluxes are possibly enhanced, we preferred to avoid overestimation, and decided to treat termite mounds as very local hot spots, with measured fluxes only representative for the collar area of 0.25 m<sup>2</sup>. On average, CH<sub>4</sub> and CO<sub>2</sub> fluxes per collar area were found to be a factor ~630 and ~16 higher when an active termite mound was present.

### **References:**

- Chambers, Jeffrey Q., et al. "Respiration from a tropical forest ecosystem: partitioning of sources and low carbon use efficiency." *Ecological Applications* 14.sp4 (2004): 72-88.
- Moura, V. S. d.: *Investigação da variação espacial dos fluxos de metano no solo em floresta de terra firme na*

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Amazônia Central, MSc thesis INPA/UEA, 2012.

- Souza, Juliana Silva de. "Dinâmica espacial e temporal do fluxo de CO<sub>2</sub> do solo em floresta de terra firme na Amazônia Central." (2005).

- Subke, Jens-Arne, et al. "Rhizosphere activity and atmospheric methane concentrations drive variations of methane fluxes in a temperate forest soil." *Soil Biology and Biochemistry* 116 (2018): 323-332.

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## **Termite weight estimates**

Following the suggestion of the reviewer we repeated the measurement and use an improved weight estimate in the revised manuscript.

We repeated the measurement as described in §2.7, with a larger sample size: we measured the weight of 4 samples of each 100 termites, which resulted in an average calculated weight of 2.832 mg, 2.986 mg, 3.085 mg and 3.141 mg. The former measurement (as given in previous manuscript) with 80 termites, gave an average weight of 3.330 mg. Averaging these 5 values results in a termite weight of 3.0748 mg (sd=0.1847).

Such variation in average termite weight can be expected, due to genetics and environmental differences during development. In addition, our values are close to the values as measured by Pequeno et al (2013), who reported a termite weight of 3.0 mg (sd=0.4) for the species *N. Brasiliensis*.

In the manuscript, we will use a termite weight of 3.07 mg (sd=0.18) for the species *N. Brasiliensis*, and we will indicate the propagated uncertainty range in the relevant calculations. The new termite weights lead to the following revised manuscript text:

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**Previous text §2.7:** Termite mass was measured in the *Laboratory of Systematics and Ecology of Soil Invertebrates* at INPA. 80 living workers of the species *N. Brasiliensis* were weighted by use of a precision scale (FA2104N). Reported individual termite mass is fresh weight per termite (mg termite<sup>-1</sup>).

**Revised text §2.7:** Termite mass was measured in the *Laboratory of Systematics and Ecology of Soil Invertebrates* at INPA. 480 living workers of the species *N. Brasiliensis* were weighted in 5 subgroups (4x n=100, 1x n=80) by use of a precision scale (FA2104N). Reported individual termite mass is fresh weight per termite (mg termite<sup>-1</sup>).

**Previous text §3.2:** The living weight of 80 workers was measured to be 0.264 g, which is 3.3 mg per worker. This value is similar to what was found by Pequeno et al. (2017), who measured 3.0 (± 0.4) mg for workers and 6.6 (± 0.3) mg for soldiers. The species *N. Brasiliensis* has a relatively low soldiers:workers ratio of 1:100 (Krishna and Araujo, 1968). For our calculations we will use an average fresh weight of 3.33 mg termite<sup>-1</sup> for the species *N. Brasiliensis*.

**Revised text §3.2:** The average weight of 5 subsets of living workers of the species *N. Brasiliensis* was determined, and found to range between 2.83 and 3.33 mg, with an average weight of 3.07 mg (sd=0.18), which is similar as what was found by Pequeno et al. (2013), who reported 3.0 mg (sd=0.4). Since the species *N. Brasiliensis* has a relatively low soldiers:workers ratio of 1:100 (Krishna and Araujo, 1968), we will use the worker weight 3.07 (sd= 0.18) mg termite<sup>-1</sup> as an average termite weight for the species *N. Brasiliensis*.

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**Possible improvement:**

- **While the manuscript is generally very well written, I would encourage the authors to focus on editing the discussion section, which reads less easily than the rest of the manuscript. Some of this could be done by shortening and streamlining this section, which is rather long and at times meandering.**

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Thank you for the suggestion. We have shortened the discussion by following the different suggestions (see revised §4.1, as shown at the end of this review). In addition, we have taken out one figure (original Figure 3: mound volume and height vs emissions), since the content of the figure did not add much to the text.

- **The authors could also improve the quality figures and tables (see below), most importantly remove the grid lines from the figures for easier readability.**

We have re-plotted all figures following your suggestions. Figure 2 (original Figure 4) is shown at the end of this review.

- **Overall, this is a very nice contribution and it was a pleasure to review it!**

Thank you once more for your review and your comments!

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**L48: 'which is around' - approximately instead of around, also better state the range in % as well given that 2-15 Tg is quite a wide range.**

We have replaced 'which is around' for 'approximately', and will express the range in %:

**Revised text:** More recent literature uses estimates in the range of 2-15 Tg CH<sub>4</sub> per year (Ciais et al., 2014; Kirschke et al., 2013; Sanderson, 1996; Saunio et al., 2020), which is approximately 0.5-4% of the total estimated natural source CH<sub>4</sub> emission (Saunio et al., 2020).

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**L64: 'termite CO<sub>2</sub> measurements' - measurements of termite CO<sub>2</sub> emissions**  
We have corrected this.

**L64: to avoid mixing weight units (gramm and tons), Pg instead of Gt**  
We will use Pg instead of Gt.

**L119: what material was your chamber built out of?**  
The large flux chamber (220L) was created from a bucket from polythene, and purchased at a common household store. The collars were made from stainless steel. The small flux chamber (4.7L) and the collars were created from a common PVC sewage pipe, purchased at a construction store.

The following text has been added to §2.3 and §2.5:

**Revised text in §2.3:** A flux chamber was created by use of a 220 L slightly cone-shaped polythene bucket.

**Revised text in §2.5:** The chamber and collars were created from a common PVC sewage pipe.

**L130: when were your measurements conducted (date, in what season?)**  
Measurements were performed in March 2020, in the wet season (stated in §2.1).

**L134: 'molar density': concentration**  
We have corrected this.

**L142: 'increase': concentration change, as you could see uptake**  
We have corrected this.

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**L176-181: The section could be improved.**  
We have corrected this. See below for the improved text.

**Revised text §3.1:** During chamber closure, the concentration changes in CH<sub>4</sub> and CO<sub>2</sub> were strongly correlated ( $R^2 > 0.95$  for each chamber closure). The ratio between the mound CH<sub>4</sub> and CO<sub>2</sub> emission (CH<sub>4</sub>/CO<sub>2</sub>) ranged between 2.1 and  $17.1 \cdot 10^{-3}$ , and showed a constant ratio when data from mound 19 (furthest away from other mounds), and mound 6 (different species) were excluded (average ratio:  $2.8 \cdot 10^{-3}$ ). The smallest mound (nr. 19) clearly showed smaller-than-average emissions, but in general no strong correlation was found between mound CH<sub>4</sub> emissions and mound height ( $R^2=0.07$ ) or volume ( $R^2=0.08$ ), and a small correlation was found between mound CO<sub>2</sub> emissions and mound height ( $R^2=0.43$ ) and mound volume ( $R^2=0.44$ ).

**L195: can you state an uncertainty of this weight per individual?**  
Please see the beginning of this review for an elaborate answer.

**L217: no need to state the original unit here, just state the values converted to the unit used in your study.**  
We have corrected this.

**L223-235: I recommend streamlining/shortening this segment. Acknowledging mount uptake is important, but it's not the focus of your study and comes out of left field here. Focus on why this is important to understanding your results.**  
We have shortened this part. The improved section §4.1 can be found at the end of this review.

**L237-241: I would move this comparison with literature data up to L215-219.**  
We have moved this part.

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**L243: To be honest, these variations among individual measurements look pretty trivial to me and may not need such extensive discussion (which ends up questioning your measurements).**

We have reduced this part, but have kept one sentence (see revised §4.1). In case someone else would like to do similar measurements, it is good to be aware of this possible minimal transport below the collar.

**L249-254: This can be tested by looking at the concentration curves within individual closures. If a relevant air exchange between chamber and ambient air occurred, concentrations should be non-linear ( $d\text{CH}_4/dt$  decreasing over time, following a  $y=a + b \cdot e^{-c \cdot t}$  function). If this is the case, fluxes should be calculated by fitting such an exponential function and calculating the slope as  $d[\text{CH}_4/dt]$  at  $t=0$ .**

We observed little variations in the linear increase, and variations were at the same moment and with the same magnitude for  $\text{CO}_2$  and  $\text{CH}_4$ . We expect that this is a result from minor air transport below the collar, possibly as a result of little disturbances (bag filling, a forest breeze, our presence close to the flux chamber). These fluctuations are not continuous, and the gradient recovers itself after a fluctuation. As can be seen in added figure (Review-Figure 1), the concentration increase still can be represented well by a linear increase with a strong  $R^2$  ( $R^2 > 0.95$ ).

**L280: it would be good to add an uncertainty range to your population estimates**

Thank you for this suggestion.

We have propagated the uncertainty of our emission factor  $0.0002985$  ( $se=1.77 \cdot 10^{-5}$ ), to define an uncertainty range in our population estimate. For example, for mound nr.

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13, a range of 89.5-100.9 thousand termites will be given.

**L288: 'contemplated': considered?**

We have corrected this.

**L291-292: 'hypothesize': don't use hypothesize that for claims you do not test. 'It is therefore likely that.'**

We have corrected this.

**L303: 'drawback': disadvantage**

We have corrected this.

**L304-305: 'is proposed': by whom? The authors? If that's that's the case, say so (ok, sorry for the snarky tone. Use active voice here - 'We propose a follow-up study to directly compare')**

Thank you for the suggestion, this is indeed unclear. We have corrected this.

**L311: 'it was decided': same here, use active voice: 'we decided .. to avoid overestimating ..'**

We have corrected this.

**L418-419: 'indicating no or very low  $\text{N}_2\text{O}$  emissions': Can you provide an uncertainty range for that estimate (e.g., limit of detection for fluxes?)**

We have calculated a detection limit of  $0.027 \text{ nmol N}_2\text{O m}^{-2} \text{ s}^{-1}$ . Here below we will:

- elaborate on how this detection limit is determined (specifications FTIR-instrument and assumptions);

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- support our statement (very low N<sub>2</sub>O emissions) with additional data;
- give the revised manuscript text.

**Detection limit of measurements:** Reviewer 2 posed a similar question, and also asked about the precision and calibration of the FTIR-instrument. For completeness, we give the information here as well.

The FTIR-instrument has the following precision ( $\sigma$ ) for 10 minute-averaged spectral analyses: 0.02  $\mu\text{mol mol}^{-1}$ , 0.2  $\text{nmol mol}^{-1}$ , 0.2  $\text{nmol mol}^{-1}$ , 0.06  $\text{nmol mol}^{-1}$ , and 0.04 permil, for respectively CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O, CO, and  $\delta^{13}\text{C}$  of CO<sub>2</sub>. Measurements performed during the campaign week were set to 5 minutes, so that a precision of  $1/\sqrt{N}$  is achieved, which is 0.09  $\text{nmol mol}^{-1}$  for N<sub>2</sub>O. The FTIR instrument has been shown to be linear for all gases in the ambient concentration range, and linearity was tested for N<sub>2</sub>O in the range 300-350 ppb. For the detection limit, we state the following:

- Assuming bag samples taken at 2, 5 and 8 minutes during chamber closure.
- Given: collar area 0.25 m<sup>2</sup>, chamber volume 220 L, mound volume 50 L, headspace volume 220-50 = 170 L.
- Assuming: Molar volume of 24.5 L mol<sup>-1</sup> (1 atm, 25 °C).
- Minimum detectable concentration difference is ( $2\sigma$ ) 0.18  $\text{nmol mol}^{-1}$ .
- A concentration difference between t=2 min and t=5 min of 0.18  $\text{nmol mol}^{-1}$ , is caused by a flux of 0.027  $\text{nmol collar/mound}^{-1} \text{ s}^{-1}$ .

The FTIR-instrument has a cross sensitivity with CO<sub>2</sub>, which is well determined for CO<sub>2</sub> <800  $\mu\text{mol mol}^{-1}$ , but is less certain for high CO<sub>2</sub> concentrations. For this

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reason, we prefer to only use the N<sub>2</sub>O headspace concentration measurements with CO<sub>2</sub> <800  $\mu\text{mol mol}^{-1}$ . Only 5 mound chamber closures had two consecutive N<sub>2</sub>O concentration points (t=2 min and t=5 min) with CO<sub>2</sub> <800  $\mu\text{mol mol}^{-1}$ , and only 3 sets of two-consecutive concentration points passed the minimum concentration difference of 0.18  $\text{nmol mol}^{-1}$ . These differences were ~0.2, ~0.3 and ~0.7  $\text{nmol mol}^{-1}$ , leading to calculated N<sub>2</sub>O fluxes ranging between ~0.03 - ~0.11  $\text{nmol mound}^{-1} \text{ s}^{-1}$ .

**Additional measurements to support statement ‘very low N<sub>2</sub>O emissions’:** In October 2020, additional valley soil N<sub>2</sub>O flux measurement were performed with the same chamber system and collars (5 collars, 3 repetitions), but with a longer closing time (35 min), without termite mounds (so lower CO<sub>2</sub>), and with 4 measurements per chamber closure. Also during these measurements, concentration increases were very low. Out of 15 measurements, 8 measurements had an R<sup>2</sup> > 0.90, and calculated fluxes ranged between 0.008-0.106  $\text{nmol m}^{-2} \text{ s}^{-1}$  (average=0.032  $\text{nmol m}^{-2} \text{ s}^{-1}$ , sd=0.33). Since the valleys are known to be low on nitrogen (Quesada et al., 2010), such low fluxes are expected, and similar N<sub>2</sub>O valley soil fluxes were found by Matson et al., (1987) in a fieldsite closeby.

**For the revised manuscript:** since the 3 calculated mound N<sub>2</sub>O flux measurements are based on only 2 consecutive headspace concentration points, no uncertainty can be given, wherefore we preferred not to state the fluxes in the previous manuscript. For the revised manuscript, we state the detection limit, explain why not all mound fluxes could be calculated, and support our observation of low N<sub>2</sub>O mound fluxes by the additional soil N<sub>2</sub>O flux measurements:

**Appendix A2:** Gas samples (3 samples per chamber closure) revealed stable N<sub>2</sub>O concentrations, and headspace concentrations ranged between 333.7 and 342.4  $\text{nmol mol}^{-1}$  over the different chamber closures. Since headspace CO<sub>2</sub> concentrations sometimes exceeded 800  $\mu\text{mol mol}^{-1}$ , and N<sub>2</sub>O-CO<sub>2</sub>

cross-sensitivity becomes uncertain at higher CO<sub>2</sub> concentrations, not all 3 headspace samples per chamber closure could be used, wherefore qualitative N<sub>2</sub>O flux estimates cannot be reported. As a back-of-the-envelope calculation, N<sub>2</sub>O fluxes were calculated if 2 consecutive headspace samples were with CO<sub>2</sub> <800 μmol mol<sup>-1</sup>, and if a minimum N<sub>2</sub>O concentration difference of 0.18 nmol mol<sup>-1</sup> was found (FTIR precision (σ) for 5 min spectra is 0.09 nmol mol<sup>-1</sup>), which gave us 3 mound flux estimates ranging between ~0.03 and ~0.11 nmol N<sub>2</sub>O mound<sup>-1</sup> s<sup>-1</sup>. Similarly low fluxes were found during additionally performed flux measurements, performed as part of a substudy, which showed valley soil fluxes ranging between 0.008-0.106 nmol N<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>. The low mound fluxes would be in agreement with a previous study which suggested that termite mound N<sub>2</sub>O emissions are dependent on the N-content of the termites diet (Brauman et al., 2015), which is expected to be low in the valleys of this ecosystem (Quesada et al., 2010).

#### References:

-Matson, Pamela A., and Peter M. Vitousek. "Cross-system comparisons of soil nitrogen transformations and nitrous oxide flux in tropical forest ecosystems." *Global Biogeochemical Cycles* 1.2 (1987): 163-170.

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## Figures

- **Remove grid lines (counter-intuitively, this makes figures easier to read), place ticks inwards.**
- **Fig 1: remove 'per mound' on the y axis, it's redundant with the unit on that axis.**
- **Fig 4: A broken axis might work better than the inserts here (if you keep the inserts, state the y axis scale). The figure could also be simplified by showing the means + SD of the four mounds instead of values for individual mounds. Also, I think the direction in which you placed the soil collars from the mound wasn't chosen deliberately, so your x axis could be just 'distance from the center of the mound', combining your flux measurements at the same distance at either side of the mound.**
- **Fig 5: number instead of amount**

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Thank you for these suggestions.

- Figure 1: we have removed 'per mound', and have removed the gridlines.
- Figure 4 (now Fig 2): we have implement a 'broken y-axes', and have added additional measurements. We have chosen to keep the mound in the middle, to better visual the actual mound, and to visually separate the emissions measured on each side of the mound.
- Figure 5 (now Fig 4): we have corrected this.

## Tables

**I recommend combining Table 2 and 3 after removing reported value and reported unit (these can be placed in a supplement) to keep the table easier to read. State the unit of the converted values in the table header. This leaves the following columns: [Study] [Study area] [CH<sub>4</sub> emission (state units)] [CO<sub>2</sub> emission (state units)] [CH<sub>4</sub>:CO<sub>2</sub> ratio (state units)] [Species]. Such a table would give a much better overview.**

Thank you for this suggestion. We have merged the two tables, and have taken some columns out. Since it might not always be clear to which value we are referring, especially when data is taken from a graph, we prefer to also state the original value and unit. Nevertheless, we have tried to improve the readability by giving this part a smaller fontsize. The new table can be found at the end of this review.

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## Revised Discussion part §4.1

### CH<sub>4</sub> and CO<sub>2</sub> emissions

Measured mound CH<sub>4</sub> emissions were of similar magnitude to emissions found by previous studies (Table 2). The termite emission factor, determined for the soil-feeding species *N. brasiliensis*, was found to be 0.35 (sd= 0.02)  $\mu\text{mol g}_{\text{termite}}^{-1} \text{h}^{-1}$ , which is similar to values found for other species in literature (Table 2, upper part), but almost two times higher than the average value reported by Martius et al (1993) for a wood-feeding species in the Amazon (0.19  $\mu\text{mol CH}_4 \text{g}_{\text{termite}}^{-1} \text{h}^{-1}$ ). Our emission rate is within the reported range of 0.1-0.4  $\mu\text{mol g}_{\text{termite}}^{-1} \text{h}^{-1}$  for soil feeders (Sugimoto et al. 2000). Mound CO<sub>2</sub> emissions and the termite CO<sub>2</sub> emission factor were similar to a little higher in comparison to the few values found in literature. Nevertheless, since mound material and termites were measured together, the contribution of *indirect* termite emissions, i.e. mound respiration, cannot be quantified, so that the direct termite-produced CO<sub>2</sub> emission is presumably lower.

There is a large variety in type of termite mounds (shape and size are dependent on, among others, species, ecosystem, climate (Noirot and Darlington, 2000)), explaining the wide range of reported termite mound CH<sub>4</sub> emissions (Table 2, middle and lower part). In-situ measurement of termite mounds gives information about the *net* CH<sub>4</sub> emission under natural conditions, but is unable to distinguish sources and sinks inside the mound. One known CH<sub>4</sub> sink in termite mounds is the uptake by methanotrophic bacteria, which are also responsible for the CH<sub>4</sub> uptake in aerobic soils. The presence and magnitude of this process have been discussed and reviewed by different studies (Khalil et al., 1990; Macdonald et al., 1998; Nauer et al., 2018; Seiler et al., 1984; Sugimoto et al., 1998a; Ho et al., 2013; Pester et al., 2007; Reuß et al., 2015). The role of possible mound CH<sub>4</sub> uptake should also be acknowledged for the measurement of individual termite emissions (Table 2, upper part): most literature values, including values from this study, are based on termite incubation in presence of mound material, with ongoing CH<sub>4</sub> uptake, wherefore actual termite CH<sub>4</sub> emission values might be higher.

Small variation in emission magnitudes was observed between measurement days. This can be caused by a variation in colony size (due to foraging activities) or termite activity, driven by fluctuations in temperature or radiation (Jamali et al., 2011a; Ohiagu and Wood, 1976; Sands, 1965; Seiler et al., 1984). However, as our termite mounds are in a tropical forest with relatively constant temperatures and only indirect daylight, strong diurnal temperature and radiation patterns are not expected. Small variation can also be caused by minimal air transport below the soil collar, through the porous upper soil layer; during preliminary tests *without* a collar, we observed that even a light forest breeze can cause chamber

C17

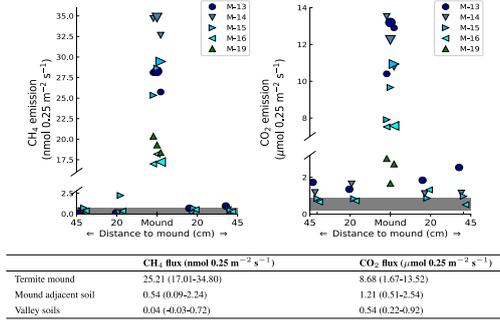
headspace variations. In case our set up was subject to minor air transport below the collar, the given mound estimates will be slightly underestimated with respect to the actual mound fluxes. Another possible underestimation is caused by the estimated corrected chamber volume, as used in Eq. (2). In this study, we considered the mound volume as a solid body. A previous study considered the solid nest volume as 10% of the actual mound volume (Martius et al. 1993), leading to a larger corrected chamber volume, and therefore to larger calculated mound emissions. By use of this approach, average calculated emissions would increase by almost 30% to be 32.7 nmol CH<sub>4</sub> mound<sup>-1</sup> s<sup>-1</sup> instead of 25.2 nmol CH<sub>4</sub> mound<sup>-1</sup> s<sup>-1</sup>.

The mound emission CH<sub>4</sub>/CO<sub>2</sub> ratio was found to be relatively constant over 4 of the 5 mounds, with an average ratio of  $2.8 \cdot 10^{-3}$ . While values in literature indicate a wide range of reported CH<sub>4</sub>/CO<sub>2</sub> ratios (Table 2), both Seiler et al. (1984) as Jamali et al. (2013) found little variation between mounds of the same species, and concluded that the CH<sub>4</sub>/CO<sub>2</sub> emission ratio is species-specific. Our overall variation of a factor of ~4 for the CH<sub>4</sub>/CO<sub>2</sub> ratio of mound emissions of the same species is of the same magnitude as what was observed in earlier studies (Seiler et al., 1984; Jamali et al., 2013).

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-384>, 2020.

C18



**Figure 2** Measured mound emissions and mound-adjacent soil fluxes for CH<sub>4</sub> (left) and CO<sub>2</sub> (right) for mound nr. 13, nr. 14, nr. 15 and nr. 16 expressed in nmol 0.25 m<sup>-2</sup> s<sup>-1</sup> for CH<sub>4</sub> and μmol 0.25 m<sup>-2</sup> s<sup>-1</sup> for CO<sub>2</sub> (collar area is 0.25 m<sup>2</sup>). Note that for CO<sub>2</sub> here the net mound emissions per collar area, not corrected for soil respiration, are shown and stated. The centrally-placed markers are the measured mound emissions (also for mound nr. 19); the larger marker indicates the day-specific mound emission when mound adjacent soil fluxes were measured. The grey bar indicates the range of additionally measured soil valley fluxes. The range and average flux for each group of measurements are given in the table. On average measured mound CH<sub>4</sub> and CO<sub>2</sub> fluxes were a factor 630 and 16 higher in comparison to the surrounding soil valley fluxes.

22

**Fig. 1.** Revised Figure 2 (previously Figure 4)

C19

28

**Table 2.** Overview of literature values for CH<sub>4</sub> and CO<sub>2</sub> emission of termites per weight (upper part), emission per termite mound (middle part), and emission per area (lower part). Values from this study are indicated in bold. If reported, the average and sd are given, otherwise a range is indicated. If multiple values were reported, measurements from higher soil-feeding termite species were selected. For each study, the graph or table where the data was found, is indicated. The CH<sub>4</sub>/CO<sub>2</sub> is given in molar ratio (10<sup>-3</sup>). a) Sawadogo et al. (2011) reported emissions per dry weight mass. To convert to fresh weight, a formula as reported by Pequeno et al. (2017) was used. With an assumed dry weight of 0.5 mg, a conversion factor of 3.14 was deducted. b) Mound emissions are divided by collar area of 0.25 m<sup>2</sup>; c) Calculated based on average values in this table; d) *Neocapritermes brasiliensis*; e) *Crenetermes albotarsalis*, *Cubitermes fungifaber*, *Cubitermes speciosus*, *Noditermes* sp., *Procapritermes* sp., *Thoracotermes macrohorax*; f) *Dicuspitermes santschii*, *Dicuspitermes nemorosus*, *Pericapritermes semarangi*, *Procapritermes* nr. *Sandakanensis*, *Homalotermes eleanorae*, *Proaculitermes* sp. A, *Pericapritermes nitobei*; g) *Coptotermes lacteus*; h) *Ancistotermes cavithorax*, *Odonotermes n. pauperans*; i) *Nasutitermes macrocephalus*, *Nasutitermes corniger*, *Nasutitermes surinamensis*, *Nasutitermes* sp., *Nasutitermes ephratae*, *Nasutitermes araujoi*; j) *Noditermes* sp., *Crenetermes albotarsalis*, *Cubitermes speciosus*, *Thoracotermes macrohorax*, *Astratotermes* sp.; k) *Macrotermes bellicosus*; l) *Microcotermes* sp., *Globitermes sulphureus*, *Termes* sp.; m) *Dicuspitermes* sp.; n) *Drepanotermes perniger*, *Nasutitermes magnus*, *Nasutitermes trididiae*, *Tumulitermes pastinator*, *Amietermes laurensis*, *Coptotermes lacteus*; o) *Bulbitermes* sp. C, *Dicuspitermes nemorosus*, *Dicuspitermes santschii*; o) *Macrotermes* and *Odonotermes* (Macrotermitinae), *Trinervitermes* (Nasutitermitinae), *Amietermes* and *Cubitermes* (Termitinae), *Hodotermes* (lower termite); p) *Cubitermes fungifaber*; q) *Microcotermes nervosus*, *Turnulitermes pastinator*, *Turnulitermes hastilis*, *Amietermes meridionalis*.

Study	Study area	Studies reporting emission per nest or mound			CH <sub>4</sub> /CO <sub>2</sub>	Species
		CH <sub>4</sub> emission (μmol g <sup>-1</sup> h <sup>-1</sup> )	CO <sub>2</sub> emission (μmol g <sup>-1</sup> h <sup>-1</sup> )	CH <sub>4</sub> /CO <sub>2</sub>		
<b>This study, Fig. 4</b>	Amazon	<b>0.25 (0.2)</b>	<b>86.8 (10.0)</b>	~42 <sup>a</sup>	Soil feeders <sup>(o)</sup>	
Brannan et al. (1992), Tab. 1	Congo	0.39-1.09	(0.36-1.09 μmol g <sup>-1</sup> h <sup>-1</sup> )	1.4-9.0	Soil feeders <sup>(f)</sup>	
Eggleton et al. (1999), Tab. 4	Australia	0.17-0.27	(0.17-0.27 μmol g <sup>-1</sup> h <sup>-1</sup> )	107 (4.5)	10-154	Soil feeders <sup>(f)</sup>
Fraser et al. (1986), Fig. 2	Australia	0.04 (0.01)	(0.07-0.2) μmol g <sup>-1</sup> h <sup>-1</sup>	107 (4.5)	~0.38 <sup>c</sup>	Wood feeders <sup>(p)</sup>
Konaté et al. (2003), Tab. 1	Ivory Coast	0.19 (0.08)	(3.0-1.3) μmol g <sup>-1</sup> h <sup>-1</sup>	31.4-133.5	(31.4-133.5 μmol g <sup>-1</sup> h <sup>-1</sup> )	Fungi feeders <sup>(h)</sup>
Martius et al. (1993), Tab. 1	Amazon	0.53-1.09	(0.53-1.09 μmol g <sup>-1</sup> h <sup>-1</sup> )	19-25	(9.4-78.4 μmol g <sup>-1</sup> h <sup>-1</sup> ) <sup>a</sup>	Wood feeders <sup>(i)</sup>
Roulund et al. (1993), Tab. 1	Congo	0.10-0.12	(0.30-0.39 μmol g <sup>-1</sup> h <sup>-1</sup> )	19-25	(9.4-78.4 μmol g <sup>-1</sup> h <sup>-1</sup> ) <sup>a</sup>	Wood feeders <sup>(i)</sup>
Sawadogo et al. (2011), Tab. 1	Burkina Faso	0.03-0.20	(3.4-26.3) μmol g <sup>-1</sup> h <sup>-1</sup>	~5 <sup>c</sup>	Wood feeders <sup>(k)</sup>	
Sugimoto et al. (1998a), Tab. 3	Thailand	0.03-0.20	(3.4-26.3) μmol g <sup>-1</sup> h <sup>-1</sup>	~5 <sup>c</sup>	Soil feeders <sup>(j)</sup>	
Study	Study area	Studies reporting emission per nest or mound			CH <sub>4</sub> /CO <sub>2</sub>	Species
		CH <sub>4</sub> emission (μmol mound <sup>-1</sup> h <sup>-1</sup> )	CO <sub>2</sub> emission (μmol mound <sup>-1</sup> h <sup>-1</sup> )	CH <sub>4</sub> /CO <sub>2</sub>		
<b>This study, Fig. 1</b>	Amazon	<b>61-125</b>	<b>4-47</b>	2.8 (0.4)	Soil feeders <sup>(o)</sup>	
Khalil et al. (1990), Fig. 4 & Tab. 3	Australia	9-135	4-92	0.12-1.11	Wood feeders <sup>(m)</sup>	
MacDonald et al. (1999), Tab. 4	Cameroon	1-11	(4.5-48) μmol mound <sup>-1</sup> h <sup>-1</sup>	0.12-1.11	Soil & wood feeders <sup>(n)</sup>	
Martius et al. (1993), Tab. 1	Amazon	125 (150)	(2.0-4.0) μmol mound <sup>-1</sup> h <sup>-1</sup>	0.07-8.7	Wood feeders <sup>(i)</sup>	
Seiler et al. (1984), Tab. 1	South Africa	1.644	(0.03-10.3) μmol mound <sup>-1</sup> h <sup>-1</sup>	0.07-8.7	Soil & wood feeders <sup>(o)</sup>	
Sugimoto et al. (1998a), Tab. 3	Thailand	0.4-1.9	(4.2-18.7) μmol mound <sup>-1</sup> h <sup>-1</sup>	0.07-8.7	Soil feeders <sup>(j)</sup>	
Study	Study area	Studies reporting emission per area			CH <sub>4</sub> /CO <sub>2</sub>	Species
		CH <sub>4</sub> emission (μmol m <sup>-2</sup> h <sup>-1</sup> )	CO <sub>2</sub> emission (mmol m <sup>-2</sup> h <sup>-1</sup> )	CH <sub>4</sub> /CO <sub>2</sub>		
<b>This study, Fig. 1</b>	Amazon	<b>245-501<sup>d</sup></b>	<b>16-187<sup>d</sup></b>	2.8 (0.4)	Soil feeders <sup>(o)</sup>	
Brümmner et al. (2009a), Fig. 5	Burkina Faso	315.7	(198.9) μmol CO <sub>2</sub> m <sup>-2</sup> h <sup>-1</sup>	~8.5 <sup>e</sup>	Soil feeders <sup>(p)</sup>	
Jamali et al. (2013), Fig. 1	Australia	32-500	(179-609) μmol CH <sub>4</sub> m <sup>-2</sup> h <sup>-1</sup>	2.7-11.0	Wood feeders <sup>(q)</sup>	
Queiroz (2004), Tab. 4	Amazon	10-24	(5.16-38) μmol m <sup>-2</sup> h <sup>-1</sup>	unknown	unknown	

**Fig. 2.** Revised Table 2 (merge of Table 2 and 3)

C20

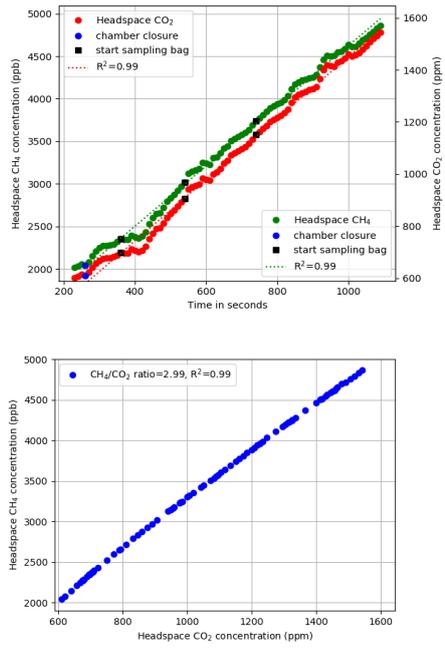


Fig. 3. Review Figure 'Review-Figure 1'

## ***Interactive comment on “The role of termite CH<sub>4</sub> emissions on ecosystem scale: a case study in the Amazon rain forest” by Hella van Asperen et al.***

**Hella van Asperen et al.**

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Received and published: 3 February 2021

### **Response to Anonymous Referee 2**

*Interactive comment on “The role of termite CH<sub>4</sub> emissions on ecosystem scale: a case study in the Amazon rain forest” by Hella van Asperen et al, Received and published: 22 December 2020*

**This manuscript presents a well thought out study to quantify methane emissions by termites in the Amazon rain forest. The authors reviewed the literature extensively and compared/discussed with their findings. I have some comments**

C1

**that I think will make the study more valuable.**

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Thank you for your kind words and the time you spent on reviewing our manuscript. We are grateful for your suggestions, which we have used to improve the manuscript. Below you will find a point to point response to each of your raised concerns and, if applicable, the corrected and improved manuscript text.

In addition, we would like to point out that the given termite emission estimates have changed due to an improved termite weight determination.

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**1. Please provide the estimate of CH<sub>4</sub> emissions by termite and put in context with the overall CH<sub>4</sub> budget globally or in the Amazon. This manuscript presents CH<sub>4</sub> emission factors only. Without knowing how many termite mounds in Amazon, it's difficult to imagine the scale of the global CH<sub>4</sub> budget. I think this is one of a key messages for readers.**

Thank you for this interesting point. Below we will:

- elaborate on our considerations regarding the termite mound-upscaling;
- provide a 'back of the envelope' estimate on the role of termite emissions in the Amazon CH<sub>4</sub> budget, and in the global CH<sub>4</sub> budget;
- show the revised manuscript text.

**Termite mound upscaling:** Based on mound density numbers, it is difficult to state

C2

a termite CH<sub>4</sub> emission estimate for the whole Amazon. As stated in the discussion (§4.3), mound density numbers vary largely between ecosystems. There is only little data available on mound density numbers, and most Amazon studies were performed in close proximity to our fieldsite (due to the research activities of local institute INPA). While this relatively large amount of local studies is unique and useful for the upscaling for our *local* ecosystem, it is unwise to assume that these mound density numbers apply to the whole Amazon. For this reason, we choose to only state a mound CH<sub>4</sub> emission estimate for our specific ecosystem, and to inform the readers about the limitations of this estimate.

**Back-of-the-envelope estimate for the global CH<sub>4</sub> budget:** By use of the data presented in the comprehensive modeling study of Kirsche et al. (2013), the following back-of-the-envelope estimate can be made:

Kirsche et al (2013) (Table 1) stated an annual global termite emission of 11 Tg CH<sub>4</sub> year<sup>-1</sup>. They state that 36% of termite emissions originate from the region 'tropical South America' (p 818, first sentence), which calculates to 3.96 Tg CH<sub>4</sub> year. Substituting the used termite emission factor of 2.8 μg CH<sub>4</sub> g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup> by the value found in our study of 5.6 μg CH<sub>4</sub> g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup>, would lead to a doubling of the regions estimated termite emission, namely 7.92 Tg instead of 3.96. The global estimate would increase from 11 Tg to 14.96 Tg.

The termite emission factor is a practical estimate of the average termite emission, which can be used for CH<sub>4</sub> budget studies. Since our study only measured one termite species, and there is likely a variation between species and ecosystems, we do not suggest that the currently used termite emission factor of 2.8 μg CH<sub>4</sub> g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup> should be replaced by our value. We do however want to show and point out that the termite emission factor is still an uncertain part in the tropical CH<sub>4</sub> budget.

C3

To include the reader in this train-of-thought, we have revised this part of the manuscript:

**Revised text in §4.3:** As a 'back-of-the-envelope' calculation, based on Kirsche et al. (2013): 36% of global termite emission (11 Tg) is expected to come from the region of 'tropical South America' (0.36\*11=3.96 Tg). Substituting the emission factor of 2.8 with the newly found 5.6 μg CH<sub>4</sub> g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup> would increase this regions estimate to 7.92 Tg, and the global estimate to 14.96 Tg.

Our study points out that termite emissions are still an uncertain source in the CH<sub>4</sub> budget, and are especially poorly quantified for the Amazon rain forest. Measurement of CH<sub>4</sub> emissions from different termite species, preferably covering species of different feeding or nesting habits, in combination with more precise termite distribution and abundance data, would allow more precise estimates and a better understanding of the role of termites in the CH<sub>4</sub> budget.

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**2. The first sentence in the Introduction section, it says "Methane (CH<sub>4</sub>) is the second most important long-lived anthropogenic greenhouse gas." I think CH<sub>4</sub> has been recognized to be "short-lived" climate pollutant.**

Thank you for pointing this out. We have changed the first sentence to:

**Revised text:** Methane (CH<sub>4</sub>) is the second most important anthropogenic greenhouse gas, but its natural sources are still not well understood.

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**3. In the Introduction section, Line 35, it says "Recently, it was shown that termites have a mitigating effect during droughts in tropical rain forests". Please elaborate what mitigating effect.**

C4

Ashton et al. (2019) performed a termite suppression experiment and found that termite activity increased during drought, resulting in accelerated litter decomposition, elevated soil moisture, greater soil nutrient heterogeneity, and higher seedling survival rates. The authors suggested different underlying mechanisms for this response such as more favorable conditions for tunneling (e.g., drier, less-waterlogged ground), increased foraging ability above ground in the absence of heavy rain, and/or reduced predation pressure from ants.

We have changed the text in the Introduction to:

**Previous text:** Recently, it was shown that termites have a mitigating effect during droughts in tropical rain forests.

**Revised text:** Recently, it was shown that termites increase their activity during droughts, resulting, among others, in enhanced litter decomposition, elevated soil moisture and higher seedling survival rates, thereby demonstrating a mitigating effect during droughts in tropical rain forests.

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**4. In the Introduction and in Appendix, the authors touched on N<sub>2</sub>O emissions from termite but didn't give conclusive results.**

We agree that this point is not sufficiently discussed. An elaboration on this subject can be found at point 7.

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C5

**5. Section 2.3, Line 129, LGR GHG analyzer was mentioned to be the instrument deployed to quantify CH<sub>4</sub> emissions in flux chambers. I think authors should add brief instrument performance specifications and details of what calibration and drift evaluation have been done in Amazon. While the absolute CH<sub>4</sub> concentrations in flux chamber measurements are not very critical, since it's to measure the CH<sub>4</sub> concentration increase, but the manuscript does not provide the measured concentrations and jumped directly to the emission factor estimates. For example, LGR UGGA precision is about 2 ppb. Does it perform the same in Amazon? Also, what CH<sub>4</sub> concentration increments measured in the flux chambers? If it was only 2 ppb, then that data would not be useful. I think it should be many times more than the instrument precision and drift.**

Thank you for raising this point. During the campaigns, we have set the Los Gatos instrument to the 10-second averaging modus. Calibration gases were measured every second day for 5 minutes, resulting in a precision ( $1\sigma$ ) of  $\sim 0.7$  ppm and  $\sim 3.0$  ppb for respectively CO<sub>2</sub> and CH<sub>4</sub>.

The concentration increases during the 20 min chamber closure were large. Concentrations were climbing from forest concentrations to concentrations of up to 5750 ppb CH<sub>4</sub>, and up to 1950 ppm CO<sub>2</sub>, thereby far exceeding the measurement precision of the Los Gatos instrument.

We have added the following lines to the revised manuscript (beginning of Results):

**Revised text in §3.1:** Headspace concentrations increased strongly during chamber closure, and chamber concentrations reached up to 5750 nmol CH<sub>4</sub> mol and 1950  $\mu$ mol CO<sub>2</sub> mol<sup>-1</sup>.

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C6

**6. Well-designed flux chambers should have a small mixing fan or internal distribution tubing to quantify fluxes. §2.5 describes how LGR sampling tubes were connected on top of a 220 L chamber, if the air inside is not well mixed, the two fittings on top of the chamber may not detect CH<sub>4</sub> at the bottom of the chamber.**

Thank you for raising this point. Below we will:

- clarify the locations of the inlet fittings;
- elaborate on why we did not install a fan, and how we ensured mixed chamber air;
- give the revised manuscript text.

The 220 L chamber had two fittings on each side of the bucket while the smaller soil chamber had the two fittings on top of the chamber. Re-reading §2.5, we agree with the reviewer that the text is confusing, and we have revised this part.

As a small side note, termite mounds emit CH<sub>4</sub> from its entire surface, thereby presenting a sphere-shaped source of 45-65 cm height *inside* the chamber head space. Therefore, we do not expect a large difference between CH<sub>4</sub> concentrations at the top and the bottom of the chamber headspace.

We were hesitant about installing a small mixing fan. On the one hand, the absence of a mixing fan might lead to an underestimation of the flux (Christiansen et al. 2011). On the other hand, a mixing fan might lead to turbulence in the head space (Janssens

C7

et al. (2000), Pumpanen (2004)), which possibly induces unrepresentatively high CH<sub>4</sub> emissions from the mound.

Since we wanted to avoid overestimation of termite mound CH<sub>4</sub> fluxes, we decided to not install a mixing fan. Instead we installed a 4 inlet vertical sampling tube inside the chamber head space, a technique to minimize the effects of gas concentration gradients in the head space (Clough et al, 2020). Inside the chamber at fitting height (~30 cm), a T-piece with two 20 cm-long Teflon tubing was positioned vertically, and two small incisions were made, so that head space air was sampled from 4 different heights (approx. at 10, 25, 35 and 50 cm height from the soil). The sampling tube was tested in the lab to verify whether air was sampled from all 4 inlets.

We have added the following lines and references to the revised manuscript:

**Revised text in §2.3:** Two one-touch fittings (1/4 inch, SMC Pneumatics) were installed on each side of the bucket. To minimize the possible effects of gas concentration gradients in the headspace, we installed a 4 inlet vertical sampling tube inside the chamber, so that air was sampled from different heights (~10, ~25, ~35 and ~50 cm) in the headspace (Clough et al, 2020).

**Revised text in §2.5:** To be able to connect the Los Gatos instrument, the soil chamber had two one-touch fittings on top.

#### References

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- Clough, Timothy J., et al. "Global Research Alliance N<sub>2</sub>O chamber methodology guidelines: Design considerations." *Journal of Environmental Quality* 49.5 (2020): 1081-1091.
- Janssens, Ivan A., et al. "Assessing forest soil CO<sub>2</sub> efflux: an in situ comparison of four techniques." *Tree physiology* 20.1 (2000): 23-32.
- Pumpanen, Jukka, et al. "Comparison of different chamber techniques for measuring soil CO<sub>2</sub> efflux." *Agricultural and Forest Meteorology* 123.3-4 (2004): 159-176

C8

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7. Appendix A1 and A2 talk about N<sub>2</sub>O calibrations and measured concentrations. The measured N<sub>2</sub>O concentrations are outside of the calibration range. While the lower range (333.7 ppb) is similar to NOAA's measurements in Brazil, the manuscript does not provide the FTIR instrument precision and therefore, it's difficult to determine whether the detected range (333.7-342.4 ppb) is within instrument drift or it's actually an increment of N<sub>2</sub>O. I don't think the authors can conclude there isn't N<sub>2</sub>O emissions.

Thank you for pointing this out. Below we will:

- explain why FTIR N<sub>2</sub>O concentration measurements outside the calibration range can be used, by stating the precision and linearity of this instrument;
- explain why we can conclude that there are low N<sub>2</sub>O emissions, by calculating the methods detection limit;
- support our statement (very low N<sub>2</sub>O emissions) with additional data.

First of all, to clarify, the mentioned range of 333.7-342.4 ppb was measured over *all* chambers during the whole week. Actual increments during individual chamber closures were a lot smaller, as discussed here below. We have clarified this in the revised manuscript text.

The FTIR-instrument has the following reported precision ( $1\sigma$ ) for 10 minute-averaged spectral analyses: 0.02  $\mu\text{mol mol}^{-1}$ , 0.2 nmol mol<sup>-1</sup>, 0.2 nmol mol<sup>-1</sup>, 0.06 nmol mol<sup>-1</sup>, and 0.04 permil, for respectively CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O, CO, and  $\sigma^{13}\text{C}$  of CO<sub>2</sub>.

C9

Measurements performed during the campaign week were set to 5 minutes, so that a precision of  $1/\sqrt{N}$  is achieved, which is 0.09 nmol mol<sup>-1</sup> for N<sub>2</sub>O.

The FTIR instrument has been shown to be linear for all gases in the ambient concentration range, and linearity was tested for N<sub>2</sub>O in the range 300-350 ppb. So while the choice of calibration gases was not optimal, we are confident that the FTIR-instrument still performs well in this concentration range.

**Detection limit of measurements:**

As also requested by reviewer 1, we calculated the minimum N<sub>2</sub>O flux detectable by this instrument and method:

- Assuming bag samples taken at 2, 5 and 8 minutes during chamber closure.
- Given: collar area 0.25 m<sup>2</sup>, chamber volume 220 L, mound volume 50 L, headspace volume 220-50 = 170 L.
- Assuming: molar volume of 24.5 L mol<sup>-1</sup> (1 atm, 25 °C).
- Minimum detectable concentration difference is ( $2\sigma$ ) 0.18 nmol mol<sup>-1</sup>.
- A concentration difference between t=2 min and t=5 min of 0.18 nmol mol<sup>-1</sup> is caused by a flux of 0.027 nmol collar/mound<sup>-1</sup> s<sup>-1</sup>.

So, given the parameters above, the chamber set up has a detection limit of 0.027 nmol mound<sup>-1</sup> s<sup>-1</sup>.

The FTIR-instrument has a cross sensitivity with CO<sub>2</sub>, which is well determined for CO<sub>2</sub> <800  $\mu\text{mol mol}^{-1}$ , but is less certain for unnaturally high CO<sub>2</sub> concentrations.

C10

For this reason, we preferred to only use the N<sub>2</sub>O headspace concentration measurements with CO<sub>2</sub> <800 μmol mol<sup>-1</sup>. Only 5 mound chamber closures had two consecutive N<sub>2</sub>O concentration points (t=2min and t=5min) with CO<sub>2</sub> <800 μmol mol<sup>-1</sup>, and only 3 sets of two-consecutive concentration points passed the minimum concentration difference of 0.18 nmol mol<sup>-1</sup>. These differences were ~0.2, ~0.3 and ~0.7 nmol mol<sup>-1</sup>, leading to a calculated N<sub>2</sub>O flux of ~0.03 - ~0.11 nmol mound<sup>-1</sup> s<sup>-1</sup>.

**Additional measurements to support statement ‘very low N<sub>2</sub>O emissions’:** In October 2020, additional valley soil N<sub>2</sub>O flux measurement were performed with the same chamber system and collars (5 collars, 3 repetitions), but with a longer closing time (35 min), without termite mounds (so lower CO<sub>2</sub>), and with 4 measurements per chamber closure. Also during these measurements, concentration increases were very low. Out of 15 measurements, 8 measurements had an R<sup>2</sup>>0.90, and calculated fluxes ranged between 0.008-0.106 nmol m<sup>-2</sup> s<sup>-1</sup> (average=0.032 nmol m<sup>-2</sup> s<sup>-1</sup>, sd=0.33). Since the valleys are known to be low on nitrogen (Quesada et al., 2010), such low fluxes are expected, and similar N<sub>2</sub>O valley soil fluxes were found by Matson et al (1987) in a field site close by.

Since the 3 calculated mound N<sub>2</sub>O flux measurements are based on only 2 consecutive headspace concentration points, no uncertainty can be given, wherefore we preferred not to state the fluxes in the previous manuscript. For the revised manuscript, we have stated the detection limit, explain why not all mound fluxes could be calculated, and support our observation of low N<sub>2</sub>O mound fluxes by the additional soil N<sub>2</sub>O flux measurements:

**Appendix A2:** Gas samples (3 samples per chamber closure) revealed stable N<sub>2</sub>O concentrations, and headspace concentrations ranged between 333.7 and 342.4 nmol mol<sup>-1</sup> over the different chamber closures. Since headspace CO<sub>2</sub> concentrations sometimes exceeded 800 μmol mol<sup>-1</sup>, and N<sub>2</sub>O-CO<sub>2</sub>

C11

cross-sensitivity becomes uncertain at higher CO<sub>2</sub> concentrations, not all 3 headspace samples per chamber closure could be used, wherefore qualitative N<sub>2</sub>O flux estimates cannot be reported. As a back-of-the-envelope calculation, N<sub>2</sub>O fluxes were calculated if 2 consecutive headspace samples were with CO<sub>2</sub> <800 μmol mol<sup>-1</sup>, and if a minimum N<sub>2</sub>O concentration difference of 0.18 nmol mol<sup>-1</sup> was found (FTIR precision (σ) for 5 min spectra is 0.09 nmol mol<sup>-1</sup>), which gave us 3 mound flux estimates ranging between ~0.03 and ~0.11 nmol N<sub>2</sub>O mound<sup>-1</sup> s<sup>-1</sup>. Similarly low fluxes were found during additionally performed flux measurements, performed as part of a substudy, which showed valley soil fluxes ranging between 0.008-0.106 nmol N<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>. The low mound fluxes would be in agreement with a previous study which suggested that termite mound N<sub>2</sub>O emissions are dependent on the N-content of the termites diet (Brauman et al., 2015), which is expected to be low in the valleys of this ecosystem (Quesada et al., 2010).

**References:**

-Matson, Pamela A., and Peter M. Vitousek. "Cross-system comparisons of soil nitrogen transformations and nitrous oxide flux in tropical forest ecosystems." *Global Biogeochemical Cycles* 1.2 (1987): 163-170.

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-384>, 2020.

C12

## ***Interactive comment on “The role of termite CH<sub>4</sub> emissions on ecosystem scale: a case study in the Amazon rain forest” by Hella van Asperen et al.***

**Hella van Asperen et al.**

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Received and published: 3 February 2021

### **Response to Anonymous Referee 3**

*Interactive comment on “The role of termite CH<sub>4</sub> emissions on ecosystem scale: a case study in the Amazon rain forest” by Hella van Asperen et al.*

This study presented a global interesting issue of termite CH<sub>4</sub>/CO<sub>2</sub> emission in an Amazonian tropical rainforest. As a case study, this in-situ measurement of termite mound emissions provided information about termite CH<sub>4</sub>/CO<sub>2</sub> production under natural conditions, it will contribution some knowledge to Biogeosciences. However,

C1

the field experiment was not well designed, and the limited data was not well analyzed. I would like to encourage the authors to revise the manuscript following my comments.

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**Thank you for your time spent on reviewing our submission!** We are grateful for your suggestions, which we have used to improve the manuscript. Below you will find a point to point response to each of your raised concerns and, if applicable, the corrected and improved manuscript text.

In addition we would like to point out that:

- we have uploaded a revised text of §4.1 (First paragraph of discussion), which is shown at the end of this review;
- we have uploaded a revised Figure 2, which is shown at the end of this review (Previous Figure 4 is Figure 2 in revised manuscript);
- we have uploaded a revised Figure 4, which is shown at the end of this review (Previous Figure 5 is Figure 4 in revised manuscript);
- we have uploaded 4 additional figures, belonging to point 7 and 8 of this review, which are shown at the end of this review;
- the given values in the text might have changed due to an improved termite weight determination.

### **General comments**

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- 1. “The blank measurements (collar with only soil and litter) showed an average**

C2

**CH<sub>4</sub> emission of 1.15 nmol collar<sup>-1</sup> s<sup>-1</sup> (L175) means the forest soil was a VERY LARGE CH<sub>4</sub> SOURCE (4.6 nmol m<sup>-2</sup> s<sup>-1</sup> or 23.2 CH<sub>4</sub> ha<sup>-1</sup> y<sup>-1</sup>). It was a FUNDAMENTAL PROBLEM! Actually, the “blank” soil should be CH<sub>4</sub> sink. Even “1.15 nmol collar<sup>-1</sup> s<sup>-1</sup>” was “-1.15 nmol collar<sup>-1</sup> s<sup>-1</sup>”, the soil CH<sub>4</sub> sink of “-23.2 kg CH<sub>4</sub> ha<sup>-1</sup> y<sup>-1</sup>” was an unbelievable large value.**

Though the reviewer correctly points out that most tropical forest soils are methane sinks, soil methane emissions in tropical ecosystems are common, especially when anaerobic conditions occur. Therefore, we disagree that this points to a fundamental problem.

In the revised manuscript we will substitute our blank collar measurement by a set of additional measurements from the surrounding area. These measurements show that the methane fluxes from the valley soil are spatially heterogeneous, but in general low. It is important to note that this heterogeneity has no impact on the given CH<sub>4</sub> emission estimates from the termite mounds, since the emissions measured from the mounds are on average a factor 627 higher than the average background soil CH<sub>4</sub> emission.

Below we will:

- provide additional information (measurements and literature) which show that soil valley CH<sub>4</sub> fluxes are heterogeneous but of low magnitude in comparison to the measured mound fluxes;
- compare the soil and mound fluxes by providing an improved Figure 4;
- provide text for the revised manuscript.

C3

#### **Additional soil valley flux measurements**

Most tropical forest soils are methane sinks (Dutaur and Verchot, 2007, Kiese et al. 2003). Nevertheless, soil methane emission in tropical ecosystems can still be observed (Carmo et al. 2006), especially when anaerobic conditions occur, such as which can be found in the valley (Sihi et al. 2020, Moura et al. 2012). This is also observed by our set of additional measurements:

***Additional measurements:*** valley soil chamber flux measurements (small chamber set up as described in §2.5), 10 soil collars, 3 repetitions, ~500 m from manuscript termite mounds, 5-50 m from igarapé (stream), measured in same week as termite mounds (March 2020), soil CH<sub>4</sub> fluxes ranged between -0.12 to 2.89 nmol m<sup>-2</sup> s<sup>-1</sup>, (median=-0.02, average=0.15, sd=0.55).

Our additional measurements show that valley soil fluxes are heterogeneous, and in general negative (median=-0.02), but that locations with relative high emissions (hotspots) can be found. Our mound adjacent soil fluxes were in general higher (0.3-8.9 nmol CH<sub>4</sub> m<sup>-2</sup> s<sup>-1</sup>, 16 soil collars), showing that mound adjacent soils are deviating from the average valley soil, likely due to the nearby presence of an active termite mound.

The magnitude of the *original* blank collar fluxes (3.9-5.4 nmol CH<sub>4</sub> m<sup>-2</sup> s<sup>-1</sup>) is quite similar to the magnitude of mound adjacent fluxes (0.3-8.9 nmol CH<sub>4</sub> m<sup>-2</sup> s<sup>-1</sup>). While the blank collar was not directly located next to a mound (~5 m of mound nr. 15), the comparison with the different datasets points at the presence of a local CH<sub>4</sub> hotspot (Subke et al. 2018), thereby not being representative as a control collar. For the revised manuscript we will use the 10 additional soil collar measurements as our ‘blank collar’ reference point.

C4

The aim of the blank 'control' measurement was to show the large difference between a 'normal' valley CH<sub>4</sub> emission (per area), and an emission (per area) when a termite mound is present. Considering the average mound emission of 25.2 nmol mound<sup>-1</sup> s<sup>-1</sup>, and the average valley soil emission of 0.03 nmol collar<sup>-1</sup> s<sup>-1</sup> (0.15 nmol m<sup>-2</sup> s<sup>-1</sup>), an average collar area emits a factor 630 more CH<sub>4</sub> when a termite mound is present. Including these complementary measurements will strengthen our message that termite mounds are hotspots in comparison to their surroundings.

We have included these additional measurements for comparison, by adapting Figure 4 (now renumbered as Figure 2, see end of this review), and by including these measurements at the following places in the manuscript:

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#### **In Methods, §2.5: Valley and mound adjacent soil fluxes**

Every mound adjacent soil flux measurement was 4 minutes, and the set of 4 collar measurements was performed once per mound, with exception of mound nr. 19. For mound nr. 13 and nr. 14, the measurements were performed on the 2<sup>nd</sup> measurement day, for mound nr. 15 and nr. 16, the measurements were done on the 3<sup>rd</sup> measurement day. Mound adjacent soil fluxes will be expressed per collar area (0.25 m<sup>2</sup>), to be better comparable to mound emissions. The same chamber set up was used in a sub study at a close by transect (~ 500 m from termite mounds) where, among others, valley soil fluxes were measured (10 collars, 3 repetitions). Measured soil fluxes from the valley will be shown for comparison.

#### **In Results, §3.1: Mound CH<sub>4</sub> and CO<sub>2</sub> emissions**

Headspace concentrations increased strongly during chamber closure, and chamber concentrations reached up to 5750 nmol CH<sub>4</sub> mol<sup>-1</sup> and 1950 μmol CO<sub>2</sub> mol<sup>-1</sup>. Mound CH<sub>4</sub> emissions ranged between 17.0 and 34.8 nmol mound<sup>-1</sup> s<sup>-1</sup> (Fig. 1), with an average emission of 25.2 nmol mound<sup>-1</sup> s<sup>-1</sup>. Additional valley measurements showed heterogeneous soil CH<sub>4</sub> fluxes with small uptake and emission taking place alongside, ranging between -0.1 and 2.9 nmol m<sup>-2</sup> s<sup>-1</sup> (med=-0.02, avg=0.15, sd=0.54). Mound adjacent CH<sub>4</sub> soil fluxes, measured at 20 and 45 cm from the mound, ranged between 0.4 and

C5

8.9 nmol CH<sub>4</sub> m<sup>-2</sup> s<sup>-1</sup> (avg=2.14, sd=2.00), and were on average enhanced in comparison to valley soils (Fig. 2). Soil valley CO<sub>2</sub> fluxes were found to range between 0.9 and 3.7 μmol m<sup>-2</sup> s<sup>-1</sup> (avg=2.14, sd=0.74) (Fig. 2). Mound adjacent soil CO<sub>2</sub> fluxes showed an average emission of 4.84 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (range=2.0-10.1, sd=2.01), thereby being enhanced with respect to the surrounding soils (Fig. 2). Mound CO<sub>2</sub> emissions, corrected for the average valley soil respiration, were ranging between 1.1 and 13.0 μmol mound<sup>-1</sup> s<sup>-1</sup>, with an average emission of 8.14 μmol mound<sup>-1</sup> s<sup>-1</sup> (Fig 1).

#### **In Discussion, §4.3:**

Valley soil CH<sub>4</sub> and CO<sub>2</sub> fluxes were similar to what was found by earlier studies (Souza (2005), Moura (2012), Chambers et al. (2004), Zanchi et al. (2012)). On average, mound adjacent soil CH<sub>4</sub> and CO<sub>2</sub> fluxes were enhanced with respect to valley soils, although differences were small, and no clear emission pattern with 'distance to mound' was observed. While mound adjacent soil fluxes are possibly enhanced, we preferred to avoid overestimation, and decided to treat termite mounds as very local hot spots, with measured fluxes only representative for the collar area of 0.25 m<sup>2</sup>. On average, CH<sub>4</sub> and CO<sub>2</sub> fluxes per collar area were found to be a factor ~630 and ~16 higher when an active termite mound was present.

#### **References:**

- Carmo, Janaina Braga do, et al. "A source of methane from upland forests in the Brazilian Amazon." *Geophysical Research Letters* 33.4 (2006).
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- Dutaur, Laure, and Louis V. Verchot. "A global inventory of the soil CH<sub>4</sub> sink." *Global biogeochemical cycles* 21.4 (2007).
- Kiese, Ralf, et al. "Seasonal variability of N<sub>2</sub>O emissions and CH<sub>4</sub> uptake by tropical rainforest soils of Queensland, Australia." *Global Biogeochemical Cycles* 17.2 (2003).
- Moura, V. S. d.: *Investigação da variação espacial dos fluxos de metano no solo em floresta de terra firme na Amazônia Central*, MSc thesis INPA/UEA, 2012.
- Sihi, Debjani, et al. "Representing methane emissions from wet tropical forest soils using microbial functional groups constrained by soil diffusivity." *Biogeosciences Discussions* (2020): 1-28.
- Souza, Juliana Silva de. "Dinâmica espacial e temporal do fluxo de CO<sub>2</sub> do solo em floresta de terra firme na Amazônia Central." (2005).
- Subke, Jens-Arne, et al. "Rhizosphere activity and atmospheric methane concentrations drive variations of methane fluxes in a temperate forest soil." *Soil Biology and Biochemistry* 116 (2018): 323-332.
- Zanchi, Fabrício B., et al. "Soil CO<sub>2</sub> exchange in seven pristine Amazonian rain forest sites in relation to soil temperature." *Agricultural and Forest Meteorology* 192 (2014): 96-107.

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C6

**2. An early study in a Southeast Asian tropical forest showed that the populations of termites was 3,000 – 4,000 m<sup>-2</sup>, 60% of which being wood-feeding termites and 30% being either litter-feeding or humus-feeding species (Chiba, 1978). This population density was supported by many recent studies showed in this manuscript (L356-358). Why this study did not include the major termite species (wood-feeding)?**

When designing this field study, we decided to focus only on 1 species, so that effects of interspecies variability could be excluded. In addition, since mound emission was one of the focus points, our preference was to look for an epigeal nest (mound) building species.

Wood-feeding termite species are most likely **not** the major termite species in the Amazon rainforest. The distribution of feeding groups within an assemblage varies around the globe, so while wood-feeding termites might be the major termite species in a Southeast Asian tropical forest (Chiba, 1978), this can be different in other tropical forests.

Jones and Eggleton (2011), compiling data of global biogeography of termites, states that soil-wood interface feeders, such as *N. Brasiliensis*, composes the most diverse and dominant group in Neotropical rainforests (page 491). In addition, the species *N. Brasiliensis* is one of the most common species in our region, and one of the most abundant among mound-builder species (Dambros et al 2016, Pequeno et al. 2013).

In the revised manuscript, we have added the following lines to the Introduction:

C7

**Revised text in Introduction:** In addition, for the Amazon, it is expected that most termites are soil-feeding (Jones and Eggleton, 2011), a group which are expected to be the strongest emitters of CH<sub>4</sub> (Bignell and Eggleton, 2000; Brauman et al., 1992).

**Revised text in Introduction:** In this paper, we are presenting a case study performed in a tropical rain forest in the Amazon, where we measured the emission of CH<sub>4</sub> and other gases of epigeal (above-ground) termite nests of the species *Neocapritermes Brasiliensis*, a soil-feeding species abundant in the Amazon (Constantino, 1992; Pequeno et al., 2013), and one of the most common species in the region (Dambros et al. 2016).

**References:**

- Dambros, Cristian S., et al. "Association of ant predators and edaphic conditions with termite diversity in an Amazonian rain forest." *Biotropica* 48.2 (2016): 237-245
- Jones, D. T., and P. Eggleton. "Global biogeography of termites: a compilation of sources. In 'Biology of Termites: A Modern Synthesis' (Eds DE Bignell, Y. Roisin and N. Lo.) pp. 477–498." (2011).

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**3. Large variations in both CH<sub>4</sub> and CO<sub>2</sub> emissions (Figure 1; L221-222, L240) among the mounds suggest that the five applicates (mounds) was not enough to represent the ecosystem level CH<sub>4</sub> and CO<sub>2</sub> emissions. From your statement (2.6: sub sample), I would guess that your CH<sub>4</sub>/CO<sub>2</sub> flux measurements were conducted for all the 19 mounds but not only 5 mounds (Figure 1). If my guess is correct, the authors should explain (in the Method) the reasons for not including the data from other mounds, for example, the other mounds were not active mounds.**

From the reviewers comment, we realize that confusion might arise about the amount of mounds sampled. Below we will:

- clarify that we measured fluxes of 5, and not 19, termite mounds;

C8

- clarify how many mound *subsamples* have been measured;
- report additional subsample measurements which confirm the termite emission factor, and present a new Figure 5, which will show these additional measurements;
- provide the improved manuscript text for §2.6, and for other parts of the manuscript.

Our mound selection procedure for the 5 mounds was as follows:

- Firstly, we searched for mounds, which were suitable for flux chamber measurements (sufficient space for collar installation, not attached to tree). We found 20 suitable and active mounds, and we sampled each mound and determined the species at the *Laboratory of Systematics and Ecology of Soil Invertebrates* at INPA. Table 1 in the manuscript gives an overview of the found species per mound.
- When further selecting individual mounds of these 20 mounds, we only choose mounds of the same species, so that effects of interspecies variation could be excluded.
- For practical reasons, we choose a set of mounds which were closely located to each other.
- With these criteria in mind, we selected the mounds from which fluxes would be measured, which were mounds nr. 13, nr. 14, nr. 15, nr. 16 and nr. 19.

C9

The choice of limiting our flux measurements to 5 mounds was based on practical considerations (hours of daylight, days in the field, distance to cover), which were especially time constrained due to our additional bag sampling measurements (Appendix A). For a possible follow up study, we would leave this element out.

**Moreover, the authors should explain why the sub sample experiment was only conducted for one mound (L161: “only one sub sample was found suitable from the all 19 mounds”).**

The sentence copied by the reviewer is different than the sentence stated at line 161, which was:

*‘From the sample from mound 19, only one suitable sub sample was found’*

To clarify: for each of the 5 selected mounds, we sampled one solid (not crumbling) piece, of which we took 3 subsamples, of which we measured emissions and counted termites. In principle, this would lead to 15 subsamples. Nevertheless, due to practical problems at mound 19, we only managed to separate 1 suitable subsample, wherefore the total amount of subsamples was 13, as shown in the original Figure 5 of the manuscript.

In the last few months, we have performed additional measurements:

- Additional measurement 1 (AM1): performed in October 2020 (dry season), with 15 subsamples of the same mounds (mounds nr. 13, nr. 14, nr. 15, nr. 16 and nr. 19). A termite emission factor of 0.0002976 (se=1.32\*10<sup>-5</sup>) CH<sub>4</sub> per termite per second was found.

C10

- Additional measurements 2 (AM2): performed in December 2020 (transition dry/wet season), with 5 subsamples, taken from a new mound of the same species. A termite emission factor of 0.0003043 (se=1.41\*10<sup>-5</sup>) CH<sub>4</sub> per termite per second was found.

For the revised manuscript, we have added these CH<sub>4</sub> termite emission measurements to the text and to Figure 5, to show the reader the consistency of the termite emission factor between mounds and seasons. Nevertheless, since we prefer to combine only measurements obtained during the same field campaign week, the manuscript estimates and derivations are based on the original determined termite emission factor of 0.0002985 nmol termite<sup>-1</sup> s<sup>-1</sup>.

We have improved Figure 5 (in revised manuscript, renumbered as Fig. 4), which we uploaded, and which can be found at the end of this review. In the text, we have made the following changes:

**Revised text caption Table 1:** Termite mounds: location, dimensions, and observed species. Termite mound volumes were estimated by Eq. (1), and mound surfaces were estimated by mathematically considering the lower part of the mound as a column, and the upper part as half a sphere. In mound nr. 1, two different termite species were found. The five mounds indicated in bold (mound nr. 13, nr. 14, nr. 15, nr. 16 and nr. 19) were the mounds selected for flux measurements.

**Revised text in §2.6:** At mound nr. 13, nr. 14, nr. 15, nr. 16 and nr. 19, after the last mound flux measurement, a mound sample was taken of approximately 1 L volume. From this, three small sub samples were taken (volume not determined).

**Revised text in §2.6:** To verify whether the termite emission factor was stable between seasons and mounds, additional measurements were performed. In October 2020 (dry season), the same type of measurements were performed on 15 subsamples of the same termite mounds, and in December 2020 (transition dry-wet season), 5 subsamples of a different mound of the same species were analysed.

C11

**Revised text in §3.2:** CH<sub>4</sub> and CO<sub>2</sub> emissions of 13 mound sub samples were measured. For each sub sample, the measured gas production was plotted over the counted termites (Fig. 4). The fitted line has a forced intercept at y=0. For CH<sub>4</sub>, an emission of 0.0002985 nmol termite<sup>-1</sup> s<sup>-1</sup> was found (se=1.77\*10<sup>-5</sup>), fitted with an R<sup>2</sup> of 0.95 (n=13). The set of additional measurements resulted in similar termite emission factors namely 0.0002976 nmol termite<sup>-1</sup> s<sup>-1</sup> (se=1.32\*10<sup>-5</sup>) and 0.0003043 nmol termite<sup>-1</sup> s<sup>-1</sup> (se=1.41\*10<sup>-5</sup>), for respectively the measurements of October and December 2020. Given estimates in this paper are based on the originally determined termite emission factor of 0.0002985 nmol termite<sup>-1</sup> s<sup>-1</sup>. For CO<sub>2</sub>, an emission of 0.1316 nmol termite<sup>-1</sup> s<sup>-1</sup> was found (se=2.59\*10<sup>-2</sup>), with an R<sup>2</sup> of 0.68 (n=13). Excluding the out liers (32, 14.9 nmol s<sup>-1</sup> and 313, 80.9 nmol s<sup>-1</sup>) gives an R<sup>2</sup> of 0.88 (n=11), with a CO<sub>2</sub> emission of 0.074 nmol termite<sup>-1</sup> s<sup>-1</sup> (se=8.5\*10<sup>-3</sup>).

**Revised text in §4.3:** Furthermore, exploratory dry season measurements of the same mounds showed emissions of the same magnitude (not shown), and additional dry season mound subsample measurements revealed very consistent termite CH<sub>4</sub> emission factors (Fig. 4). We therefore do not expect that mound CH<sub>4</sub> emissions are only of importance in the valleys, or only present in the wet season.

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**4. In tropical forest, the termite mounds have different size and different shapes, and many are already not active mounds. This study only selected the relatively small size of termite mound (Table 1), thus it is not surprised that the authors gave the conclusion of weak correlation between CH<sub>4</sub> emission and mound size (3.1; Fig. 3).**

For this study, we only measured active termite mounds; but during our search in the first phase of the research, no abandoned epigeal mounds were found, and only 1 abandoned tree nest was found.

Furthermore, we also point out to the readers that termite mounds appear in many

C12

different sizes and shapes (§4.1). Because we are aware that different species build different type of nests, we only searched for a *species-specific* correlation between mound size and mound emission.

It is common that a certain species-specific correlation is found between mound size and mound population (Lepage and Darlington, 2000, Pequeno et al. 2013), wherefore it is also reasonable to expect a relationship between mound size and mound emission. Nevertheless, as Pequeno et al. (2013) pointed out, mounds from the species *N. Brasiliensis* have been shown to **not** present a strong correlation between mound size and mound population. Therefore, it is not surprising that we also did not find a strong relationship between mound size and mound emission.

To shorten the manuscript, we have decided to remove the original Figure 3, and only report our findings in the text. The discussion on variation in termite mounds and shapes, and on correlation between emission and mound size, can be found in the Discussion in §4.1 and §4.2:

**Revised text in §4.1:** There is a large variety in type of termite mounds (shape and size are dependent on species, ecosystem, climate (Noirot and Darlington, 2000)), explaining the wide range of reported termite mound CH<sub>4</sub> emissions (Table 2, middle and lower part).

**Revised text in §4.2:** Interestingly, Pequeno et al. (2013) concluded that mound volume is a weak indicator for population size for nests of the species *N. brasiliensis*, as also indicated by the weak correlation we found between mound volume and mound CH<sub>4</sub> emissions .

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## 5. This in-situ measurement could not be able to partition the contribution of

C13

**mound soil (CO<sub>2</sub> source but CH<sub>4</sub> sink) from termite, thus the termite CH<sub>4</sub> emission could be underestimated but termite CO<sub>2</sub> emission could be overestimated. The results should be calibrated, because the structure and nutrients of the mound-soil are different from the normal soil (blank soil in this study).**

Based on in-situ mound measurements as conducted here, it is impossible to partition the contribution of mound material vs termites. This was not done in any comparable studies. As the reviewer correctly points out, for CH<sub>4</sub> this will lead to an underestimation, and for CO<sub>2</sub> to an overestimation of estimated termite emissions. However, studies like ours determine the overall termite-induced emissions and this is the aim of our study.

Below we will:

- evaluate the impact of soil and mound emissions/uptake on our CH<sub>4</sub> and CO<sub>2</sub> termite estimates;
- elaborate on direct and indirect termite CO<sub>2</sub> emissions (termite-induced CO<sub>2</sub> emissions);
- show how we improved this part in the manuscript.

### **The impact of mound emissions/uptake on our CH<sub>4</sub> and CO<sub>2</sub> termite estimates**

**For mound CH<sub>4</sub> emission:** overestimation is not expected: surrounding valley soils show heterogeneous but in general low magnitude (negative) fluxes, ranging between -0.03 to 0.72 nmol collar<sup>-1</sup> s<sup>-1</sup> (median=-0.01, average=0.03, sd=0.55, collar= 0.25 m<sup>-2</sup>). Considering the average mound emission (25.2 nmol collar<sup>-1</sup> s<sup>-1</sup>),

C14

the contribution of an average soil CH<sub>4</sub> flux to the mound emission would lead to an overestimation of < 1%.

As the reviewer correctly points out, underestimation is more likely, due to the uptake of CH<sub>4</sub> by mound material, as also discussed in the manuscript. To give a lower bound assessment, we have used the *net* mound CH<sub>4</sub> emissions for our ecosystem estimates.

**For mound CO<sub>2</sub> emission:** we cannot be sure which part of the mound emitted CO<sub>2</sub> derives directly from termites and which part derives from soil and mound respiration. To account for soil respiration, the most attainable approach is to determine the average CO<sub>2</sub> emission of the surrounding soils and subtract this value from the measured mound CO<sub>2</sub> emissions. Values shown in the manuscript are the corrected values.

Mound respiration however is an indirect effect of termite activity, and thereby a termite-*induced* emission. Partitioning direct and indirect termite CO<sub>2</sub> emissions is difficult, and impossible to determine without disturbing the mound. We will therefore clearly state this in the manuscript, and discuss that *direct* termite-emitted CO<sub>2</sub> emissions are presumably lower.

The topic of soil and mound respiration is discussed in the following places of the revised manuscript:

**Revised text in §2.3, last sentence:** Unless mentioned otherwise, given mound CO<sub>2</sub> emissions are corrected for the estimated contribution of soil respiration, by subtracting the average valley soil CO<sub>2</sub> emission (see §2.5).

C15

**Revised text in §3.1:** Soil valley CO<sub>2</sub> fluxes were found to range between 0.9 and 3.7 μmol m<sup>-2</sup> s<sup>-1</sup> (avg=2.14, sd=0.74) (Fig. 2). Mound adjacent soil CO<sub>2</sub> fluxes showed an average emission of 4.84 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (range= 2.0 - 10.1, sd=2.01), thereby being enhanced with respect to the surrounding soils (Fig. 2). Mound CO<sub>2</sub> emissions, corrected for the average valley soil respiration, were ranging between 1.1 and 13.0 μmol mound<sup>-1</sup> s<sup>-1</sup>, with an average emission of 8.14 μmol mound<sup>-1</sup> s<sup>-1</sup>.

**Revised text in §4.1:** Mound CO<sub>2</sub> emissions and the termite CO<sub>2</sub> emission factor were similar, or a little higher, in comparison to the few values found in literature. Nevertheless, since mound material and termites were measured together, the contribution of *indirect* termite emissions, i.e. mound respiration, cannot be quantified, so that the *direct* termite-produced CO<sub>2</sub> emission is presumably lower.

**Revised text in §4.3:** Nevertheless, since the 'emission per mound' as well as the 'termite emission factor' are both affected by indirect effects of termite activity (mound respiration), the contribution of *direct* termite-emitted CO<sub>2</sub> into the ecosystem is presumably smaller.

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**6. Chamber volume (CV in L145; L159-163, L258-262) is a major parameter for calculation of flux rate (Equation 2). If the exact volume of the sample mound was not known, means CV was not known, based on the calculation using equation 2, the estimated both CH<sub>4</sub> and CO<sub>2</sub> fluxes (Table 2, 3; L218-222, L241-243) would be absolutely under- or over-estimated.**

In all our assumptions, we have followed literature (Clough et al. (2019), Kirschke et al. (2013), Krishna and Araujo (1968), Pequeno et al. (2013), Ribeiro (1997), Sanderson (1996)), and have tried to aim for a lower bound appraisal. For example, for mound volume estimation, we have chosen to use the equation given by Pequeno (2013). Furthermore, we considered the mound as a solid body, even if a previous comparable study did not (Martius et al. 1993), thereby possible underestimating our mound emissions by ~ 30% (see text in §4.1, copied below).

C16

So even if CV is an uncertain parameter, by communicating this clearly to the reader, and by demonstrating that our estimate is lower bound, our message, that termite mounds and termites are important in this ecosystem, remains strong.

**Revised text in §4.1:** An additional possible underestimation is caused by the estimated corrected chamber volume, as used in Eq. (2). In this study, we considered the mound volume as a solid body. A previous study considered the solid nest volume as 10% of the actual mound volume (Martius et al., 1993), leading to a larger corrected chamber volume, and therefore to larger calculated mound emissions. By use of this approach, average measured emissions would increase by almost 30% to be  $32.7 \text{ nmol CH}_4 \text{ mound}^{-1} \text{ s}^{-1}$  instead of  $25.2 \text{ nmol CH}_4 \text{ mound}^{-1} \text{ s}^{-1}$ .

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**7. In my experience, this  $R^2 > 0.95$  (L178 and other places) was non-believable. The chamber was relatively (or very) large (220 L), UGGA internal (pump) flow was only about  $350 \text{ mL min}^{-1}$ , the chamber air could not be mixed without installing one or two micro fans inside the chamber, because it takes about 630 min to replace the chamber air if only depending on the UGGA internal pump. Particularly, the chamber was about 1 m high, the emitted  $\text{CH}_4$  and  $\text{CO}_2$  was not be able to be mixed inside the chamber if only depending on both diffusion and UGGA internal pump. Moreover, based on the bag sampling (A1),  $\text{CH}_4$  flux could be estimated. The authors are suggested to compare the result with that of mound chamber and sub sample.**

Thank you for raising this topic, which we will answer point by point (7.1, 7.2, 7.3):

7.1: Mixing in the chamber, where we explain why we did not install a fan, and how we

C17

ensured mixed chamber air;

7.2: Linearity of headspace concentration increase, where we show that, despite small fluctuations, linear regression ( $d\text{CO}_2/dt$ ,  $d\text{CH}_4/dt$  and  $d\text{CH}_4/d\text{CO}_2$ ) was performed with an  $R^2 > 0.95$ ;

7.3: FTIR bag measurements, where we elaborate on estimation of mound  $\text{CH}_4$  fluxes based on bag measurements.

**7.1) Concerning the mixing of the chamber**, first a small side note: termite mounds emit  $\text{CH}_4$  from its entire surface, thereby presenting a sphere-shaped source of 45-65 cm height *inside* the chamber head space. Therefore, we do not expect a large difference between  $\text{CH}_4$  concentrations at the top and the bottom of the chamber head space.

We were hesitant about installing a small mixing fan. On the one hand, the absence of a mixing fan might lead to an underestimation of the flux (Christiansen et al. 2011). On the other hand, a mixing fan might lead to turbulence in the head space (Janssens et al. (2000), Pumpanen (2004)), which might induce unrepresentatively high  $\text{CH}_4$  emissions from the mound.

Since we wanted to avoid overestimation of termite mound  $\text{CH}_4$  fluxes, we decided to not install a mixing fan. Instead we installed a 4 inlet vertical sampling tube inside the chamber, a technique to minimize the effects of gas concentration gradients in the head space (Clough et al, 2020). Inside the chamber at fitting height ( $\sim 30$  cm), a T-piece with two 20 cm-long Teflon tubing was positioned vertically, and two small incisions were made, so that head space air was sampled from 4 different heights (approx. at 10, 25, 35 and 50 cm height from the soil). The sampling tube was tested in the lab to verify whether air was sampled from all 4 inlets.

C18

We have added the following lines to the manuscript, and have added the following references to the manuscript.

**Revised text in §2.3:** Two one-touch fittings (1/4 inch, SMC Pneumatics) were installed on each side of the bucket. On the inside of the bucket, a 4 inlet vertical sampling tube was placed, so that air was sampled from different heights ( 10, 25, 35 and 50 cm) in the headspace (Clough et al, 2020).

**References:**

- Christiansen, Jesper Riis, et al. "Assessing the effects of chamber placement, manual sampling and headspace mixing on CH<sub>4</sub> fluxes in a laboratory experiment." *Plant and soil* 343.1-2 (2011): 171-185.
- Clough, Timothy J., et al. "Global Research Alliance N<sub>2</sub>O chamber methodology guidelines: Design considerations." *Journal of Environmental Quality* 49.5 (2020): 1081-1091.
- Janssens, Ivan A., et al. "Assessing forest soil CO<sub>2</sub> efflux: an in situ comparison of four techniques." *Tree physiology* 20.1 (2000): 23-32.
- Pumpanen, Jukka, et al. "Comparison of different chamber techniques for measuring soil CO<sub>2</sub> efflux." *Agricultural and Forest Meteorology* 123.3-4 (2004): 159-176

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**7.2) Concerning the linear regressions of dCH<sub>4</sub>/dt with R<sup>2</sup> > 0.95**, first of all, we would like to rectify two details from the manuscript. At line 132, we state that chambers were closed for 25 minutes, but this should have been 20 minutes. In addition, we state that we are correcting for sampling bag dilution (line 147), a decision we later reversed because gradients were only calculated over headspace concentrations after bag filling: this sentence should have been deleted.

In the figure (Review-Figure 7.2a, end of this review) we show the *last* 10 minutes (of total chamber closure) of five headspace chamber increases, measured on one day. As can be seen, even while fluctuations occur, the linear regression line still captures the shape of the line well, and still an R<sup>2</sup> > 0.95 can be found.

C19

To further clarify the Review-Figure 7.2a: chamber closures were for 20 minutes, and sample bag filling (Appendix A of manuscript) was done at minute 3, 5 and 8. To determine the actual headspace concentration increase, we used the increment *after* the first 10 minutes, when the chamber was less disturbed by the bag sampling. The fluctuations, clearly visible for mound nr. 14 and nr. 15, take place at the 'beginning' of this second time window. Part of this might be explained by the remaining effect of the bag sampling, but we also expect that our presence close to the flux chamber (when closing and labeling the sampling bags) might have had an effect: in a different experiment, we saw headspace fluctuations, which disappeared when we distanced ourselves from the chamber. This is something we should keep in mind for a possible next experiment.

We realize that this part of the *Material and Methods* needs to be improved, and we have revised the text in §2.4 to:

**Revised text in §2.4:** Linear regression was used to derive the concentration increase, and given error bars are the propagated standard error of the linear regression slope. Concentration increases were calculated over the last 10 minutes of the chamber closure, to avoid possible effects of the bag filling. Nevertheless, if clear headspace concentration fluctuations were observed in the beginning of this time window, possibly by a remaining effect of the bag filling, the window was shortened by a maximum of 2 minutes (leaving a time window of 8 minutes). All calculated dC/dt increases showed a R<sup>2</sup> > 0.95.

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**Concerning the linear regressions of dCH<sub>4</sub>/dCO<sub>2</sub> with R<sup>2</sup> > 0.95**, at line 178 we stated:

*The CH<sub>4</sub> and CO<sub>2</sub> concentration increases inside the closed flux chamber were*

C20

strongly correlated ( $R^2 > 0.95$  for each chamber closure).

This statement is true: during all chamber closures, fluctuations in  $\text{CH}_4$  and  $\text{CO}_2$  concentrations were strongly correlated, with  $R^2 > 0.95$ .

As also discussed in §4.1, both gases are showing a strong correlation, AND showing fluctuations of the same magnitude and at the same moment. We therefore assume that these fluctuations are caused by an external factor, like wind or human disturbance, sucking/pushing out high-concentration air from the chamber. This can also be seen in the figure below (Review-Figure 7.2b), where some fluctuations seem to happen when bag filling is performed. Nevertheless, it can also be seen that the gradient recovers after each fluctuation. In addition, if chamber air is diluted, the gradient will be underestimated, thereby not weakening the message of our paper.

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**7.3) Concerning the FTIR bag measurements**, bag samples were aimed to be sampled at 2, 5 and 8 minutes after chamber closure ( $\Delta t=3$  min). Nevertheless, during the field campaign, variation in  $\Delta t$  occurred, such as due to changing pump performance (due to varying battery voltage), or due to timing inconsistencies. Since  $\Delta t$  between bag samples is not known with certainty, a flux based on the bag samples *alone* cannot be given. As described in the manuscript, we have used the Los Gatos fluxes to deduct the FTIR fluxes.

**Revised text in A2:** To calculate the fluxes of  $\text{N}_2\text{O}$  and  $\text{CO}$ , FTIR-measured bag concentrations of  $\text{N}_2\text{O}$ ,  $\text{CO}$  and  $\text{CO}_2$  were used. For each chamber closure, the  $d\text{N}_2\text{O}/dt$ ,  $d\text{CO}/dt$  and  $d\text{CO}_2/dt$  were calculated so that the ratios  $d\text{N}_2\text{O}/d\text{CO}_2$  and  $d\text{CO}/d\text{CO}_2$  could be derived. To calculate the fluxes of  $\text{N}_2\text{O}$  and  $\text{CO}$ , the ratios were combined with the in-situ measured mound  $\text{CO}_2$  flux, as measured by the Los Gatos instrument. This approach was chosen because the intended 3 min bag sampling interval was not always

C21

accomplished, so that a fixed  $\Delta t$  could not be assumed with certainty.

For the reviewers interest, here below (Review-Figure 7.3) we show one example of bag concentrations, measured by the FTIR, in comparison to Los Gatos concentrations. During this measurement, sampling with 3 minutes interval was close to accomplished, so that  $\Delta t$  approximated 3 min.

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**8. Data was too limited; I strongly encourage the authors to show the data measured in the dry season (L348-350) and compare it with that of wet season showed in this manuscript.**

The measurements in the dry season were performed as an *exploratory* measurement, to see whether the mounds were still active, and fluxes were similar as in the wet season. Nevertheless, due to time limitations, measurements were only performed once. For this reason, we do not show them in the manuscript.

For the reviewers interest, we can show the additional measurements from October 2020 here in the review (Review-Figure 8, dark red bars). Measurements from mound nr. 13, nr. 15 and nr. 16 were in the same range as measured in March 2020, while fluxes from mound nr. 14 and nr. 19 were deviating. Considering the long time period which passed (~6 months), the change could be due to increased/decreased population size and/or activity, or (in case of mound nr. 14) a collar which was not well installed. Since it was outside the scope of the presented research, we have not structurally looked into the reasons for the difference, and prefer not to speculate too much. Nevertheless, these measurements confirm that the mounds are also active in the dry season, and remain hotspots in the ecosystem.

C22

Additional 'dry season' measurements of mound *sub* samples, used to determine the termite emission factor, were performed in two sets. For the revised manuscript, the new figure and revised text can be found at point 3 of this review.

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**9. Overall, using the limited data to scale up it to ecosystem (4.3) and global (4.4) levels would no doubt create large uncertainty. The authors are suggested to cancel or at least shorten these two issues.**

**For our upscaling to ecosystem level (§4.3):** while this estimate is based on limited data, it is important to note that up scaling was only done for our *local* ecosystems CH<sub>4</sub> budget.

In addition, our fieldsite is situated in a geographical unique region: due to the nearby-presence of the institute INPA (which has been doing Amazon research since the 50's), many termite and ecosystem studies have been performed closeby (see bulletpoints below). Therefore, assumptions (mound density numbers, termite abundance) and comparisons (available ecosystem CO<sub>2</sub> and CH<sub>4</sub> fluxes) can be stated with more certainty than anywhere else in the Amazon. So, because of this strong complementary local dataset, we can estimate and evaluate the role of termites for our *local* CH<sub>4</sub> budget

**Local studies:**

- 5 local studies (< 50 km) reported mound density numbers (Queiroz, (2004), Oliveira et al., (2016), Dambros et al., (2016), (de Souza and Brown, (1994), Ackerman et al., (2007);
- 1 local study (< 50 km) studied the weight and mound-population dynamics of the same termite species (Pequeno 2013);
- Several studies focussing on ecosystem CO<sub>2</sub> and CH<sub>4</sub> were performed at the exact same fieldsite (Chambers et al.,

C23

(2004), Moura. (2012), de Souza (2005), Zanchi et al. (2014), Querino et al. (2011).

**For our upscaling to global levels,** we have followed the method and assumptions as described by Kirschke et al. (2013). To clarify, we only have substituted the 'termite emission factor' value, all the other upscaling has been adapted from Kirschke et al. (2013). In addition, it is important to make the link to the global levels, which informs the reader about the important role of model parameters (termite density and termite emission factors), thereby clearly showing that this is an uncertain part of the CH<sub>4</sub> budget.

As suggested by Reviewer 2, the text on the global estimate has been extended and improved:

*Termites contribution to tropical South America CH<sub>4</sub> budget (in §4.3)*

In current CH<sub>4</sub> budget studies, a termite emission factor of  $2.8 \mu\text{g CH}_4 \text{ g}_{\text{termite}}^{-1} \text{ h}^{-1}$  is used for 'Tropical ecosystems and Mediterranean shrub lands' (Kirschke et al., 2013; Saunio et al., 2020), which is mainly based on field studies in Africa and Australia (Brümmer et al., 2009a; Jamali et al., 2011a, b; Macdonald et al., 1998; MacDonald et al., 1999). The only termite emission factor measured for the Amazon rain forest is by Martius et al. (1993) ( $3.0 \mu\text{g g}_{\text{termite}}^{-1} \text{ h}^{-1}$ ) for a wood-feeding termite species, which are expected to emit less CH<sub>4</sub> than soil-feeding termites (Bignell and Eggleton, 2000; Brauman et al., 1992). As a 'back-of-the-envelope' calculation, based on (Kirschke et al., 2013): 36% of global termite emission (11 Tg) is expected to come from the region of 'tropical South America' ( $0.36 \cdot 11 = 3.96$  Tg). Substituting the emission factor of 2.8 with the newly found  $5.6 \mu\text{g CH}_4 \text{ g}_{\text{termite}}^{-1} \text{ h}^{-1}$  would increase this regions estimate to 7.92 Tg, and the global estimate to 14.96 Tg.

Our study points out that termite emissions are still an uncertain source in the CH<sub>4</sub> budget, and are especially poorly quantified for the Amazon rain forest. Measurement of CH<sub>4</sub> emissions from different termite species, preferably covering species of different feeding or nesting habits, in combination with more precise termite distribution and abundance data, would allow more precise estimates and a better understanding of the role of termites in the CH<sub>4</sub> budget.

C24

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## Minor Comments:

**L10 (L211, L284): Reads are easily be confused by the colony size and population, also the colony size of 50-120 thousands individuals and  $54.6-116.6 \times 10^3$  termites per mound should be unified.**

Thank you for this point. We have improved this, and have now tried to use words for large numbers (as advised by the guidelines of Biogeosciences). We have unified this in the revised manuscript.

**L120: Change "mound 15" to "mound #15".**

We have made all mound numbering consistent by adding 'nr' every time a specific mound is mentioned, and we have used '#' when discussing a measurement repetition (For example, measurement #1, #2, and #3 of mound nr. 13.)

**L120: Only one control (blank) made this result (also see above) weaker.**

We have revised this part of the manuscript, as demonstrated at point 1 in this review.

**L130: The distance between the UGGA and chamber was 2 m.**

This tubing was of 2 meter length, but the distance was usually a little less. Two meter length was chosen to have some flexibility about where to place the UGGA.

**L131: It is about 350 mL/min (from LGR).**

We have corrected this.

C25

**L150-157 (§2.5): Soil flux chamber had no mixing fan would have the same problem with the mound chamber (see above)**

For the small flux chamber, the volume is only 4.7 L, wherefore the circular LGR flow of 0.35 L/min induces basic chamber mixing. In addition, as found by different studies, a fan might induce unnatural turbulence, leading to an overestimation of the flux (Janssens et al. 2000, Pumpanen et al. 2004). Since we wanted to avoid overestimation of our fluxes, and since our CO<sub>2</sub> fluxes (without a fan), measured in different places in the ecosystem, are quite close to earlier studies (Chamber et al. 2004, Souza 2005, Zanchi et al. 2014), we decided to not install a small fan inside this chamber.

### References

- Chambers, Jeffrey Q., et al. "Respiration from a tropical forest ecosystem: partitioning of sources and low carbon use efficiency." *Ecological Applications* 14.sp4 (2004): 72-88.
- Janssens, Ivan A., et al. "Assessing forest soil CO<sub>2</sub> efflux: an in situ comparison of four techniques." *Tree physiology* 20.1 (2000): 23-32.
- Pumpanen, Jukka, et al. "Comparison of different chamber techniques for measuring soil CO<sub>2</sub> efflux." *Agricultural and Forest Meteorology* 123.3-4 (2004): 159-176.
- Souza, Juliana Silva de. "Dinâmica espacial e temporal do fluxo de CO<sub>2</sub> do solo em floresta de terra firme na Amazônia Central." (2005).
- Zanchi, Fabrício B., et al. "Soil CO<sub>2</sub> exchange in seven pristine Amazonian rain forest sites in relation to soil temperature." *Agricultural and Forest Meteorology* 192 (2014): 96-107.

**L177: Soil CO<sub>2</sub> emission of  $0.47 \mu\text{mol collar}^{-1} \text{s}^{-1}$  ( $1.87 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was too small. The authors are suggested to compare it with other studies in tropical forests.**

Tropical soils usually emit more than  $1.87 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , although wet soils with anaerobic properties, such as our local valley soils, have been shown to emit lower magnitudes (Souza, 2004).

We have extended and improved our soil CO<sub>2</sub> emission estimate by reporting valley

C26

soil CO<sub>2</sub> emissions from 10 soil collars (3 repetitions), which gave an average valley emission of 2.15 μmol m<sup>-2</sup> s<sup>-1</sup> (sd=0.74), which is similar to what was found by Chamber et al. (2004), and Zanchi et al. (2014).

The revised manuscript text concerning these additional measurements can be found at point 5 of this review.

**L187-189: Move to the Method, and L189-192 move to the caption of Figure 4.**

Thank you for the suggestion, we have corrected this.

**L252-257: The statement of “air flow below the soil collar” does not make sense.**

We have rephrased the sentence.

**Equation 2: not completed; missed chamber pressure and chamber temperature.**

Since we are stating dC/dt in μmol m<sup>-3</sup> s<sup>-1</sup>, and not in μmol mol<sup>-1</sup> s<sup>-1</sup>, the pressure and chamber temperature term in this equation become redundant. We have chosen for this equation form, since we assume a stable temperature, as stated §2.4.

**L311: The statement of “Mound adjacent soil flux measurements showed no enhanced CH<sub>4</sub> and CO<sub>2</sub> fluxes in comparison to soils in the blank collar” does not consist with the results. For example, adjacent CO<sub>2</sub> flux (1.3) was almost three times of blank soil (0.47).**

Thank you for pointing this out. The revised manuscript text for this part is given in the beginning of this review (review point 5).

C27

**L337: 11 g is the maximum value; the variation range should be listed. Consequently, the following value of 0.5-1.0 nmol m<sup>-2</sup> s<sup>-1</sup> was overestimated.**

The biomass value of 11 g m<sup>-2</sup> has been stated and used as a standard for tropical rainforests in different previous studies (Bignell and Eggleton 2000, Sanderson, 1996, Sugimoto et al. 1998).

In addition, for our *local* ecosystem, the termite biomass estimate of 11 g termite m<sup>-2</sup> is **not** considered a maximum value, and possibly even an underestimation:

A recent paper links the termite biomass to GPP, thereby correcting the termite biomass estimate for less active tropical ecosystems (see figure S6 in Kirsche et al. 2013). Since we are only using the termite biomass estimate for our *local* ecosystem, for which the GPP has been estimated to be 3000 g C m<sup>-2</sup> year<sup>-1</sup> (Chambers et al. 2004), based on Figure S6 we deduced that the termite biomass is even higher than 11 g m<sup>-2</sup>. This is also confirmed by a *local* study, performed in a fieldsite close by, where a termite biomass of 14-17 g m<sup>-2</sup> was found (Martius, 1998).

While the termite biomass is likely higher than 11 g m<sup>-2</sup> in our ecosystem, we prefer to stay in sync with previous studies on tropical ecosystems, and will continue with this lower bound appraisal for termite biomass.

#### References

- Bignell, D. E. and Eggleton, P.: Termites in ecosystems, in: Termites: evolution, sociality, symbioses, ecology, pp. 363–387, Springer, 2000.
- Martius, Christopher. "Occurrence, body mass and biomass of *Syntermes* spp. (Isoptera: Termitidae) in reserva Ducke, Central Amazonia." Volume 28, Número 3, Pags. 319-319 (1998).
- Sanderson, M.: Biomass of termites and their emissions of methane and carbon dioxide: A global database, *Global Biogeochemical Cycles*, 10, 543–557, 1996.
- Sugimoto, Atsuko, et al. "Methane oxidation by termite mounds estimated by the carbon isotopic composition of methane." *Global Biogeochemical Cycles* 12.4 (1998): 595-605.

C28

#### **L415: Check the grammar.**

Thank you for pointing this out. We have revised this part to:

**Revised text in A1:** For calibration of the instrument, 2 calibration gases were used: Gas 1 with values 381.8  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ , 2494.9 nmol  $\text{CH}_4 \text{ mol}^{-1}$ , 336.6 nmol  $\text{N}_2\text{O mol}^{-1}$ , 431.0 nmol  $\text{CO mol}^{-1}$ , and -7.95 permil  $\sigma^{13}\text{C}$  of  $\text{CO}_2$ , and gas 2 with 501.6  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ , 2127.0 nmol  $\text{CH}_4 \text{ mol}^{-1}$ , 327.8 nmol  $\text{N}_2\text{O mol}^{-1}$ , 256.7 nmol  $\text{CO mol}^{-1}$ , and -14.41 permil for  $\sigma^{13}\text{C}$  of  $\text{CO}_2$ .

#### **A3: Shorten or discuss the scientific meaning of $^{13}\text{CO}_2$ in this study.**

We have shortened this part, and moved a part of the information to the figures caption. The new text is as follows:

**Revised text in A3:** For each chamber measurement, a mound-specific  $\sigma^{13}\text{C}$  value of the  $\text{CO}_2$  flux was determined. Figure A2 shows the Keeling plot intercepts, wherein error bars represent the standard errors of the intercept. In general, the values were more depleted than values found by De Araujo et al. (2008), who found a  $\sigma^{13}\text{C}$  of -30.1 permil for valley litter during the dry season (August 2004). To investigate whether our values are representative for other mounds or soils in the valley, and to investigate whether an isotopic difference exists between mound and soil emitted  $\text{CO}_2$ , more measurements would be needed.

#### **Unify the concentration unit of ppm and $\mu\text{mol mol}^{-1}$ .**

We have corrected this.

C29

### **Revised Discussion part §4.1**

#### **$\text{CH}_4$ and $\text{CO}_2$ emissions**

Measured mound  $\text{CH}_4$  emissions were of similar magnitude to emissions found by previous studies (Table 2). The termite emission factor, determined for the soil-feeding species *N. brasiliensis*, was found to be 0.35 (sd= 0.02)  $\mu\text{mol g}_{\text{termite}}^{-1} \text{ h}^{-1}$ , which is similar to values found for other species in literature (Table 2, upper part), but almost two times higher than the average value reported by Martius et al (1993) for a wood-feeding species in the Amazon (0.19  $\mu\text{mol CH}_4 \text{ g}_{\text{termite}}^{-1} \text{ h}^{-1}$ ). Our emission rate is within the reported range of 0.1-0.4  $\mu\text{mol g}_{\text{termite}}^{-1} \text{ h}^{-1}$  for soil feeders (Sugimoto et al. 2000). Mound  $\text{CO}_2$  emissions and the termite  $\text{CO}_2$  emission factor were similar to a little higher in comparison to the few values found in literature. Nevertheless, since mound material and termites were measured together, the contribution of *indirect* termite emissions, i.e. mound respiration, cannot be quantified, so that the direct termite-produced  $\text{CO}_2$  emission is presumably lower.

There is a large variety in type of termite mounds (shape and size are dependent on, among others, species, ecosystem, climate (Noirot and Darlington, 2000)), explaining the wide range of reported termite mound  $\text{CH}_4$  emissions (Table 2, middle and lower part). In-situ measurement of termite mounds gives information about the *net*  $\text{CH}_4$  emission under natural conditions, but is unable to distinguish sources and sinks inside the mound. One known  $\text{CH}_4$  sink in termite mounds is the uptake by methanotrophic bacteria, which are also responsible for the  $\text{CH}_4$  uptake in aerobic soils. The presence and magnitude of this process have been discussed and reviewed by different studies (Khalil et al., 1990; Macdonald et al., 1998; Nauer et al., 2018; Seiler et al., 1984; Sugimoto et al., 1998a; Ho et al., 2013; Pester et al., 2007; Reuß et al., 2015). The role of possible mound  $\text{CH}_4$  uptake should also be acknowledged for the measurement of individual termite emissions (Table 2, upper part): most literature values, including values from this study, are based on termite incubation in presence of mound material, with ongoing  $\text{CH}_4$  uptake, wherefore actual termite  $\text{CH}_4$  emission values might be higher.

Small variation in emission magnitudes was observed between measurement days. This can be caused by a variation in colony size (due to foraging activities) or termite activity, driven by fluctuations in temperature or radiation (Jamali et al., 2011a; Ohiagu and Wood, 1976; Sands, 1965; Seiler et al., 1984).. However, as our termite mounds are in a tropical forest with relatively constant temperatures and only indirect daylight, strong diurnal temperature and radiation patterns are not expected. Small variation can also be caused by minimal air transport below the soil collar, through the porous upper soil layer;

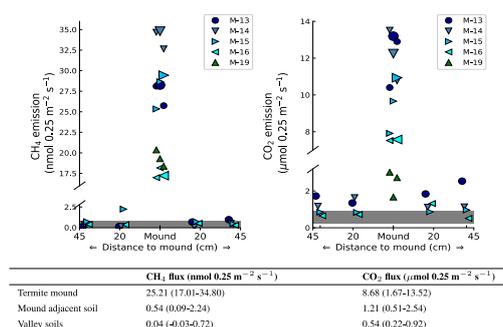
C30

during preliminary tests *without* a collar, we observed that even a light forest breeze can cause chamber headspace variations. In case our set up was subject to minor air transport below the collar, the given mound estimates will be slightly underestimated with respect to the actual mound fluxes. Another possible underestimation is caused by the estimated corrected chamber volume, as used in Eq. (2). In this study, we considered the mound volume as a solid body. A previous study considered the solid nest volume as 10% of the actual mound volume (Martius et al. 1993), leading to a larger corrected chamber volume, and therefore to larger calculated mound emissions. By use of this approach, average calculated emissions would increase by almost 30% to be  $32.7 \text{ nmol CH}_4 \text{ mound}^{-1} \text{ s}^{-1}$  instead of  $25.2 \text{ nmol CH}_4 \text{ mound}^{-1} \text{ s}^{-1}$ .

The mound emission  $\text{CH}_4/\text{CO}_2$  ratio was found to be relatively constant over 4 of the 5 mounds, with an average ratio of  $2.8 \times 10^{-3}$ . While values in literature indicate a wide range of reported  $\text{CH}_4/\text{CO}_2$  ratios (Table 2), both Seiler et al. (1984) as Jamali et al. (2013) found little variation between mounds of the same species, and concluded that the  $\text{CH}_4/\text{CO}_2$  emission ratio is species-specific. Our overall variation of a factor of  $\sim 4$  for the  $\text{CH}_4/\text{CO}_2$  ratio of mound emissions of the same species is of the same magnitude as what was observed in earlier studies (Seiler et al., 1984; Jamali et al., 2013).

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-384>, 2020.

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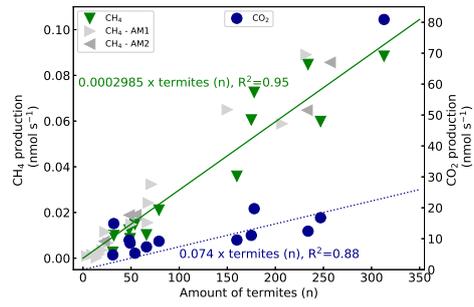


**Figure 2.** Measured mound emissions and mound-adjacent soil fluxes for  $\text{CH}_4$  (left) and  $\text{CO}_2$  (right) for mound nr. 13, nr. 14, nr. 15 and nr. 16 expressed in  $\text{nmol } 0.25 \text{ m}^{-2} \text{ s}^{-1}$  for  $\text{CH}_4$  and  $\mu\text{mol } 0.25 \text{ m}^{-2} \text{ s}^{-1}$  for  $\text{CO}_2$  (collar area is  $0.25 \text{ m}^2$ ). Note that for  $\text{CO}_2$  here the net mound emissions per collar area, not corrected for soil respiration, are shown and stated. The centrally-placed markers are the measured mound emissions (also for mound nr. 19); the larger marker indicates the day-specific mound emission when mound adjacent soil fluxes were measured. The grey bar indicates the range of additionally measured soil valley fluxes. The range and average flux for each group of measurements are given in the table. On average measured mound  $\text{CH}_4$  and  $\text{CO}_2$  fluxes were a factor 630 and 16 higher in comparison to the surrounding soil valley fluxes.

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**Fig. 1.** Revised Figure 2 (previously Figure 4)

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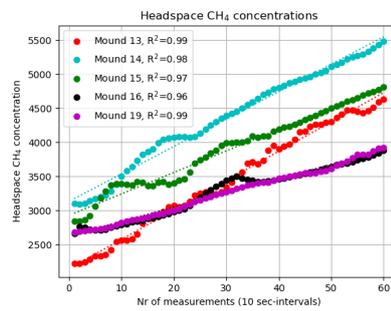


**Figure 4.** CH<sub>4</sub> production (left axis, green triangles) and CO<sub>2</sub> production (right axis, blue circles), measured in the closed small flux chamber, over counted termites. The lines (green solid for CH<sub>4</sub>, blue dashed for CO<sub>2</sub>) represent a linear regression fit with forced intercept at y=0. For CH<sub>4</sub>, a production of 0.0002985 nmol termite<sup>-1</sup> s<sup>-1</sup> (se=1.77\*10<sup>-5</sup>, R<sup>2</sup>=0.95) was found, and for CO<sub>2</sub>, a production of 0.1316 nmol termite<sup>-1</sup> s<sup>-1</sup> (se=2.59\*10<sup>-3</sup>, R<sup>2</sup>=0.68) was found. Excluding the outliers (32, 14.9 nmol s<sup>-1</sup> & 313, 80.9 nmol s<sup>-1</sup>) gives an R<sup>2</sup> of 0.88 (n=11), with a CO<sub>2</sub> emission of 0.074 nmol termite<sup>-1</sup> s<sup>-1</sup> (se=8.5\*10<sup>-5</sup>). For comparison, two sets of additional subsample CH<sub>4</sub> emission measurements are shown. The first additional measurements (AM1, light grey triangles) resulted in a termite emission factor of 0.0002976 nmol termite<sup>-1</sup> s<sup>-1</sup> (se=1.32\*10<sup>-5</sup>); one point (599 termites, 0.165 nmol s<sup>-1</sup>) is not shown in this figure. The second set (AM2, dark grey triangles) gave a termite emission factor of 0.0003043 nmol termite<sup>-1</sup> s<sup>-1</sup> (se=1.41\*10<sup>-5</sup>).

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**Fig. 2.** Revised Figure 4 (previously Figure 5)

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**Fig. 3.** Review Figure 'Review-Figure 7.2a'

C34

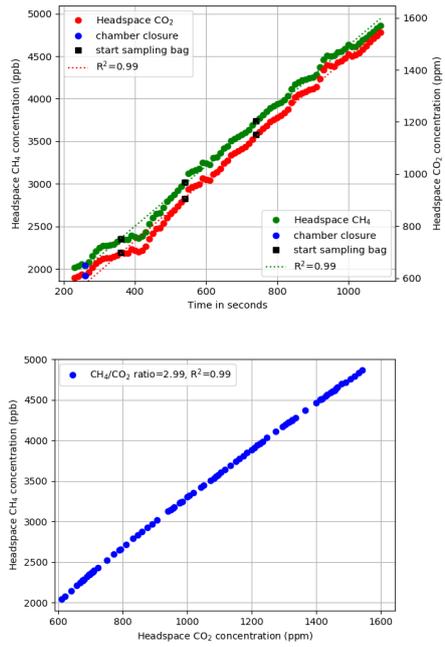


Fig. 4. Review Figure 'Review-Figure 7.2b'

C35

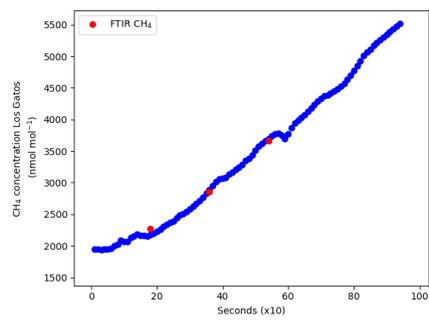


Fig. 5. Review Figure 'Review-Figure 7.3'

C36

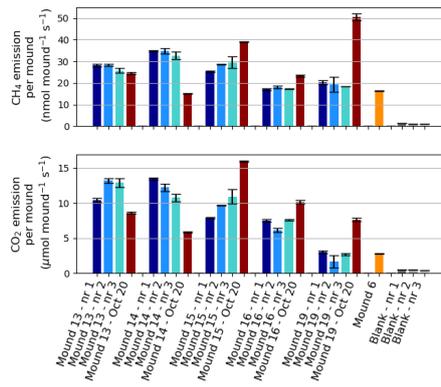


Fig. 6. Review Figure 'Review-Figure 8