

# The role of termite CH<sub>4</sub> emissions on ecosystem scale: a case study in the Amazon rain forest

Hella van Asperen<sup>1</sup>, João Rafael Alves-Oliveira<sup>2</sup>, Thorsten Warneke<sup>1</sup>, Bruce Forsberg<sup>3</sup>,  
Alessandro Carioca de Araújo<sup>4,5</sup>, and Justus Notholt<sup>1</sup>

<sup>1</sup>Institute of Environmental Physics (IUP), University of Bremen, Otto-Hahn-Allee 1, Bremen, 28359, Germany

<sup>2</sup>Coordenação de Pesquisas em Entomologia (CPEN), Instituto Nacional de Pesquisas da Amazônia (INPA), Av. André Araújo, 2936, Aleixo, AM 69060-001, Manaus, Brazil

<sup>3</sup>Coordenação de Dinâmica Ambiental (CODAM), Instituto Nacional de Pesquisas da Amazônia (INPA), Av. André Araújo, 2936, Petrópolis, AM 69067-375, Manaus, Brazil, (currently at Vermont Agency of Natural Resources, Department of Environmental Conservation, Vermont-USA)

<sup>4</sup>Programa de Grande Escala da Biosfera-Atmosfera na Amazônia (LBA), Instituto Nacional de Pesquisas da Amazônia (INPA), Av. André Araújo, 2936, Aleixo, AM 69060-001, Manaus, Brazil

<sup>5</sup>Brazilian Agricultural Research Corporation (EMBRAPA), Embrapa Amazônia Oriental, Tv. Dr. Enéas Piheiro, s/n, Marco, PA 66095-903, Caixa postal 48, Belém, Brazil

**Correspondence:** Hella van Asperen (v\_asperen@iup.physik.uni-bremen.de)

**Abstract.** The magnitude of termite methane (CH<sub>4</sub>) emissions is still an uncertain part of the global CH<sub>4</sub> budget and current emission estimates are based on limited field studies. We present in-situ CH<sub>4</sub> emission measurements of termite mounds and termite mound sub samples, performed in the Amazon rain forest. Emissions of five termite mounds of the species *Neocapritermes brasiliensis* were measured by use of a large flux chamber connected to a portable gas analyser, measuring CH<sub>4</sub> and CO<sub>2</sub>.

5 In addition, the emission of mound sub samples was measured, after which termites were counted, so that a termite CH<sub>4</sub> and CO<sub>2</sub> emission factor could be determined.

Mound emissions were found to range between 17.0 and 34.8 nmol mound<sup>-1</sup> s<sup>-1</sup> for CH<sub>4</sub> and between 1.1 and 13.0 μmol mound<sup>-1</sup> s<sup>-1</sup> for CO<sub>2</sub>. A termite emission factor of 0.35 μmol CH<sub>4</sub> g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup> was found, which is almost twice as high as the only other reported value for the Amazon. By combining mound emission measurements with the termite emission factor,

10 colony sizes could be estimated, which were found to range between 55-125 thousand individuals. Estimates were similar to literature values, and we therefore propose that this method can be used as a quick non-intrusive method to estimate termite colony size in the field.

The role of termites in the ecosystems CH<sub>4</sub> budget was evaluated by use of two approaches. Termite mound emission values were combined with local mound density numbers, leading to an estimate of 0.15-0.71 nmol CH<sub>4</sub> m<sup>-2</sup> s<sup>-1</sup> on average emitted

15 by termite mounds. In addition, the termite CH<sub>4</sub> emission factor from this study was combined with termite biomass numbers, resulting in an estimate of termite-emitted CH<sub>4</sub> of ~1.0 nmol m<sup>-2</sup> s<sup>-1</sup>. Considering the relatively low net CH<sub>4</sub> emissions previously measured at this ecosystem, we expect that termites play an important role in the CH<sub>4</sub> budget of this Terra Firme ecosystem.

## 20 1 Introduction

Methane (CH<sub>4</sub>) is one of the most important greenhouse gases, but its natural sources are still not well understood. Anaerobic decomposition processes in wetlands are expected to represent the largest natural CH<sub>4</sub> source, but estimates remain a large source of uncertainty (Kirschke et al., 2013; Saunio et al., 2020). Recently, alternative CH<sub>4</sub> production mechanisms and their possible important role on ecosystem scale have been proposed, such as the CH<sub>4</sub> production by living vegetation (Bruhn et al., 25 2012; Wang et al., 2014), the CH<sub>4</sub> emission due to photo and thermal degradation (Lee et al., 2012), or the transport of anaerobic soil-produced CH<sub>4</sub> through wetland trees (Pangala et al., 2015; Rice et al., 2010). An additional known CH<sub>4</sub> source in tropical ecosystems is the emission by termites.

Termites (isoptera) can mostly be found between 45°N and 45°S, and are especially abundant in warm ecosystems (Bignell, 2006; Brian and Brian, 1978; Gomati et al., 2011; Wood, 1988). They are highly socialised insects, living in large commu- 30 nities of up to several million individuals (Wood, 1988). Termites are considered ‘ecosystem engineers’: they are known for decomposing organic substances, and moving and mixing organic and mineral materials, thereby enhancing humus formation, modifying soil structure, and improving soil fertility (Bignell, 2006; Brian and Brian, 1978; Bignell and Eggleton, 2000; Mishra et al., 1980; De Bruyn and Conacher, 1990; Wood, 1988). In addition, they are able to modify their environment to their needs: most termite species live in complex above or (partly) below-ground nests where temperature and moisture remain 35 stable (Bignell, 2019; Noirot and Darlington, 2000; Wood, 1988). Recently, it was shown that termites increase their activity during droughts, resulting in, among others, enhanced litter decomposition, elevated soil moisture and higher seedling survival rates, thereby demonstrating a mitigating effect during droughts in tropical rain forests (Ashton et al., 2019).

Three main groups of termites can be distinguished, based on their main feeding habits: soil-feeding (humiverous) termites, who can mainly be found in and on the soil, decomposing decayed organic soil material, xylophagous termites, feeding on (de- 40 composed) wood, which can also be found in living trees, and fungus-feeding termites, which live in a symbiotic relationship with fungus (Eggleton, 2000; Sanderson, 1996).

CH<sub>4</sub> production by termites was first described and measured by Cook (1932). Follow up studies found that methane is produced by almost all termite species, and that its production takes place in the termite gut: in higher termites (dominant in 45 tropical forests, more evolved species with respect to diet and community complexity) CH<sub>4</sub> production is caused by symbiotic bacteria, and in lower termites the production is caused by flagellate protozoa (Bignell et al., 1997; Brune, 2018; Lee et al., 1971). In a laboratory experiment Zimmerman et al. (1982) measured the emission strength of individual termites and, by use of termite biomass numbers, presented a global termite emission estimate of 150 Tg CH<sub>4</sub> yr<sup>-1</sup>, which was estimated to be 40% of the global natural CH<sub>4</sub> emissions. Different estimates followed, resulting in lower values, such as by Seiler et al. (1984) of 50 2-5 Tg yr<sup>-1</sup>, by Fraser et al. (1986) of 14 Tg yr<sup>-1</sup>, by Khalil et al. (1990) of 12 Tg yr<sup>-1</sup>, and by Martius et al. (1993) of 26 Tg yr<sup>-1</sup>. 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; Saunois et al., 2020), which is approximately 0.5-4% of the total estimated natural source CH<sub>4</sub> emission (Saunois et al., 2020). While on global scale termite emissions can be considered small in comparison to natural sources like wetland emissions (~147 Tg yr<sup>-1</sup>) or fresh water emissions (~159 Tg yr<sup>-1</sup>) (Saunois et al., 2020), the question remains what  
55 their role can be in the CH<sub>4</sub> budget of a local tropical ecosystem.

Estimates of global termite CH<sub>4</sub> emissions are based on field and laboratory measurements. To estimate global CH<sub>4</sub> termite emissions, most commonly the CH<sub>4</sub> emission per termite (mg CH<sub>4</sub> termite<sup>-1</sup> h<sup>-1</sup>) or termite mass (mg CH<sub>4</sub> g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup>) is measured, whereby termite mass can either be measured directly or be taken from literature (Sanderson, 1996). The disadvantage of this approach is that termites are removed from their natural environment, thereby possibly changing their emission and behaviour. Another approach is to measure termite nest CH<sub>4</sub> emissions in-situ in the field. In this case, emissions are expressed per mound or nest (mg CH<sub>4</sub> mound<sup>-1</sup> h<sup>-1</sup>). While this method does not disturb the natural environment, correct estimation of termite nest colony size is challenging, wherefore values are hard to convert to emission-per-termite values (Jones et al., 2005). Besides CH<sub>4</sub>, termite emissions of other gases have also been investigated, such as for CO<sub>2</sub>, O<sub>2</sub>, CO, H<sub>2</sub>, CHCl<sub>3</sub>, N<sub>2</sub>O and  
65 different hydrocarbons (Cook, 1932; Khalil et al., 1990; Zimmerman et al., 1982). In previous studies, measurements of termite CO<sub>2</sub> emissions were often performed alongside CH<sub>4</sub> emission measurements, and generally a clear relationship between CH<sub>4</sub> and CO<sub>2</sub> emissions was found, of which the ratio is expected to be species dependent (Seiler et al., 1984; Jamali et al., 2013). For termite-emitted CO<sub>2</sub>, reported global estimates are 50 Pg yr<sup>-1</sup> (Zimmerman et al., 1982), 4 Pg yr<sup>-1</sup> (Khalil et al., 1990), and 3.5 Pg yr<sup>-1</sup> (Sanderson, 1996) (1 Pg= 1000 Tg). In addition, Khalil et al. (1990) observed mound CO uptake  
70 and emissions, but reported them to be irregular and small. Strong termite mound N<sub>2</sub>O emissions have also been detected (Brümmer et al., 2009b; Brauman et al., 2015), although they were also found to be very irregular or undetectable (Khalil et al., 1990; Zimmerman et al., 1982). Brauman et al. (2015) suggested that termite mound N<sub>2</sub>O emissions occur if N-rich organic matter is available.

75 Current global termite CH<sub>4</sub> emission estimates are based on relatively few studies, and there is still a lack of data on termite CH<sub>4</sub> emission rates (Brune, 2018). In addition, existing studies have mostly focused on Australian or Asian species (Eggleton et al., 1999; Fraser et al., 1986; Jamali et al., 2011a, b, 2013; Khalil et al., 1990; Macdonald et al., 1998; Sugimoto et al., 1998a, b) or African species (Brauman et al., 1992; Brümmer et al., 2009a; Macdonald et al., 1998; Rouland et al., 1993; Sawadogo et al., 2011, 2012; Seiler et al., 1984). To our knowledge, only two studies focused on CH<sub>4</sub> emission of termites  
80 in the Amazon (Martius et al., 1993; Queiroz, 2004), and only one study reported CH<sub>4</sub> emission values for Amazonian termites (Martius et al., 1993). Martius et al. (1993) performed field measurements on wood-feeding termites by semi-field and laboratory measurements, and suggested that Amazonian termites release more methane than species in other regions. In addition, for the Amazon, it is expected that most termites are soil-feeding (Jones and Eggleton, 2010), a group which are expected to be the strongest emitters of CH<sub>4</sub> (Bignell and Eggleton, 2000; Brauman et al., 1992).

85

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<sup>1</sup> abundant in the Amazon (Constantino, 1992; Pequeno et al., 2013), and one of the most common species in the region (Dambros et al., 2016). In addition we measured the CH<sub>4</sub> emission of countable groups of termites. The goal of our research was twofold. Firstly, we are providing the first CH<sub>4</sub> and other gas emission measurements of the species *N. brasiliensis*, thereby expanding the limited literature on CH<sub>4</sub> emissions from Amazonian termites. Secondly, we are aiming to quantify the role of termite emissions in the CH<sub>4</sub> budget of this specific ecosystem, as part of a larger ecosystem CH<sub>4</sub> budget study (van Asperen et al., in preparation). In addition, we are presenting a possible quick non-intrusive field method to estimate termite colony size in-situ.

95

## 2 Material and methods

### 2.1 Study site

The study was conducted at the experimental field site Reserva Biológica do Cuieiras – ZF2 (2 °36' 32.67 S, 60 °12'33.48 W, 40-110 m above sea level (a.s.l.), managed by the *Instituto Nacional de Pesquisas da Amazônia* (INPA), located ~50 km northwest of Manaus (Brazil). Field site ZF2 consists of plateaus and valleys with typical terra firme forest with tree heights of 35-40 m on the plateaus and 20-35 m in the valleys. Soils on the plateau are clayey and can be classified as Oxisols and Ultisols. Soils in the valleys contain more sand and can be classified as Spodosols (Luizão et al., 2004; Zanchi et al., 2014). The field site has a strong seasonality, with a wet season from December to April, and a dry season from June to September. Annual average temperatures range between 26-28 °C, and annual average precipitation is around 2400 mm. More information about the field site can be found in Araújo et al. (2002); Chambers et al. (2004); Luizão et al. (2004); Quesada et al. (2010); Zanchi et al. (2014). Measurements took place at the end of the wet season (March 2020).

105

### 2.2 Selection of termite mounds

In the study area, two main trails exist, following the topography from valley to plateau, and termite nests in vicinity of these trails were inventoried. For practical reasons, only free-standing epigeal (above-ground) nests were considered, from here on called mounds. Twenty termite mounds were selected for further research, and of each mound the termite species was determined. For flux chamber measurements, five mounds with the same termite species were selected (nr. 13, nr. 14, nr. 15, nr. 16, and nr. 19); for practical reasons, chosen mounds were in close proximity of each other, and all located in the valley. As an exploratory measurement, an additional mound was selected of a different species on the plateau (nr. 6). Of each mound,

110

---

<sup>1</sup>The species *Neocapritermes brasiliensis* is a wood/soil interface feeding species. Species feeding on extremely decomposed wood are in the centre of the 'wood-soil decomposition gradient' termite classification (Bourguignon et al., 2011), but are classified as soil-feeders according to Eggleton and Tayasu (2001).

115 height and perimeter were measured. Termite mound volumes were estimated by use of the following formula, as also used in  
Ribeiro (1997) and in Pequeno et al. (2013):

$$V = \frac{\pi HWT}{6} \quad (1)$$

wherein V is the mound volume (cm<sup>3</sup>), H is the height (cm), W is the width (cm), and T is the thickness (cm) of the mound.  
Termite mound surface was estimated by mathematically considering the lower part of the mound as a column, and the upper  
120 part as half a sphere. Details of each mound (dimensions, species, location) are given in Table 1.

### 2.3 Mound flux chamber set up

Collars (stainless steel, 15 cm height, 56.5 cm diameter) were placed around the five selected termite mounds a week before the  
start of the measurements. Collars were inserted for approximately 5 cm into the soil/litter layer. A flux chamber was created  
125 by use of a 220 L slightly cone-shaped polyethylene bucket, with a diameter of 57.5 cm. A strip of closed-pore foam (1 cm  
x 1 cm x 57.5 cm) was attached over the whole inner perimeter, so that if the bucket was placed on the collar, the foam strip  
would seal the part between the bucket and the collar. 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). The set up (chamber and tubing) was  
130 tested for internal emissions of all measured gases. For CO (see Appendix), an internal emission of <0.014 nmol s<sup>-1</sup> was  
found: presented CO fluxes are not corrected for this possible internal emission.

CH<sub>4</sub> and CO<sub>2</sub> concentrations were measured with a Los Gatos Ultraportable Greenhouse Gas Analyser. The instrument was  
connected in a closed loop with the flux chamber (2 x 2 meter PTFE tubing, 1/4 inch). For air circulation, the internal pump  
135 of the Los Gatos instrument was used, with a flow of ~0.35 L min<sup>-1</sup>. The instrument measures concentrations every second;  
10-sec averaged concentrations were saved and used for flux calculations. For each measurement, the flux chamber was closed  
for 20 minutes, during which time concentrations were measured continuously. All five mounds were always measured on the  
same day and in the same order. Over one week, each mound was measured three times, each time at approximately the same  
hour of the day.

140

### 2.4 Flux calculations

Fluxes were calculated as follows. By use of the ideal gas law, mole fractions (nmol/ $\mu$ mol mol<sup>-1</sup>) were converted to concentra-  
tions (nmol/ $\mu$ mol m<sup>-3</sup>). For chamber temperature, a standard temperature of 25 °C was assumed. For chamber volume (CV),  
the termite mound volume (Table 1) was deducted from the bucket volume (220 L).

145

Fluxes could be calculated as follows:

$$F = \frac{dC}{dt} * \frac{CV}{A} \quad (2)$$

150 wherein  $\frac{dC}{dt}$  is the concentration change ( $\text{nmol}/\mu\text{mol m}^{-3} \text{ s}^{-1}$ ), CV the corrected chamber volume ( $\text{m}^3$ ), and A the collar area ( $0.25 \text{ m}^2$ ). Linear regression was used to derive the concentration change, 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 (see Appendix). 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  
155 minutes (leaving a time window of 8 minutes). All calculated  $dC/dt$  increases showed a  $R^2 > 0.95$ . Unless mentioned otherwise, given mound  $\text{CO}_2$  emissions are corrected for the estimated contribution of soil respiration, by subtracting the average valley soil emission (see §2.5). For mound nr. 6, the average plateau soil emission was subtracted.

## 2.5 Valley and mound adjacent soil fluxes

160 To quantify the  $\text{CH}_4$  and  $\text{CO}_2$  emissions of the soils adjacent to the termite mounds, four soil collars were installed around each mound: two soil collars were placed at 20 and 45 cm distance from the mound (distance between mound collar and middle of soil collar), and two additional soil collars were placed on the opposite side of the mound at the same distances. The soil collars were of 20 cm diameter, with a height of 10 cm, and were inserted for 5 cm into the soil. The flux chamber height was 15 cm, so that the soil chamber volume was 4.7 L. To be able to connect the Los Gatos instrument, the soil chamber had two  
165 one-touch fittings on top. The chamber and collars were created from a common PVC sewage pipe. 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 mound-collar area ( $0.25 \text{ m}^2$ ), to be better comparable to mound emissions. The same chamber set up was also used in a sub  
170 study at a close by transect ( $\sim 500 \text{ m}$  from termite mounds) where, among others, valley soil (10 collars) and plateau soil (10 collars) fluxes were measured (3 repetitions). Measured soil fluxes from the valley will be shown for comparison.

## 2.6 Termite mound sub sample emission measurements

After each last mound flux measurement, a mound sample was taken of approximately 1 L volume. From this, three small sub  
175 samples were taken (volume not determined). When selecting a piece, we tried to look for solid not crumbling pieces, so that the inside of the sub sample was undisturbed. From the sample from mound nr. 19, only one suitable sub sample was found.

Each sub sample was placed in a small closed box (12.6 cm x 19.2 cm x 6.8 cm), with two one-touch fittings, functioning as a small closed flux chamber. A blank measurement was made with the small box, and no internal emissions were found. Each mound sub sample was measured with the Los Gatos instrument for 5 minutes, to determine the CH<sub>4</sub> and CO<sub>2</sub> production in the chamber over time. After each measurement, the mound sample was carefully broken open and termites were counted, so that the CH<sub>4</sub> and CO<sub>2</sub> emission per termite (the termite emission factor) could be calculated. The measurements took place next to the mound, and time between sampling and measuring was always less than 15 min. 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), measurements were performed on 5 subsamples of a different mound of the same species.

## 2.7 Termite mass measurement

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, 1 x n=80) by use of a precision scale (FA2104N). Reported individual termite mass is fresh weight per termite (mg termite<sup>-1</sup>).

## 3 Results

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

Headspace concentrations increased strongly during chamber closure, and chamber concentrations climbed up to 5750 nmol CH<sub>4</sub> mol<sup>-1</sup> and up to 1950 μmol CO<sub>2</sub> mol<sup>-1</sup>. CH<sub>4</sub> emissions of mounds nr. 13-19 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 soil CH<sub>4</sub> fluxes, measured at 20 and 45 cm distance 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), and the average plateau soil CO<sub>2</sub> emission was 4.03 μmol m<sup>-2</sup> s<sup>-1</sup> (sd=1.36). Mound adjacent soil CO<sub>2</sub> fluxes showed an average emission of 4.81 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (range=2.0-10.1, sd=2.04), thereby being enhanced with respect to the surrounding valley soils (Fig. 2). Mound CO<sub>2</sub> emissions, corrected for the average valley and plateau 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> (average of mounds nr. 13-19).

During chamber closure, the concentration changes in CH<sub>4</sub> and CO<sub>2</sub> were strongly correlated (R<sup>2</sup> >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 \*10<sup>-3</sup> (Fig. 3), and showed a constant ratio when data from mound nr. 19 (furthest away from other mounds), and mound nr 6 (different

species) were excluded (average ratio:  $2.8 \cdot 10^{-3}$ ,  $sd=0.4$ ). The smallest mound (nr. 19) clearly showed smaller-than-average emissions, but in general no strong correlation was found between mound  $CH_4$  emissions and mound height ( $R^2=0.07$ ) or volume ( $R^2=0.08$ ), and a small correlation was found between mound  $CO_2$  emissions and mound height ( $R^2=0.43$ ) and mound volume ( $R^2=0.44$ ).

### 3.2 Termite weight, individual termite emission, and colony size estimation

The average weight of 5 subsets of living workers of the species *N. brasiliensis* was determined, and was found to range between 2.83 and 3.33 mg, with an average weight of 3.07 mg ( $sd=0.18$ ), which is similar to 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 mg ( $sd=0.18$ ) as an average termite weight for the species *N. brasiliensis*.

$CH_4$  and  $CO_2$  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  $CH_4$ , an emission of  $0.0002985 \text{ nmol termite}^{-1} \text{ s}^{-1}$  was found ( $se=1.77 \cdot 10^{-5}$ ), fitted with an  $R^2$  of 0.95 ( $n=13$ ). The set of additional measurements resulted in similar termite  $CH_4$  emission factors, namely  $0.0002976 \text{ nmol termite}^{-1} \text{ s}^{-1}$  ( $se=1.32 \cdot 10^{-5}$ ) and  $0.0003043 \text{ nmol termite}^{-1} \text{ s}^{-1}$  ( $se=1.41 \cdot 10^{-5}$ ), for respectively the measurements of October and December 2020. Given estimates in this paper will be based on the termite emission factor of  $0.0002985 \text{ nmol } CH_4 \text{ termite}^{-1} \text{ s}^{-1}$ . For  $CO_2$ , an emission of  $0.1316 \text{ nmol termite}^{-1} \text{ s}^{-1}$  was found ( $se=2.59 \cdot 10^{-2}$ ), with an  $R^2$  of 0.68 ( $n=13$ ). Excluding the outliers (32,  $14.9 \text{ nmol s}^{-1}$  and 313,  $80.9 \text{ nmol s}^{-1}$ ) gave an  $R^2$  of 0.88 ( $n=11$ ), with a  $CO_2$  emission of  $0.074 \text{ nmol termite}^{-1} \text{ s}^{-1}$  ( $se=8.5 \cdot 10^{-3}$ ). Converting the emission rates from termite to termite-mass (fresh weight), and from seconds to hourly rates gives a termite emission factor of  $0.35 \mu\text{mol g}_{\text{termite}}^{-1} \text{ h}^{-1}$  ( $se=0.02$ ) for  $CH_4$  and of  $86.8 \mu\text{mol g}_{\text{termite}}^{-1} \text{ h}^{-1}$  ( $se=10.0$ ) for  $CO_2$  (Table 2).

By combining the termite  $CH_4$  emission factor with the termite mound  $CH_4$  emissions, colony sizes were estimated. Colony size estimates were based on highest measured emissions and were found to range between 55-125 thousand individuals (Table 3). Colony size can also be estimated by use of mound volume or mound external surface. Table 3 shows the colony size estimates, based on values as given by Lepage and Darlington (2000) for termites in general, and also shows the estimates based on the 'mound volume-termite biomass' relation found by Pequeno et al. (2013), specifically for the species *N. brasiliensis*.



## 4 Discussion

### 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, middle and lower part). The termite emission factor, determined for the soil-feeding species *N. brasiliensis*, was found to be 0.35 μmol g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup> (sd=0.02), which is similar to values found for other species (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 μmol g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup>). Our emission rate is within the reported range of 0.1-0.4 μmol g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup> 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 (Table 2). 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 (Ho et al., 2013; Khalil et al., 1990; Macdonald et al., 1998; Nauer et al., 2018; Seiler et al., 1984; Sugimoto et al., 1998a; 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 mound 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 headspace variations. In case our set up was subject to minor air transport below the collar, the given mound emission estimates will be slightly underestimated with respect to the actual mound emissions. Another possible underestimation is caused by the estimated corrected chamber volume CV, 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 mound emissions

270 would increase by almost 30% to be 32.7 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. While values in literature indicate a wide range of 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 ratio is species-specific. Our average variation of a factor ~4  
275 between mounds of the same species is of the same magnitude as what was observed in earlier studies (Seiler et al., 1984; Jamali et al., 2013).

#### 4.2 Colony size estimate

To estimate colony sizes of (epigeal) nest building termites, different methods exist. One method is by fumigation of the nest (to prevent colony evacuation) followed by excavation, after which termites can be removed from the nest debris by flotation  
280 in water. This process is labour intensive, and can take five persons up to three weeks to finish one nest (Darlington, 1984; Jones et al., 2005). A faster method is by sub sampling known volumes of the mound, counting the termites in the sub sample, and extrapolating this to the total mound volume. Termite mounds can have irregular shapes, wherefore volume estimates strongly depend on which volume estimation approach is used (Jones et al., 2005).

285 The population estimation method we tested combined CH<sub>4</sub> mound emissions with a termite emission factor, measured in-situ at the field site. We estimated colony sizes ranging between 57.6 and 124.0 thousand termites per mound. For all mounds, our population estimate was in the estimated range based on mound volume or external surface area, as taken from literature equations (Table 3). Comparison to estimates based on a *N. brasiliensis* species-specific equation shows an average difference of 20% (Pequeno et al., 2013): it should be noted that the relation found between mound volume and colony biomass  
290 by Pequeno et al. (2013) was quite weak ( $R^2=0.41$ ), and our estimates would fit in the general spread they observed in their data. 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. The influence of mound CH<sub>4</sub> uptake on our population estimate method should be considered: mound methanotrophic CH<sub>4</sub> uptake probably decreases the net mound CH<sub>4</sub> emission, resulting in an underestimation of the colony size when linking it to  
295 termite emission factors, as also suggested by Nauer et al. (2018). However, our termite emission factor was determined inside small pieces of undisturbed mound material, so that the materials CH<sub>4</sub> uptake rate was presumably only little affected. It is therefore likely that our termite emission factor is underestimated to the same degree as our mound emissions, wherefore both values can still be combined.

300 Overall, our colony size estimation approach can be considered as a test case for a quick population estimation method. The combination of one mound flux measurement (15 minutes) in combination with 5 sub sample measurements (5x5 minutes) can be performed within 1 hour, thereby being faster than the original methods. Also, the method is applicable to epigeal mounds of all species, independent of internal mound structure (Josens and Soki, 2010) or species characteristics (Pequeno et al., 2013).

In addition, the method is not strongly dependent on a correct mound volume estimate, which remains a source of uncertainty (Jones et al., 2005), and which has been shown to be a weak indicator of population size for some species (Pequeno et al., 2013; Josens and Soki, 2010). Moreover, mounds can also be measured several times in a row before sub sample measurement, so that colony size dynamics over time can be studied noninvasively. A disadvantage of this method is that it is only applicable to freestanding epigeal mounds, at least with the current type of chamber set up. For a possible follow up study, we propose a set up wherein the different methods are compared.

310

### 4.3 Role of termites on ecosystem scale

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., 2014). 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.

To estimate the role of termites on ecosystem scale, one approach is to combine mound emission values with termite mound density numbers. A local study reported a density value of 21.6 mounds ha<sup>-1</sup> for the species *N. brasiliensis* specifically (Pequeno, 2014), which deducts to an average CH<sub>4</sub> emission of 0.05 nmol m<sup>-2</sup> s<sup>-1</sup> caused by mounds of this species alone. Non-species specific mound densities are known to vary strongly between and within ecosystems (Ackerman (2006), Appendix B8). We found five *local* studies reporting mound (epigeal nest) density values, which were ~100 mounds ha<sup>-1</sup> (Queiroz, 2004), 193 mounds ha<sup>-1</sup> (Oliveira et al., 2016), 250 mounds ha<sup>-1</sup> (Dambros et al., 2016), 60 and 280 mounds ha<sup>-1</sup> (de Souza and Brown, 1994), and even 760 mounds ha<sup>-1</sup> (Ackerman et al., 2007). When excluding the strong outlier of 760 mound ha<sup>-1</sup>, the emission of termite mounds on ecosystem scale was estimated to range between 0.15-0.71 nmol m<sup>-2</sup> s<sup>-1</sup> for CH<sub>4</sub> and between 0.05-0.23 μmol m<sup>-2</sup> s<sup>-1</sup> for CO<sub>2</sub>. Since (epigeal) mounds only represent a part of the total termite community, and not the termites located in the subsoil, in dead wood or on trees (arboreal nests), this emission value underestimates the actual role of termites on ecosystem scale. To our knowledge, only Bandeira and Torres (1985) (as given in Martius et al. (1996)) assessed the ratio between nest-building vs total termite biomass, and estimated it to be ~0.16. Considering the limited literature on this subject, we prefer to not further extrapolate our mound emission measurements.

A more comprehensive approach is to use termite biomass estimates and combine them with termite emission factors, a method which is commonly used for global CH<sub>4</sub> budget studies (Kirschke et al., 2013; Saunois et al., 2020). For active tropical ecosystems, generally a termite biomass of ~11 g termite m<sup>-2</sup> is assumed (Bignell and Eggleton, 2000; Kirschke et al., 2013; Sanderson, 1996; Saunois et al., 2020). Considering the previously found value of 0.19 μmol CH<sub>4</sub> g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup> for wood-feeding termites in the Amazon (Martius et al., 1993), and our newly found termite emission factor of 0.35 μmol CH<sub>4</sub> g<sub>termite</sub><sup>-1</sup>

h<sup>-1</sup> for a soil-feeding termite, a termite-derived ecosystem CH<sub>4</sub> emission range of 0.6-1.1 nmol m<sup>-2</sup> s<sup>-1</sup> can be calculated. For CO<sub>2</sub>, our termite emission factor of 86.8 μmol CO<sub>2</sub> g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup> leads to a termite-induced ecosystem CO<sub>2</sub> emission of  
340 ~0.27 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>.

An overview of the different estimates is given in Table 4. Each of these estimates are based on measurements from mounds and termites found in the valley, which were only measured during the wet season. Nevertheless, an exploratory measurement of a small mound of a different species on the plateau (mound nr. 6) indicated CH<sub>4</sub> fluxes of a similar magnitude in comparison  
345 to a similar-sized mound in the valley (mound nr. 19). Furthermore, exploratory dry season measurements of the same mounds showed emissions of similar 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.

To put the estimates in perspective, not-termite specific ecosystem CH<sub>4</sub> and CO<sub>2</sub> fluxes, measured at this field site during earlier studies, are given. Ecosystem termite CO<sub>2</sub> emissions were estimated to range between 0.05-0.27 μmol m<sup>-2</sup> s<sup>-1</sup>, which is approximately ~1-3% of the estimated total ecosystem respiration (7.8 μmol m<sup>-2</sup> s<sup>-1</sup> (Chambers et al., 2004)). 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. For  
350 CH<sub>4</sub>, we rather expect an underestimation than an overestimation of our termite and mound emission values, wherefore we expect that these ecosystem estimates are lower bound. For CH<sub>4</sub>, it is difficult to judge the role on ecosystem scale, since the earlier measured CH<sub>4</sub> flux (above canopy EC measurements, ~2.0 nmol m<sup>-2</sup> s<sup>-1</sup> (Querino et al., 2011)), is a net flux of uptake and emission processes with relatively unknown individual magnitudes. Nevertheless, considering the magnitude of our estimated termite-emitted CH<sub>4</sub> emissions (0.15-1.1 nmol m<sup>-2</sup> s<sup>-1</sup>), it is expected that termites play a significant role in this  
355 Terra Firme ecosystem.

#### *Termites contribution to tropical South America CH<sub>4</sub> budget*

In current CH<sub>4</sub> budget studies, a termite emission factor of 2.8 μg CH<sub>4</sub> g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup> is used for ‘*Tropical ecosystems and Mediterranean shrub lands*’<sup>2</sup> (Kirschke et al., 2013; Saunois et al., 2020), which is mainly based on field studies in Africa  
365 and Australia (Brümmer et al., 2009a; Jamali et al., 2011a, b; Macdonald et al., 1998; MacDonald et al., 1999; Sanderson, 1996). The only termite emission factor measured for the Amazon rain forest is by Martius et al. (1993) (3.0 μg g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup>) for a wood-feeding species, which are expected to emit less CH<sub>4</sub> than soil-feeding species (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\*11=3.96 Tg). Substituting the emission factor  
370 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 thereby the global

---

<sup>2</sup>Kirschke et al. (2013) and Saunois et al. (2020) stated a termite emission factor 2.8 (±1.0) mg CH<sub>4</sub> (g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup>). Correspondence with the authors clarified that a termite emission factor of 2.8 (±1.0) μg CH<sub>4</sub> (g<sub>termite</sub><sup>-1</sup> h<sup>-1</sup>) was meant.

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  
375 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.

## 5 Conclusions

In-situ measurement of termite mound CH<sub>4</sub> and CO<sub>2</sub> emissions confirmed that mounds are important local hot spots, playing  
380 a considerable role on ecosystem scale. Measured mound emissions of the species *N. brasiliensis* were of similar magnitude to observed emissions for different soil and wood-feeding species, and mounds showed a relatively constant CH<sub>4</sub>/CO<sub>2</sub> emission ratio. By performing emission measurements on small groups of termites, we derived a termite CH<sub>4</sub> emission factor, so far only the second value reported for the Amazon rain forest. The newly found termite emission factor, measured for a soil-feeding species, is almost twice as high as the previously reported average value for the Amazon, which was determined for a wood-  
385 feeding species. By combining mound emissions and termite emission factors, mound colony sizes were estimated, and values were similar to estimates based on literature review. Considering the quick, wide applicable and non-destructive nature of this approach, we propose that it can be used as an alternative to the traditional methods, that are intrusive and time-consuming. Assessment of the magnitude of termite-emitted CH<sub>4</sub> on ecosystem scale was attempted by two approaches. Mound emission values were combined with mound density numbers, leading to an estimate of 0.15-0.71 nmol CH<sub>4</sub> m<sup>-2</sup> s<sup>-1</sup> emitted by mounds  
390 on average; since this estimate neglects emission from termite activity outside mounds, the number is likely an underestimation. The CH<sub>4</sub> termite emission factor from this study, and from the only other Amazon field study, were combined with termite biomass numbers, resulting in an estimate of termite-emitted CH<sub>4</sub> of 0.6-1.1 nmol m<sup>-2</sup> s<sup>-1</sup>. Considering the relatively low CH<sub>4</sub> emissions previously measured at this ecosystem, we expect that termites play an important role in the CH<sub>4</sub> budget of this Terra Firme ecosystem.

## 395 Appendix A: Termite mounds: N<sub>2</sub>O, CO, and δ<sup>13</sup>C of CO<sub>2</sub>

### A1 Methodology

In addition to the direct mound CH<sub>4</sub> and CO<sub>2</sub> emission measurements (performed with the Los Gatos instrument), mound N<sub>2</sub>O and CO fluxes and the δ<sup>13</sup>C of the mound CO<sub>2</sub> flux were determined by the following method. Three bags (5L inert foil, Sigma-Aldrich) were sampled consecutively during chamber closure. The bags were measured on the same or the consecutive  
400 day with a Spectronus FTIR analyser, which can quantify concentrations of CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O and CO, and can determine the δ<sup>13</sup>C of CO<sub>2</sub>. The N<sub>2</sub>O and the δ<sup>13</sup>C of CO<sub>2</sub> measurements of the FTIR analyser have a cross sensitivity for CO<sub>2</sub> concen-

trations, which is well quantified for the CO<sub>2</sub> range 380-800 ppm (Hammer et al., 2013). In order to sample air with CO<sub>2</sub> concentrations <800 μmol mol<sup>-1</sup>, air samples were taken in the first minutes after chamber closure (2 min, 5 min, 8 min). Out of the 45 taken bag samples, 2 bag samples could not be used.

405

Before measurement of the bag sample, sample lines were flushed with bag sample air. Air samples were dried by a Nafion dryer and by a column of magnesium perchlorate. Measurements were corrected for pressure and temperature variations as well as for cross-sensitivities (Hammer et al., 2013). For more information on this instrument, please refer to Griffith et al. (2012). For calibration of the instrument, 2 calibration gases were used: gas 1 with values 381.8 μmol CO<sub>2</sub> mol<sup>-1</sup>, 2494.9  
410 nmol CH<sub>4</sub> mol<sup>-1</sup>, 336.6 nmol N<sub>2</sub>O mol<sup>-1</sup>, 431.0 nmol CO mol<sup>-1</sup>, and a δ<sup>13</sup>C of CO<sub>2</sub> of -7.95 ‰, and gas 2 with values 501.6 μmol CO<sub>2</sub> mol<sup>-1</sup>, 2127.0 nmol CH<sub>4</sub> mol<sup>-1</sup>, 327.8 nmol N<sub>2</sub>O mol<sup>-1</sup>, 256.7 nmol CO mol<sup>-1</sup>, and a δ<sup>13</sup>C of CO<sub>2</sub> of -14.41 ‰.

To calculate the fluxes of N<sub>2</sub>O and CO, FTIR-measured bag concentrations of N<sub>2</sub>O, CO and CO<sub>2</sub> were used. For each chamber closure, the  $\frac{dN_2O}{dt}$ ,  $\frac{dCO}{dt}$  and  $\frac{dCO_2}{dt}$  were calculated, so that the ratios  $\frac{dN_2O}{dCO_2}$  and  $\frac{dCO}{dCO_2}$  could be derived. To calculate  
415 the fluxes of N<sub>2</sub>O and CO, the ratios were combined with the in-situ determined mound CO<sub>2</sub> flux, as measured by the Los Gatos instrument. This approach was chosen because the intended 3 min bag sampling interval was not always accomplished, so that an exact Δt could not be assumed with certainty. To determine the δ<sup>13</sup>C of the CO<sub>2</sub> emitted by the termite mounds, Keeling plots were used (Pataki et al., 2003).

## A2 Mound N<sub>2</sub>O and CO fluxes

420 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> cross-sensitivity becomes uncertain at higher CO<sub>2</sub> concentrations (Hammer et al., 2013), 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 headspace samples were with CO<sub>2</sub> <800 μmol  
425 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 *soil* flux measurements, performed as part of a substudy, which showed valley soil fluxes ranging between 0.008-0.106 nmol 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.,  
430 2015), which is expected to be low in the valleys of this ecosystem (Quesada et al., 2010).

Chamber CO concentrations ranged between 120 and 220 nmol mol<sup>-1</sup>, and showed a clear uptake on all days and for all mounds, ranging between -0.04 to -0.78 nmol mound<sup>-1</sup> s<sup>-1</sup> (Fig. A1). Termite mound uptake has been observed before by Khalil et al. (1990). We expect that the observed uptake is caused by aerobic CO-oxidising bacteria in the mound, which are  
435 also responsible for the CO uptake in (tropical) soils (Conrad, 1996; Kisselle et al., 2002; Liu et al., 2018; Potter et al., 1996;

Whalen and Reeburgh, 2001; Yonemura et al., 2000a). Soil CO uptake is dependent on atmospheric CO and therefore often limited by low soil diffusivity (Sun et al., 2018; Yonemura et al., 2000b). The dry porous mound material (Martius et al., 1993) is therefore a suitable place for CO uptake.

### 440 **A3 $\delta^{13}\text{C}$ of the mound-emitted $\text{CO}_2$**

For each chamber measurement, a mound-specific  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  of  $-30.1\text{‰}$  for valley litter during the dry season (August 2004). However, for our measurements, at least one sample bag per chamber closure was with  $\text{CO}_2 > 800\ \mu\text{mol mol}^{-1}$ , so  
445 that the  $\text{CO}_2$  cross sensitivity correction for these samples was less certain. Intercepts based on only the first 2 concentrations points, which were generally lower (or around)  $800\ \mu\text{mol mol}^{-1}$ , resulted on average in less depleted ( $\sim 1\text{‰}$ )  $\delta^{13}\text{C}$  values. To investigate if these values are representative for other mounds, and to investigate whether an isotopic difference exists between mound and soil-emitted  $\text{CO}_2$ , more measurements would be needed.

450 *Author contributions.* HA designed and performed the field experiment, and wrote the paper, JA was responsible for the determination of the termite species, and gave input on the entomology part of the research, BF and AA provided access to the logistics and infrastructure of the field site, JA, TW, BF, AA and JN reviewed and commented on the paper.

*Competing interests.* The authors declare that they have no conflict of interest.

*Acknowledgements.* The study was funded by the DFG-project 'Methane fluxes from seasonally flooded forests in the Amazon basin' (project nr. 352322796). We are thankful for the support of the crew of the experimental field site ZF2, the research station managed by INPA-LBA (National Institute for Amazonian Research (INPA)- The Large Scale Biosphere-Atmosphere Research Program in the Amazon (LBA)). We would also like to express our gratitude to the staff of LBA, for providing logistics, advice, and support during different phases of this research. In addition, we would like to thank Thiago de Lima Xavier and Leonardo Ramos de Oliveira for their advice in planning the technical parts of the experiment. Furthermore, we would like to acknowledge the group 'Department of Aquatic Biology and Limnology'  
460 (working group MAUA, INPA) for lending us an additional Los Gatos instrument. Last but not least, we would like to thank Sipko Bulthuis for his assistance and ongoing support during the challenging field measurements days.

## References

- Ackerman, I. L.: Termites in ecosystems of central Amazonia: species composition, soil properties, and nutrient cycling, Cornell University, Aug., 2006.
- 465 Ackerman, I. L., Teixeira, W. G., Riha, S. J., Lehmann, J., and Fernandes, E. C.: The impact of mound-building termites on surface soil properties in a secondary forest of Central Amazonia, *Applied soil ecology*, 37, 267–276, 2007.
- Araújo, A., Nobre, A., Kruijt, B., Elbers, J., Dallarosa, R., Stefani, P., Von Randow, C., Manzi, A., Culf, A., Gash, J., et al.: Comparative measurements of carbon dioxide fluxes from two nearby towers in a central Amazonian rainforest: The Manaus LBA site, *Journal of Geophysical Research: Atmospheres*, 107, LBA–58, 2002.
- 470 Ashton, L., Griffiths, H., Parr, C., Evans, T., Didham, R., Hasan, F., Teh, Y., Tin, H., Vairappan, C., and Eggleton, P.: Termites mitigate the effects of drought in tropical rainforest, *Science*, 363, 174–177, 2019.
- Bandeira, F. and Torres, M.: Abundancia e distribuicao de invertebrados do solo em ecossistemas da Amazonia Central. O papel ecologico dos cupins, *Boletim do Museu Paraense Emilio Goeldi Serie Zoologia*, 1985.
- Bignell, D. E.: Termites as soil engineers and soil processors, in: *Intestinal microorganisms of termites and other invertebrates*, pp. 183–220, 475 Springer, 2006.
- Bignell, D. E.: Termite Ecology in the First Two Decades of the 21st Century: A Review of Reviews, *Insects*, 10, 60, 2019.
- Bignell, D. E. and Eggleton, P.: Termites in ecosystems, in: *Termites: evolution, sociality, symbioses, ecology*, pp. 363–387, Springer, 2000.
- Bignell, D. E., Eggleton, P., Nunes, L., and Thomas, K. L.: Termites as mediators of carbon fluxes in tropical forest: budgets for carbon dioxide and methane emissions, *Forests and insects*, pp. 109–134, 1997.
- 480 Bourguignon, T., Šobotník, J., Lepoint, G., MARTIN, J.-M., Hardy, O. J., Dejean, A., and Roisin, Y.: Feeding ecology and phylogenetic structure of a complex neotropical termite assemblage, revealed by nitrogen stable isotope ratios, *Ecological Entomology*, 36, 261–269, 2011.
- Brauman, A., Kane, M. D., Labat, M., and Breznak, J. A.: Genesis of acetate and methane by gut bacteria of nutritionally diverse termites, *Science*, 257, 1384–1387, 1992.
- 485 Brauman, A., Majeed, M. Z., Buatois, B., Robert, A., Pablo, A.-L., and Miambi, E.: Nitrous oxide (N<sub>2</sub>O) emissions by termites: does the feeding guild matter?, *PloS one*, 10, 2015.
- Brian, M. V. and Brian, M.: *Production ecology of ants and termites*, vol. 13, Cambridge University Press, 1978.
- Bruhn, D., Møller, I. M., Mikkelsen, T. N., and Ambus, P.: Terrestrial plant methane production and emission, *Physiologia Plantarum*, 144, 201–209, 2012.
- 490 Brümmer, C., Papen, H., Wassmann, R., and Brüggemann, N.: Fluxes of CH<sub>4</sub> and CO<sub>2</sub> from soil and termite mounds in south Sudanian savanna of Burkina Faso (West Africa), *Global Biogeochemical Cycles*, 23, 2009a.
- Brümmer, C., Papen, H., Wassmann, R., and Brüggemann, N.: Termite mounds as hot spots of nitrous oxide emissions in South-Sudanian savanna of Burkina Faso (West Africa), *Geophysical Research Letters*, 36, 2009b.
- Brune, A.: Methanogenesis in the digestive tracts of insects and other arthropods, *Biogenesis of Hydrocarbons. Handbook of Hydrocarbon and Lipid Microbiology*. Cham: Springer International Publishing, pp. 1–32, 2018.
- 495 Chambers, J. Q., Tribuzy, E. S., Toledo, L. C., Crispim, B. F., Higuchi, N., Santos, J. d., Araújo, A. C., Kruijt, B., Nobre, A. D., and Trumbore, S. E.: Respiration from a tropical forest ecosystem: partitioning of sources and low carbon use efficiency, *Ecological Applications*, 14, 72–88, 2004.

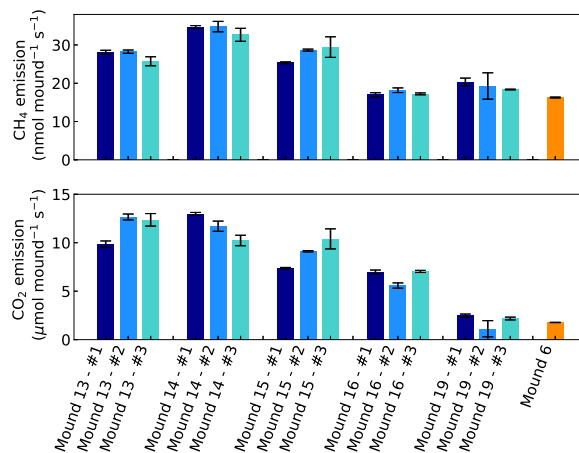


- Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., et al.: Carbon and other biogeochemical cycles, in: *Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, pp. 465–570, Cambridge University Press, 2014.
- Clough, T. J., Rochette, P., Thomas, S. M., Pihlatie, M., Christiansen, J. R., and Thorman, R. E.: *Global Research Alliance N<sub>2</sub>O chamber methodology guidelines: Design considerations*, *Journal of Environmental Quality*, 49, 1081–1091, 2020.
- Conrad, R.: Soil microorganisms as controllers of atmospheric trace gases (H<sub>2</sub>, CO, CH<sub>4</sub>, OCS, N<sub>2</sub>O, and NO), *Microbiological reviews*, 60, 609–640, 1996.
- Constantino, R.: Abundance and diversity of termites (Insecta: Isoptera) in two sites of primary rain forest in Brazilian Amazonia, *Biotropica*, pp. 420–430, 1992.
- Cook, S.: The respiratory gas exchange in *Termopsis nevadensis*, *The Biological Bulletin*, 63, 246–257, 1932.
- Dambros, C. S., Morais, J. W., Vasconcellos, A., Souza, J. L., Franklin, E., and Gotelli, N. J.: Association of ant predators and edaphic conditions with termite diversity in an Amazonian rain forest, *Biotropica*, 48, 237–245, 2016.
- Darlington, J. P.: A method for sampling the populations of large termite nests, *Annals of Applied Biology*, 104, 427–436, 1984.
- De Araujo, A., Ometto, J., Dolman, A., Kruijt, B., and Ehleringer, J.: Implications of CO<sub>2</sub> pooling on  $\delta^{13}\text{C}$  of ecosystem respiration and leaves in Amazonian forest, *Biogeosciences*, 5, 779–795, 2008.
- De Bruyn, L. and Conacher, A. J.: The role of termites and ants in soil modification—a review, *Soil research*, 28, 55–93, 1990.
- de Souza, O. F. F. and Brown, V. K.: Effects of habitat fragmentation on Amazonian termite communities, *Journal of Tropical Ecology*, pp. 197–206, 1994.
- Eggleton, P.: Global patterns of termite diversity, in: *Termites: evolution, sociality, symbioses, ecology*, pp. 25–51, Springer, 2000.
- Eggleton, P. and Tayasu, I.: Feeding groups, lifetypes and the global ecology of termites, *Ecological research*, 16, 941–960, 2001.
- Eggleton, P., Homathevi, R., Jones, D., MacDonald, J., Jeeva, D., Bignell, D., Davies, R., and Maryati, M.: Termite assemblages, forest disturbance and greenhouse gas fluxes in Sabah, East Malaysia, *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 354, 1791–1802, 1999.
- Fraser, P., Rasmussen, R., Creffield, J., French, J., and Khalil, M.: Termites and global methane—another assessment, *Journal of Atmospheric Chemistry*, 4, 295–310, 1986.
- Gomati, V., Ramasamy, K., Kumar, K., Sivaramaiah, N., and Mula, R.: Green house gas emissions from termite ecosystem, *African Journal of Environmental Science and Technology*, 5, 56–64, 2011.
- Griffith, D., Deutscher, N., Caldow, C., Kettlewell, G., Riggenbach, M., and Hammer, S.: A Fourier transform infrared trace gas and isotope analyser for atmospheric applications, *Atmospheric Measurement Techniques*, 5, 2481–2498, 2012.
- Hammer, S., Griffith, D., Konrad, G., Vardag, S., Caldow, C., and Levin, I.: Assessment of a multi-species in-situ FTIR for precise atmospheric greenhouse gas observations, *Atmospheric Measurement Techniques*, 6, 1153–1170, 2013.
- Ho, A., Erens, H., Mujinya, B. B., Boeckx, P., Baert, G., Schneider, B., Frenzel, P., Boon, N., and Van Ranst, E.: Termites facilitate methane oxidation and shape the methanotrophic community, *Appl. Environ. Microbiol.*, 79, 7234–7240, 2013.
- Jamali, H., Livesley, S. J., Dawes, T. Z., Cook, G. D., Hutley, L. B., and Arndt, S. K.: Diurnal and seasonal variations in CH<sub>4</sub> flux from termite mounds in tropical savannas of the Northern Territory, Australia, *Agricultural and Forest Meteorology*, 151, 1471–1479, 2011a.
- Jamali, H., Livesley, S. J., Dawes, T. Z., Hutley, L. B., and Arndt, S. K.: Termite mound emissions of CH<sub>4</sub> and CO<sub>2</sub> are primarily determined by seasonal changes in termite biomass and behaviour, *Oecologia*, 167, 525–534, 2011b.

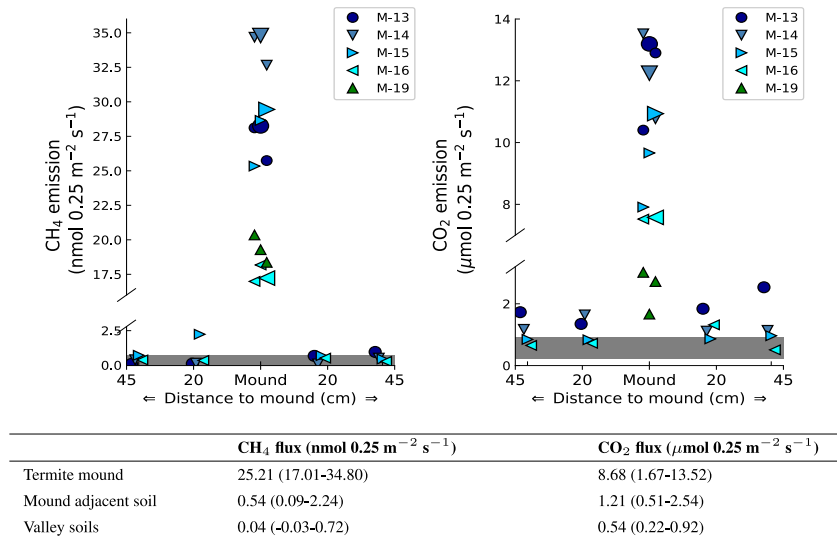
- Jamali, H., Livesley, S., Hutley, L. B., Fest, B., and Arndt, S.: The relationships between termite mound CH<sub>4</sub>/CO<sub>2</sub> emissions and internal concentration ratios are species specific, *Biogeosciences*, 10, 2229–2240, 2013.
- Jones, D. T. and Eggleton, P.: Global biogeography of termites: a compilation of sources, in: *Biology of termites: a modern synthesis*, pp. 477–498, Springer, 2010.
- 540 Jones, D. T., Verkerk, R. H., and Eggleton, P.: Methods for sampling termites, *Insect sampling in forest ecosystems*, pp. 221–253, 2005.
- Josens, G. and Soki, K.: Relation between termite numbers and the size of their mounds, *Insectes sociaux*, 57, 303–316, 2010.
- Khalil, M., Rasmussen, R., French, J., and Holt, J.: The influence of termites on atmospheric trace gases: CH<sub>4</sub>, CO<sub>2</sub>, CHCl<sub>3</sub>, N<sub>2</sub>O, CO, H<sub>2</sub>, and light hydrocarbons, *Journal of Geophysical Research: Atmospheres*, 95, 3619–3634, 1990.
- Kirschke, S., Bousquet, P., Ciais, P., Saunoy, M., Canadell, J. G., Dlugokencky, E. J., Bergamaschi, P., Bergmann, D., Blake, D. R., Bruhwiler, L., et al.: Three decades of global methane sources and sinks, *Nature geoscience*, 6, 813–823, 2013.
- 545 Kisselle, K. W., Zepp, R. G., Burke, R. A., de Siqueira Pinto, A., Bustamante, M. M., Opsahl, S., Varella, R. F., and Viana, L. T.: Seasonal soil fluxes of carbon monoxide in burned and unburned Brazilian savannas, *Journal of Geophysical Research: Atmospheres*, 107, LBA–18, 2002.
- Konaté, S., Roux, X. L., Verdier, B., and Lepage, M.: Effect of underground fungus-growing termites on carbon dioxide emission at the point-and landscape-scales in an African savanna, *Functional Ecology*, 17, 305–314, 2003.
- 550 Krishna, K. and Araujo, R.: A revision of the neotropical termite genus *Neocapritermes* (Isoptera, Termitidae, Termitinae). *Bulletin of the AMNH*; v. 138, article 3, 1968.
- Lee, H., Rahn, T., and Throop, H.: An accounting of C-based trace gas release during abiotic plant litter degradation, *Global Change Biology*, 18, 1185–1195, 2012.
- 555 Lee, K. E., Wood, T. G., et al.: *Termites and soils.*, Termites and soils., 1971.
- Lepage, M. and Darlington, J. P.: Population dynamics of termites, in: *Termites: evolution, sociality, symbioses, ecology*, pp. 333–361, Springer, 2000.
- Liu, L., Zhuang, Q., Zhu, Q., Liu, S., Van Asperen, H., and Pihlatie, M.: Global soil consumption of atmospheric carbon monoxide: an analysis using a process-based biogeochemistry model, *Atmospheric Chemistry and Physics (Online)*, 18, 2018.
- 560 Luizão, R. C., Luizão, F. J., Paiva, R. Q., Monteiro, T. F., Sousa, L. S., and Kruijt, B.: Variation of carbon and nitrogen cycling processes along a topographic gradient in a central Amazonian forest, *Global Change Biology*, 10, 592–600, 2004.
- MacDonald, J., Jeeva, D., Eggleton, P., Davies, R., Bignell, D., Fowler, D., Lawton, J., and Maryati, M.: The effect of termite biomass and anthropogenic disturbance on the CH<sub>4</sub> budgets of tropical forests in Cameroon and Borneo, *Global change biology*, 5, 869–879, 1999.
- Macdonald, J. A., Eggleton, P., Bignell, D. E., Forzi, F., and Fowler, D.: Methane emission by termites and oxidation by soils, across a forest disturbance gradient in the Mbalmayo Forest Reserve, Cameroon, *Global Change Biology*, 4, 409–418, 1998.
- 565 Martius, C., Waßmann, R., Thein, U., Bandeira, A., Renneberg, H., Junk, W., and Seiler, W.: Methane emission from wood-feeding termites in Amazonia, *Chemosphere*, 26, 623–632, 1993.
- Martius, C., Fearnside, P. M., Bandeira, A. G., and Wassmann, R.: Deforestation and methane release from termites in Amazonia, *Chemosphere*, 33, 517–536, 1996.
- 570 Mishra, S., Sen-Sarma, P., et al.: Humic acids in faecal matter, nest material and fungus comb of termites., *Bulletin of Entomology*, 21, 122–125, 1980.
- 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.

- 575 Nauer, P. A., Hutley, L. B., and Arndt, S. K.: Termite mounds mitigate half of termite methane emissions, *Proceedings of the National Academy of Sciences*, 115, 13 306–13 311, 2018.
- Noirot, C. and Darlington, J. P.: Termite nests: architecture, regulation and defence, in: *Termites: evolution, sociality, symbioses, ecology*, pp. 121–139, Springer, 2000.
- Ohiagu, C. and Wood, T.: A method for measuring rate of grass-harvesting by *Trinervitermes geminatus* (Wasmann)(Isoptera, Nasutitermitinae) and observation on its foraging behaviour in Southern Guinea Savanna, Nigeria, *Journal of applied Ecology*, pp. 705–713, 1976.
- 580 Oliveira, J. R. A. d. et al.: Padrões de distribuição e variação temporal de térmitas (Blattodea: Isoptera): uso como bioindicadores na amazônia central, 2016.
- Pangala, S. R., Hornibrook, E. R., Gowing, D. J., and Gauci, V.: The contribution of trees to ecosystem methane emissions in a temperate forested wetland, *Global Change Biology*, 21, 2642–2654, 2015.
- Pataki, D., Ehleringer, J., Flanagan, L., Yakir, D., Bowling, D., Still, C., Buchmann, N., Kaplan, J., and Berry, J.: The application and interpretation of Keeling plots in terrestrial carbon cycle research, *Global biogeochemical cycles*, 17, 2003.
- 585 Pequeno, P. A., Baccaro, F. B., Souza, J. L., and Franklin, E.: Ecology shapes metabolic and life history scalings in termites, *Ecological Entomology*, 42, 115–124, 2017.
- Pequeno, P. A. L.: Negative effects of Azteca ants on the distribution of the termite *Neocapritermes braziliensis* in central Amazonia, *Sociobiology*, 59, 893–902, 2014.
- 590 Pequeno, P. A. L., Franklin, E., Venticinque, E. M., and Serrao Acioli, A. N.: The scaling of colony size with nest volume in termites: a role in population dynamics?, *Ecological Entomology*, 38, 515–521, 2013.
- Pester, M., Tholen, A., Friedrich, M. W., and Brune, A.: Methane oxidation in termite hindguts: absence of evidence and evidence of absence, *Appl. Environ. Microbiol.*, 73, 2024–2028, 2007.
- Potter, C. S., Klooster, S. A., and Chatfield, R. B.: Consumption and production of carbon monoxide in soils: a global model analysis of spatial and seasonal variation, *Chemosphere*, 33, 1175–1193, 1996.
- 595 Queiroz, J. M. T. d.: Fluxo de metano em cupinzeiros epígeos em florestas e ambientes alterados na Amazônia Central, MSc thesis INPA/UFAM, 2004.
- Querino, C., Smeets, C., Vigano, I., Holzinger, R., Moura, V., Gatti, L., Martinewski, A., Manzi, A., De Araújo, A., and Röckmann, T.: Methane flux, vertical gradient and mixing ratio measurements in a tropical forest, *Atmospheric chemistry and physics*, 11, 7943–7953, 2011.
- 600 Quesada, C., Lloyd, J., Schwarz, M., Patino, S., Baker, T., Czimeczik, C., Fyllas, N., Martinelli, L., Nardoto, G., Schmerler, J., et al.: Variations in chemical and physical properties of Amazon forest soils in relation to their genesis, *Biogeosciences*, 7, 1515–1541, 2010.
- Reuß, J., Rachel, R., Kämpfer, P., Rabenstein, A., Küver, J., Dröge, S., and König, H.: Isolation of methanotrophic bacteria from termite gut, *Microbiological research*, 179, 29–37, 2015.
- 605 Ribeiro, J.: Ecologia de *Labiotermes labralis* (Isoptera: Termitidae) em Floresta e Terra firme na Amazônia, Ph.D. thesis, Dissertation, Manaus, INPA/FUA, 1997.
- Rice, A. L., Butenhoff, C. L., Shearer, M. J., Teama, D., Rosenstiel, T. N., and Khalil, M. A. K.: Emissions of anaerobically produced methane by trees, *Geophysical Research Letters*, 37, 2010.
- Rouland, C., Brauman, A., Labat, M., and Lepage, M.: Nutritional factors affecting methane emission from termites, *Chemosphere*, 26, 610 617–622, 1993.

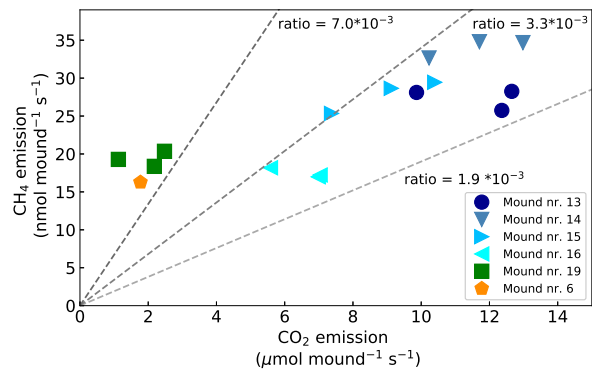
- Sanderson, M.: Biomass of termites and their emissions of methane and carbon dioxide: A global database, *Global Biogeochemical Cycles*, 10, 543–557, 1996.
- Sands, W.: Mound population movements and fluctuations in *Trinervitermes ebenerianus* Sjöstedt (Isoptera, termitidæ, nasutitermitinæ), *Insectes Sociaux*, 12, 49–58, 1965.
- 615 Saunois, M., Stavert, A. R., Poulter, B., Bousquet, P., Canadell, J. G., Jackson, R. B., Raymond, P. A., Dlugokencky, E. J., Houweling, S., Patra, P. K., et al.: The global methane budget 2000–2017, *Earth system science data*, 12, 1561–1623, 2020.
- Sawadogo, J., Traoré, A., and Dianou, D.: Seasonal CO and CH Emissions from Termite Mounds, *differences*, 4, 2, 2012.
- Sawadogo, J. B., Dianou, D., Traoré, A. S., et al.: Effects of temperature and termite's substrate on methane and carbon dioxide emissions from *Macrotermes bellicosus* and *Microcerotermes dubius* cultures, *Int. J. Biol. Chem. Sci.*, 9, 75–83, 2011.
- 620 Seiler, W., Conrad, R., and Scharffe, D.: Field studies of methane emission from termite nests into the atmosphere and measurements of methane uptake by tropical soils, *Journal of Atmospheric Chemistry*, 1, 171–186, 1984.
- Souza, J. S. d.: Dinâmica espacial e temporal do fluxo de CO<sub>2</sub> do solo em floresta de terra firme na Amazônia Central, 2005.
- Sugimoto, A., Inoue, T., Kirtibutr, N., and Abe, T.: Methane oxidation by termite mounds estimated by the carbon isotopic composition of methane, *Global Biogeochemical Cycles*, 12, 595–605, 1998a.
- 625 Sugimoto, A., Inoue, T., Tayasu, I., Miller, L., Takeichi, S., and Abe, T.: Methane and hydrogen production in a termite-symbiont system, *Ecological Research*, 13, 241–257, 1998b.
- Sugimoto, A., Bignell, D. E., and MacDonald, J. A.: Global impact of termites on the carbon cycle and atmospheric trace gases, in: *Termites: evolution, sociality, symbioses, ecology*, pp. 409–435, Springer, 2000.
- Sun, W., Kooijmans, L. M., Maseyk, K., Chen, H., Mammarella, I., Vesala, T., Levula, J., Keskinen, H., and Seibt, U.: Soil fluxes of carbonyl sulfide (COS), carbon monoxide, and carbon dioxide in a boreal forest in southern Finland, *Atmospheric Chemistry and Physics*, 18, 1363–1378, 2018.
- 630 Wang, Y., Chen, H., Zhu, Q., Peng, C., Wu, N., Yang, G., Zhu, D., Tian, J., Tian, L., Kang, X., et al.: Soil methane uptake by grasslands and forests in China, *Soil Biology and Biochemistry*, 74, 70–81, 2014.
- Whalen, S. and Reeburgh, W.: Carbon monoxide consumption in upland boreal forest soils, *Soil Biology and Biochemistry*, 33, 1329–1338, 2001.
- 635 Wood, T.: Termites and the soil environment, *Biology and fertility of soils*, 6, 228–236, 1988.
- Yonemura, S., Kawashima, S., and Tsuruta, H.: Carbon monoxide, hydrogen, and methane uptake by soils in a temperate arable field and a forest, *Journal of Geophysical Research: Atmospheres*, 105, 14 347–14 362, 2000a.
- Yonemura, S., Yokozawa, M., Kawashima, S., and Tsuruta, H.: Model analysis of the influence of gas diffusivity in soil on CO and H<sub>2</sub> uptake, *Tellus B: Chemical and Physical Meteorology*, 52, 919–933, 2000b.
- 640 Zanchi, F. B., Meesters, A. G., Kruijt, B., Kesselmeier, J., Luizão, F. J., and Dolman, A. J.: Soil CO<sub>2</sub> exchange in seven pristine Amazonian rain forest sites in relation to soil temperature, *Agricultural and Forest Meteorology*, 192, 96–107, 2014.
- Zimmerman, P., Greenberg, J., Wandiga, S., and Crutzen, P.: Termites: a potentially large source of atmospheric methane, carbon dioxide, and molecular hydrogen, *Science*, pp. 563–565, 1982.



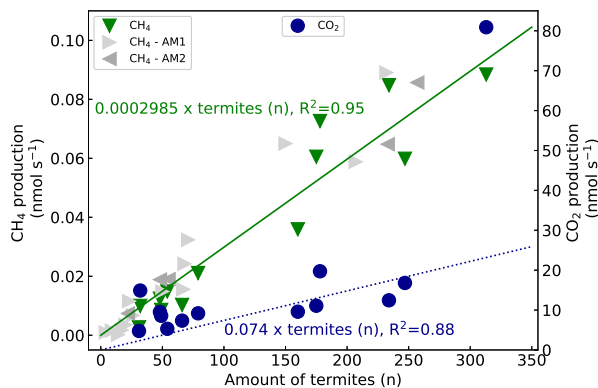
**Figure 1.** CH<sub>4</sub> and CO<sub>2</sub> emissions of mounds nr. 13 -19 (in valley) and of mound nr. 6 (on plateau), expressed in nmol and μmol mound<sup>-1</sup> s<sup>-1</sup>, which represents a collar area of 0.25 m<sup>2</sup>. All mounds (except mound nr. 6) were measured 3 times during one week, and each series-nr (#) was measured on the same day and in the same order. Error bars are propagated standard errors of the linear regression slope, as described in §2.4.



**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 total 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.

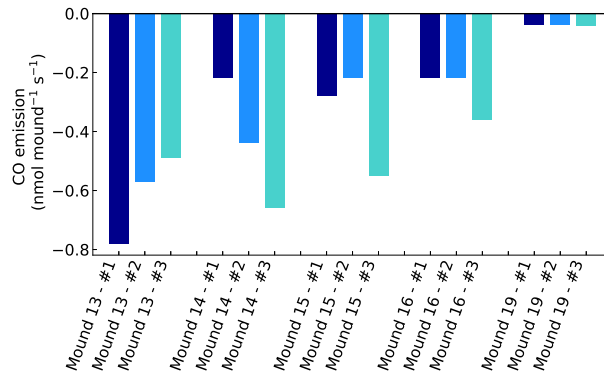


**Figure 3.** Mound CO<sub>2</sub> emissions ( $\mu\text{mol mound}^{-1} \text{s}^{-1}$ ) versus mound CH<sub>4</sub> emissions ( $\text{nmol mound}^{-1} \text{s}^{-1}$ ). Dotted lines indicate the different dCH<sub>4</sub>/dCO<sub>2</sub> emission ratios.

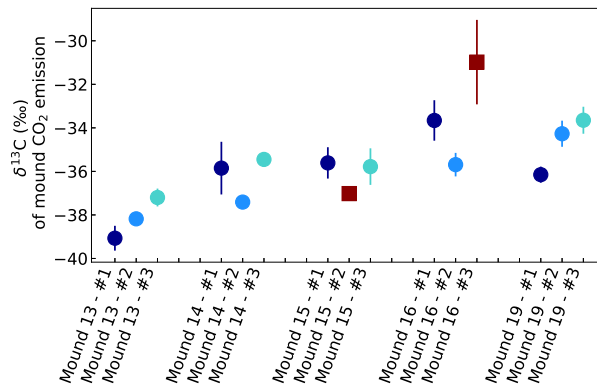


**Figure 4.** CH<sub>4</sub> production (left axis, green triangles) and CO<sub>2</sub> production (right axis, blue circles), over counted termites. The lines (green solid for CH<sub>4</sub>, blue dashed for CO<sub>2</sub>) represent a linear regression fit with forced intercept at y=0. For CH<sub>4</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>-2</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>-3</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 measurement 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>).





**Figure A1.** CO emissions of valley mounds nr. 13 -19, expressed in  $\text{nmol mound}^{-1} \text{s}^{-1}$ , which represents a collar area of  $0.25 \text{ m}^2$ . All mounds were measured 3 times during one week, and each series-nr (#) was measured on the same day and in the same order.



**Figure A2.**  $\delta^{13}\text{C}$  of  $\text{CO}_2$  emitted by mounds nr. 13 -19, derived by use of Keeling plots. Error bars represent the standard error of the linear regression intercept. Red squares indicate intercepts based on linear regression fits with  $R^2 < 0.99$ , or based on linear regression with only 2 instead of 3 sample points. All mounds were measured 3 times during one week, and each series-nr was measured on the same day and in the same order. Per mound, an average was calculated, which were  $-38.1\text{‰}$  (mound nr. 13,  $\text{se}=0.9$ ),  $-36.2\text{‰}$  (mound nr. 14,  $\text{se}=1.0$ ),  $-35.7\text{‰}$  (mound nr. 15,  $\text{se}=0.1$ ),  $-34.7\text{‰}$  (mound nr. 16,  $\text{se}=1.4$ ), and  $-34.7\text{‰}$  (mound nr. 19,  $\text{se}=1.3$ ). For calculation of these averages, values with a linear regression of  $R^2 < 0.99$  or values based on a linear regression of only two measurements (indicated as dark red squares), were excluded.

**Table 1.** Termite mounds: location, dimensions, and observed species. Volume is estimated mound volume as calculated by Eq. (1), and surface is estimated mound surface by mathematically considering the lower part of the mound as a column, and the upper part as half a sphere. In mound 1, two different termite species were found. *N. bra* stands for *Neocapritermes brasiliensis*, *H. ten* for *Heterotermes tenuis*, *R. bra* for *Rotunditermes bracantinus*, and *E. neo* for *Enbiratermes neotenicus*. 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.

Nr	Location	Height	Perimeter	Volume	Surface	Species
1	valley	50 cm	128 cm			<i>N. bra, H. ten</i>
2	slope	45 cm	145 cm			<i>N. bra</i>
3	plateau	35 cm	128 cm			<i>N. bra</i>
4	plateau	55 cm	138 cm			<i>N. bra</i>
5	plateau	45 cm	148 cm			<i>R. bra</i>
<b>6</b>	<b>plateau</b>	<b>47 cm</b>	<b>99 cm</b>	<b>33.8 L</b>	<b>4653 cm<sup>2</sup></b>	<b><i>E. neo</i></b>
7	plateau	50 cm	160 cm			<i>E. neo</i>
8	slope	35 cm	160 cm			<i>E. neo</i>
9	valley	37 cm	105 cm			<i>N. bra</i>
10	valley	50 cm	94 cm			<i>N. bra</i>
11	valley	45 cm	111 cm			<i>N. bra</i>
12	valley	65 cm	125 cm			<i>N. bra</i>
<b>13</b>	<b>valley</b>	<b>65 cm</b>	<b>150 cm</b>	<b>77.6 L</b>	<b>9750 cm<sup>2</sup></b>	<b><i>N. bra</i></b>
<b>14</b>	<b>valley</b>	<b>54 cm</b>	<b>118 cm</b>	<b>48.0 L</b>	<b>6372 cm<sup>2</sup></b>	<b><i>N. bra</i></b>
<b>15</b>	<b>valley</b>	<b>58 cm</b>	<b>121 cm</b>	<b>50.5 L</b>	<b>7018 cm<sup>2</sup></b>	<b><i>N. bra</i></b>
<b>16</b>	<b>valley</b>	<b>58 cm</b>	<b>120 cm</b>	<b>49.7 L</b>	<b>6960 cm<sup>2</sup></b>	<b><i>N. bra</i></b>
17	valley	55 cm	157 cm			<i>N. bra</i>
18	valley	75 cm	130 cm			<i>N. bra</i>
<b>19</b>	<b>valley</b>	<b>45 cm</b>	<b>105 cm</b>	<b>38.0 L</b>	<b>4725 cm<sup>2</sup></b>	<b><i>N. bra</i></b>
20	slope	30 cm	92 cm			<i>N. bra</i>

**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 found, measurements from higher soil-feeding termite species were selected. For each study, the graph or table where the data was found, is given. 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 fresh weight of 1.57 mg is 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., *Procupitermes* sp., *Thoracotermes macrothorax*; f) *Dicuspidermes santschii*, *Dicuspidermes nemorosus*, *Pericapritermes semarangi*, *Procapritermes* nr. *Sandakanensis*, *Homallotermes eleanorae*, *Proaciculitermes* sp. A, *Pericapritermes nitobei*; g) *Coptotermes lacteus*; h) *Ancistrotermes cavithorax*, *Odontotermes n. pauperans*; i) *Nasutitermes macrocephalus*, *Nasutitermes corniger*, *Nasutitermes surinamensis*, *Nasutitermes* sp., *Nasutitermes ephratae*, *Nasutitermes araujoii*; j) *Noditermes* sp., *Crenetermes albotarsalis*, *Cubitermes speciosus*, *Thoracotermes macrothorax*, *Astratotermes* sp.; k) *Macrotermes bellicosus*; l) *Microcerotermes* sp., *Globitermes sulphureus*, *Termes* sp., *Dicuspidermes* sp.; m) *Drepanotermes perniger*, *Nasutitermes magnus*, *Nasutitermes triodiae*, *Tumulitermes pastinator*, *Amitermes laurensis*, *Coptotermes lacteus*; n) *Bulbitermes* sp. C, *Dicuspidermes nemorosus*, *Dicuspidermes santschii*; o) *Macrotermes* and *Odontotermes* (Macrotermitinae), *Trinervitermes* (Nasutitermitinae), *Amitermes* and *Cubitermes* (Termitinae), *Hodotermes* (lower termite); p) *Cubitermes fungifaber*; q) *Microcerotermes nervosus*, *Turnulitermes pastinator*, *Turnulitermes hastilis*, *Amitermes meridionalis*.

Studies reporting emission per gram termite							
Study	Study area	CH <sub>4</sub> emission (μmol g <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )	CO <sub>2</sub> emission (μmol g <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )	CH <sub>4</sub> /CO <sub>2</sub>	Species		
<b>This study, Fig. 4</b>	<b>Amazon</b>	<b>0.35 (0.2)</b>	(0.002985 nmol tm <sup>-1</sup> s <sup>-1</sup> )	<b>86.8 (10.0)</b>	(0.074 nmol tm <sup>-1</sup> s <sup>-1</sup> )	~4 <sup>c</sup>	<b>Soil feeders</b> <sup>(d)</sup>
Brauman et al. (1992), Tab. 1	Congo	0.39-1.09	(0.39-1.09 μmol g <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )				Soil feeders <sup>(e)</sup>
Eggleton et al. (1999), Tab. 4	Australia	0.17-0.27	(0.17-0.27 μmol g <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )	1.4-9.0	(1.4-36.4 μmol g <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )	10-154	Soil feeders <sup>(f)</sup>
Fraser et al. (1986), Fig. 2	Australia	0.04 (0.01)	(0.67 (0.2) mg kg <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )	107 (4.5)	(4.7 (0.2) g kg <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )	~0.38 <sup>c</sup>	Wood feeders <sup>(g)</sup>
Konaté et al. (2003), Tab. 1	Ivory Coast			31.4-133.5	(31.4-133.5 nmol mg <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )		Fungi feeders <sup>(h)</sup>
Martius et al. (1993), Tab. 1	Amazon	0.19 (0.08)	(3.0 (1.3) μg g <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )				Wood feeders <sup>(i)</sup>
Rouland et al. (1993), Tab. 1	Congo	0.53-1.09	(0.53-1.09 μmol g <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )				Soil feeders <sup>(j)</sup>
Sawadogo et al. (2011), Tab. 1	Burkino Faso	0.10-0.12	(0.30-0.39 μmol g <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> ) <sup>a</sup>	19-25	(59.4-78.4 μmol g <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> ) <sup>a</sup>	~5 <sup>c</sup>	Wood feeders <sup>(k)</sup>
Sugimoto et al. (1998a), Tab. 3	Thailand	0.03-0.20	(3.4-20.3*10 <sup>-8</sup> mol g <sub>tm</sub> <sup>-1</sup> h <sup>-1</sup> )				Soil feeders <sup>(l)</sup>
Studies reporting emission per nest or mound							
Study	Study area	CH <sub>4</sub> emission (μmol mound <sup>-1</sup> h <sup>-1</sup> )	CO <sub>2</sub> emission (mmol mound <sup>-1</sup> h <sup>-1</sup> )	CH <sub>4</sub> /CO <sub>2</sub>	Species		
<b>This study, Fig. 1</b>	<b>Amazon</b>	<b>61-125</b>	(17.0-34.8 nmol mound <sup>-1</sup> s <sup>-1</sup> )	<b>4-47</b>	(1.1-13.0 μmol mound <sup>-1</sup> s <sup>-1</sup> )	2.8 (0.4)	<b>Soil feeders</b> <sup>(d)</sup>
Khalil et al. (1990), Fig. 4 & Tab. 3	Australia	9-135	(0.04-0.6 μg mound <sup>-1</sup> s <sup>-1</sup> )	4-92	(0.05-1 μg mound <sup>-1</sup> s <sup>-1</sup> )	0.12-11	Wood feeders <sup>(m)</sup>
MacDonald et al. (1999), Tab. 4	Cameroon	1-11	(4.5-49 ng mound <sup>-1</sup> s <sup>-1</sup> )				Soil & wood feeders <sup>(n)</sup>
Martius et al. (1993), Tab. 1	Amazon	125 (150)	(2.0 (2.4) mg nest <sup>-1</sup> h <sup>-1</sup> )				Wood feeders <sup>(i)</sup>
Seiler et al. (1984), Tab. 1	South Africa	1-644	(0.02-10.3 mg nest <sup>-1</sup> h <sup>-1</sup> )	0.7-241	(0.03-10.6 g nest <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*10 <sup>-7</sup> mol nest <sup>-1</sup> h <sup>-1</sup> )				Soil feeders <sup>(l)</sup>
Studies reporting emission per area							
Study	Study area	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>	Species		
<b>This study, Fig. 1</b>	<b>Amazon</b>	<b>245-501<sup>b</sup></b>	(17.0-34.8 nmol mound <sup>-1</sup> s <sup>-1</sup> )	<b>16-187<sup>b</sup></b>	(1.1-13.0 μmol mound <sup>-1</sup> s <sup>-1</sup> )	2.8 (0.4)	<b>Soil feeders</b> <sup>(e)</sup>
Brümmer et al. (2009a), Fig. 5	Burkino Faso	315.7	(3788.9 μg CH <sub>4</sub> -C m <sup>-2</sup> h <sup>-1</sup> )	37.3	(447.0 mg CO <sub>2</sub> -C m <sup>-2</sup> h <sup>-1</sup> )	~8.5 <sup>c</sup>	Soil feeders <sup>(p)</sup>
Jamali et al. (2013), Fig. 1	Australia	32-500	(379-6000 μg CH <sub>4</sub> -C m <sup>-2</sup> h <sup>-1</sup> )	0-129	(0-1550 mg CO <sub>2</sub> -C m <sup>-2</sup> h <sup>-1</sup> )	2.7-11.0	Wood feeders <sup>(q)</sup>
Queiroz (2004), Tab. 4	Amazon	10-24	(0.16-0.38 mg m <sup>-2</sup> h <sup>-1</sup> )				unknown

**Table 3.** Colony size estimates (CSE) based on different methods, values given per thousand (\*10<sup>3</sup>). ‘Mound volume’ is estimated mound volume as given in Table 1, and ‘Mound emission’ is highest measured emission per individual mound. a) CSE based on highest measured mound CH<sub>4</sub> emission, and combined with emission factor of 0.0002985 nmol CH<sub>4</sub> termite<sup>-1</sup> s<sup>-1</sup> (se=1.77 \*10<sup>-5</sup>); b) CSE based on mound volume, by use of mound termite density values (0.2-5.6 termite cm<sup>-3</sup> (Lepage and Darlington, 2000)); c) CSE based on mound surface area (given in Table 1), by use of mound termite surface values (5.6-16.7 termite cm<sup>-2</sup> (Lepage and Darlington, 2000)); d) CSE based on mound volume, by species-specific volume-population equation of  $y=47.94*x^{0.47}$  (x is mound volume (L), y is colony biomass (g)), as given by Pequeno et al. (2013); for termite weight 3.07 mg (sd=0.18) was used. Since mound nr. 6 was of a different species, it is not included in this table.

<b>Mound Nr</b>	<b>Mound volume</b>	<b>Mound emission</b>	<b>CSE by emission<sup>a</sup></b>	<b>CSE by volume<sup>b</sup></b>	<b>CSE by surface area<sup>c</sup></b>	<b>CSE by species-specific volume<sup>d</sup></b>
13	77.6 L	28.3 nmol mound <sup>-1</sup> s <sup>-1</sup>	89.6 - 100.9	15.5 - 434.6	54.6-162.8	114.0 - 128.2
14	48.0 L	34.8 nmol mound <sup>-1</sup> s <sup>-1</sup>	110.1 -124.0	9.6 - 268.8	35.7-106.4	91.0 - 102.3
15	50.5 L	29.5 nmol mound <sup>-1</sup> s <sup>-1</sup>	93.4 - 105.1	10.1 - 282.8	39.3-117.2	93.2 - 104.8
16	49.7 L	18.2 nmol mound <sup>-1</sup> s <sup>-1</sup>	57.6 - 64.9	9.9 - 278.3	39.0-116.2	92.5 - 104.0
19	38.0 L	20.4 nmol mound <sup>-1</sup> s <sup>-1</sup>	64.6 - 72.7	7.6 - 212.8	26.5-78.9	81.5 - 91.7

**Table 4.** Overview of termite-induced CH<sub>4</sub> and CO<sub>2</sub> emissions, based on two different approaches. For comparison, the lowest row shows total (not termite-specific) ecosystem CH<sub>4</sub> and CO<sub>2</sub> flux values, measured at the same field site by previous studies. a) Querino et al. (2011) performed above-canopy Eddy Covariance CH<sub>4</sub> flux measurements, and reported an average CH<sub>4</sub> flux of  $\sim 2 \text{ nmol m}^{-2} \text{ s}^{-1}$ ; b) Chambers et al. (2004) quantified different respiratory CO<sub>2</sub> sources in this ecosystem, and estimated the total ecosystem respiration to be  $7.8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ .

<b>Estimation approach</b>	<b>CH<sub>4</sub> (nmol m<sup>-2</sup> s<sup>-1</sup>)</b>	<b>CO<sub>2</sub> (μmol m<sup>-2</sup> s<sup>-1</sup>)</b>
1) Mounds per hectare * emission per mound (mol mound <sup>-1</sup> s <sup>-1</sup> )	0.15-0.71	0.05-0.23
2) Termite biomass estimate (g m <sup>-2</sup> ) * termite emission factor (mol g <sub>termite</sub> <sup>-1</sup> s <sup>-1</sup> )	0.5-1.1	0.27
Total (not termite-specific) ecosystem fluxes	$\sim 2^a$	$7.8^b$