ARE TERMITE MOUNDS BIOFILTERS FOR METHANE?

Challenges and new approaches to quantify methane oxidation in termite mounds

Philipp A. Nauer[1], Lindsay B. Hutley[2], Mila Bristow[2] and Stefan K. Arndt[1]*

BACKGROUND

ISOTOPE MODELS

PHYSICAL CHARACTERISATION

In tropical regions, termites release major amounts of the greenhouse gas methane into the atmosphere. However, the contribution of termites to the global methane budget is relatively uncertain. This ongoing project aims to improve our understanding of the processes governing these emissions by investigating microbial methane oxidation (MOX) inside termite mounds (TMs).

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INHIBITORS

TRACER TESTS

FIELD SURVEY

[1]University of Melbourne
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The main challenge for quantification of MOX in TMs is to separate production and consumption of CH_4, as well as gas transport inside the mound. The only field study conducted on the topic employed an approach based on isotope fractionation. However, results may be biased when not accounting for gas transport effects on the isotopic signature of CH_4.

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We propose two independent approaches to quantify MOX in TMs: i) the use of specific inhibitor gases to separate production and consumption of CH_4; and ii) the use of tracers to account for gas transport in TMs. Preliminary results are presented that demonstrate the feasibility of these methods. In a later phase we will employ the methods in a comprehensive field survey to investigate key factors that determine MOX in TMs.

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A further challenge for accurate scaling of fluxes and turnover rates is the highly irregular shape and internal structure of TMs. We are working on an innovative approach based on image-based 3D reconstruction of TMs to estimate bulk volume, surface area and density, and CT scanning to estimate species-specific internal void volume and porosity.

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Termites and their Mounds

Termites are nest- or mound-building social insects that live in colonies throughout the tropics and subtropics. Termite mounds are considered part of the extended organism (the colony), thus can be found in an impressive variety of sizes, shapes and internal structures highly specific to the respective species. Besides fortification, homeostasis and food storage, maintaining an efficient exchange of respiratory gases is one of the key functions of a termite mound.

Special mound types found in Northern Australia.

Image source: Korb (2011)

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Korb, J. (2011). Termite mound architecture, from function to construction. In D. E. Bignell, Y. Roisin, & N. Lo (Eds.), *Biology of Termites: a Modern Synthesis* (pp. 349–373). Springer Netherlands.

Some typical mound types found in Northern Australia.

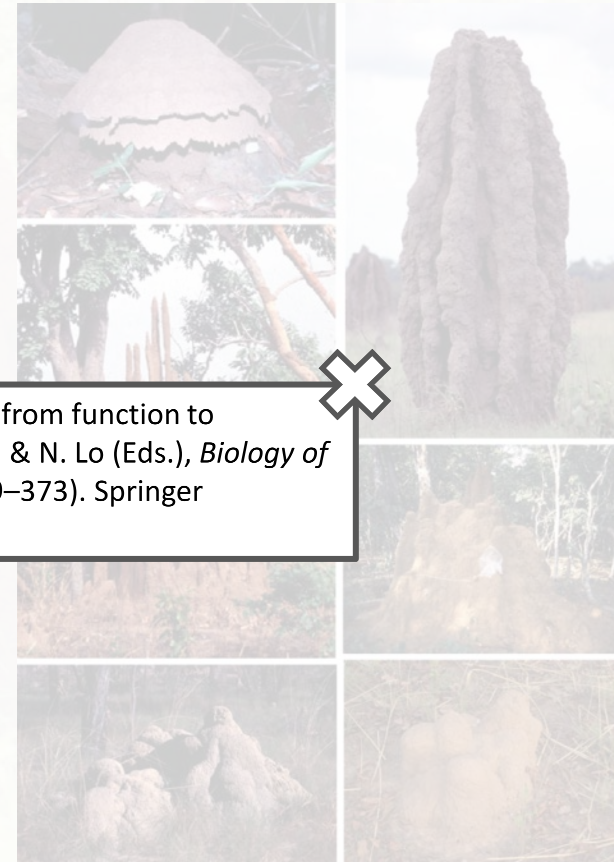


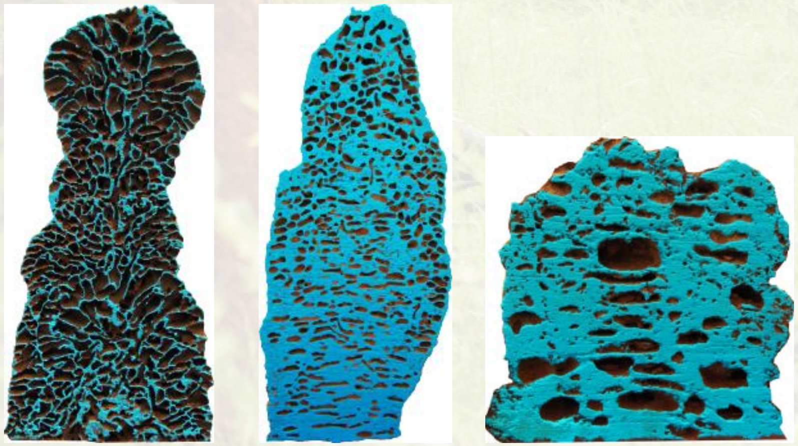
Image source: Korb, J. (2011)

Gas Transport in Termite Mounds

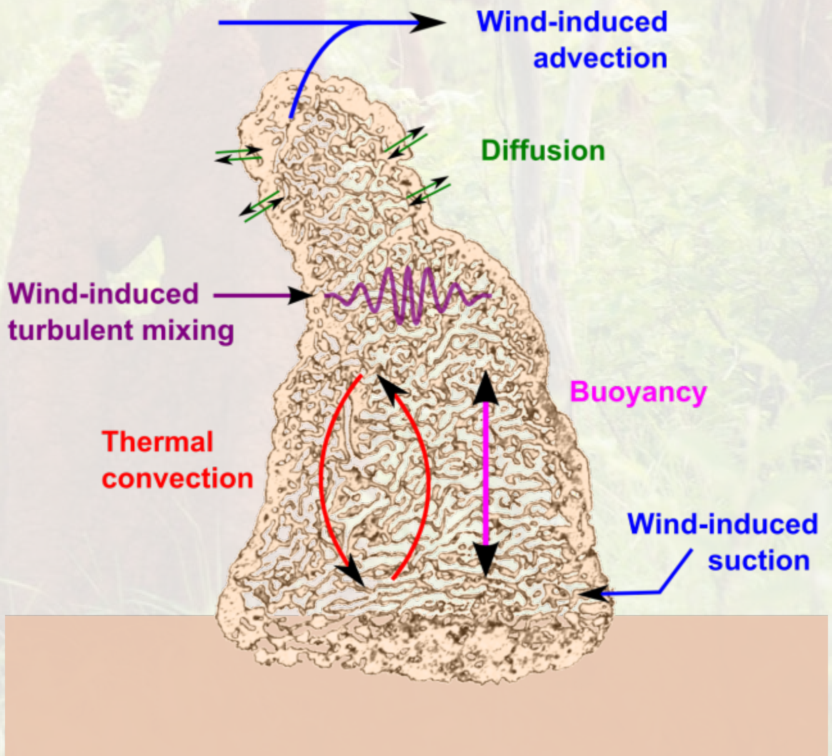
Gas transport in termite mounds can be driven by several possible mechanisms:

- Advection (pressure gradients)
- Diffusion (concentration gradients)
- Convection (temperature gradients)
- Buoyancy (density gradients)

The relative contribution to actual gas exchange varies between species. Advection and diffusion seem to be the dominant mechanisms for exchange through the outer wall.



Painted X-sections of TMs highlighting the diversity of internal structures.



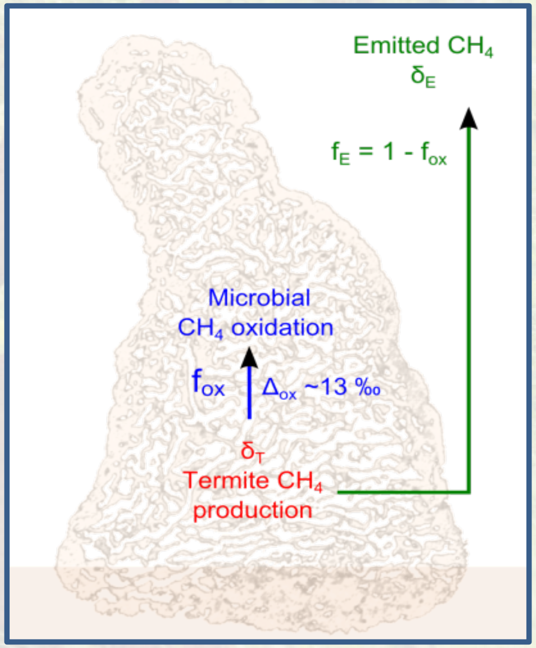
Scheme illustrating the possible mechanisms for gas exchange in TMs.

Isotope Fractionation Method to Estimate MOX in TMs

In principle, the oxic environment in a TM constitutes an ideal habitat for MOB because of the steep O_2/CH_4 counter-gradients. However, up to now only one field study directly estimated MOX in termite mounds. The fraction of oxidised CH_4, f_{ox}, was determined using a closed-system isotope fractionation model. While relatively simple in it's application, small errors in Δ_{ox} may lead to large uncertainty in f_{ox} due to its power law. Further bias from gas transport fractionation highlights the need for different approaches.

sample	δ_T	δ_E	f_E	f_{ox}
1	-89.0	-80.1	0.47	0.53
2	-85.4	-71.8	0.32	0.68
3	-93.5	-72.1	0.17	0.83
4	-97.1	-80.9	0.25	0.75
5	-91.3	-82.4	0.47	0.53
6	-93.8	-70.9	0.15	0.85
7	-91.7	-74.4	0.23	0.77

Stable carbon isotope ratios of produced (δ_T) and emitted (δ_E) CH_4 of 7 TMs measured by Sugimoto et al. (1998), and corresponding f_{ox}. (Emission factor $f_E = 1 – f_{ox}$)



$$\ln(f_E) = \frac{1}{\Delta_{ox}/1000} ln\left(\frac{\delta_E/1000 + 1}{\delta_T/1000 + 1}\right)$$

Illustration of the closed-system isotope fractionation model for a TM following the Rayleygh equation.

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Sugimoto, A., Inoue, T., Kirtibutr, N., & Abe, T. (1998). Methane oxidation by termite mounds estimated by the carbon isotopic composition of methane. *Global Biogeochemical Cycles*, *12*(4), 595–605.

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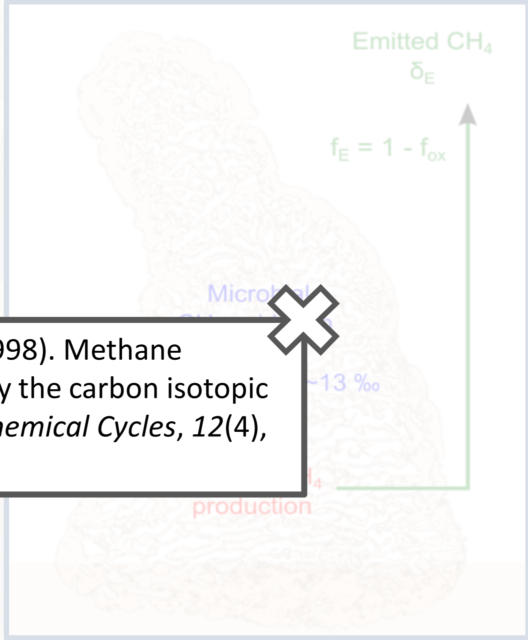

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Closed-system isotope fractionation model following the Rayleygh distillation equation, as employed by Sugimoto et al. (1998).

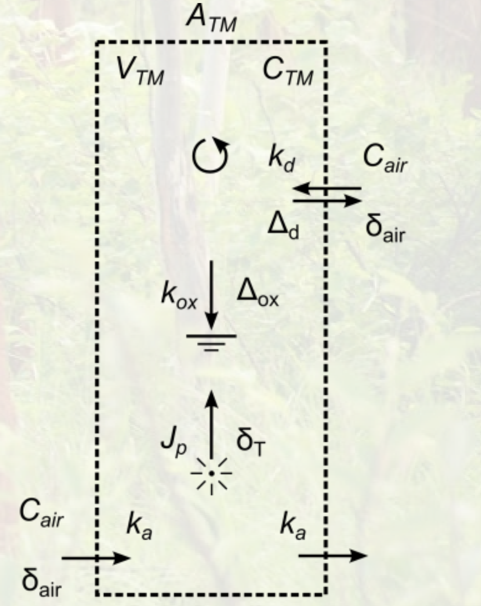
The Challenge with Isotope Fractionation Models

Using a normalised steady-state mass-balance model (flow-through reactor with diffusive boundary) to simulate CH_4 concentrations inside a TM, we illustrate how isotopic shifts of CH_4 ($\delta_T - \delta_\infty$) may occur from physical transport (diffusion and advection) alone. The extent of the shift depends on the relative magnitude of the respective rate coefficients k_i and the production J_p. Thus, in a TM designed for efficient gas exchange, the isotope fractionation method may lead to large biases without further knowledge on gas transport.

Input			Steady-state results ($t = \infty$)		
k_c	k_d	k_a	C_{TM}	δ_∞ (‰)	f_{ox}
1	0	0	1	-77	1
0	1	0	2	-59	0
0	0	1	2	-68	0
1	1	0	1	-62	0.5
0	1	1	1.5	-58	0
1	0	1	1	-62	0.5
1	1	1	1	-57	0.33

Results from simulations with heavy and light CH_4 isotopomers represented as separate chemical species. Rate coefficients are set to unity and switched on or off for the various scenarios.

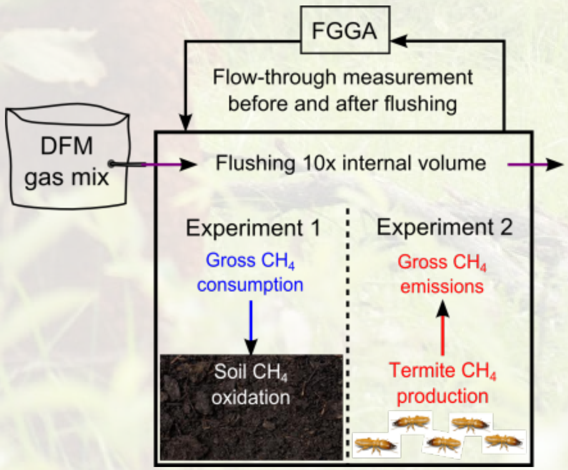
Constant input	
C_0	1
C_{air}	1
J_p	1
δ_T	-89 ‰
δ_{air}	-47 ‰
Δ_{ox}	13 ‰
Δ_d	19 ‰



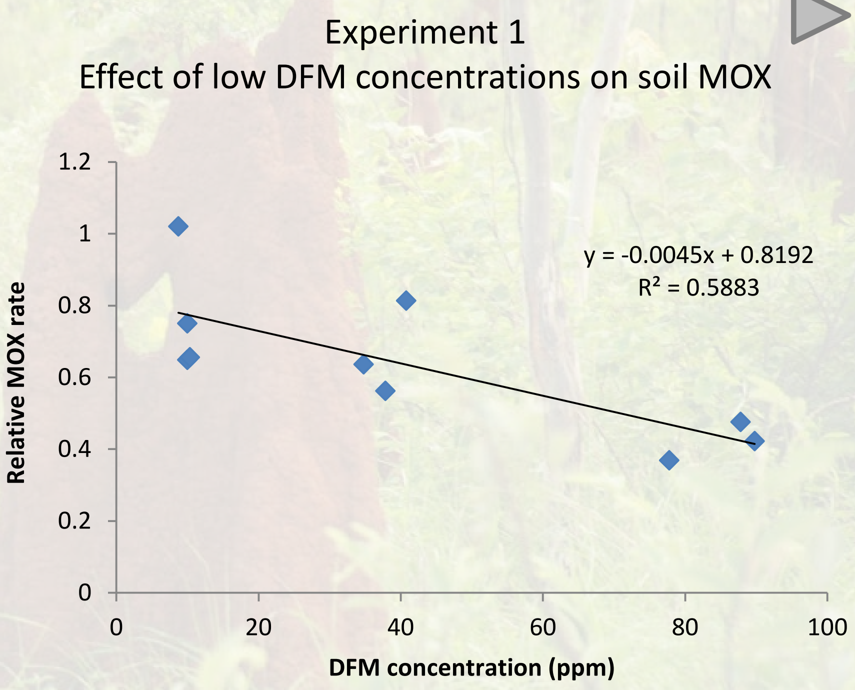
TM: termite mound
d: diffusion
p: production
a: advection

Testing Inhibitors for Injection into TMs

Inhibition of MOX by a selective inhibitor is an effective way of separating source and sink contributions to net CH_4 emissions. We decided to work with the difluoromethane (DFM), the only inhibitor selective for MOX in a wide concentration range. We tested the effect of low DFM concentrations on soil MOX, and high concentrations on CH_4 production rate of termites, with incubation experiments. Methane consumption or production rates were compared before and after application of DFM.



Incubation experiments to test the immediate response of DFM application on CH_4 reaction rates. FGGA: Fast greenhouse-gas analyzer



Soil methane oxidation rates (relative to control rates before DFM injection) decreased with DFM concentrations. Concentrations >100 ppm should effectively inhibit MOX. For field tests 1000ppm-1 vol% will be used due to dilution.

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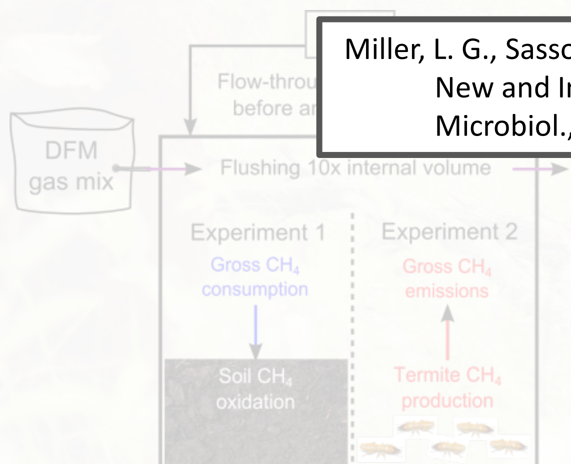

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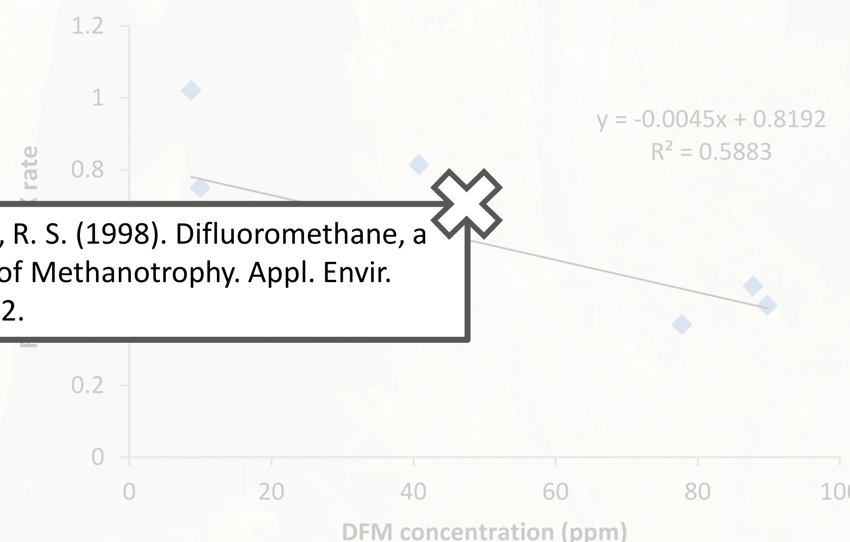
Experiment 1

Effect of low DFM concentrations on soil MOX



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Miller, L. G., Sasson, C., & Oremland, R. S. (1998). Difluoromethane, a New and Improved Inhibitor of Methanotrophy. Appl. Envir. Microbiol., 64(11), 4357–4362.

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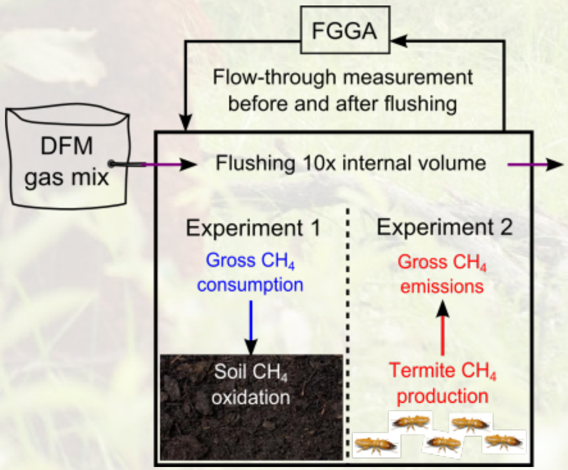

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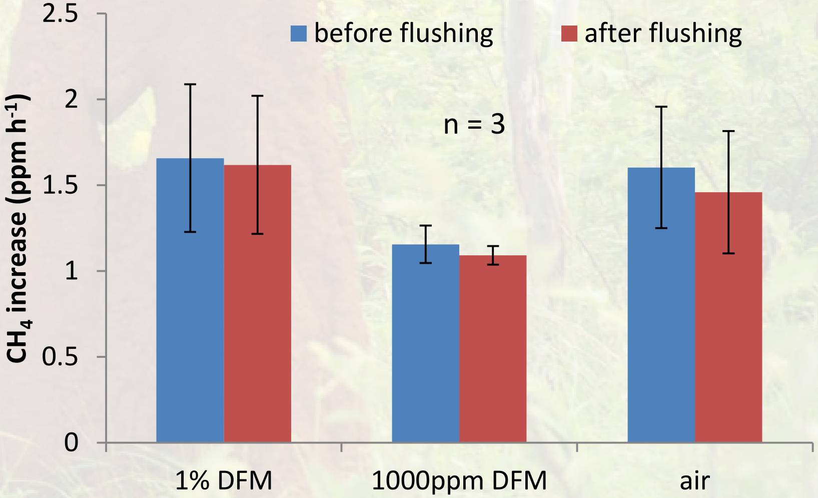
Experiment 2

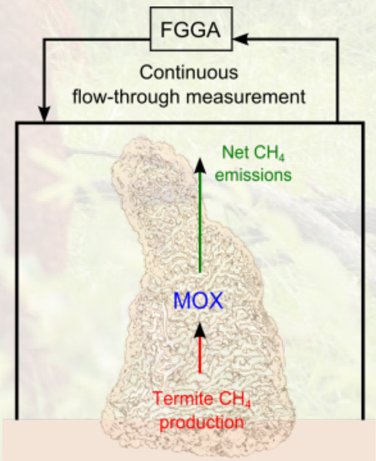
Effect of high DFM concentrations on termites



Termite methane production rates before and after flushing with DFM show no significant difference compared to flushing with air. The application of DFM up to 1 vol% does not affect termites or methane production. Error bars denote one standard deviation.

First Field Application of DFM in TMs

Methane flux of three TMs were compared before and after the injection of DFM in the TM (test 1) or the chamber headspace (test 2). The CH_4 fluxes were corrected for natural fluctuations during the test using CO_2 fluxes. Methane fluxes increased ~20 % and ~60 % in TM1 and TM2, respectively, consistent in both tests. TM3 showed no apparent MOX activity. The fraction of oxidised CH_4 in TM1 and TM2 was estimated to be 0.2 and 0.4.



Field test setup before injection of DFM (closed dynamic chamber; FGGA: Fast Greenhouse-Gas Analyzer)

Mound	Gas	Flux of CO_2 (mg m^{-2} h^{-1}) and CH_4 (µg m^{-2} h^{-1}): before DFM injection	after DFM injection	relative change	corrected change*	MOX in TM	Fraction of oxidized CH_4
Test 1: injection of 8 L of 1000 ppm DFM into TM							
TM1	CO_2	0.22	0.30	33%			
	CH_4	0.50	0.83	66%	25%	0.16	0.2
TM2	CO_2	0.74	0.27	-64%			
	CH_4	4.7	2.8	-40%	66%	1.11	0.4
TM3	CO_2	0.54	0.49	-10%			
	CH_4	0.45	0.38	-15%	-6%	-	
Test 2: injection of 10L of 1 vol% DFM into chamber							
TM1	CO_2	0.36	0.32	-11%			
	CH_4	0.68	0.72	5.5%	18%	0.11	0.2
TM2	CO_2	0.53	0.20	-62%			
	CH_4	3.3	2.01	-39%	59%	0.75	0.4

* Relative change of CH_4 flux compared to change in CO_2 flux

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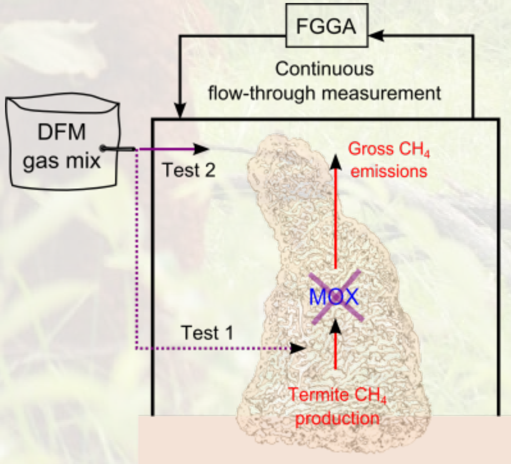

After injection of DFM, MOX should be inhibited and gross CH_4 production is measured.

Mound	Gas	Flux of CO_2 ($mg\ m^{-2}\ h^{-1}$) and CH_4 ($\mu g\ m^{-2}\ h^{-1}$): before DFM injection	after DFM injection	relative change	corrected change*	MOX in TM	Fraction of oxidized CH_4
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Chamber and TM3 with injection tube. The tube was inserted some days prior to the test and sealed by termites.

Mound	Gas	Flux of CO_2 ($mg\ m^{-2}\ h^{-1}$) and CH_4 ($\mu g\ m^{-2}\ h^{-1}$) before DFM injection	after DFM injection	relative change	corrected change*	MOX in TM	Fraction of oxidized CH_4
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Injection of DFM into TM, while gas concentrations in the chamber headspace are monitored with the FGGA.

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Photogrammetric Measurement of Volume and Surface Area

The low-cost software solution Agisoft Photoscan can reconstruct full 3D models from a set of unordered images taken with a conventional digital camera. This approach has been applied and tested for 3D registration of archaeological sites. Currently we are evaluating this technique to accurately determine volume and surface area of above-ground parts of TMs from reconstructed 3D models. Verification will be performed by reconstructing irregular objects (e.g. large rocks) with known volume.

Selected mounds were cross-sectioned and the above-ground part was weighted to estimate the bulk density of the TM

One of approx. 30-40 photos used in the 3D reconstruction of TM1.

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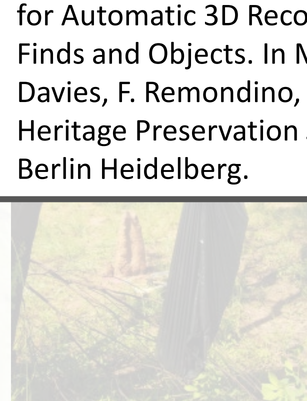
Kersten, T., & Lindstaedt, M. (2012). Image-Based Low-Cost Systems for Automatic 3D Recording and Modelling of Archaeological Finds and Objects. In M. Ioannides, D. Fritsch, J. Leissner, R. Davies, F. Remondino, & R. Caffo (Eds.), Progress in Cultural Heritage Preservation SE - 1 (Vol. 7616, pp. 1–10). Springer Berlin Heidelberg.



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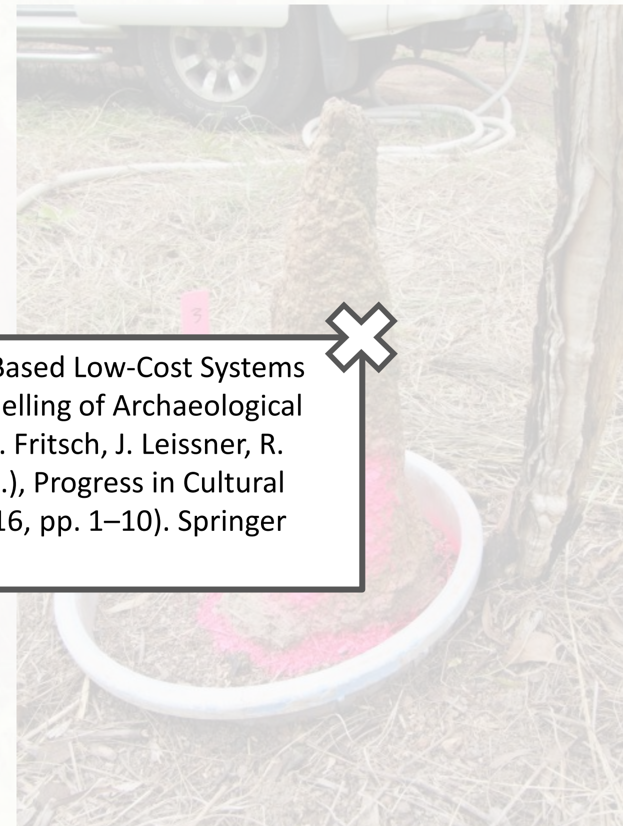

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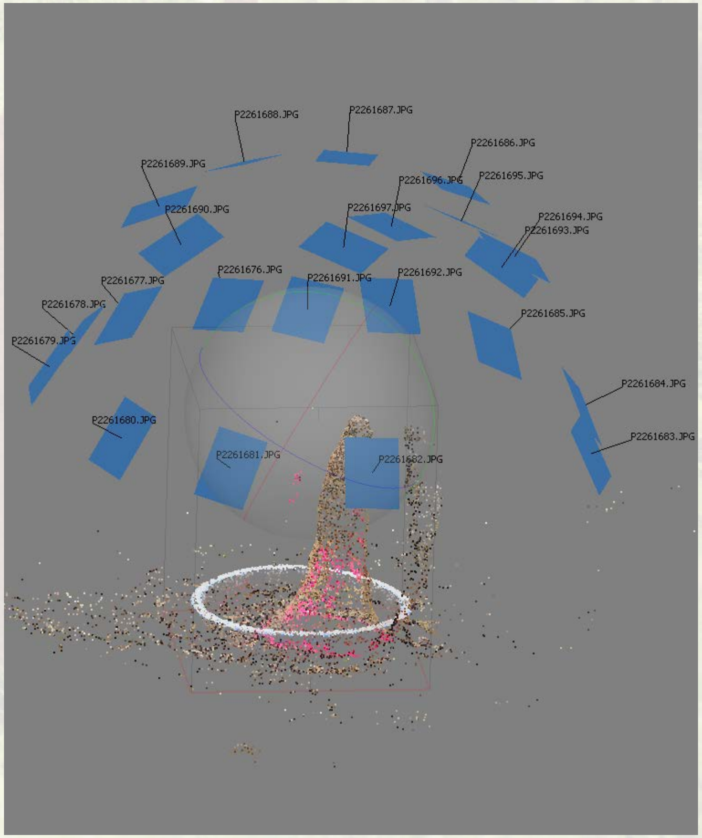

Camera positions reconstruction from aligned photos with Photoscan.

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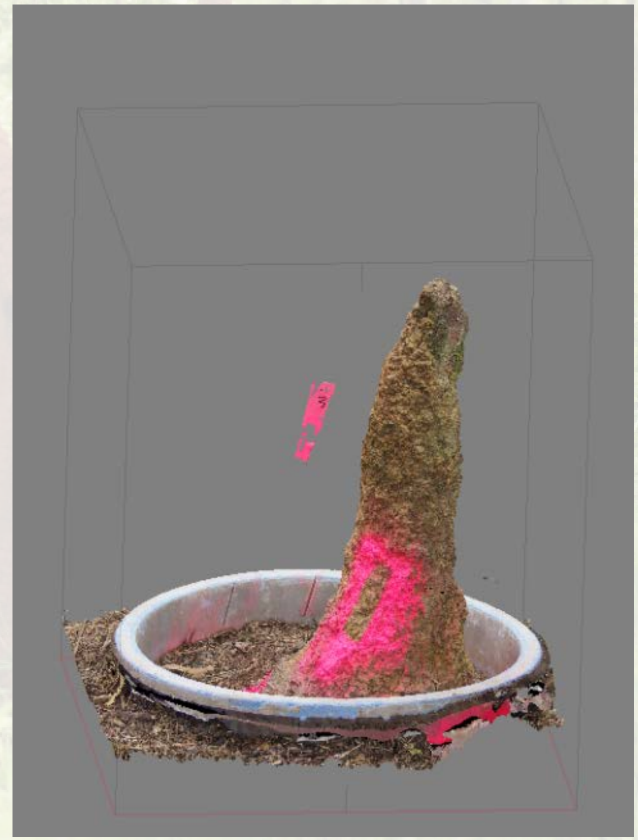
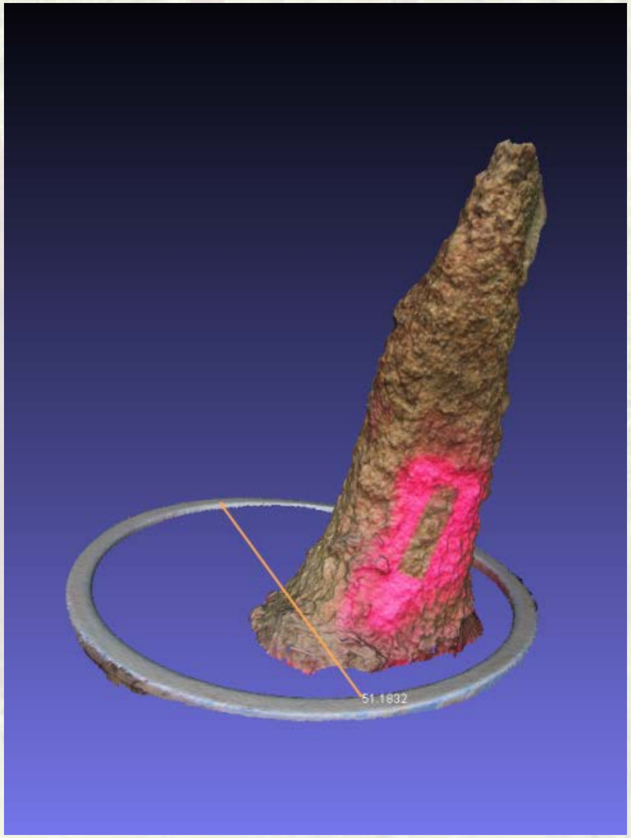


Dense point cloud and surface mesh reconstruction with Photoscan.

Photogrammetric Measurement of Volume and Surface Area

The low-cost software solution Agisoft Photoscan can reconstruct full 3D models from a set of unordered images taken with a conventional digital camera. This approach has been applied and tested for 3D registration of archaeological sites. Currently we are evaluating this technique to accurately determine volume and surface area of above-ground parts of TMs from reconstructed 3D models. Verification will be performed by reconstructing irregular objects (e.g. large rocks) with known volume.

Selected mounds were cross-sectioned and the above-ground part was weighted to estimate the bulk density of the TM



Scaling, volume and surface calculation were performed with Meshlab.

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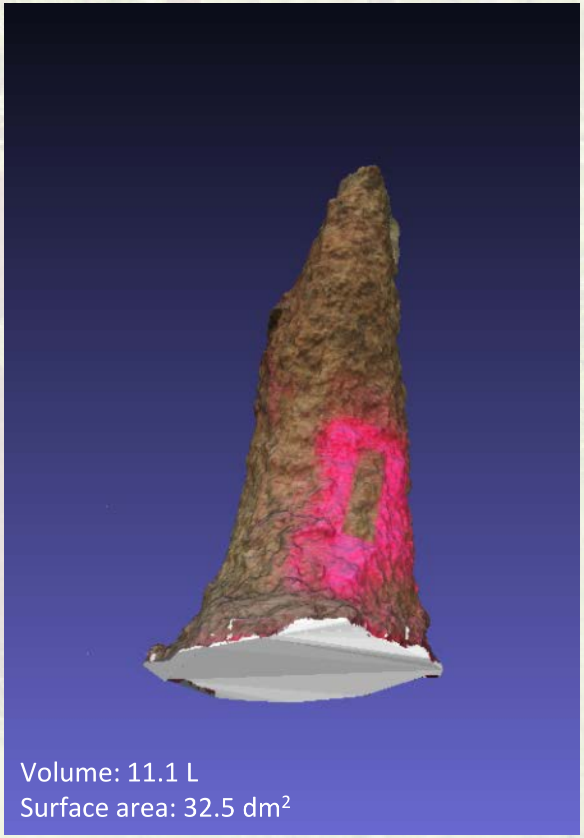


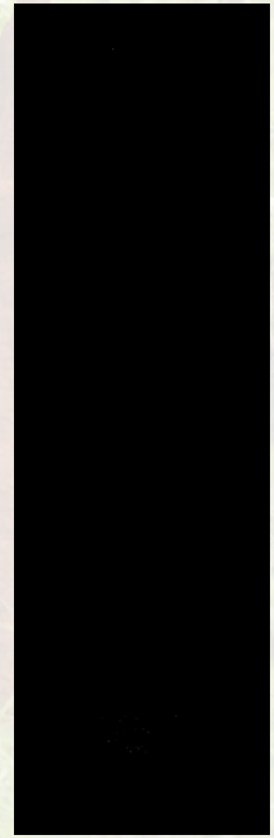
Volume measurement on closed model in Meshlab.

BACKGROUND
ISOTOPE MODELS
PHYSICAL CHARACTERISATION
INHIBITORS
TRACER TESTS
FIELD SURVEY

Computer Tomography Scanning of TMs

Scanning of termite mounds by computer tomography (CT) has been applied before to estimate connectivity of internal chambers. We tested CT scanning of TM1 and TM2 as a tool to determine their internal volume and porosity. Volume measurement of TM2 solid and void spaces was performed with ImageJ after binarization. This allowed determination of bulk and solid density, as well as macro- and micro-porosity. Further scans of several mounds per species may yield species-specific porosities for gas turnover calculations.

CT scanning could be performed at Darwin Medical Imaging Centre, thanks to David de Sousa. Digging out the TMs was not without risks...



Video of the slices of TM1 reconstructed with ImageJ software.

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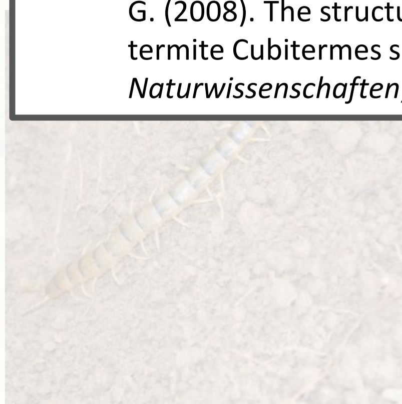
Perna, A., Jost, C., Couturier, E., Valverde, S., Douady, S., & Theraulaz, G. (2008). The structure of gallery networks in the nests of termite Cubitermes spp. revealed by X-ray tomography. *Die Naturwissenschaften*, *95*(9), 877–84.



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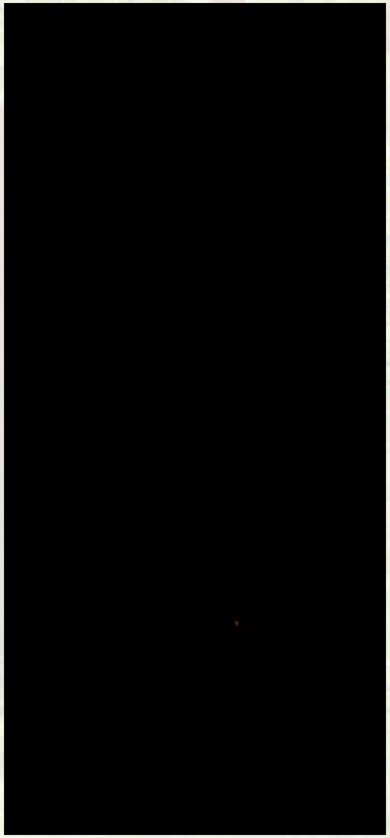


TM2 measures

Solid volume	13.0 L
Total volume	23.1 L
Wall density	2.0 kg L^{-1}
Bulk density	1.1 kg L^{-1}
Micro-porosity	0.24
Macro-porosity	0.43
Total porosity	0.67

Video of the slices of TM2 reconstructed with ImageJ software.

Tracer Tests in Inactive TM

As an alternative to inhibitors, conservative, gaseous tracers can be injected into a TM to elucidate gas transport parameters. By using an appropriate model to fit measured breakthrough curves, tracer tests may yield time-resolved kinetic parameters, e.g. first-order rate coefficient k_{ox}, or Michaelis-Menten parameters, in addition to total reacted mass. In this test we injected pulses of CH_4 and CO_2 directly into inactivated TM2, thus using the gases as tracers to calibrate a two-compartment mass balance model.

TM2 was excavated and dried for 72h at 105°C to kill termites and inactivate microorganisms. The mound was then placed in a sandbox with a closed-dynamic chamber connected to a CH_4/CO_2 analyser

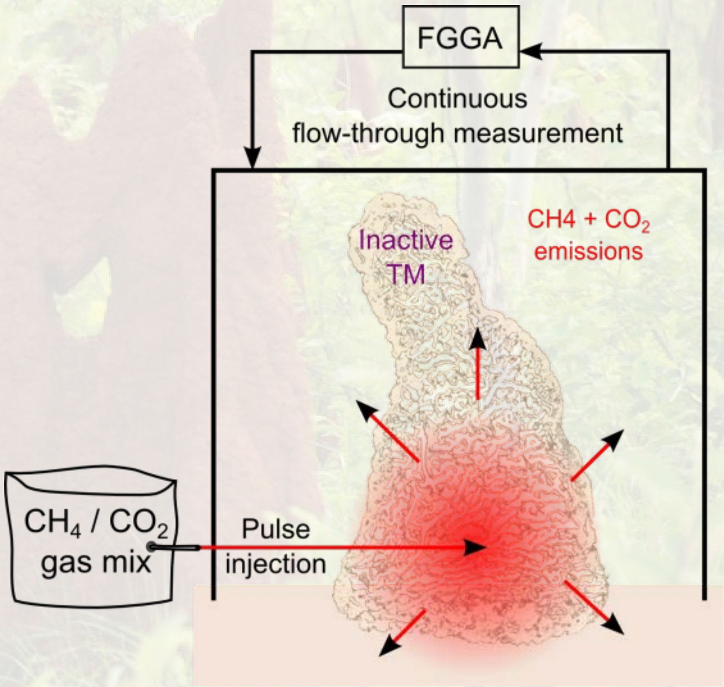
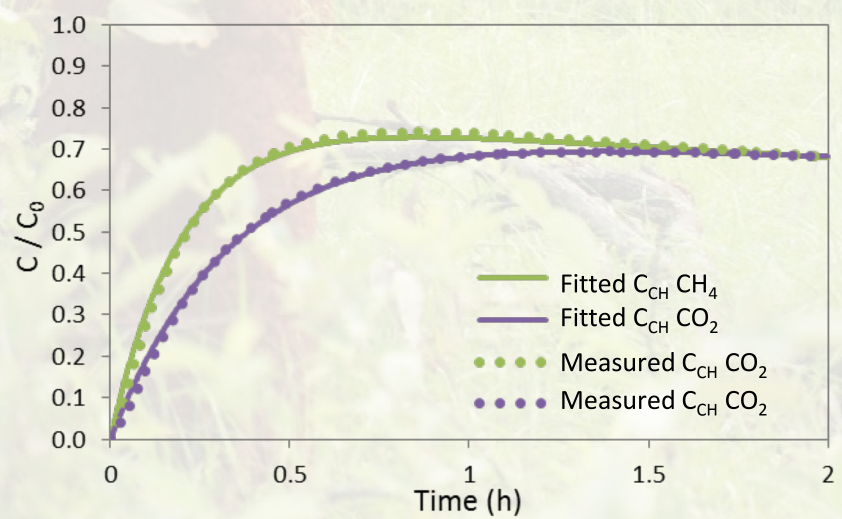
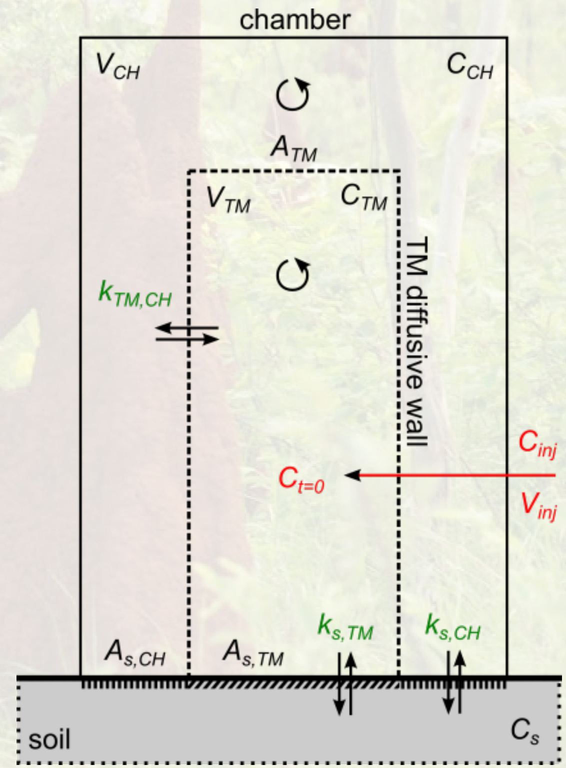


Illustration of the performed pulse injections of CH_4 and CO_2 into inactivated TM2. Injected pulses of 50-500 mL total volume varied in concentrations from 8 ppm – 10 vol%. Measured concentrations were corrected for background and normalized to injected concentrations.

Calibrated Mass-Balance Model

Results from a series of pulse injections in TM2 yielded breakthrough curves that allowed finding optimal values for rate coefficients of diffusion of the employed two-compartment mass-balance model (i.e. two coupled, well-mixed flow-through reactors). Model and measured data agreed well for both gases (see graph below), although different tests yielded slightly different coefficients for the same TM.

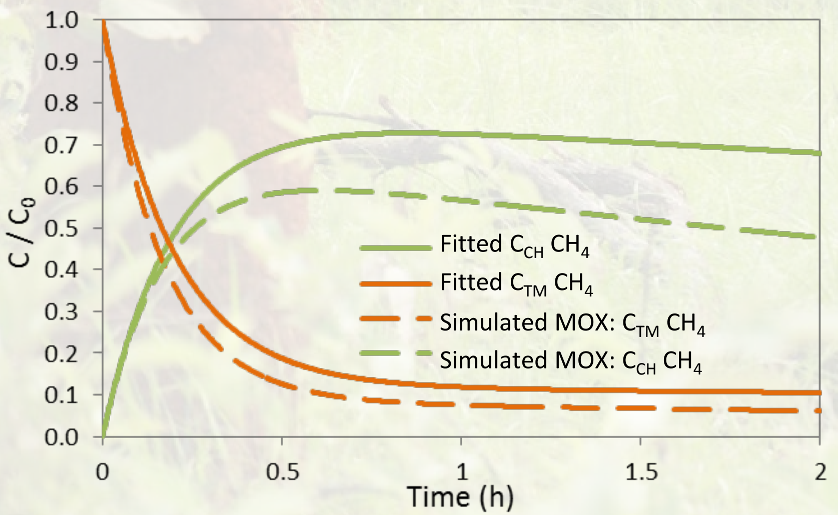


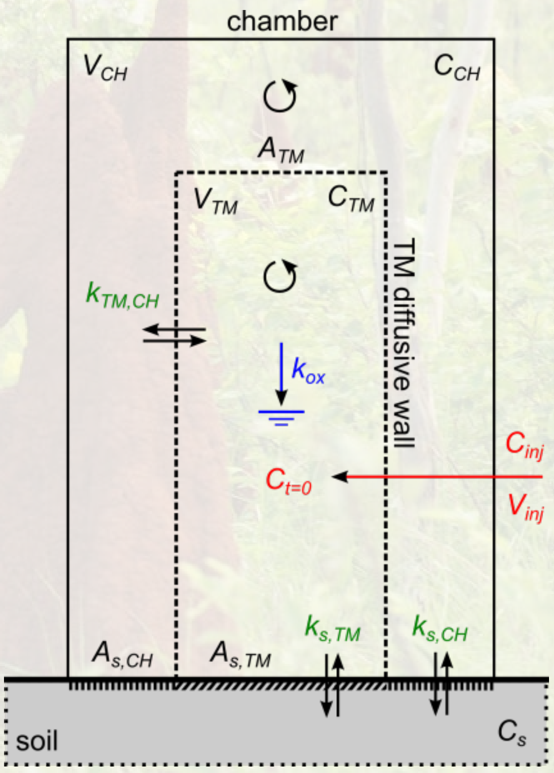


Representation of the two-compartment mass-balance model for a TM. The inside of the mound is assumed well-mixed, with a hypothetical, diffusive "bottleneck" boundary. The data of the tracer tests were used to optimize transport reaction coefficients highlighted in green.

Concept of a Tracer Test in Active TMs

In principle, the described tracer test can be used to estimate kinetic parameters of MOX when co-injecting CH_4 with a tracer of similar physical properties. In a first step the normalised data from the tracer is used to optimize transport coefficients, and in a second step data from injected CH_4 to estimate k_{ox}. Any production of CH_4 during the test may be neglected if injected CH_4 >> background-CH_4. Suitable tracers for CH_4 include argon and carbon monoxide. Both gases will be evaluated for the use in TMs.



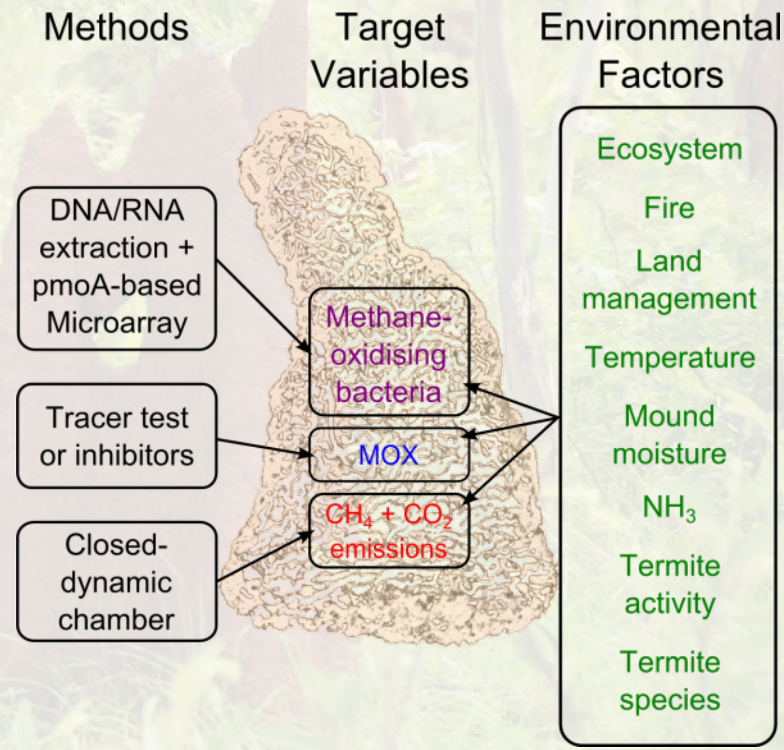


Expanded mass-balance model with first-order rate coefficient k_{ox}. Including k_{ox} = 1 h^{-1} in the previously optimized model leads to the simulated dashed curves (graph on the left).

Outlook on Planned Field Survey

In a further phase of the project, the presented methods to quantify MOX in TMs will be applied in a comprehensive field survey in Australian savanna and forest ecosystems. We aim to gain insights into major driving factors, and possibly diurnal or seasonal patterns, of MOX in TMs. Furthermore, we seek to characterise the associated community of CH_4 oxidising bacteria through molecular tools. By selecting representative field sites we may be able to elucidate influence of fire and land-management practices.

Field measurements of CH_4 and CO_2 emissions, and termite sampling in Northern Australian savannah in the wet season.



Target variables of the planned field survey are net CH_4+CO_2 emissions, amount and reaction rate of MOX, and the community of methane-oxidising bacteria. We seek correlations and possibly functional relations with a number of environmental factors highlighted in green.