ACCESS TO RESEARCH INFRASTRUCTURES

Nitrous oxide (N_2O) and methane (CH_4) fluxes from stems of different tropical tree species in Mare Longue Nature Reserve (NOME-TROPITREES)

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- Introduction and motivation

Tropical forests are considered a natural source of nitrous oxide (N_2O) and a natural sink for methane (CH_4), both of which are important greenhouse gases (GHGs). The net N_2O emission from the tropical forests is estimated to be 4.76 kg ha^{-1} y^{-1}, the net CH_4 deposition seems to lie by -3.86 kg ha^{-1} y^{-1} (Dalal and Allen 2008). To date, N_2O and CH_4 fluxes between forests and the atmosphere have been mostly calculated only based on GHGs exchange at the soil–atmosphere interface, and they do not include exchange activity of other ecosystem compartments as trees and other vegetation.

N_2O is naturally produced in soils in a wide range of nitrogen turnover processes, including aerobic nitrification, anaerobic dissimilatory nitrate reduction to ammonium, and anaerobic denitrification processes. The denitrification processes are the only processes known to reduce N_2O to N_2 (Smith et al. 2003). CH_4 is produced by strictly anaerobic methanogenesis in water saturated soils and oxidized by methanotrophic bacteria (Smith et al. 2003).

Both gases can be released into the atmosphere by gas diffusion at the soil surface and ebullition. However, plants can also contribute to ecosystem N_2O and CH_4 exchange by (a) taking up both gases from soil by the root system and transporting them into the atmosphere through the transpiration stream, and/or aerenchyma system and enlarged intercellular spaces (Machacova et al. 2013); (b) producing N_2O and CH_4 directly in plant tissues (Smart and Bloom 2001, Keppler et al. 2006); (c) consuming N_2O and CH_4 from the atmosphere by a non-specified mechanism (Sundqvist et al. 2012; Machacova et al. 2016b, 2017); and (d) altering the nitrogen and carbon turnover processes in adjacent soil (Menyailo and Hungate 2005). Moreover, the cryptogamic stem covers (i.e. photoautotrophic organisms associated with the tree bark as lichens, liverworts, mosses and/or ferns) seem to also contribute to N_2O and CH_4 exchange of the trees/forest ecosystem (Lenhart et al. 2015, Machacova et al. 2017).

Recent research has revealed that not only soils, but also trees of tropical, temperate and boreal zones can be significant sources of N_2O and CH_4 (e.g. Machacova et al. 2013; 2016a,b; 2017; Pangala et al. 2017; Maier et al. 2018; Welch et al. 2018). Pangala et al. (2017) showed that tropical trees adapted to high soil water level are responsible for up to half of the CH_4 emissions from the Amazon floodplain, the largest natural CH_4 source in the tropics. However, to our knowledge, there is only little known about CH_4 exchange capacities of tropical trees grown under low soil water content typical for upland forests or forests growing on lava flows (Welch et al. 2018). Moreover, the N_2O exchange of mature trees grown under their natural field conditions is world-wide rarely studied (Díaz-Pinés et al. 2015; Machacova et al. 2016a, 2017; Wen et al. 2017), the information for tropical regions is almost missing.

- Scientific objectives

The project objective was to quantify N_2O, CH_4 and additionally carbon dioxide (CO_2, indicator of physiological activity) exchange of common tree species in Mare Longue Nature Reserve at Reunion Island (*Syzygium borbonicum*, *Doratoxylon apetalum*, *Antirhea borbonica*, *Homalium paniculatum*, *Mimusops balata* and *Labourdonnaisia calophylloides*), and of adjacent soil. The aim was to determine whether and to which extent these trees, growing on lava flow covered with thin and irregular organic soil layer, exchange N_2O and CH_4 with the atmosphere. Moreover, the gas exchange capacity of common cryptogamic stem covers (*Pyrrhobryum spiniforme*, *Leucoloma* sp., *Leucophanes* sp.) was studied. The overall aim of the project was to clarify the role of the studied forest components in N_2O and CH_4 exchange of the tropical lowland rain forest.

- Methodology and experimental set-up

The experiment was performed in the Mare Longue Nature Reserve (21°21'S, 55°45'E), located in the southeast of Reunion Island, South West Indian Ocean. The studied mixed forest is a tropical lowland rain forest characterized with endemic vegetation typical for the volcanic islands in the Mascarene Archipelago. The studied experimental plot (size of 1 ha, established in 1999 by the University of Reunion Island) is located on a slope at 180 - 200 m a. s. l., and situated on an approx. 400 years old pahoehoe basaltic lava flow. The soil cover is irregular and thin, and consists mostly from organic matter (Kirman et al. 2007).

The measurement period (9.10.–6.11.2018) was characterized by air temperature in range of 24-30°C and rapidly increasing air humidity due to the coming warm and humid season.

The exchange of N_2O, CH_4 and CO_2 from stems was studied on six tree species common for the Mare Longue Nature Reserve (in total 24 mature trees): *Syzygium borbonicum* J. Guého et A.J. Scott (n=5), *Doratoxylon apetalum* (Poir.) Radlk. var. *apetalum* (n=5), *Antirhea borbonica* J.F. Gmel (n=5), *Homalium paniculatum* (Lam.) Benth. (n=3), *Mimusops balata* (Aubl.) C.F. Gaertn. (n=3), *Labourdonnaisia calophylloides* Bojer (n=3). The exchange of GHGs from the adjacent soil was measured close to each selected individual tree (in total 24 soil positions). Finally, cryptogamic stem covers typical for the selected trees (*Pyrrhobryum spiniforme*, *Leucoloma* sp., *Leucophanes* sp.) were collected for gas flux measurements.

The fluxes of N_2O, CH_4 and CO_2 were measured separately from stems and soil using stem and soil chamber enclosure methods (see below, *explaining remark made by the reviewers*). The measurements were performed "in pairs", i.e. the stem gas flux measurement of an individual tree directly followed the gas flux measurement of the adjacent soil to ensure measurements under similar environmental and climatic conditions. All fluxes were determined between 9 a.m. and 6 p.m. to prevent possible effect of diurnal cycle. Acquiring one measurement set from all selected trees and soil required approx. 1.5-2 weeks, all the forest components were measured two times.

Stem flux measurements

The stem fluxes of N_2O, CH_4 and CO_2 were measured at the bottom part of the stems (approximately 0.2 m above the soil) by all selected tree species and tree individuals. The vertical profile of the stem fluxes (measurements at three stem heights of approximately 0.2, 1.0 and 1.8 m above the soil) was studied by 3 individual trees of each of the following three species: *S. borbonicum*, *D. apetalum* and *A. borbonica*. The stem fluxes were measured manually using static stem chamber systems installed at the beginning of the measurement campaign. The box chambers consisted of transparent plastic containers with removable airtight lids (Lock & Lock, Anaheim, CA, USA) and a neoprene sealing frame. They were gas-tightly affixed to the carefully smoothed bark surface using silicone. Per each tree except *S. borbonicum*, two rectangular box chambers (total area of 0.0108 m^2, total internal system volume of 0.0021 m^3) were installed at one stem height on opposite sides of the stem and interconnected with polyurethane tubes into one flow-through chamber system (for more details see Machacova et al. 2017). Due to large stem diameter of *S. borbonicum* trees, three box chambers (total area of 0.0162 m^2, total internal volume of 0.0028 m^3) were installed at one stem height in the same way. Gas-tightness of all the chambers was regularly tested.

Soil flux measurements

Soil N_2O, CH_4 and CO_2 fluxes were measured using manual static soil chamber systems. The soil collars were installed in the vicinity of the investigated trees at the beginning of the research stay to reduce soil disturbances. Due to very thin soil layer on the lava flow, the bottom part of the collars was imbedded max. 2.5 cm deep into the soil. The soil chambers were made of sewage pipes (area of 0.0083 m^2, total internal system volume of approx. 0.0015 m^3 depending on soil depth). During measurements, the soil chambers were closed by a lid and sealed with water between the body of the chamber and the lid (Machacova et al. 2013).

Flux measurements of cryptogamic stem covers

Common cryptogams were collected from the bark of *S. borbonicum* trees. To prevent any disruption of the bark-microcosm within the stem chambers, the cryptogams were sampled from the bark outside these chambers. After collection, fresh samples were placed into 3.9 l plastic gas-tight incubation boxes (Lock &

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Lock, USA) connected to gas analyser (see below). Gas exchange measurements were performed under field conditions during the day (day-time measurements) and under laboratory conditions in the night (night-time measurements).

Flux measurements and calculation

The stem and soil chamber systems, and the incubation boxes were left open between the individual measurements. For measurements, the chambers/boxes were closed by lids, and the concentration changes of N_2O, CH_4 and CO_2 in chamber/box headspace were measured using a portable Fourier transform infrared (FTIR) gas analyser (DX-4015, GASMET, Finland; Warlo et al. 2018). The zero point calibration of the analyser was done with nitrogen (purity 5.2) every morning. One measurement run of stem/soil gas fluxes lasted approx. 45 minutes, the measurement time for cryptogams was between 4 to 10 hours depending on flux rates. The internal pump (2 l min^{-1}) of the analyser ensured the air mixing inside the chamber systems.

The gas exchange of stems, soil and cryptogams will be quantified based on the linear changes in N_2O, CH_4 and CO_2 concentrations in the chamber/box headspace over time (for equations see Machacova et al. 2016a). The fluxes from tree stems and soil will be roughly up-scaled to the ecosystem level based on tree and forest characteristics (Machacova et al. 2016a). The contribution of cryptogams to the stem GHGs fluxes will be estimated based on upscaling of the flux rates to unit of stem surface area (Machacova et al. 2017).

The flux data will be further statistical processed. Correlation analyses between stem and soil fluxes, and the fluxes and continuously measured environmental parameters at the permanent plot (soil and air temperature/humidity, precipitation) will be performed. The fluxes will be further used for specification of certain parameters of the biosphere-atmosphere exchange models for N_2O, CH_4 and CO_2 over the Reunion island (data from Maïdo atmospheric observatory, in close cooperation with Dr. Valentin Duflot, Laboratory for the Study of the Atmosphere and Cyclones, University of Reunion Island).

Figure 1: Example of an experimental set-up for measurement of N_2O, CH_4 and CO_2 exchange of tree stems and adjacent soil using a portable FTIR analyser in Mare Longue Nature Reserve (for more information see "Methodology and experimental set-up").

- **Preliminary results and conclusions**

We present here first preliminary results based on the real-time observation of the concentration changes of N_2O and CH_4 in chamber system headspace, as the extensive data processing will last for several months.

The majority of stems of the tropical tree species studied were **weak sinks for CH_4** with some trees showing no detectable fluxes. The stem deposition rates of CH_4 seem to decrease within the measurement campaign probably due to coming wet and warm season, and with it connected change in activity of methanogenic and methanotrophic microorganisms. To our knowledge, this is the **first study showing a deposition potential for CH_4 by stems of tropical tree species**. The current studies report tree stems as CH_4 emitters, especially in Amazon floodplain forests, where the highest known CH_4 emission rates for trees were detected (Pangala et al. 2017). First studies in boreal forests, however, report that tree stems can also

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take up CH_4 from the atmosphere, especially under low soil water content (Sundqvist et al. 2012, Machacova et al. 2016b). However, the mechanism and fate of this CH_4 uptake by trees is still not understood.

Our test incubation experiments showed that the **cryptogamic stem covers as weak sinks for CH_4 might be partly responsible for the CH_4 uptake in stem chambers**. Machacova et al. (2017) detected similar situation for N_2O, where stems of beech trees (*Fagus sylvatica*) consistently deposited N_2O from the atmosphere and the cryptogams were identified as significant N_2O sinks too.

Even though the studied forest is a tropical rain forest with mean annual rainfall of 4000 mm, **the forest soil was a significant sink for CH_4**. This might be explained by the hillside situation and high porosity of the lava stones, both resulting in fast runoff of the rain water from the studied forest. These results in the aerobic conditions in the thin soil layer connected with predominant CH_4 oxidation by methanotrophic microorganisms. However, it seems that the soil might evince also potential for methanogenic activity under high soil water content (e.g. in period of extreme precipitation events), as one studied small-scaled wet soil area was characterized by clear CH_4 emissions over the studied time period.

The fluxes of N_2O from stems and soil seem to be very low and not significantly different from zero. One explanation might be low soil nitrogen content and the fact that the studied forest did not show any biomass increment over the last 10 years (nutrient limitation?, Ah-Peng C., oral information).

Concluded, the tropical lowland rain forest seems to be a significant sink for CH_4 and plays only a rudimental role in the global N_2O exchange.

- **Multidisciplinary approach**

Our project was/is an interdisciplinary project **integrating bio-ecosphere and atmosphere ENVRIplus environmental domains.** The project was carried out at the **multi-disciplinary research infrastructure platform OSUR at Reunion Island including the forest station in Mare Longue Nature Reserve and the atmospheric observatory Maïdo. Our study of GHG exchange of the tropical lowland rain forest is the first study of its kind at the island.** The aims were to 1) get a first overview of the role of trees, soil and cryptogams in CH_4 and N_2O exchange of the tropical lowland rain forest situated on a lava flow, 2) roughly estimate the GHGs balance of this forest (sink vs. source), and 3) validate some aspects of the parameterisation of **biosphere-atmosphere CH_4 and N_2O exchange over the Reunion Island** (measured at observatory Maïdo, in close cooperation with Dr. V. Duflot, Laboratory for the Study of the Atmosphere and Cyclones, University of Reunion Island).

Within our research stay, fruitful **meetings with researchers from different research fields** (Dr. Claudine Ah-Peng and her colleagues from St. Pierre; Dr. V. Duflot, Dr. Jean-Pierre Cammas, and their colleagues from St. Dennis; University of Reunion Island) took place. The aim was to introduce the research field of forest ecosystem GHGs exchange, to present and discuss our preliminary results and its future usage, and to discuss the **intensification of future cross-domain cooperation** (C. Ah-Peng, V. Duflot) with the aim to improve our understanding of GHGs exchange from the natural ecosystems at the island (needed for modelling of GHGs exchange over the Reunion Island).

K. Machacova gave a **talk on the topic** of "Nitrous oxide (N_2O) and methane (CH_4) exchange of trees as a missing component in greenhouse gas balance of forest ecosystems." at the University of Reunion Island in St. Pierre (30.10.2018). All project members visited the **atmospheric observatory Maïdo for an extensive excursion** organised by V. Duflot.

- **Outcome and future studies**

Our project was/is a first case study of its kind at the island. We found out that the tropical lowland rain forest grown on a lava flow seems to be a significant sink for CH_4 and plays only a minor role in the global N_2O exchange. After an extensive data processing, the obtained **results will be presented at an international conference (EGU 2019, Vienna, Austria) and published in an impact journal.**

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The project has **established new cooperation** with the researchers from the University of Reunion Island, which will be a **basis for future common experiments**. The results of this case study need to be verified by a larger future project directed to studying spatial and seasonal variability in CH_4 and N_2O exchange of soil and trees, as well as to understanding the role of cryptogams in the GHGs exchange under different climatic conditions. The future studies should involve **measurements of CH_4 and N_2O exchange of soil and trees, if present, in different natural ecosystems in the whole altitudinal profile at the island** incl. high elevated mountain ecosystems. Such measurements are crucial for the **future estimation of CH_4 and N_2O budget of the volcanic Reunion Island**, and therefore of global GHGs flux inventories.

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Note: We do not wish to make this scientific report public. Thanks for understanding.