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# Spatial variations of nitrogen trace gas emissions from tropical mountain forests in Nyungwe, Rwanda

**N. Gharahi Ghehi<sup>1</sup>, C. Werner<sup>2</sup>, L. Cizungu Ntaboba<sup>1</sup>, J. J. Mbonigaba Muhinda<sup>3</sup>,  
E. Van Ranst<sup>4</sup>, K. Butterbach-Bahl<sup>5</sup>, R. Kiese<sup>5</sup>, and P. Boeckx<sup>1</sup>**

<sup>1</sup>Faculty of Bioscience Engineering, Isotope Bioscience Laboratory – ISOFYS, Ghent University, Belgium

<sup>2</sup>LOEWE Biodiversity and Climate Research Centre (BIK-F), Frankfurt, Germany

<sup>3</sup>National University of Rwanda (NUR), Department of Soil and Environmental Management (SEM), Rwanda

<sup>4</sup>Department of Geology and Soil Science, Laboratory of Soil Science, Ghent University, Belgium

<sup>5</sup>Karlsruhe Institute of Technology, Institute for Meteorology and Climate Research, Atmospheric Environmental Research, Garmisch-Partenkirchen, Germany

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Correspondence to: N. Gharahi Ghehi (nasrin.gharahighehi@ugent.be)

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**BGD**

8, 11631–11660, 2011

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## Abstract

Globally, tropical forest soils represent the second largest source of N<sub>2</sub>O and NO. However, there is still considerable uncertainty on the spatial variability and soil properties controlling N trace gas emission. To investigate how soil properties affect N<sub>2</sub>O and NO emission, we carried out an incubation experiment with soils from 31 locations in the Nyungwe tropical mountain forest in southwestern Rwanda. All soils were incubated at three different moisture levels (50, 70 and 90 % water filled pore space (WFPS)) at 17 °C. Nitrous oxide emission varied between 4.5 and 400 µg N m<sup>-2</sup> h<sup>-1</sup>, while NO emission varied from 6.6 to 265 µg N m<sup>-2</sup> h<sup>-1</sup>. Mean N<sub>2</sub>O emission at different moisture levels was 46.5 ± 11.1 (50 % WFPS), 71.7 ± 11.5 (70 % WFPS) and 98.8 ± 16.4 (90 % WFPS) µg N m<sup>-2</sup> h<sup>-1</sup>, while mean NO emission was 69.3 ± 9.3 (50 % WFPS), 47.1 ± 5.8 (70 % WFPS) and 36.1 ± 4.2 (90 % WFPS) µg N m<sup>-2</sup> h<sup>-1</sup>. The latter suggests that climate (i.e. dry vs. wet season) controls N<sub>2</sub>O and NO emissions. Positive correlations with soil carbon and nitrogen indicate a biological control over N<sub>2</sub>O and NO production. But interestingly N<sub>2</sub>O and NO emissions also showed a negative correlation (only N<sub>2</sub>O) with soil pH and a positive correlation with free iron. The latter suggest that chemo-denitrification might, at least for N<sub>2</sub>O, be an important production pathway. In conclusion improved understanding and process based modeling of N trace gas emission from tropical forests will not only benefit from better spatial explicit trace gas emission and basic soil property monitoring, but also by differentiating between biological and chemical pathways for N trace gas formation.

## 1 Introduction

Nitrogen oxide emissions from soil are of major concern because of their significant impact on atmospheric chemistry and as a driver for global climate (Crutzen, 1979; WMO, 2006; Mosier et al., 1998; Breuer et al., 2000). Nitrous oxide (N<sub>2</sub>O) is one of the main contributors to global warming and becoming the main ozone destroyer in stratospheric

**BGD**

8, 11631–11660, 2011

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Spatial variations of  
nitrogen trace gas  
emissions**

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



ozone destruction (Meehl et al., 2007). Nitric oxide (NO) acts as a catalyst in the synthesis of tropospheric ozone (Delmas et al., 1997; Holland and Lamerque, 1997) an important component in ecosystem and human health issues. Nitrous oxide and NO are produced in soils by microbial processes of nitrification, denitrification, nitrifier-denitrification (e.g. Davidson et al., 2000). Besides agricultural soils, tropical forest soils are considered as the most important source for atmospheric N<sub>2</sub>O (Bouwman et al., 1993; Matson et al., 1990; Mosier et al., 1998; Breuer et al., 2000; Butterbach-Bahl et al., 2004; Kiese et al., 2003; Werner et al., 2006). Also with regard to NO, tropical forest soils may represent a significant source within the global atmospheric budget of this trace gas (Butterbach-Bahl et al., 2004; Gut et al., 2002). However, current estimates of the global source strength are still highly uncertain because detailed measurements, in particular, for tropical forest soils are still scarce. Kroeze et al. (1999) and Mosier et al. (1998) estimated the contribution of N<sub>2</sub>O from tropical forest soils to be in the range of 2.2–3.7 Tg N<sub>2</sub>O-N yr<sup>-1</sup>. The mean estimate of 3.0 Tg N<sub>2</sub>O-N yr<sup>-1</sup> accounts for about 18 % of all the sources of global atmospheric N<sub>2</sub>O (Prather and Ehhal, 2001) whereas Werner et al. (2007a), using a GIS coupled mechanistic biogeochemical model (ForestDNDC-tropica), provided a revised estimate of N<sub>2</sub>O emission from tropical forests of 1.34 Tg N<sub>2</sub>O-N yr<sup>-1</sup> (0.88–2.37 Tg N<sub>2</sub>O-N yr<sup>-1</sup>).

On the basis of limited available field measurements, the global contribution of tropical forest soils to the global NO budget is estimated at 1.1 Tg NO-N yr<sup>-1</sup> (Davidson and Kingerlee, 1997). However, a detailed study of Butterbach-Bahl et al. (2004) indicates that NO emission from tropical rain forest soils might be up to 3 Tg NO-N yr<sup>-1</sup> strong.

Further, simulation results, using an N isotopic coupled mechanistic biogeochemical model, show that total gaseous losses, including N<sub>2</sub>, from tropical rain forest soils in Hawaii contributed for ~26–48 % of total N losses from natural ecosystems (Bai and Houlton, 2009).

So far current research and policy programs for tropical forests mainly focus on C storage and emission (Stickler et al., 2009; Defries et al., 2010). Though, tropical forest ecosystems play an important role in the global C balance, also non-CO<sub>2</sub> greenhouse

gases contribute to the net greenhouse gas balance from tropical forest ecosystems. For this reason, N<sub>2</sub>O and NO emission inventories in tropical forest are required as an additional decision tool for sustainable forest management and closing global trace gas budgets.

5 Despite the importance of tropical rain forest soils as source of atmospheric N<sub>2</sub>O and NO, there are only few available datasets available (Serca et al., 1994; Breuer et al., 2000; Kiese et al., 2005; Butterbach-Bahl et al., 2004; Gut et al., 2002; Werner et al., 2007b). The majority of N<sub>2</sub>O measurements were conducted in the Amazon, Central America (e.g. Keller and Reiners, 1994; Verchot et al., 1999) and in tropical  
10 regions of Australia (e.g. Kiese et al., 2003; Butterbach-Bahl et al., 2004). The only dataset of N<sub>2</sub>O emissions from tropical rain forest soils of Africa was reported by Serca et al. (1994) and Werner et al. (2007b), who worked in the Mayombe forest in the DR Congo and the Kakamega forest in Kenya, respectively. Furthermore, most studies on NO were carried out in the Amazon (Gut et al., 2002) and Queensland, Australia  
15 (Butterbach-Bahl et al., 2004). To our knowledge except for Serca et al. (1994), no reports on soil NO emissions from African tropical forest soils are available to date, indicating a need for an improved data availability of N-trace gas exchange for African forest soils in particular.

It is widely accepted that the magnitude of soil N<sub>2</sub>O emissions, as well as NO, is  
20 highly variable and strongly influenced by environmental conditions, like soil properties and soil moisture (e.g. Davidson, 1993; Breuer et al., 2000). Additionally, knowledge of how soil properties and changes in soil moisture regulate the turnover of these N oxide gases is essential for realistic modeling of soil-atmosphere N trace gas exchange in tropical forest region. Even though variations in soil moisture are considered as the  
25 main driver of temporal variations in N oxide emissions, it does not explain observed spatial variations from site to regional scales. Based on current knowledge it is obvious that observed spatial variations in N trace gas emissions must be closely linked to variations in soil properties, though individual effects of soil properties on N trace gas emissions are still remaining largely unexplored, especially in the tropics. So far, only a

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

few studies are available where variations in soil properties have been explicitly linked to spatial variation of N<sub>2</sub>O and NO emissions from tropical soils (e.g. Breuer et al., 2000; Ishizuka et al., 2005; Keller et al., 2005; van Haren, 2010).

However, more detailed information on the linkage between soil properties and N oxide emissions are needed to better understand spatial variability of N trace gas emissions in tropical forest regions. Furthermore, this information may be used for improving the parameterization and for validating predictions of mechanistic biogeochemical model such as the ForestDNDC-tropica (Werner et al., 2007a), which have recently been used to estimate global N<sub>2</sub>O emissions from tropical rainforest soils.

In-situ N-trace gas fluxes for plot-based studies are usually derived with static (N<sub>2</sub>O) or dynamic (NO) chamber measurements (see e.g. Kiese and Butterbach-Bahl, 2002). However, for regional scale flux estimates this approach is not feasible (especially not for remote areas) since it concentrates measurements only on a few sites with limitations to cover all climatic, soil and vegetation characteristics of a given region. Soil incubation studies in the laboratory have the advantage to allow for measuring N-trace gas emissions from a high number of samples, thereby allowing to covering regional variability of soils. Several previous studies reported N-trace gas fluxes, which were derived from soil incubation experiments and which agreed well with field-derived fluxes determined via chambers (Gut et al., 1999; Otter et al., 1999; Ludwig et al., 2001; van Dijk et al., 2002).

In view of the fact that few results on N<sub>2</sub>O and NO fluxes from African tropical rain forest soils have been reported on the one hand and the inaccessibility of the terrain due to logistic and safety constrains on the other hand, we present results on N<sub>2</sub>O and NO emissions from incubated soil samples from the Nyungwe tropical mountain forest, a national park situated in southwestern Rwanda. Our aims were (a) to quantify the magnitude and spatial variability of soil N<sub>2</sub>O and NO emissions, and (b) to determine the importance of different soil properties for explaining the spatial variability of the N<sub>2</sub>O and NO emissions from this central African tropical mountain forest.

**BGD**

8, 11631–11660, 2011

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## 2 Methods

### 2.1 Site description and soil sampling

The study was conducted in the Nyungwe National Park covering an area of about 1000 km<sup>2</sup> of tropical mountain forest, located between 2°17'–2°49' S and 29°03'–29°29' E at altitudes of 1485–2925 m in southwestern Rwanda (Fig. 1). Nyungwe is one of the largest mountainous rainforest areas remaining in Africa. It divides the Nile and the Congo river basins. The soils have been developed mainly from schists, micaschists, quartzitic schists and granites (UGent/Minagri, 2000a, b, c, d). The eastern part of the forest, with an altitude exceeding 2000 m, is dominated by micaschists, whereas the western area featuring lower altitudes (<2000 m) shows schists as dominant parent material. Figure 1 illustrates the difference in elevation and parent material from the western to eastern part of the forest.

The forest contains various ecosystems ranging from dense forest, bamboo groves to marshes, and contains approximately 1105 plant species, as well as high biodiversity of fauna. Many species are endemic for the area and the central African Highlands (Graham et al., 1995; Sun et al., 1996; Masozera and Alavalapati, 2004; Plumptre et al., 2007; Fischer and Killmann, 2008).

The average annual precipitation from 1974 to 1989 as measured by the seven weather stations located in the vicinity of the Nyungwe forest (Fig. 1a) is 1660 mm (ranging from 1308 to 2071 mm). In the dry seasons (June to August) monthly precipitation is below 80 mm per month and generally above 130 mm during the other months. The average monthly minimum and maximum temperature is 11 and 23 °C, respectively. The average annual temperature was 17 °C, with small seasonal variations (monthly mean range: 17.0 °C in June to 17.6 °C in April) (Minagri and CTB/BTS, 1993a, b). Weather data was not available for more recent years, but an automatic climate station has been established inside the Nyungwe forest (2°28'–43.3" S, 29°12'00" E) on February 2007 (Fig. 1a). The average annual mean temperature and precipitation at this location is 14.7 °C and 1706 mm, respectively (Nsabimana, 2009).

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Soil sampling in the Nyungwe forest has been performed during September 2009 at 31 different locations (Fig. 2) covering all major soil types and climate conditions in the region. Soil core samples were taken from 0 to 10 cm depth. The soil samples were composite samples consisting of 5 different subsamples per location, which were taken in an X-shaped pattern (in each leg head and in the center of the X) of two meters from each head with a length of ~2.8 m for each leg. All soil samples were immediately air-dried to reduce microbial activity during transportation to laboratory facilities in Belgium.

Total soil C and N content were measured by an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS) (20-20, SerCon, Crewe, UK). Soil texture was determined by the Andreasen pipette method by sieving (Allen, 1975). The pH was measured by suspending dried soil samples in water (1:10 soil/water) and a glass electrode. Free iron (Fe oxides from outside the silicate lattices) was measured upon sodium dithionite extraction (Mehra and Jackson, 1960) and measured via optical emission spectrometer (Varian ICP-OES) (720 ES, Mulgrave VIC 3170, Australia).

## 2.2 Laboratory experimental conditions for soil N<sub>2</sub>O and NO measurements

After air-drying the soil was homogenized by sieving (2 mm mesh size). The soil samples were incubated in the laboratory in tubes of 2.6 cm diameter, 9 cm height and 47.78 cm<sup>3</sup> volume. The mass of dry soil needed was calculated from the measured field bulk density ( $\rho_b$ ) with the known volume ( $\rho_b = \frac{\text{mass of dry soil}}{47.78 \text{ cm}^3}$ ). The soil moisture content was brought to three levels (50, 70 and 90 %) of water-filled pore space (WFPS) and kept constant during the incubation. The lowest water content in this study was 50 %, which is e.g. equivalent to lowest WFPS for Kakamega forest soils in Kenya (~40 % WFPS with daily precipitation only by <5 mm) (Werner et al., 2007b). The air temperature during the incubation was kept constant at 17 °C. To avoid that flux measurements are biased by the exponential increase of microbial activity upon remoistening the soil samples, all the soil incubation tubes were first pre-incubated at the three moisture levels at 17 °C for 7 days.

### Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





## 2.3 Measurements of soil N<sub>2</sub>O and NO production

The incubation tubes were placed into airtight sealed glass containers with a volume of 1200 ml. Measurements of N oxide gases were performed at day 1, 3 and 5 upon 7 days pre-incubation. The headspace concentration of NO of each incubation tube sample was measured four times (0, 40, 80 and 120 min after closing the glass containers) during each measuring day.

NO concentrations in the sample air were determined by use of an NO analyzer (CLD 77AM, Eco Physics, Switzerland) with a continuous flow rate of 55 ml min<sup>-1</sup>. The detection is based on the chemoluminescence of the oxidation of NO to NO<sub>2</sub> in the presence of ozone (O<sub>3</sub>).

The NO analyzer was calibrated using an NO reference gas, with a known concentration of 9.8 ppmv ± 0.5 in N<sub>2</sub>. Following measuring NO concentrations at 0, 40, 80 and 120 min, the same containers were used to sample N<sub>2</sub>O gas. Immediately after the NO measurements, 12 ml gas sample was withdrawn from the headspace by a syringe. The gas sample was transferred to a 12 ml vacutainer tube and stored therein for later measurements of N<sub>2</sub>O concentration. Since less than 10% of the headspace was used during the measurement the effect of change in pressure has been ignored in view of the large variability of observed NO and N<sub>2</sub>O fluxes.

As a check on the reliability of NO measurements at each moment in time containers with known initial concentrations (24.5, 49.8 and 99.7 ppb) of NO were measured during the experiment to determine the leakiness of the glass containers, and the soil measurements were corrected accordingly with a correction magnitude of less than ca. 13%. The N<sub>2</sub>O concentration was determined via a Gas Chromatograph (14B, Shimadzu, Japan) equipped with an electron capture detector ECD). One ml from the 12 ml vacutainer was injected into the GC with a Hamilton airtight syringe. The GC analysis of the samples was always accompanied by measurements of varying amounts of a reference gas with a known concentration of 2.46 ± 0.12 ppmv in order to construct a calibration curve for N<sub>2</sub>O.

**BGD**

8, 11631–11660, 2011

### Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





The ideal gas law in combination with the molecular weight of  $\text{N}_2\text{O}$  and  $\text{NO}$  was used to calculate the fluxes in  $\mu\text{g N}_2\text{O l}^{-1}$  and  $\mu\text{g NO l}^{-1}$ . Those values are then recalculated to  $\mu\text{g N}_2\text{O h}^{-1} \text{kg}^{-1}$  dry soil and  $\mu\text{g NO h}^{-1} \text{kg}^{-1}$  dry soil by use of changing in headspace concentrations over time using a linear regression approach. The  $\text{NO}$  and  $\text{N}_2\text{O h}^{-1} \text{kg}^{-1}$  dry soil fluxes were finally recalculated to  $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$  and  $\mu\text{g NO m}^{-2} \text{h}^{-1}$  with a known soil surface area.

## 2.4 Statistical analysis

Statistical analysis was performed using the statistical package SPSS 16 (SPSS inc., 2007). Using the Kolmogorov-Smirnov goodness-of-fit test, each N oxide gas emission rate showed a log-normal distribution. Therefore, log-transformed data were used to make comparisons between groups and all data were tested at the 5% significance level. All data given in tables and figures (error bars) are accompanied by the standard error.

## 3 Results

### 3.1 $\text{N}_2\text{O}$ and $\text{NO}$ production rates

All  $\text{N}_2\text{O}$  and  $\text{NO}$  fluxes showed significant effects for soil moisture content. Multiple comparison of all  $\text{N}_2\text{O}$  fluxes (log-transformed data) from all sites showed a significant difference (Table 1) between the three applied levels of soil WFPS.. In all cases the highest fluxes were observed for the 90 % WFPS. A significant positive correlation was observed between  $\text{N}_2\text{O}$  fluxes and WFPS for the entire dataset (Fig. 3). The  $\text{N}_2\text{O}$  emission rate averaged over the 31 locations for day 1, day 3 and day 5 after pre-incubation were 40.2, 43.2 and 56.3  $\mu\text{g N m}^{-2} \text{h}^{-1}$ , respectively for 50 % WFPS, 70.7, 70.9 and 73.6  $\mu\text{g N m}^{-2} \text{h}^{-1}$  for 70 % WFPS and 88.3, 109.6 and 98.3  $\mu\text{g N m}^{-2} \text{h}^{-1}$  for 90 % WFPS (Table 1).

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



In contrast to N<sub>2</sub>O emissions, NO emissions decreased with increasing soil moisture, with significant differences between the three applied levels of soil WFPS. Consequently, the NO emission rates were significantly negatively correlated with WFPS (Fig. 3). The mean NO emission rate for day 1, day 3 and day 5 after pre-incubation were 59.9, 61.3 and 86.6 μg N m<sup>-2</sup> h<sup>-1</sup>, respectively for 50 % WFPS, 40.3, 43.3 and 57.6 μg N m<sup>-2</sup> h<sup>-1</sup> for 70 % WFPS and 33.9, 33.3 and 41.0 μg N m<sup>-2</sup> h<sup>-1</sup> for 90 % WFPS (Table 1).

### 3.2 Variation of soil properties and N<sub>2</sub>O and NO gas emission rates

The soil textural classes at the sampled sites were classified (USDA classification system) as clay (C), clay loam (CL), sandy loam (SL), silt loam (SiL), loamy sand (LS), silty clay (SiC) and silty clay loam (SiCL). Table 1 and Fig. 2 show summary statistics and distribution for soil characteristics of the studied sites. The sites in the eastern and northeastern corners of the forest (Fig. 2a) showed high clay content (>37%). The highest silt contents were found at sites in the northern part of the forest with values above 50%. The sites in the northwestern corners had a very low fraction of sand (<14%). The forest is dominated by strongly leached acid soils (pH ranging from 2.9 to 4.8). Areas with high soil organic carbon (OC) contents (>4%, Fig. 2e), corresponding with high total nitrogen (TN) contents, were found in the northern and northeastern parts of the forest. OC content were in general high and ranged from 3.6 to 11.9%, total N contents ranged from 0.08 to 0.89% and C:N ratio ranged from 7.7 to 16.3 in the analyzed samples. Free iron ranged from 0.1 to 5.5% and high free iron contents (>3%, Fig. 2g) were mostly present in sites of the eastern part of the forest, where altitude is higher and micaschists dominate the soilscape (Fig. 1). The variation in silt, sand, clay content and free iron (formed through weathering) reflects the lithological variation in the forest. Coefficients of variation (CV) ranged between 45 and 72% for all soil variables except for pH (CV = 9%) and C:N ratio (CV = 11%).

The N<sub>2</sub>O and NO emission rates showed a large spatial variation and a highly skewed distribution. Coefficients of variation for the N<sub>2</sub>O emission rates were 133.3,

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



89.9 and 92.5 %, respectively for 50, 70 and 90 % WFPS. Coefficients of variation of the NO emission rates for 50, 70 and 90 % WFPS were 75.5, 68.8 and 65.2 %, respectively. The N<sub>2</sub>O emission rates ranged from 4.5 to 400.5 µg N m<sup>-2</sup> h<sup>-1</sup> and the NO emission rates from 6.6 to 265.5 µg N m<sup>-2</sup> h<sup>-1</sup>. The range and skewness of the N<sub>2</sub>O emission rates was higher than the NO emission rates. The majority of soil samples showed relatively low N<sub>2</sub>O emission rates, with the exception of a few sites (large skewness). Figure 5 shows the spatial variation of the N<sub>2</sub>O and NO emission rates for the three WFPS levels considered. It also illustrates that measured N<sub>2</sub>O emissions varied substantially over the study region. Compared to N<sub>2</sub>O, the spatial variability of NO emission rates was more or less opposite to that of N<sub>2</sub>O (Fig. 4).

The NO:N<sub>2</sub>O emission ratio (NO:N<sub>2</sub>O) varied on average from 3.3 to 0.9 and 0.5, respectively for 50, 70 and 90 % WFPS. The NO:N<sub>2</sub>O ratio showed no marked trend for soil samples taken across the study region (data not shown). Increased moisture levels resulted in a decrease of the measured NO:N<sub>2</sub>O ratio (Fig. 3).

### 3.3 Correlation between N<sub>2</sub>O and NO gas emission rates and soil properties

For co-variation analysis we only used N trace gas data at 50 % WFPS, since spatial differences between sampling sites were most pronounced for this treatment. The variation in N<sub>2</sub>O emissions was positively correlated with clay and free iron content and negatively with pH and silt content (Table 2). No significant correlation was observed for OC and TN contents. In contrast, NO emission rates were significantly positively correlated with OC, TN and free iron content and showed a weak significant negative correlation with C:N ratio.

These contradictory results could be due to the large variance in N<sub>2</sub>O and NO emission. Nitrous oxide emission rates for sites at low altitude (<2000 m) in the western part of the forest (where schists dominate the soilscape) were relatively low (e.g. 8.8–130 µg N m<sup>-2</sup> h<sup>-1</sup> for 50 % WFPS) compared to higher N<sub>2</sub>O emission rates (e.g. up to 238 µg N m<sup>-2</sup> h<sup>-1</sup> for 50 % WFPS) for samples taken from higher altitude in the eastern

**BGD**

8, 11631–11660, 2011

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



part (where micaschists dominate the soilscape). High NO emissions were distributed among sampling sites with high OC and TN content.

This result indicates that variation in parent material and topography (Fig. 1b, c), which largely control variation in soil properties, exerts a primary control on N<sub>2</sub>O emission. Therefore, we separately investigated how correlation between N<sub>2</sub>O and NO emissions and soil properties varied for low altitude, where schists dominate and high altitude where micaschists dominate. We did this by creating a different sub dataset according to variation in parent material and altitude: data subset 1: locations at high altitude and micaschists as dominant parent material; and data subset 2: locations at low altitude and schists as dominant parent material.

For data subset 1, the N<sub>2</sub>O emission rate was significantly and positively correlated with OC and clay content and negatively correlated with pH. The NO emission rate showed significantly negative correlation with C:N ratio ( $p < 0.05$ ) and positive correlation with OC and TN. Both N<sub>2</sub>O and NO correlated positively with free iron content but not significant.

For data subset 2, N<sub>2</sub>O emissions were significantly and positively correlated with the OC, TN clay and free iron content. NO emission was positively, significantly correlated with TN and free iron. The correlation was also positive with OC but not significant.

## 4 Discussion

To our knowledge, our N<sub>2</sub>O and NO flux estimates from 31 different locations in the Nyungwe tropical forest in southwestern Rwanda represent the first large spatial dataset of potential N<sub>2</sub>O and NO gas exchange, on the African continent.

The N<sub>2</sub>O emission rates from Nyungwe were comparable in magnitude with N<sub>2</sub>O fluxes from field measurements at the Kakamega forest sites in Kenya (1.1–324.8  $\mu\text{g N m}^{-2} \text{h}^{-1}$ ; Werner et al., 2007b). With mean N<sub>2</sub>O emission rates ranging from 46.3 to 98.8  $\mu\text{g N m}^{-2} \text{h}^{-1}$ , the Nyungwe forest emitted comparable levels of N<sub>2</sub>O as reported by Serca et al. (1994) for the Mayombe forest soils in Democratic Republic of

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Congo (mean flux in the rainy season:  $19.6 \mu\text{g N m}^{-2} \text{h}^{-1}$  and mean flux at the end of rainy season:  $207 \mu\text{g N m}^{-2} \text{h}^{-1}$ ). Furthermore, with an average clay, silt and OC content of 26 %, 44 % and 4.4 %, respectively, the Nyungwe forest soils are similar to the Kakamega (Kenya) rainforest soils (Werner et al., 2007b), (34 % clay, 23 % silt and 3.5 % C content) and the Mayombe forest soils (1.7–4.5 % C content).

In our study we measured individual  $\text{N}_2\text{O}$  fluxes up to  $558.5 \mu\text{g N m}^{-2} \text{h}^{-1}$ , which is of similar magnitude as the reported maximum  $\text{N}_2\text{O}$  fluxes of  $492.1 \mu\text{g N m}^{-2} \text{h}^{-1}$  and  $570.8 \mu\text{g N m}^{-2} \text{h}^{-1}$  for Australian rain forest soils following periods of intensive rainfalls (Breuer et al., 2000; Kiese and Butterbach-Bahl, 2002). The coefficient of variation for  $\text{N}_2\text{O}$  emission rates of three WFPS at the Nyungwe forest between different sampling sites were in good agreement with results from Werner et al. (2007b), who reported values of 52.8–147.9 % for the spatial variation of  $\text{N}_2\text{O}$  fluxes for the Kakamega forest in Kenya.

Compared to other  $\text{N}_2\text{O}$  emission published data in other tropical rain forest ecosystems (except Amazon tropical rainforest), the observed variability of  $\text{N}_2\text{O}$  emission rate at the Nyungwe forest was high. Kiese and Butterbach-Bahl (2002) and Breuer et al. (2000) reported coefficients of variation for  $\text{N}_2\text{O}$  emission rate in tropical forest ecosystems in Australia, between 36.5–60.2 % and 16.6–67.6, respectively. However, these authors analyzed the spatial variability of fluxes at a given site on the basis of chamber measurements at different plots, while in our study we analyzed the spatial variability of fluxes along an approx. 40 km long transect with 31 sampling sites.

The observed NO emission rates were higher than previously reported maximum emissions for the Amazon rainforest:  $60.2 \mu\text{g N m}^{-2} \text{h}^{-1}$  (Bakwin et al., 1990),  $16.9 \mu\text{g N m}^{-2} \text{h}^{-1}$  for the Amazon tropical rainforest site at Reserva Biologica Jaru, Brazil (Gut et al., 2002), and  $14.4 \mu\text{g N m}^{-2} \text{h}^{-1}$  at the end of a rainy season (mean flux rainy season  $4.1 \mu\text{g N m}^{-2} \text{h}^{-1}$ ) for the Mayombe forest in DR Congo. However, the observed NO emission rates for the Nyungwe forest were lower than reported fluxes (mean NO emission =  $207.1 \mu\text{g N m}^{-2} \text{h}^{-1}$ , range:  $0.1\text{--}773.8 \mu\text{g N m}^{-2} \text{h}^{-1}$  with coefficient variation of 45.4–93.1) for rain forests in Queensland, Australia at the onset of the rainy

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

season (Butterbach-Bahl et al., 2004). It must be pointed out that the latter results of  $N_2O$  and  $NO$  emissions from the Australian tropical rain forest were field measurements at the start of the rainy season. As the authors point out accumulated litter may have been rapidly mineralized, thereby providing plenty of substrates for microbial  $N$  and  $C$  turnover and, thus, fueling microbial  $NO$  production and emission (Butterbach-Bahl et al., 2004).

Nitrogen trace gas emissions in tropical environments are predominantly governed by WFPS (Davidson, 1991; Kiese and Butterbach-Bahl, 2002; Werner et al., 2007b). The observed decrease of the  $NO:N_2O$  ratio with increasing WFPS (Fig. 3) indicates that denitrification or nitrifier-denitrification processes were possibly the main pathway of  $N_2O$  gas production rather than nitrification. This assumption is in good agreement with previous observations in rain forest ecosystems in Australia (Kiese and Butterbach-Bahl, 2002; Butterbach-Bahl et al., 2004) and rain forests in Kenya (Werner et al., 2007b). Based on previous studies, maximum  $N_2O$  emission rates are reported at WFPS values between 50 and 80 %, though other studies suggest slightly higher ranges of 60 to 90 % (Davidson, 1991; Kiese and Butterbach-Bahl, 2002; Werner et al., 2007b). In our study maximum  $N_2O$  emission occurred at 90 % WFPS, conditions whereby the denitrification process normally favors  $N_2$  formation. The relationship between  $N_2O$  emissions and WFPS was suggested to be linear for Australian rain forest soils (WFPS ranging from 10 to 50 % (Butterbach-Bahl et al., 2004)) and exponential for Kakamega rain forest soils in Kenya (WFPS ranging from 40 to 75 % (Werner et al., 2007b)). For our data, using a linear function (Fig. 3), 99 % of the variability of the  $N_2O$  emissions could be explained by changes in soil moisture content. Keller et al. (2005) also found significant relationship between  $N_2O$  emissions and soil moisture for tropical forest site in Brazil.

Maximum  $NO$  emissions can be expected at WFPS <50 % depending on soil physical properties. A quadratic function was reported for the relation between  $NO$  emissions and WFPS for Australian rain forest soils (Butterbach-Bahl et al., 2004) (WFPS levels ranging from <5 % to 50 %).

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[⏪](#)[⏩](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

For our data a linear function was able to describe 96% of the relation between average NO emission rates and the three WFPS levels (Fig. 3). The NO fluxes at low moisture content (50% WFPS) were higher than those at medium (70% WFPS) and pronounced higher than at high soil moisture (90% WFPS) contents. These results are supportive to several other studies which were showing a strong reduction in soil NO production if soil moisture is increasing to values >50% WFPS (e.g. Butterbach-Bahl et al., 2004).

In contrast to NO emission, correlation between N<sub>2</sub>O emission and OC and TN only appeared in the data subsets. In the western part of the forest, where altitude is lower and schists dominate the soilscape, both OC, but also TN showed a correlation with N<sub>2</sub>O emissions. In the eastern part of the forest (higher altitude and micaschists) both OC and TN were correlated with NO emissions. Further, pH seems to be an important controlling factor as it appeared in the entire data set and sub-dataset 1.

Positive correlations of N<sub>2</sub>O emissions with OC and TN are in agreement with Booth et al. (2005), who showed a direct effect OC and TN on N<sub>2</sub>O emission for soils from a wide range of ecosystems. It is well known that low pH decreases the activity of the N<sub>2</sub>O-reductase, thereby increasing production of N<sub>2</sub>O from denitrification (Nömmik, 1956; Weier and Gillam, 1986; Granli and Bockman, 1994). For nitrification, it has also been demonstrated that low pH values favor N<sub>2</sub>O production (Sitaula and Bakken, 1993; Martikainen and De Boer, 1993; Kesik et al., 2006). Furthermore, it has been shown by Kiese and Butterbach-Bahl (2002) that low pH was a crucial factor driving high N<sub>2</sub>O emissions from coastal lowland soils in an Australian rainforest.

The importance of soil N for explaining N<sub>2</sub>O and NO fluxes may be explained by the fact that nitrogen availability is one of the primary controlling parameters for organic matter mineralization, whereby mineralization is delivering inorganic N for nitrifying and denitrifying bacteria. Nitrogen content, as an important controlling parameter for NO emission, was also clearly indicated in the study of Pilegaard et al. (1999) analyzing NO emission rates from temperate forest soils across a wide range of sites in Europe. The negative effect of the C:N ratio on the NO emission rate is similar to that reported

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)



by Stark et al. (2002), indicating that the C:N ratio affected the NO emission rate from forest soils of Western North America by controlling N mineralization.

Breuer et al. (2000), investigating different rain forest sites in tropical Queensland, Australia, reported that nitrate concentration and WFPS content at Kauri Creek; soil pH and nitrate concentration at Lake Eacham; and for all sites CO<sub>2</sub> emission, C:N ratio and WFPS content were the most influential factors driving small scale (<100 m) spatial variations in soil N<sub>2</sub>O emissions. Furthermore, Werner et al. (2007b) explained site differences in N<sub>2</sub>O emissions for different sites in the tropical rainforest of Kakameka, Kenya, by differences in the C:N ratio and clay content. Mapanda et al. (2010) also indicated that variability of N<sub>2</sub>O emissions from Miombo woodland in Zimbabwe is controlled by changes of soil moisture, mineral N and pH.

Acidic soils of the Nyungwe forest in combination with high free iron contents could favor chemo-denitrification (auto-decomposition of nitrites) for NO and N<sub>2</sub>O production in addition to microbial processes, (Van Cleemput and Baert, 1984). All the favorable conditions for this phenomenon to occur are present: very low pH, abundance of clay and reduced metals (e.g. Fe<sup>2+</sup>) (Nelson and Bremner, 1970; Wullstein and Gilmour, 1964). Positive correlations of N<sub>2</sub>O and NO emissions with clay content (for all the data subsets) and free iron for the entire data and data subsets and negative correlations with soil pH (N<sub>2</sub>O only) (Table 2) give further supporting evidence that chemo-denitrification might play a role. Serca et al. (1994) also found that chemo-denitrification in acid Mayombe forest soils is a potentially important cause of N oxide gases production.

It would be interesting and useful to define and distinguish chemo-denitrification from related pathway and processes in acidic soils of the Nyungwe forest. However, this is outside the scope of this paper, though further research will be carried out dealing with these aspects.

Again, variation in parent material and topography caused average free iron content (4.1 ± 0.2 %) to be higher in the eastern part of the forest, where micaschists dominate the soilscape, than the average free iron content (2.5 ± 0.2 % free iron) at western part,

**BGD**

8, 11631–11660, 2011

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



where chists dominate the soilscape.

Our results indicate that potential factors controlling spatial variation of N<sub>2</sub>O and NO emission from Nyungwe forest soils are controlled by both geological (free iron, pH) and biogeochemical drivers (OC, TN). Reducing the uncertainty in N trace gas emission estimates requires constrained information of soil properties at a spatial scale that is fine enough to capture the spatial variability over the forest. Of course, our incubation study cannot unambiguously improve N trace gas source strength estimates for tropical forests since all results are based on incubations for a few days only. Nonetheless, our study reveals aspects of spatial variability and control of N<sub>2</sub>O and NO emissions that may assist to the development of baseline information required for reducing emissions from deforestation and degradation (REDD) activities designed to limit greenhouse gas emissions from tropical forests from developing countries (Stickler et al., 2009). Finally, an additional benefit of the obtained results is their potential to validate the mechanistic biogeochemical (e.g. ForestDNDC-tropica, Werner et al., 2007a) models and close gaps in global trace gas budgets.

## 5 Conclusion

This laboratory study provided a unique and large spatial explicit data set of N<sub>2</sub>O and NO fluxes for tropical rain forest soils. Nitrous oxide and NO emissions rates were in the range with those reported for other tropical rain forests, but showed a large spatial variation. Soil water content was found to affect NO and N<sub>2</sub>O emission differently. The relationship between soil properties and N<sub>2</sub>O and NO emission rates was somewhat scale-dependent and spatial information on topography and parent material distribution helped to determine biogeochemical and geological controls on N<sub>2</sub>O and NO emission. Positive correlations with soil carbon and nitrogen indicate a biological control over N<sub>2</sub>O and NO production. But, a negative correlation (only N<sub>2</sub>O) with soil pH and a positive correlation with free iron suggest that chemo-denitrification might an important production pathway. We, therefore, recommend that future research on N trace gas research

## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



in tropical forests include efforts to differentiate biological and chemical pathways for N trace gas emission.

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## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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### Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Table 1.** Range, median, mean, standard error (SE), skewness and kurtosis of soil characteristics<sup>a</sup>, N<sub>2</sub>O and NO flux rates (measured at day 1, 3, and 5 after pre-incubation). The letters indicate significant differences ( $P < 0.05$ ) for mean N trace gas fluxes between different water-field pore space (WFPS).

		Range	Median	Mean	SE	Skewness	Kurtosis
N <sub>2</sub> O ( $\mu\text{g N m}^{-2} \text{h}^{-1}$ )		<i>n</i> = 31					
WFPS 50 %	Day 1	5.8–226.6	21.5	40.2	10.4	2.5	5.7
	Day 3	4.5–249.2	19.0	43.2	11.4	2.2	3.9
	Day 5	7.7–244.2	34.1	56.3	12.7	1.9	2.5
WFPS 70 %	Day 1	38.2–297.5	46.7	70.7	11.7	2.5	5.6
	Day 3	31.1–245.9	51.2	70.9	11.1	1.9	2.5
	Day 5	15.9–276.6	56.4	73.6	12.7	1.8	2.3
WFPS 90 %	Day 1	45.5–400.5	55.6	88.3	16.4	2.9	8.2
	Day 3	41.0–400.5	81.8	109.6	17.0	2.3	4.7
	Day 5	31.8–400.0	80.3	98.3	17.0	2.2	4.2
average 1st, 3rd, 5th day							
WFPS 50 %	CV <sup>b</sup> 133.3	8.8–238.4	23.6	46.5a	11.1	2.1	3.2
WFPS 70 %	CV 89.9	36.6–266.6	47.0	71.7b	11.5	2.2	3.4
WFPS 90 %	CV 92.5	46.2–400.2	66.0	98.8c	16.4	2.5	5.3
NO ( $\mu\text{g N m}^{-2} \text{h}^{-1}$ )		<i>n</i> = 31					
WFPS 50 %	Day 1	0.7–260.8	55.7	59.9	8.2	2.8	12.1
	Day 3	0.8–231.1	52.1	61.3	7.9	1.9	6.2
	Day 5	2.6–265.5	64.5	86.4	13.5	1.2	0.3
WFPS 70 %	Day 1	2.6–118.6	33.4	40.3	5.1	1.2	1.0
	Day 3	4.3–113.7	36.2	43.3	5.1	0.99	0.4
	Day 5	2.3–173.5	46.0	57.6	8.1	1.3	1.3
WFPS 90 %	Day 1	3.4–127.5	28.8	33.9	4.7	1.9	4.9
	Day 3	2.8–72.1	28.7	33.3	3.0	0.6	–0.2
	Day 5	0.9–144.5	41.4	41.0	5.7	1.7	4.1
average 1st, 3rd, 5th day							
WFPS 50 %	CV 75.5	1.3–252.5	53.3	69.3a	9.3	1.6	3.7
WFPS 70 %	CV 68.8	4.6–128.8	36.4	47.1b	5.8	1.2	1.0
WFPS 90 %	CV 65.2	2.9–108.9	31.7	36.1c	4.2	1.5	2.8
Soil properties							
Clay (%)	CV 47.4	3.0–51.0	28.0	26.1	2.2	–0.1	–1.0
Sand (%)	CV 72.3	7.0–86.0	26.0	30.4	3.9	0.7	–0.3
OC (%)	CV 50.9	1.0–11.9	3.9	4.5	0.4	1.0	2.1
TN (%)	CV 51.8	0.08–0.89	0.35	0.4	0.4	0.6	–0.2
C:N	CV 11.2	7.7–16.3	11.1	11.5	0.4	0.4	–0.5
pH	CV 9.1	2.9–4.89	4.2	4.1	0.6	–0.92	0.82
Fe (%)	CV 52.1	0.1–5.5	3.04	3.03	0.3	–0.24	–1.02

<sup>a</sup> OC = organic carbon, TN = total nitrogen, C:N = organic carbon to total N ratio, Fe = free iron (Fe oxides from outside the silicate lattices)

<sup>b</sup> CV = coefficient of variation in %

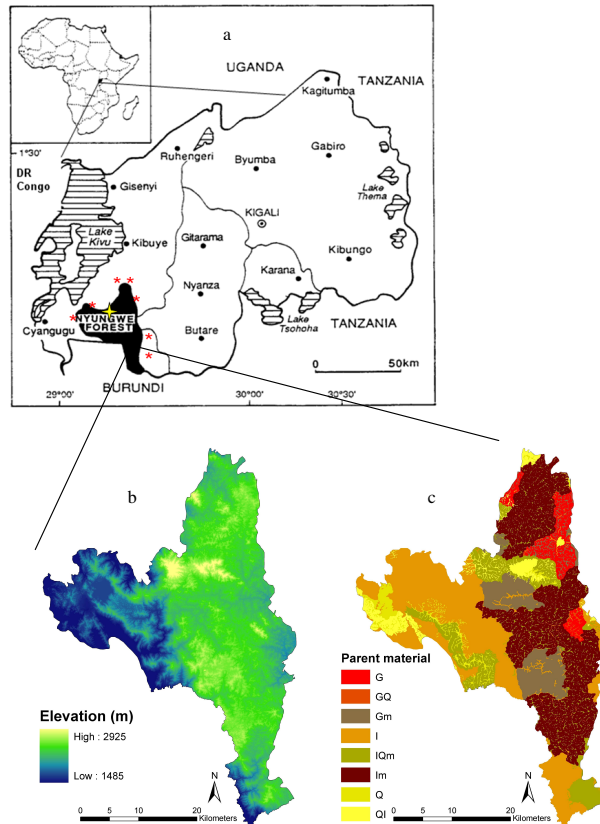
## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.

**Table 2.** Correlation coefficients for N<sub>2</sub>O and NO emission rates and soil properties (silt and clay content; organic carbon (OC), total nitrogen (TN), C:N ratio pH, and free iron (Fe)) for two different data subsets data subset 1: locations at high altitude and micaschists as dominant parent material; and data subset 2: locations at low altitude and schists as dominant parent material. In this analysis we only include N oxide emission data from the 50 % WFPS; \*\* indicates significant correlations at  $P < 0.01$  and \* indicates significant correlations at  $P < 0.05$ .

		Clay	Silt	Sand	OC	TN	C:N	pH	Fe
Data Subset 1	N <sub>2</sub> O	0.59*	-0.57	0.07	0.68*	0.25	0.65	-0.77*	0.26
	NO	0.19	0.58	-0.56	0.61*	0.81**	-0.63*	-0.09	0.42
Data Subset 2	N <sub>2</sub> O	0.51*	-0.09	-0.14	0.50*	0.63**	-0.12	-0.35	0.50*
	NO	0.31	-0.07	-0.08	0.39	0.49*	-0.07	0.21	0.53*
Entire dataset	N <sub>2</sub> O	0.58*	-0.52*	0.16	0.02	-0.15	0.45	-0.59**	0.38*
	NO	0.13	0.27	-0.33	0.49**	0.63**	-0.36*	0.02	0.40*

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[◀](#)
[▶](#)
[◀](#)
[▶](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)

**Fig. 1.** Location of the Nyungwe National forest in southwestern Rwanda; seven climate stations around the forest are shown by red stars; the recent climate station in the Nyungwe forest is shown by a yellow star **(a)**, elevation map **(b)** and parent material map of the Nyungwe forest: Q = quartzite; QI = quartzite intercalated with schists; IQm = quartzite intercalated with micaschists; GQ = granitic and quartzitic rocks; G = acid rocks (granite); Gm = micaceous acid rocks (granitoides); Im = micaschists; I = schists **(c)**.

**Spatial variations of nitrogen trace gas emissions**

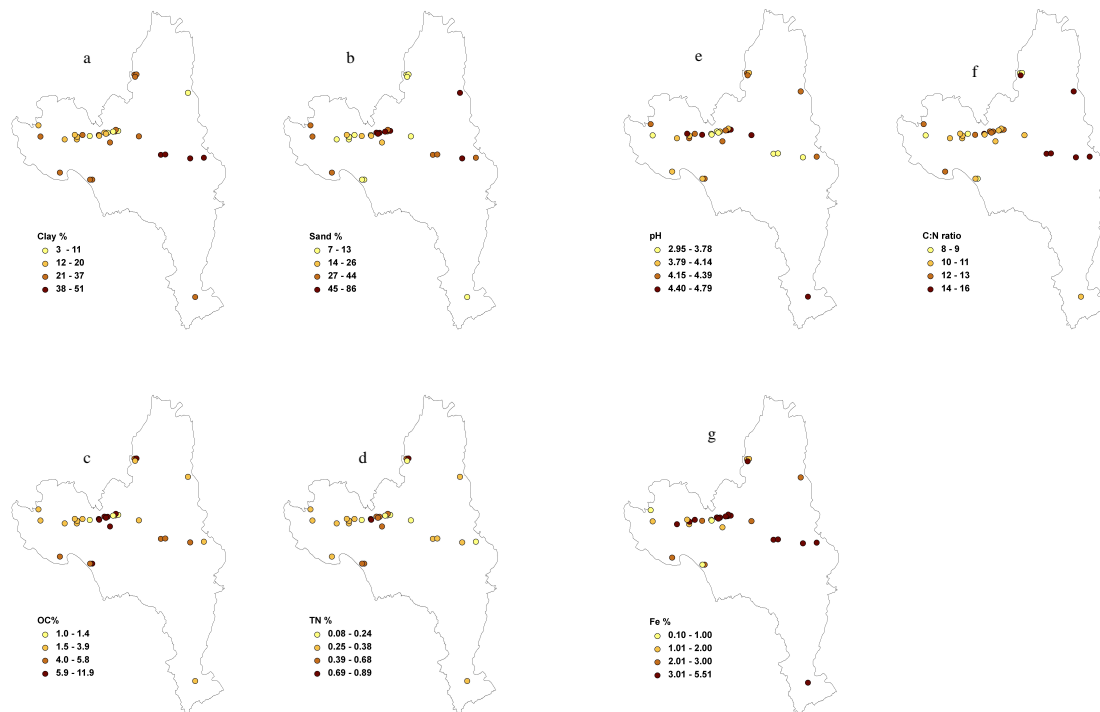
N. Gharahi Ghehi et al.

Title Page	
Abstract	Introduction
Conclusions	References
Tables	Figures
◀	▶
◀	▶
Back	Close
Full Screen / Esc	
Printer-friendly Version	
Interactive Discussion	



## Spatial variations of nitrogen trace gas emissions

N. Gharahi Ghehi et al.



**Fig. 2.** Spatial pattern of the soil characteristics of the samples sites in the Nyungwe forest: clay (a), sand (b), organic carbon (OC) (c), total nitrogen (TN) (d), pH (e), C:N ratio (f), free iron (Fe) (g).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

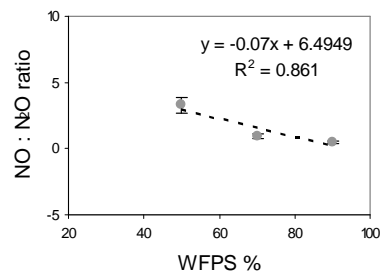
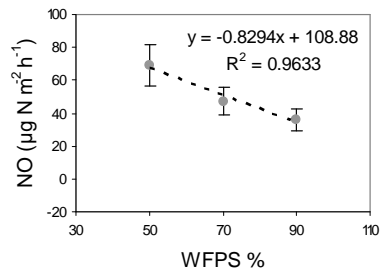
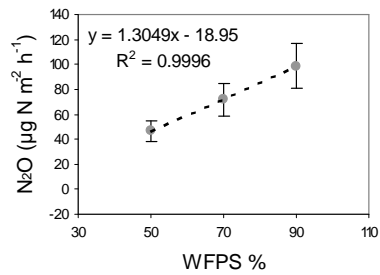
Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





**Fig. 3.** Correlation of water-filled pore space (WFPS) and N<sub>2</sub>O, NO emission rates and NO:N<sub>2</sub>O ratio (mean of 31 sites, error bars indicate plus minus standard error).

**Spatial variations of nitrogen trace gas emissions**

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

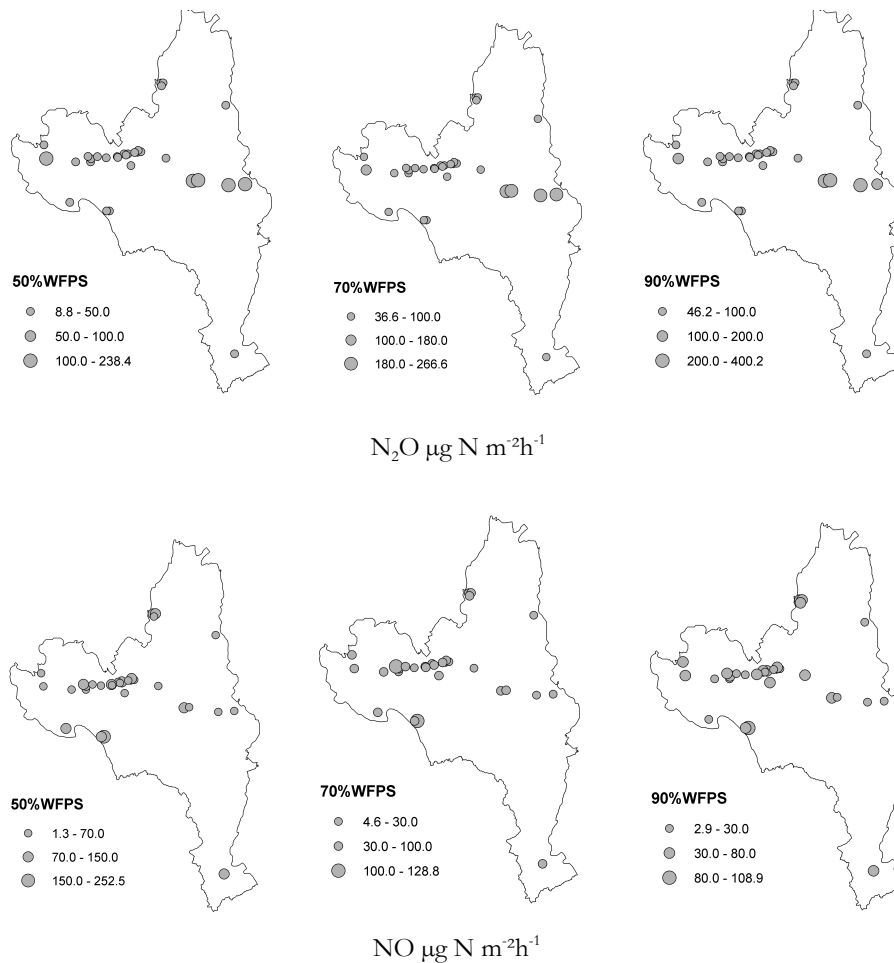
Full Screen / Esc

Printer-friendly Version

Interactive Discussion







**Fig. 4.** Spatial distribution of  $\text{N}_2\text{O}$  (top) and  $\text{NO}$  (bottom) emission rates in the Nyungwe forest.

**Spatial variations of nitrogen trace gas emissions**

N. Gharahi Ghehi et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

