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Quantifying the contribution of land use to N2O, NO and CO2 fluxes in a montane forest ecosystem of Kenya

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Corresponding Author:	Eugenio Díaz-Pinés, Ph.D Universitat fur Bodenkultur Wien Wien, AUSTRIA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universitat fur Bodenkultur Wien
Corresponding Author's Secondary Institution:	
First Author:	Cristina Arias-Navarro
First Author Secondary Information:	
Order of Authors:	Cristina Arias-Navarro
	Eugenio Díaz-Pinés, Ph.D
	Pablo Zuazo
	Mariana C Rufino
	Louis V Verchot
	Klaus Butterbach-Bahl
Order of Authors Secondary Information:	

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	Climate Food and Farming Research Network	Mrs Cristina Arias-Navarro
	Helmholtz Research School MICMoR	Mrs Cristina Arias-Navarro
	Bundesministerium für Bildung und Forschung (01DG13012)	Dr. Eugenio Díaz-Pinés Dr Klaus Butterbach-Bahl

Abstract: Increasing demand for food and fibre by the growing human population is driving significant land use (LU) change from forest into intensively managed land systems in tropical areas. But empirical evidence on the extent to which such changes affect the soil-atmosphere exchange of trace gases is still scarce, especially in Africa. We investigated the effect of LU on soil trace gas production in the Mau Forest Complex region, Kenya. Intact soil cores were taken from natural forest, commercial and smallholder tea plantations, eucalyptus plantations and grazing lands, and were incubated in the lab under different soil moisture conditions. Soil fluxes of nitrous oxide (N2O), nitric oxide (NO) and carbon dioxide (CO2) were quantified, and we approximated annual estimates of soil N2O and NO fluxes using soil moisture values measured in situ.
Forest and eucalyptus plantations yielded annual fluxes of 0.3-1.3 kg N2O-N ha-1 a-1 and 1.5-5.2 kg NO-N ha-1 a-1. Soils of commercial tea plantations, which are highly fertilized, showed higher fluxes (0.9 kg N2O-N ha-1 a-1 and 4.3 kg NO-N ha-1 a-1) than smallholder tea plantations (0.1 kg N2O-N ha-1 a-1 and 2.1 kg NO-N ha-1 a-1) or grazing land (0.1 kg N2O-N ha-1 a-1 and 1.1 kg NO-N ha-1 a-1). High soil NO fluxes

	were probably the consequence of long-term N fertilization and associated soil acidification, likely promoting chemodenitrification. Our experimental approach can be implemented in understudied regions, with the potential to increase the amount of information on production and consumption of trace gases from soils.
Response to Reviewers:	Response to the reviewers' comments COMMENTS TO THE AUTHOR: Reviewer #1: The authors use a core incubation method at different soil moisture levels to determine emissions of N2O, NO and CO2 from soils across several intact and disturbed land use types in montane Kenya. These values are then combined with in situ measures of soil moisture to scale to annual emissions and compare cover types. Overall, I think that this paper is a valuable contribution, especially in the context of the low number of trace gas studies from Africa, and will be of interested to readers of Biogeochemistry. This study appears to be methodologically comprehensive and meticulous. In studies of this kind, it is fairly common that there are numerous small methodological aspects upon which many researchers do not agree. However in my experience the specific choices made here are justifiable, and I know based on the ongoing body of work from these authors that this measurement approach and analytical system are the result of long-term and ongoing optimization. A second issue to note in that regard is the difficulty of frequent field sampling in remote areas, which also gives context to some of the authors decisions- eg to perform core incubations rather than direct field emissions and to scale by moisture alone. Though this leads to some uncertainty, this is acknowledged by the authors and acceptable given the context. Thank you very much for the positive and useful comments to the manuscript. We fully agree with the statement that when conducting trace gas measurements, there is a critical decision process involving many of the steps of the whole procedure. We have applied our experience and know-how to come up to the best solutions and to minimize potential measurement bias. Special care was taken to handle all the soil cores in the same way, to allow time for equilibration That said, at least one or a small number of static chamber sampling events (for N2O and CO2- unfortunately field sampling of NO is much more involved) would have been a useful addition to this study to ground truth incubation fluxes. Though the authors cite several studies that show good agreement between field and core methods, I suspect that there are many more studies that find discrepancies, especially in the context of longer term incubations where plant roots have been removed. As the reviewer has already pointed out, gaining field data, even for soil “only” CO2 and N2O fluxes, is highly challenging in tropical areas with large logistical restrictions. For this reason, it was not possible to feed our dataset with in situ measurements. We have acknowledged the limitations of this approach, and addressed possible sources of under- or overestimations (e.g. L464-470) Overall the data is clearly presented, the discussion is thorough and the conclusions are appropriate. I have primarily minor comments, outlined below, and if accepted I would like to see a couple of statistical and discussion points clarified prior to publication. Line 54: Change 'on to which extent' to 'on the extent to which' Thanks. We have amended the text (L54). Line 147: Expense rather than extent? Test amended (now L150). Line 175: How long between extraction and analysis? How dry was 'air dry'? After air-drying WFPS of the cores was 10-15 %. Slightly varying from one plot to the other (since soil sampling lasted a couple of days), 4 months elapsed between sampling and incubation in the lab. This information has been now included in the text (L 179-182)

Line 274: How was error propagation performed? How did you generate your uncertainty estimates for annual fluxes?
The uncertainty estimates corresponds to the uncertainty of the curve fitting. For annual fluxes, we cumulatively added the sum of the prediction uncertainty of each individual day (L292-295).

Specifically for the linear models (NO), we have used the function predict.lm (R package stats). For nonlinear fits (N2O) we followed A Monte Carlo approach to nonlinear error propagation using the function predictNLS (R package propagate):

We have cited the R packages and indicated the functions used in the methods section (L XXX). Since documentation for R packages can be found at the global open-source repository CRAN, we don't consider necessary to include more details.

Line 330: Be consistent with capitalization of eucalyptus

Thanks. Capitalization of eucalyptus has been united throughout the manuscript)L 338).

Line 304: is the +/- one SD?

This is correct. And it has been included in the text (L290-291)

Line 348: Without reporting stats, this doesn't actually conclusively indicate that the value is different from zero.

The statement has been deleted in the manuscript following the suggestion (L357).

Line 283: Wouldn't a mixed effects model be a better way to test relative explanatory power of different variables? Instead of testing each independently as a regression?

It is true that mixed models provide a powerful method to analyze a multivariate dataset. We run both methods and they yielded very similar results. Furthermore, we wanted to identify the effect of individual variables, since soil variables are very often highly correlated, which would reduce the applicability of a mixed effects model. For the sake of simplicity, we decided to test each variable independently in single variable regression models.

Line 394: Sometimes annual values are presented without error and sometimes with. Add errors here and please present throughout

Thanks. Errors have been added and presented throughout the manuscript for both internal and external data, with the exception of those references where authors didn´t report errors in their publications (i.e. Butterbach-Bahl et al (2004) and Gharahi Ghehi et al (2014)).

Line 431: awkward wording

Since the reviewer found the wording awkward, and the information provided is indeed repetitive because we have previously mentioned (L419-423), we have deleted the statement.

Line 440: I think some papers by Veldkamp report higher values from Peruvian and Costa Rican montane rainforests.

We have now included some other publications reporting N2O fluxes from tropical montane forests, e.g. Müller et al. 2015, Koehler et al. 2009. We have accordingly modified the ranges of previously reported N2O estimates for those ecosystems (from 0.8 to 1.3 kg N ha-1 a-1, L453); however, our argumentation that montane forests emit less N2O that lowland forests still holds true, since the upper range for montane forests is still lower than the lower range for lowland forests (1.3 and 1.9 kg N ha-1 a-1, respectively).

Line 444: Not necessarily. See low emissions in Wieder et al 2011 (GCB) in Costa Rica

We have now acknowledged the work from Wieder et al (2011), L460. Nevertheless, we consider this study is an exception, given the large number of studies showing high N2O fluxes from lowland tropical rain forests. Further, our intention is to point out that the montane tropical rainforests have characteristically low net mineralization rates, suggesting that those forests are possibly N limited compared to lowland forests.

	Line 448: I would think overestimates from litter removal might be countered by removing live plant roots as a sink. This point at least merits a mention as a caveat on core incubations. This is a very interesting point which has now been included (L467-470) Line 452: See Koehler et al 2009 (GCB) for some higher NO estimates from Keller Results from Koehler et al (2009) have been now included (L475), although it doesn´t affect the range of emissions we provide Line 484: They aren't much lower in the general scheme of things. The statement now reads: that our estimations are somewhat lower than those estimates from Rosenstock et al. (2015), (514) Line 500: But don't these emission occur primarily immediately after wetting only, after which the nitrite pool is quickly depleted? I'm not sure that I buy that there is evidence for sustained chemodenitrification that could account for most of the elevated NO you saw over a long period of time. We have slightly restructured our discussion with regard to chemo-denitrification. We have discussed that adequate conditions for denitrification may take place (low pH, high clay content), so it is reasonable to argue that the process may take place, and this is in agreement with previous research (L488-491). In relation to the commercial tea plantations, we speculate that due to high fertilization rates and even lower pH, chemo-denitrification might be more important than in the forest or the smallholder farms (L544-547). In either case, we argue that chemo-denitrification may have played an important role in the emissions of NO, but we don´t attribute to this process the majority of the NO fluxes observed. Accordingly, we have been very careful to avoid such statements. Line 510: Or Fe-ammox (sensu Yang 2012)? Would produce N2 but not N2O? We agree that direct dinitrogen production can be the dominant Fe-ammox pathway, short-circuiting the nitrogen cycle and resulting in ecosystem nitrogen losses as demonstrated by Yang et al (2012). However, since we don’t have any information about iron content or predominant form in our soils, we prefer to avoid speculation to that regard. Line 514: You mean primarily ammonia deposition? However it looks like there is significantly higher soil N in forests near SH farms than TE? How do you reconcile that? Any speculation at to why the difference in C and N between the two forests? We acknowledge that the last paragraph of the discussion is highly speculative, and it contradicts the observation of higher soil N contents in SH-forests compared to the tea state ones. Thus, we have decided to remove the sentence. We hope to gather more empirical evidence in the near future to reconcile those differences.

Running head

Soil trace gas fluxes in tropical montane land uses

Article type

General research

Title

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Authors

Arias-Navarro, C[1, 2, 3]; Díaz-Pinés, E[1, 4,*]; Zuazo, P[1]; Rufino, M C[5]; Verchot, L V[6]; Butterbach-Bahl, K [1, 3]

Affiliations

[1] Institute of Meteorology and Climate Research, Atmospheric Environmental Research (IMK-IFU), Karlsruhe Institute of Technology (KIT), Kreuzeckbahnstr. 19, 82467 Garmisch-Partenkirchen, Germany.

[2] Center for International Forestry Research (CIFOR), P.O. Box 30677, Nairobi 00100, Kenya

[3] Mazingira Centre, Environmental Research and Educational Facility, International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi 00100, Kenya

[4] Present address: Institute of Soil Research, University of Natural Resources and Life Sciences (BOKU), Peter-Jordan-Strasse 82, 1190, Vienna, Austria.

[5] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, United Kingdom

[6] International Center for Tropical Agriculture (CIAT), Km 17, Recta Cali–Palmira CP 763537, Apartado Aéreo 6713, Cali, Colombia

* Corresponding author:

eugenio.diaz-pines@boku.ac.at

Institute of Soil Research, University of Natural Resources and Life Sciences (BOKU)

Peter-Jordan-Strasse 82, 1190 – Vienna, Austria

Telephone: +43 1 47654-91120

Fax +41 1 47654-91109

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Keywords

Carbon dioxide; land use change; nitric oxide; nitrous oxide; soils; tropical forests

Abstract

Increasing demand for food and fibre by the growing human population is driving significant land use (LU) change from forest into intensively managed land systems in tropical areas. But empirical evidence on the extent to which such changes affect the soil-atmosphere exchange of trace gases is still scarce, especially in Africa. We investigated the effect of LU on soil trace gas production in the Mau Forest Complex region, Kenya. Intact soil cores were taken from natural forest, commercial and smallholder tea plantations, eucalyptus plantations and grazing lands, and were incubated in the lab under different soil moisture conditions. Soil fluxes of nitrous oxide (N_2O), nitric oxide (NO) and carbon dioxide (CO_2) were quantified, and we approximated annual estimates of soil N_2O and NO fluxes using soil moisture values measured *in situ*.

Forest and eucalyptus plantations yielded annual fluxes of 0.3-1.3 kg N_2O-N ha^{-1} a^{-1} and 1.5-5.2 kg NO-N ha^{-1} a^{-1}. Soils of commercial tea plantations, which are highly fertilized, showed higher fluxes (0.9 kg N_2O-N ha^{-1} a^{-1} and 4.3 kg NO-N ha^{-1} a^{-1}) than smallholder tea plantations (0.1 kg N_2O-N ha^{-1} a^{-1} and 2.1 kg NO-N ha^{-1} a^{-1}) or grazing land (0.1 kg N_2O-N ha^{-1} a^{-1} and 1.1 kg NO-N ha^{-1} a^{-1}). High soil NO fluxes were probably the consequence of long-term N fertilization and associated soil acidification, likely promoting chemodenitrification. Our experimental approach can be implemented in understudied regions, with the potential to increase the amount of information on production and consumption of trace gases from soils.

1. Introduction

Nitrous oxide (N_2O) and carbon dioxide (CO_2) are important greenhouse gases (GHG) contributing directly to climate change (IPCC 2014), while nitric oxide (NO) is a key substance involved in the tropospheric production of ozone, which is also a potent GHG (Chameides et al. 1992). Soils are the dominating source of atmospheric CO_2 (Raich et al. 2002) and N_2O (Butterbach-Bahl et al. 2013) and contribute considerably to the atmospheric budget of NO (Conrad 1996; Butterbach-Bahl et al. 2009). The exchange of these gases between soil and atmosphere is influenced by several factors such as land use (LU) and land use change (LUC), temperature and precipitation, N input and soil properties (Butterbach-Bahl et al. 2013).

Land use change from natural forest ecosystems into other more intensively managed LU is driven by the increasing demand for food and fibre, especially in tropical regions, where population growth fosters agriculture encroachment in forested areas (IPCC 2007). In East Africa, where agriculture is the primary LU, tropical montane forests are particularly endangered because they are located in areas highly suitable for agricultural production; thus, the expansion of cropland, grazing areas, and forest plantations at the expense of natural forests and other natural ecosystems is expected to continue in the future (Potting and Bakkes 2004). For Kenya's montane forests, deforestation was approximately 50,000 ha for the 2000-2010 period, with the encroachment of tea cultivation areas being an important driver for LUC (UNEP 2012; Mutugi and Kiiru 2015).

Kenya is the third largest tea producer and the first black tea exporter worldwide (Monroy et al. 2013). The tea sector is divided in two production systems: the large-scale monoculture tea plantations (also called tea estates) and the local-scale smallholders, who traditionally cultivate tea to supplement subsistence agriculture. The commercial tea plantations have usually higher yields compared to those managed by smallholder producers, as more fertilizers are used and management is optimised (Kenya Human Rights Commision 2008). Following increased tea

demand, the area devoted for tea production in Kenya has grown in the past decades, primary by the smallholders (Monroy et al. 2013).

Both land use and land management play a significant role in the C and N cycling, potentially influencing the exchange rates of trace gases between the soil and the atmosphere. For example, N fertilization usually promotes both N_2O and NO production in the soil due to enhanced substrate availability for microbial utilization (Stehfest and Bouwman 2006). Changes in micro-climate (moisture, temperate) and soil bulk density, porosity, mineral N content and pH following LUC (Farquharson and Baldock 2008) influence the consumption and production of trace gases in the soil (Davidson et al. 2000b; Saiz et al. 2006; Tang et al. 2006a). However, studies of the soil-atmosphere trace gas exchange in tropical ecosystems are still scarce, especially in Africa (Kim et al. 2016). The lack of evidence translates into considerable uncertainty on the impact of LUC on soil trace gas emissions (van Lent et al. 2015).

Understanding land use effects on soil GHG fluxes remains difficult due to high spatio-temporal variations of fluxes. Therefore, the design of experiments and sampling strategies for evaluating land use change effects on soil GHG fluxes is crucial (Arias-Navarro et al. 2017). Unfortunately, *in situ* measurements with high spatial and temporal resolution in remote tropical ecosystems are constrained by limited infrastructure and therefore high costs. To overcome these constrains, soil samples can be taken to the laboratory for targeted incubation experiments, e.g. for studying spatial and temporal variability of fluxes in dependence of changes of environmental conditions. Fluxes from soil cores determined in the laboratory usually agree well with fluxes determined via field chambers (Otter et al. 1999; Ludwig et al. 2001; Gut et al. 2002; van Dijk 2002; Yao et al. 2010).

In this study, we quantified soil CO_2, N_2O, and NO fluxes of representative land uses of the Mau Forest Complex in Kenya. We used a fully-automated laboratory incubation and monitoring system to study fluxes from intact soil cores at different soil moisture contents. The objectives of this study were:

(1) To evaluate the effect of LU on the soil N_2O, NO and CO_2 fluxes

(2) To analyse effects of tea plantation management (commercial versus smallholder) on the soil N_2O, NO and CO_2 fluxes.

(3) To quantify the importance of soil moisture as a driver of soil trace gas emissions.

We hypothesised that tea plantations would emit higher amounts of N-trace gases than other LUs and that N fluxes from soils taken from commercial tea plantations are higher than those from soils of smallholder farmers. Moreover, we hypothesized that soil water content could be used for approximating the seasonality of soil trace gas emissions and for calculating annual fluxes.

2. Material and Methods

2.1 Study Area

The Mau Forest Complex in Kenya is the largest indigenous Afromontane forest of East Africa covering an area of about 417,000 ha. The study site lies in the Southwest Mau part, east of Kericho town (-0° 22' 3" S, 35° 16' 59" E) (Figure 1) at approximately 2500 m a.s.l. The climate is cool and humid tropical with a mean annual precipitation between 1800 and 1950 mm (1979-2009) (Omumbo et al. 2011). The region has a bi-modal rainfall pattern, with the “long rains” falling between April and August and “short rains” between October and December, while January and February are generally the driest months. The mean annual temperature ranges from 15.7 to 18°C (1979-2009) (Omumbo et al. 2011) with modest (approx. 7°C) seasonal variations. The geology substrate is formed by Tertiary lavas from the mid-Miocene (Blackie and Edwards 1979). The soils are well drained, deeply weathered, dark reddish-brown, clayey, and with an acidic humic topsoil (Krhoda 1988; Jaetzold et al. 2010). Soils are classified as Andic Humic Nitisols (IUSS Working Group WRB 2015).

In the last decades the Mau Forest region has experienced a loss of forest cover of about 25 % at the expense of other LUs (Government of Kenya 2010). In the region, tea is produced in both

large estates and smallholder farms. Smallholder farms are typically less than half a hectare, with most of the land planted with tea and only 20 % of the land reserved for food crops and grazing (Milder et al. 2015). Commercial tea estates grow eucalyptus woodlots in addition to tea, as source of firewood for the tea factories.

2.2 Experimental Design

In this experiment, two contrasting tea-growing areas were investigated. The first area is located in Kapkorech Estate, a large tea estate (hereafter, TE) owned by a private company. Nearly 120 ha of commercial plantations of tea (*Camelia sinensis var. sinensis* L.) were established more than 60 years ago after clearance of the native forest. Approximately 20 ha were designated to grow eucalyptus (*Eucalyptus grandis* L.). Aerial application of NPK 26:5:5 fertilizer to the tea fields is conducted 2-3 times a year (300-400 kg N ha^{-1} a^{-1}; personal communication). The second tea-growing area is managed by smallholder farmers (hereafter, SH). Smallholder tea plantations are fertilized with NPK 26:5:5 at an approximate rate of 150 kg N ha^{-1} a^{-1} (personal communication). In addition to tea cultivation, a significant share of the land is devoted to livestock grazing.

At the SH area, we monitored plots under tea (T), grazing (G) and the contiguous natural forest (F) (Figure 1a). At the TE area, we investigated plots under tea (T), eucalyptus plantations (P) and the adjacent natural forest (F) (Figure 1b). Therefore, the experimental design comprised six experimental sites (SH-T, SH-G, SH-F, TE-T, TE-P and TE-F) each of them replicated three times, making a total of 18 experimental plots, with an area of 0.25 ha each, approximately.

2.3 Soil sampling

We used intact soil cores to minimize the disturbance of the soil structure. Five soil cores were collected at three random locations within each plot. At each location, the uppermost layer of litter with visible undecomposed material (leaves, twigs, etc) was removed before PVC tubes

(5 cm inner diameter; 10 cm height) were driven into the soil with the help of a wooden block and rubber hammer. The filled PVC cores were carefully removed and immediately air-dried at 25 °C for three days. After this period, the soils had a water filled pore space (WFPS) of approximately 10-15 %. Subsequently, the soils were transported to the laboratory at IMK-IFU (Garmisch-Partenkirchen, Germany) and stored at ambient temperature until laboratory incubations were conducted, approximately four months after sampling.

2.4 Soil incubation

The soil cores were incubated using a custom-built, temperature-regulated automatic gas sampling system encompassing 18 incubation chambers. Each chamber consists of a poly-methylmethacrylate cylinder (126 mm inner diameter; 240 mm height) acting as a steady-state dynamic chamber (Pumpanen et al. 2004; Pihlatie et al. 2013). A cylindrical piece of 7 cm height was placed at the bottom of each chamber, on top of which we placed three intact soil cores, one from each of replicate within a plot. The air volume between the cores was filled with quartz sand up to the upper edge of the soil cores. The sand was covered with a metallic sheet (2 mm thick), so that only the soil surface of the cores was directly exposed to the headspace. This design allowed small chamber headspace (374 cm^3) without dead volumes. The chamber was then closed with a gas-tight lid equipped with an inlet and an outlet.

During the incubation, background air was continuously supplied through the inlet to all the chambers, allowing for a permanent equilibrium state of the headspace (Pape et al. 2009)(Figure 2a). The sampling from the incubation chambers and the background air was controlled through electromechanically operated solenoid valves (Bürkert GmbH & Co. KG, Ingelfingen, Germany) in 180-minute long cycles in which a measure of the concentration at the outlet of each incubation chamber was gained. Further details on the custom-build system can be found in Zuazo (2016).

Nitrous oxide and CO_2 concentrations were determined using cavity ring-down spectroscopy (G2508, Picarro, Santa Clara, CA, USA). The gas analyser was calibrated every measuring cycle using a gas blend containing defined concentrations of N_2O (408 ppbv) and CO_2 (406 ppmv) in synthetic air (Air Liquide GmbH, Düsseldorf, Germany). Nitric oxide concentrations were quantified by a chemiluminescence detector (CLD88p, Eco Physics AG, Duernten, Switzerland) calibrated daily with four different NO concentrations in synthetic air: 0, 50, 200 and 500 ppbv NO. These blends were prepared by mixing a stable concentrated preparation (4 ppm NO in N_2; Air Liquide GmbH, Germany) with synthetic air (20 % O_2 + 80 % N_2) using a multi-gas calibration system (series 6100; Environics Inc., Tolland, CT, USA).

The soil-headspace exchange rate of each trace gas was calculated from the mass balance between the inlet and outlet concentrations assuming mass flow equilibrium conditions (Pape et al. 2009).

$$F_{chamb} = \frac{Q}{A} * \rho(\mu_{chamb-}\mu_{amb})$$

where F_{chamb} stands for the trace gas flux (nmol m^2 s^{-1}); A denotes the soil surface of the three soil cores (m^2); Q is the headspace air flow rate (m^3 s^{-1}). μ_{cham} and μ_{amb} are the trace gas mixing ratios (nmol mol^{-1}) of the inflowing ambient air and of the outflowing chamber air, respectively; and ρ is the molar density of dry air molecules (mol m^{-3}).

Because seasonal fluctuations of mean daily air temperatures at the study area are < 7 °C, our experiment focused on the effects of soil moisture changes on the trace gas fluxes. The incubation temperature throughout the experiment was set to the average annual temperature in the study area (i.e. 18 °C). We chose an air relative humidity of 70 % to avoid excessive drying of the soil during the measuring cycle. Soil water content of the cores was determined gravimetrically prior to the experiment using a replicate intact soil core. Bulk density (BD) was determined gravimetrically using oven-dry (105 °C) soil weight divided by the core volume.

BD was used to calculate the pore volume and consequently the amount of water required to reach 20, 30, 50, 70 and 90 % WFPS using the equation:

$$WFPS\ (\%) = \frac{W_{vol}}{\left(\frac{1 - BD}{2.65}\right)}$$

where W_{vol} is the volumetric water content (g cm^{-3}), BD is soil bulk density (g cm^{-3}) and 2.65 is the soil particle density (g cm^{-3}).

Incubations at different WFPS levels were performed with independent soil cores to avoid potential bias associated with substrate depletion due to sequential incubation. Targeted WFPS was achieved by adding a standard rain solution (Breuer et al. 2002).

Each incubation run (30, 50, 70 and 90 % WFPS, respectively), was divided in three periods (Figure 2b) as follows:

Dry conditions (2 days): Trace gas fluxes from the soil cores were measured prior to soil re-wetting.

20 % WFPS (3 days): Re-wetting of dry soils usually leads to a short-lasting, over-proportionate increase of emissions (initial pulse, Figure 2b), with the magnitude of this response being dependent on moisture soil conditions prior to re-wetting (Borken and Matzner 2009; Liang et al. 2015). For this reason, we adjusted the initial moisture content to 20 % WFPS to purposely homogenize the soil moisture status before setting the soils to the final targeted WFPS % treatment. Trace gas fluxes were measured for three consecutive days, after which we observed that soil trace gas fluxes stabilized.

Targeted WFPS % (10 days): Water was added to the soil surface until the targeted WFPS % was achieved and the soil trace gas fluxes were measured for 10 consecutive days. To avoid the short-term interference typically observed as a consequence of the pulse of NO, N_2O and CO_2 occurring after rewetting of soil we excluded the data of the four days after rewetting (second pulse, Figure 2b) to analyse the effects of soil moisture on trace gas fluxes for the different land uses (post-pulse area, Figure 2b).

Because the incubation system allowed to obtain flux measurements for each chamber every 3 hours and the incubation run for 15 days in total, approximately 120 individual flux measurements were generated per incubation chamber (N=18). Cumulative N_2O, NO and CO_2 emissions were calculated by linear interpolation between two consecutive sampling events.

2.5 Determination of soil properties

At the end of the incubation, the soils were air-dried and sieved (2 mm). We mixed 20 g from each replicate soil core to obtain a composite sample, which was sent to a commercial laboratory (Landwirtschaftliches Labor Dr. Janssen GmbH, Gillersheim, Germany) for analysis. Total nitrogen (TN) content was determined by dry combustion (DIN ISO 13878). Carbonates were removed beforehand by acid application, and the organic C content was determined by dry combustion (DIN ISO 10694). Soil pH was determined in water (10 g soil + 25 ml solution) as detailed in the VDLUFA (1991, section A, 5.1, 1). Soil texture was determined according to DIN ISO 18123.

2.6 Monitoring of environmental parameters on site.

Environmental data were collected from July 2015 to July 2016 from a near weather station located at the Kenya Forest Service Kericho forest station (-0° 21' 5" S, 35° 21' 5" E, 2184 m a.s.l.). Moisture and temperature in 10 cm soil depth were measured using a combined water potential and temperature sensor (Decagon 5TM, Decagon Devices, Inc., Pullman, WA, USA). Rainfall was measured with a rain gauge (Decagon ECRN-50, Decagon Devices, Inc.). Data were logged in 10 min intervals on a digital data logger and downloaded periodically (Decagon Em50 series, Decagon Devices, Inc.). Daily average soil volumetric water content (W_{vol}) monitored at the weather station was used to calculate the daily average WFPS of each

experimental site using the respective BD values (Table 1) assuming that W_{vol} did not vary across sites.

Because soil temperature does not vary greatly in many tropical forests, soil water content is often found to be a more significant factor affecting temporal variation of soil trace gases (Butterbach-Bahl et al. 2004). Thus, estimated daily values of WFPS were used for approximating the seasonality of soil trace gas emissions and for calculating annual fluxes on basis of the regression curves describing the relationship between soil moisture and the trace gas fluxes observed in our laboratory experiments (see 3.3).

2.7 Data processing, data analysis

All statistical analyses and plotting were carried out using R 3.1.3 (R Core Team 2016). Downloading and formatting Google Maps images was done with the ggmap package (Kahle and Wickham 2013). One-way ANOVA was used to test differences in soil properties across experimental sites. Trace gas fluxes among different sites and soil moisture levels were compared using a two-way ANOVA. We used the Fisher LSD method to compare individual means. Descriptive statistics are reported as the mean of the three soil core replicates along with the standard deviation. The contribution of soil parameters to the variance of N_2O, NO and CO_2 gas fluxes was studied using regression analysis. Uncertainty of the curve fitting for linear models was calculated using the function "predict.lm" (R package stats), which produces predicted values, obtained by evaluating the regression function and calculates the standard errors of the predictions. For exponential models we used the function "predictNLS" (R package propagate, Spiess 2014) which propagates the error using Monte Carlo simulation. Significance level was established at $p \leq 0.05$.

3. Results

3.1 Soil parameters

All topsoils had a clayey texture, with clay contents in the range of 56-67% (Table 1). Topsoil bulk density (BD) values were below 1 g cm^{-3}, with the lowest values observed for soils sampled at the natural forests (SH-F and TE-F) and at the commercial tea plantations (TE-T). Soil organic carbon (SOC) contents were in the range of 58-79 g kg^{-1}. Soils taken from SH-F had the highest SOC content although differences are not significant from TE-T and SH-G. Nitrogen contents ranged between 5.6 and 8.1 g kg^{-1}. Soils taken from SH-F had as well the highest total nitrogen (TN) content. Soils were acidic (4.2-6.0), with the lowest soil pH value observed for soils from TE-T (Table 1).

3.2 Soil trace gas fluxes

Soil N_2O fluxes

We measured a very low N_2O uptake from dried soil cores (average: -9 ± 31 µg N m^{-2} h^{-1}). Initial re-wetting of soil cores to 20% WFPS did not cause N_2O emission pulses, regardless of the site. After setting the soil cores to the targeted WFPS, substantial N_2O fluxes (> 50 µg N_2O-N m^{-2} h^{-1}) were measured only when WFPS was finally set to 90%. The peak maximum for these cores was observed after two to four days, depending on the experimental sites (Figure 3). The only significant differences in soil N_2O fluxes were found between 90% WFPS and the other soil moisture levels. With an average post-pulse N_2O flux of 131 ± 61 µg N_2O-N m^{-2} h^{-1}, TE-F tended to emit more N_2O than soils from other sites, although strong variation precluded significant differences (Table 2).

Pulse fluxes contributed to more than half (initial pulse: 22 ± 9 %, second pulse: 34 ± 16 %) of the total time-weighted average cumulative soil N_2O rates during the 15 day-incubation period for the different sites and levels of WFPS (Figure 4a). Cumulative soil N_2O fluxes during the second pulse were significantly higher for soils moistened to 90% WFPS (159 ± 258 g N_2O-N ha^{-1}) compared to soils adjusted to other moisture levels (70% WFPS: 27 ± 28 g N_2O-N ha^{-1};

50% WFPS: 27 ± 9 g N_2O-N ha^{-1}; 30 % WFPS: 27 ± 11 g N_2O-N ha^{-1}). The second pulse was not significantly different between sites.

Soil NO fluxes

Dry-soil NO fluxes were very low for all sites (average 5 ± 7 µg NO-N m^{-2} h^{-1}). Large NO pulses (average 99 ± 100 µg NO-N m^{-2} h^{-1}) occurred already following the first re-wetting event, when soil moisture was adjusted to 20 % WFPS (Figure 5). The highest peak fluxes following the first initial re-wetting were observed for soils from commercial tea plantations, while for soils from smallholder grazing plots this first pulse was negligible (TE-T: 259 ± 111 µg NO-N m^{-2} h^{-1}; SH-G: 23 ± 13 µg NO-N m^{-2} h^{-1}). Soil NO fluxes after the second soil re-wetting to the target moisture were not significantly different across soil moisture levels (Table 3). Nitric oxide emissions were significantly higher for soils from tea plantations (TE-T and SH-T) and natural forests (TE-F and SH-F) compared to those taken from eucalyptus plantations (TE-P) or grazing land (SH-G) (Table 3).

Pulse fluxes contributed to more than 80 % of the total time-weighted average cumulative soil NO rates during the 15 day-incubation period for the different sites and levels of % WFPS (initial pulse: 31 ± 14%, second pulse: 51 ± 9 %) (Figure 4b). The highest time-weighted average cumulative NO fluxes during the initial pulse (20 % WFPS) were those for soils from TE-T (196 ± 78 g NO-N ha^{-1}) followed by TE-F sites (127 ± 65 g NO-N ha^{-1}). The initial pulse was not significantly different for the rest of the sites (TE-P: 47 ± 19 g NO-N ha^{-1}; SH-T: 44 ± 21 g NO-N ha^{-1}; SH-G: 18 ± 19 g NO-N ha^{-1}; SH-F: 13 ± 9 g NO-N ha^{-1}). After setting the soil cores to the final targeted WFPS, average cumulative NO fluxes during the second pulse followed the same pattern, i.e., significant highest emissions were found for soils from TE-T and TE-F (201 ± 46 g NO-N ha^{-1} and 200 ± 68 g NO-N ha^{-1} respectively) although in this case, fluxes were not significant different from SH-T (147 ± 99 g NO-N ha^{-1}). The second pulse at SH-T and at the rest of the sites were no significantly different (TE-P: 78 ± 31 g NO-N ha^{-1};

SH-F: 67 ± 19 g NO-N ha^{-1}; SH-G: 65 ± 55 g NO-N ha^{-1}). Moisture content did not have a significant effect on the magnitude of the second pulse.

Soil CO_2 fluxes

The CO_2 emissions during the dry-incubation period were on average 2 ± 1 mg C m^{-2} h^{-1}. Following the initial re-wetting of soils to 20% WFPS small (< 40 mg C m^{-2} h^{-1}) and short-lived pulse fluxes of CO_2 were observed, which returned to pre-incubation levels in less than 24 hours (Figure 6). Following rewetting to target moisture levels, the soil CO_2 fluxes gradually increased with increasing WFPS for all sites. No significant differences in CO_2 fluxes were found between 70 and 90% WFPS. Soils taken from forests had significantly higher CO_2 fluxes than the rest of the soils (Table 4). The contribution to the total time-weighted average cumulative soil CO_2 rates of the 15 day-incubation period of soil CO_2 fluxes from the initial pulse event after re-wetting of soils was 12 ± 10 % (Figure 4c) and no significant differences were found among sites. The second pulse event, on the other hand, contributed with 58 ± 7% (Figure 4c) and was significantly higher for soils set to 90 and 70% WFPS (92 ± 40 kg CO_2-C ha^{-1} and 79 ± 40 kg CO_2-C ha^{-1} respectively) compared to the pulse obtained when the soils were set to 50 and 30% WFPS (45 ± 24 kg CO_2-C ha^{-1} and 23 ± 16 kg CO_2-C ha^{-1} respectively). The second pulse event was significantly higher for soils from forests (SH-F: 81 ± 53 kg CO_2-C ha^{-1}; TE-F: 69 ± 32 kg CO_2-C ha^{-1}) compared to TE-T (34 ± 11 kg CO_2-C ha^{-1}). No significant differences were found between forests and the rest of the soils (SH-G: 64 ± 60 kg CO_2-C ha^{-1} SH-T: 57 ± 37 kg CO_2-C ha^{-1}, TE-P: 53 ± 29 kg CO_2-C ha^{-1}).

3.3 Relationship between soil properties and trace gas fluxes.

Average post-pulse soil N_2O and CO_2 fluxes were significantly and positively correlated (R^2=0.4). There were no correlations between N_2O or CO_2 soil fluxes with soil properties.

374 Average post-pulse soil NO emissions were negatively correlated with pH (R^2=0.3), BD
375 (R^2=0.3) and silt content (R^2=0.2) and positively correlated with clay content (R^2=0.2).
376
377 3.3 Estimates of annual emissions
378 In this incubation study, N_2O emissions increased exponentially with WFPS, while the
379 relationship between WFPS and NO followed a 2th degree polynomial (Figure 7). In the case of
380 TE-T, no satisfactory model was found for N_2O; therefore, the mean N_2O flux across WFPS
381 levels was used for calculating the annual soil N_2O fluxes.
382 Measured rainfall at our weather station in the Mau region showed a bi-modal pattern, with
383 rains falling between April-June and between October-December. The total annual rainfall for
384 the period July 2015-July 2016 was 1956 mm and the mean air temperature was 16.7 °C
385 (min=13.7 °C, max=20.9 °C) (Figure 8).
386 Soil moisture at 10 cm soil depth ranged from 10.5 to 78.5 % WFPS with an average value of
387 25.3 % for SH-F, TE-F, TE-T sites and between 11.6 to 86.6 % with a mean value of 28 % for
388 SH-T, SH-G and TE-P sites. The daily values of WFPS were finally used to calculate annual
389 emissions on basis of the relationships between WFPS and trace gas fluxes (Figure 9 shows
390 SH-F as an example).
391 N_2O fluxes through the year were generally below 50 µg N_2O-N m^{-2} h^{-1} (mean value: 11.2 µg
392 N_2O-N m^{-2} h^{-1}) with peak emissions only when the soil moisture content was above 60 %
393 WFPS. Daily soil NO emissions were on average 31.8 µg NO-N m^{-2} h^{-1}, decreasing when the
394 WFPS levels were below 25 %. Daily estimated soil NO and N_2O fluxes and WFPS% at the
395 SH-F site is shown in figure 9.
396 Table 5 summarizes calculated annual flux rates for the different sites. Across sites, NO fluxes
397 contributed 60-95% to the total N-oxide losses (N_2O + NO losses). Highest annual fluxes of N-
398 oxides were observed for the TE-F and TE-T sites, with emissions of 5.5 ± 0.9 kg N ha^{-1} a^{-1} and

399 5.2 ± 0.5 kg N ha^{-1} a^{-1} respectively. At the smallholder site the estimated annual soil N emissions
400 for SH-F and SH-T were 3.9 ± 0.9 kg N ha^{-1} a^{-1} and 2.2 ± 0.3 kg N ha^{-1} a^{-1}, respectively.
401 In general, lowest annual N_2O emissions were estimated at the smallholder tea plantations and
402 grazing sites (SH-T: 0.1 ± 0.2 kg N_2O-N ha^{-1} a^{-1}; SH-G: 0.1 ± 0.3 kg N_2O-N ha^{-1} a^{-1}). Soil from
403 the grazing sites showed also the lowest annual NO emissions (SH-G: 1.1 ± 0.3 kg NO-N ha^{-1}
404 a^{-1}). Highest annual NO emissions were estimated from the natural forest sites and tea
405 plantations in the TE area (TE-F: 5.2 ± 0.7 kg N ha^{-1} a^{-1}; TE-T: 4.3 ± 0.2 kg NO-N ha^{-1} a^{-1}).
406

407 ## 4. Discussion

408 Soil moisture is a key governing parameter in the production and consumption of N oxides in
409 the soil as it controls both soil gas diffusion and oxygen (O_2) availability for microbial use
410 (Davidson et al. 2000a). Whereas both NO and N_2O may be produced through the same
411 processes (i.e. nitrification and denitrification), the ratios of the two products may vary strongly
412 depending on O_2 availability (Pilegaard 2013). Soil NO production during nitrification requires
413 O_2 as electron acceptor, while N_2O is more commonly produced by reductive processes –i.e.
414 under O_2-limiting environmental conditions- such as denitrification or nitrifier-denitrification
415 (Butterbach-Bahl et al. 2013). Therefore, highest emission rates for NO have been frequently
416 observed at soil moisture contents below field capacity, which for many soils is about 60 %
417 WFPS. With regard to N_2O, maximum emission rates are reported at values between 50 and 90
418 % WFPS, depending on soil properties (Davidson et al. 1991; Breuer et al. 2002; Werner et al.
419 2007) and at WFPS > 90 % dominant soil anaerobiosis favours complete denitrification,
420 yielding N_2 as product.
421 In our study, N_2O fluxes increased exponentially if soil cores were wetted at WFPS higher than
422 70 %, but were relatively low at 30–50 % WFPS. These findings are in agreement with many
423 other studies which have reported that N_2O production and emission increase exponentially
424 with soil water content (Garcia-Montiel et al. 2001; Arai et al. 2008). We further found high

425 soil CO_2 rates, and a significant correlation between N_2O fluxes and soil respiration rates at 90
426 % WFPS. This correlation has also been described in field studies conducted by Castaldi et al.
427 (2012) at a rainforest site in Ghana and by Werner et al. (2007) for a lowland rain forest in
428 Kenya. While soil moisture contents close to water saturation may favour the development of
429 anaerobiosis and therefore reduce organic matter decomposition and the soil CO_2 efflux (e.g.
430 Smith 1990), it seems that our soils were predominantly aerobic even at 90 % WFPS due to the
431 low bulk density of the topsoils (< 1 g cm^{-3}). Similarly, in Chinese montane, subtropical
432 rainforests Zhou et al. (2013) found that soil CO_2 fluxes significantly increase with soil moisture
433 at constant temperatures due to improved substrate availability for microbial respiration.
434 In our study, optimum soil moisture content for NO emission for different sites and land uses
435 varied, which was probably an effect not only of land use but also of varying soil properties.
436 The effect of WFPS on soil NO emissions was markedly lower than on N_2O fluxes, leading to
437 a decrease of the NO: N_2O ratio with increasing WFPS, suggesting that denitrification or
438 nitrifier-denitrification processes took over nitrification as source process for N trace gases with
439 increasing soil moisture levels (Breuer et al. 2002; Butterbach-Bahl et al. 2004; Werner et al.
440 2007).
441 For the forest sites, our approach yielded flux rate estimates of 0.3 ± 0.2 kg N_2O-N ha^{-1} a^{-1} and
442 1.3 ± 0.2 kg N_2O-N ha^{-1} a^{-1} for TE-F and SH-F, respectively. This is similar to what has been
443 measured *in situ* at other tropical montane forests (0.3 – 1.3 kg N_2O-N ha^{-1} a^{-1}) (Riley et al.
444 1995; Ishizuka et al. 2002; Purbopuspito et al. 2006; Koehler et al. 2009; Müller et al. 2015),
445 but lower than most of the studies performed in tropical lowland forests, which range between
446 1.9 and 6.1 kg N_2O-N ha^{-1} a^{-1} (Keller et al. 1993; Serca et al. 1994; Keller and Reiners 1994;
447 Verchot et al. 1999; Breuer et al. 2000; Melillo et al. 2001; Garcia-Montiel et al. 2003; Werner
448 et al. 2007; Castaldi et al. 2013), with some studies showing exceptionally lower N_2O fluxes
449 (e.g. Wieder et al. 2011, ca. 0.75 kg N_2O-N ha^{-1} a^{-1}). Some evidence suggests that in contrast to
450 tropical lowland forests, tropical montane forests may be N limited, as they e.g. show low net

N mineralization rates (Purbopuspito et al. 2006; Nottingham et al. 2015; Gütlein et al. 2016). Our results may be a conservative estimate since we removed the litter layer, which may have contributed to additional N_2O losses (e.g. Wang et al. 2014) and we only incubated the top 10 cm of soil, while N_2O fluxes of tropical soils have been shown to have their N_2O production optima at 5-20 cm soil depth (Nobre et al. 2001). On the contrary, no effective plant N uptake in the soil cores may have led to higher microbial N availability and thus, to a modest overestimate of N_2O fluxes compared to field conditions (Brumme 1995).

Fluxes of NO from soils of tropical montane forests have so far only rarely been reported, but the annual flux which we estimated (3.9 ± 1.8 kg NO-N ha^{-1} a^{-1}) seems to be higher than in previous studies in montane (0.03 – 0.4 kg NO-N ha^{-1} a^{-1}) (Johansson et al. 1988; Riley et al. 1995; Davidson and Kingerlee 1997; Purbopuspito et al. 2006; Koehler et al. 2009) and in lowland tropical forests (0.7 – 1.5 kg NO-N ha^{-1} a^{-1}) (Keller et al. 1993; Serca et al. 1994; Keller and Reiners 1994; Verchot et al. 1999). However, Butterbach-Bahl et al. (2004) found that soils of a tropical lowland rainforest in Australia emitted approx. 3.0 kg NO-N ha^{-1} within a three-month period following the rewetting of soils after a drought period, suggesting that the emission of NO from tropical rain forest soils may still be underestimated. Our estimate is however very similar to the 3.0 kg NO-N ha^{-1} a^{-1} model-estimated by Gharahi Ghehi et al.(2014) for a tropical montane forest in Rwanda. The high NO fluxes we found might be related to the high clay content, the high SOC and the low soil pH. Soil organic C content has been found to be positively correlated with NO emissions (Bouwman et al., 2002), and rates of microbial transformation of N are expected to be higher in soils with high SOC content (Matson et al. 1990; Li et al. 2005). Further, chemodenitrification (abiotic reduction of nitrite) may have contributed to some NO production due to the low soil pH found in our sites, as this process has been observed to occur under strongly acidic conditions, both in temperate (Venterea et al. 2003) and tropical environments (Serca et al. 1994, Gharahi Ghehi et al. 2014).

Annual estimates of soil N_2O emissions from Eucalyptus plantations (TE-P) were, with 1 ± 0.02 kg N_2O-N ha^{-1} a^{-1}, similar, though towards the lower end, to those in other studies covering measurements of N_2O fluxes from tropical rainforest forest soils. Forest conversion to pasture influences strongly soil N cycling (mineralization, nitrification, and denitrification) and therefore also soil N_2O and NO fluxes (Davidson et al. 2000b). In our study, soils from smallholder grazing sites (SH-G) showed relative low N_2O (except at 90% WFPS) and low NO fluxes, in line with previous studies in tropical ecosystems (Keller et al. 1993; Veldkamp et al. 1999; Davidson and Verchot 2000; Garcia-Montiel et al. 2001; Melillo et al. 2001).

Background N_2O and NO emissions occurring in non-fertilized control areas are crucial for developing robust national emission inventories of nitrogenous gases and corresponding emission factors (Zheng et al. 2004); nevertheless, direct measurements of background emissions in tea plantations, especially measurements covering an entire year, have been rarely reported (Akiyama et al. 2006). The few studies available from commercial tea plantations are from Asia where large amounts of N fertilizers are applied (up to 2600 kg ha^{-1} a^{-1} Fu et al. 2012; Han et al. 2013; Li et al. 2013), while at the commercial tea plantation in our study 300 kg N ha^{-1} a^{-1} was applied. The calculated annual N_2O emission rate for commercial tea plantations in our study (0.9 ± 0.3 kg N_2O-N ha^{-1} a^{-1}) is relatively low compared to the 4-7 kg N_2O-N ha^{-1} a^{-1} estimated from "zero N-control" tea plantations in China and Japan (Akiyama et al. 2006; Fu et al. 2012; Yao et al. 2015) although those flux estimates were likely highly affected by the previous application of large amounts of N fertilizer (average of 553 kg N ha^{-1} a^{-1}). Regarding smallholder tea plantations, our annual N_2O estimations (0.1 ± 0.2 kg N_2O-N ha^{-1} a^{-1}) are somewhat lower than those estimates from Rosenstock et al. (2015) who reported annual N_2O fluxes of 0.4 ± 0.2 kg N_2O-N ha^{-1} $a^{-1}$and 0.7 ± 0.5 kg N_2O-N ha^{-1} a^{-1} kg N ha^{-1} a^{-1} in similar systems in Kenya and Tanzania, respectively. They further suggested that the emission factor from N application in smallholder tea systems would be below 1 % of N applied.

Beyond N_2O, our work shows that tea plantations are a major source for NO. The estimated

502 annual NO flux of 4.3 ± 0.7 Kg ha^{-1} a^{-1} and 2.1 ± 1.1 kg ha^{-1} a^{-1} for TE-T and SH-T, respectively
503 is higher than the annual NO fluxes reported by Yao et al. (2015) for a tea plantation in China
504 (1.6 ± 0.4 kg NO-N ha^{-1} a^{-1}, no N fertilizer application).
505 The higher N_2O and NO emissions (N_2O + NO) from soils of the commercial tea plantation
506 (5.2 ± 0.5 kg N ha^{-1} a^{-1}) compared to soils of tea plantations from smallholder farmers (2.2 ±
507 0.3 kg N ha^{-1} a^{-1}) are very likely due to the long-term N fertilization and the subsequent soil
508 acidification (Tokuda and Hayatsu 2004; Yamamoto et al. 2014). Soil from the commercial tea
509 plantation (TE-T) in our study showed a mean pH value of 4.2, which was significantly lower
510 than soils from tea plantations of smallholders (SH-T, mean: 5.0). Enhanced soil acidity is an
511 important factor affecting biotic and abiotic processes and consequently promoting N losses,
512 by e.g. inducing chemodenitrification and therefore NO losses but also N_2O (Venterea et al.
513 2003; Kesik et al. 2006; Medinets et al. 2015; Yao et al. 2015). Chemodenitrification has been
514 suspected to be an important source of NO emissions from soils after drying and wetting of soil,
515 and in excessively fertilized soils, as it is the case in the soils from the commercial tea
516 plantations, where nitrite can accumulate (Davidson 1992; Neff et al. 1995; Verchot et al. 1999).
517 Thus, while relevant chemodenitrification may already occur in the forest and in the small
518 holder tea areas, high rates of fertilization in combination with very low soil pH values may
519 have further increased the contribution of chemodenitrification to the total NO efflux in the
520 commercial tea plantations.
521
522 ## 5. Conclusions
523 Large uncertainties still exist with regard to the magnitude of soil NO and N_2O emissions from
524 tropical African terrestrial ecosystems. Our observations contribute to a growing body of
525 empirical evidence on soil trace gas emissions from different land uses in the African tropics
526 and their governing parameters. Temporal upscaling solely based on soil moisture carries
527 additional uncertainty, since we were not able to include spatial variations in soil C and N

availability for microbial processes. We present a conservative upscaling of flux rates which do not include the effect of consecutive watering-drying cycles. For a robust understanding of the trace gas exchange processes in tropical ecosystems, long-term observations at multiple sites are strongly required. Our results reveal aspects of control of N_2O, NO and CO_2 emissions that may assist to the development of baseline information required to develop land use and agricultural practices and management approaches aiming to ensure sustainable increases in productivity while reducing the contribution of agriculture to climate change.

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Table caption list

Table 1 Topsoil (0-10 cm) properties of individual sites (mean ± 1 standard deviation, n=3). Values followed by different letters indicate significant differences ($p \leq 0.05$) within columns.

Table 2 Average post-pulse fluxes (mean ± 1 standard deviation, n=3) of N_2O at different soil moisture levels (WFPS: water-filled pore space). Same letters indicate no significant differences ($p > 0.05$) between sites (uppercase) and between % WFPS (lowercase)

Table 3 Average post-pulse fluxes (mean ± 1 standard deviation, n=3) of NO at different soil moisture levels (WFPS: water-filled pore space). Same letters indicate no significant differences ($p > 0.05$) between sites (uppercase) and between % WFPS (lowercase)

Table 4 Average post-pulse fluxes (mean ± 1 standard deviation, n=3) of CO_2 at different soil moisture levels (WFPS: water-filled pore space). Same letters indicate no significant differences ($p > 0.05$) between sites (uppercase) and between % WFPS (lowercase)

Table 5 Estimated annual emission (± standard error of the estimate) without pulse emission contribution for different sites (SH: smallholder, TE: tea estate; F: forest, T: tea, G: grazing and P: eucalyptus plantations)

Figure caption list

Fig 1 Location of the experimental plots at a) the smallholders area and b) at the tea estate area. Different symbols denote different land uses

Fig 2 Schematic overview of the experiment. a) Incubation chamber. b) Incubation setup. Dots represent flux measurements for a given gas for an incubation chamber. Blue arrows represent watering events. Temperature (T) and relative humidity (RH) were kept constant at 18°C and 70% .c) Soil analysis. The incubation procedure outlined here was replicated four times for separate soil cores (once for each of the four soil moisture levels, WFPS: water-filled pore space)

Fig 3 Temporal evolution of the soil fluxes of nitrous oxide (N_2O) for different soil moisture levels (WFPS %) and different sites (SH: smallholder, TE: tea estate; F: forest, T: tea, G: grazing and P: eucalyptus plantations). Vertical bars indicate standard deviations of the three spatial replicates. Water was applied at day 2 and at day 5 to reach 20 % WFPS and targeted WFPS, respectively. Grey area indicates measurements used to calculate the mean post-pulse fluxes (day 9 to 12 of the incubation run)

Fig 4 Time-weighted average cumulative soil a) N_2O, b) NO and c) CO_2 emission rates during the different incubation periods and different levels of water filled pore space (WFPS) for different land uses (SH: smallholder, TE: tea estate; F: forest, T: tea, G: grazing and P: eucalyptus plantations)

590 **Fig 5** Dynamics of soil fluxes of nitric oxide (NO) at different water-filled pore space (%
591 WFPS) and for different sites (SH: smallholder, TE: tea Estate; F: forest, T: tea, G: grazing and
592 P: eucalyptus plantation). Vertical bars indicate standard deviations of three spatial replicates.
593 Water was applied at day 2 and at day 5 to reach 20 % WFPS and targeted WFPS, respectively.
594 Grey area indicates measurements at the post-pulse period (day 9 to 12 of the incubation cycle)
595
596 **Fig 6** Dynamics of soil fluxes of carbon dioxide (CO_2) at different water-filled pore space (%
597 WFPS) and for different sites (SH: smallholder, TE: tea estate sites; F: forest, T: tea, G: grazing
598 and P: eucalyptus plantation). Vertical bars indicate standard deviations of three spatial
599 replicates. Water was applied at day 2 and at day 5 to reach 20 % WFPS and targeted WFPS,
600 respectively. Grey area indicates measurements at the post-pulse period (day 9 to 12 of the
601 incubation cycle)
602
603 **Fig 7** Relationships between soil WFPS and N_2O (upper panel) and NO (lower panel) fluxes
604 determined in laboratory experiments. The curve fits were used for calculating annual flux
605 estimates for different sites (SH: smallholder, TE: tea estate; F: forest, T: tea, G: grazing and P:
606 eucalyptus plantations) using observed *in situ* daily WFPS values at our meteorological
607 observation site. The grey areas indicate the 95 % confidence intervals for the individual curve
608 fits. Vertical bars indicate standard errors of three spatial replicates
609
610 **Fig 8** Daily a) mean air temperature and b) cumulative rainfall from July 215 to July 2016
611
612 **Fig 9** Daily values of a) soil NO, b) soil N_2O flux estimations and c) % WFPS at the natural
613 forest site in the smallholder area (SH-F) from July 2015 to July 2016
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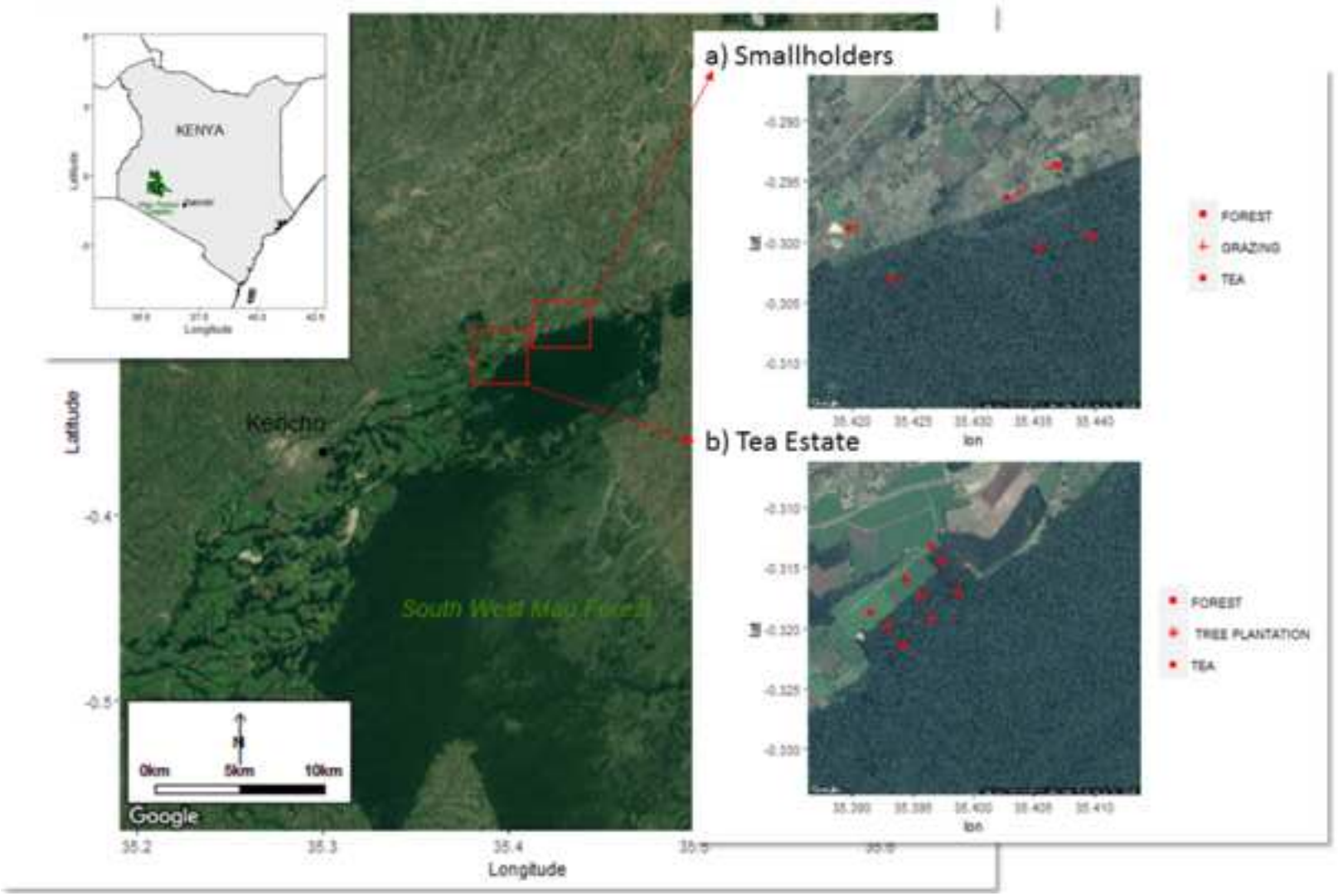

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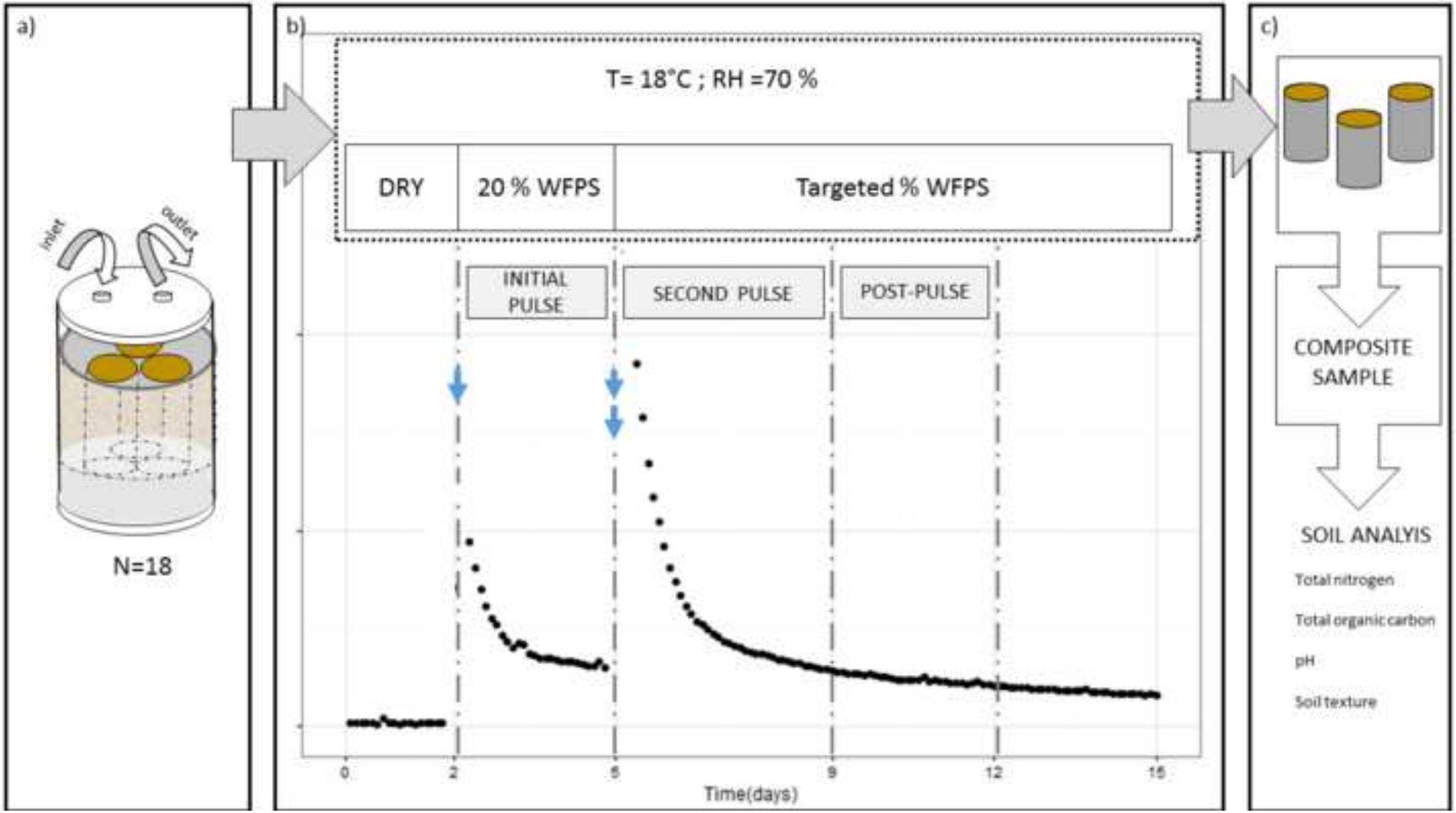

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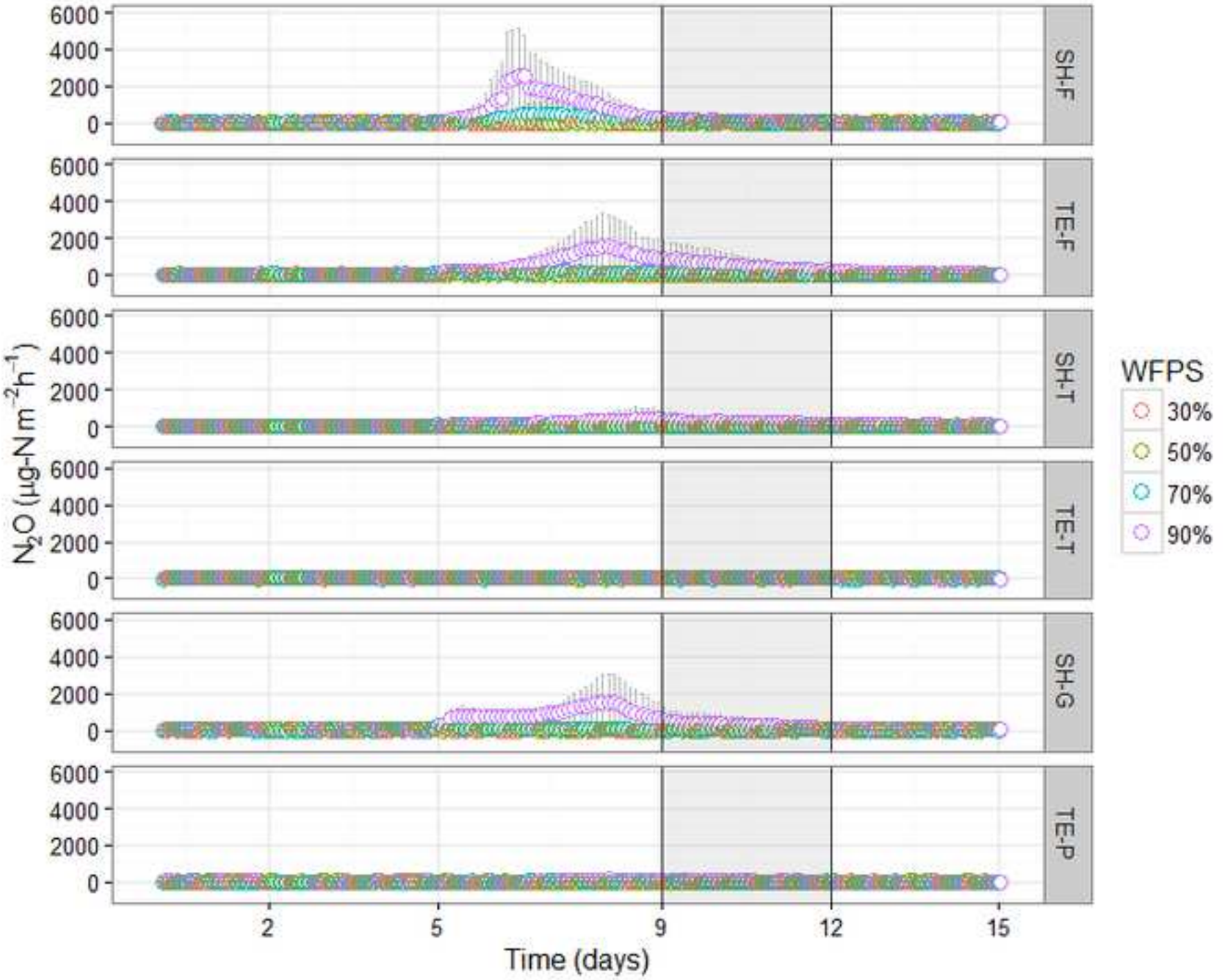

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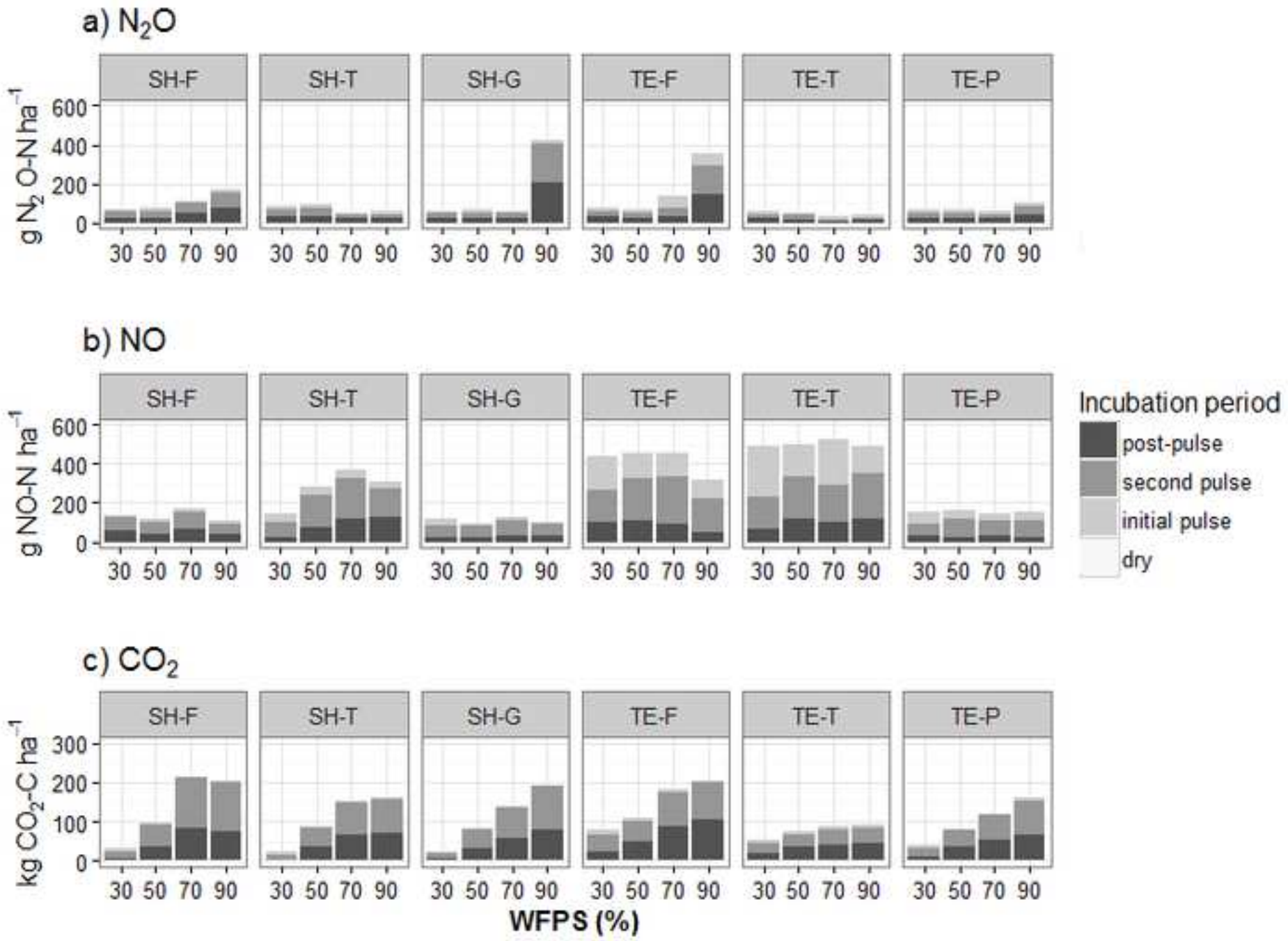

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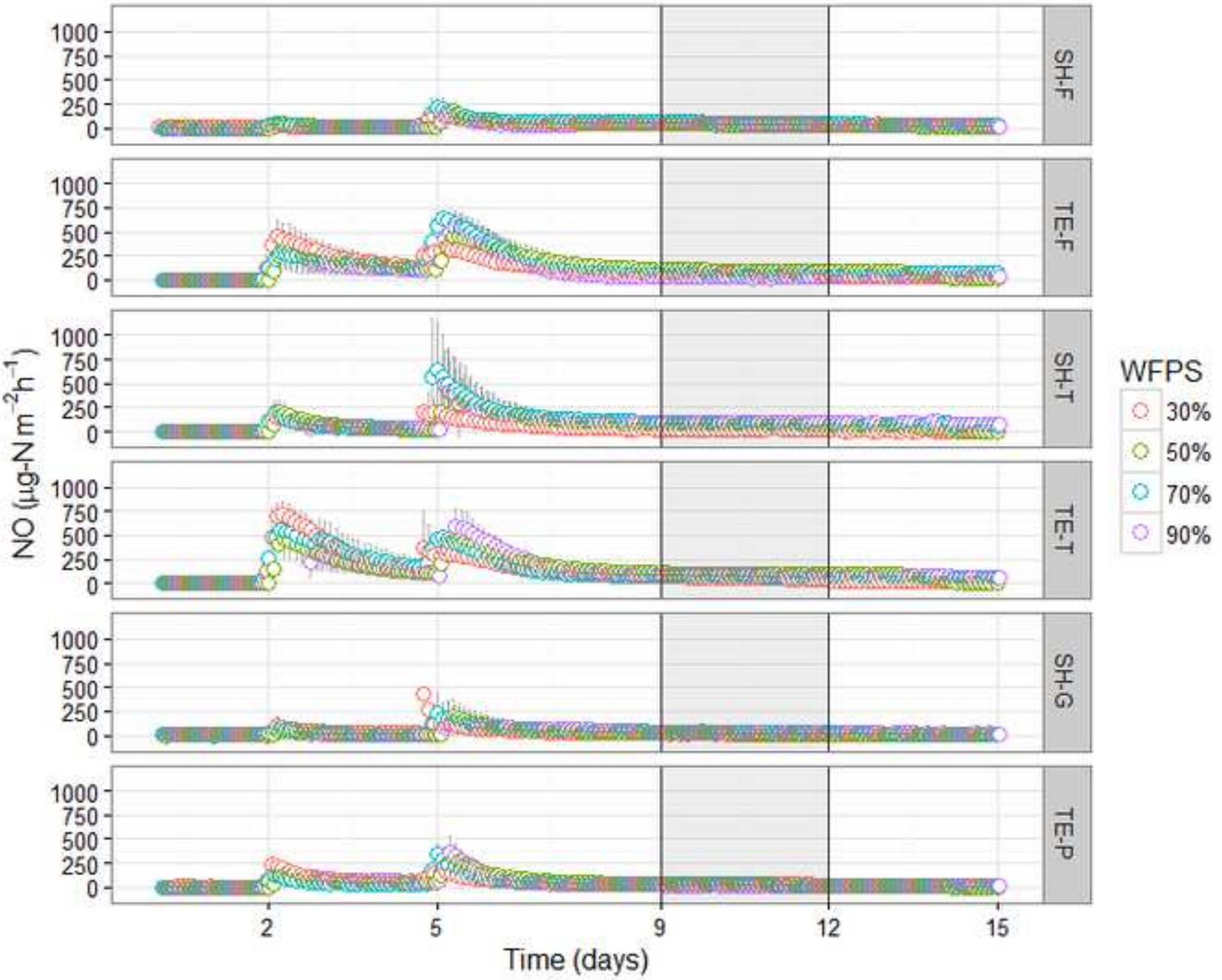

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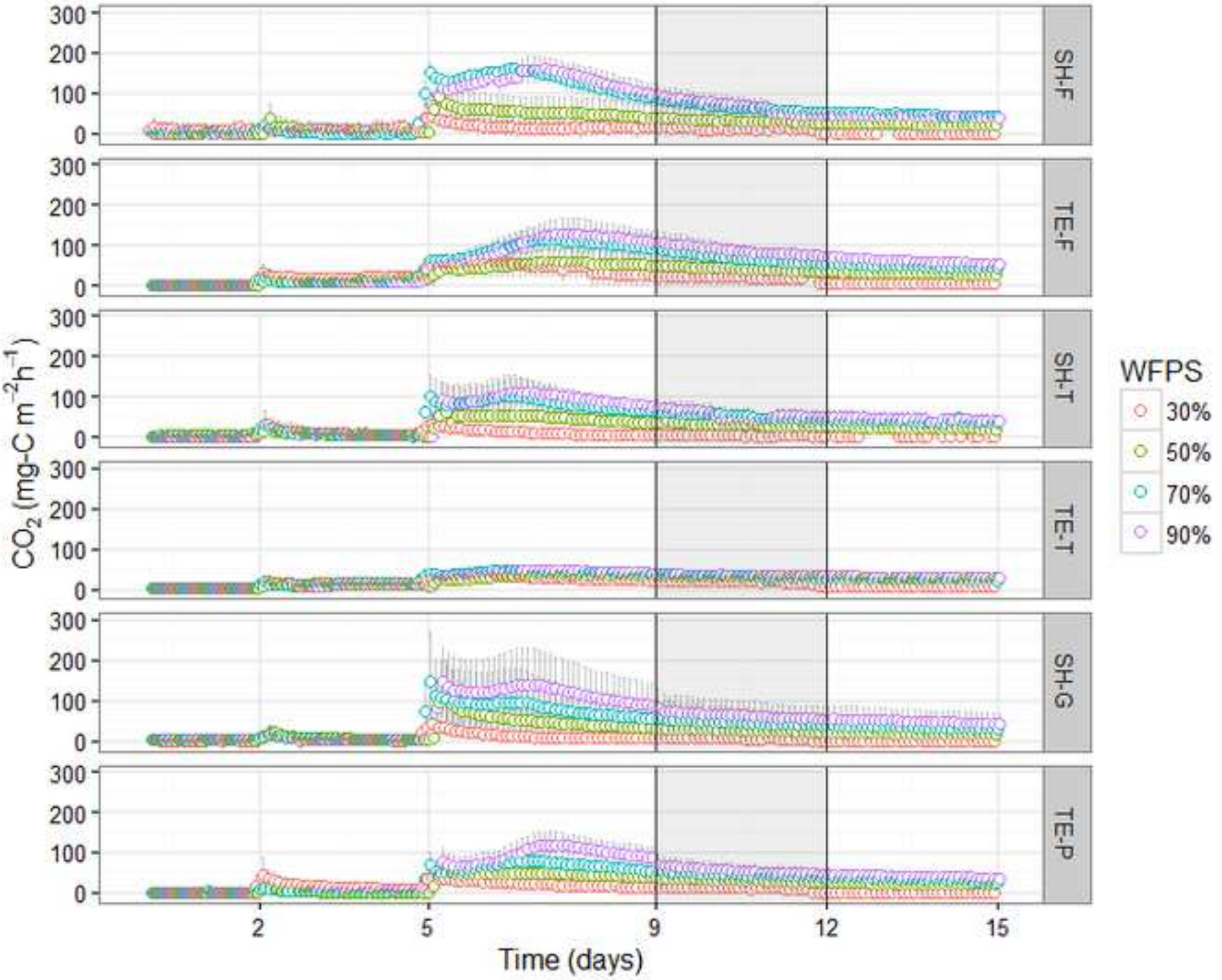

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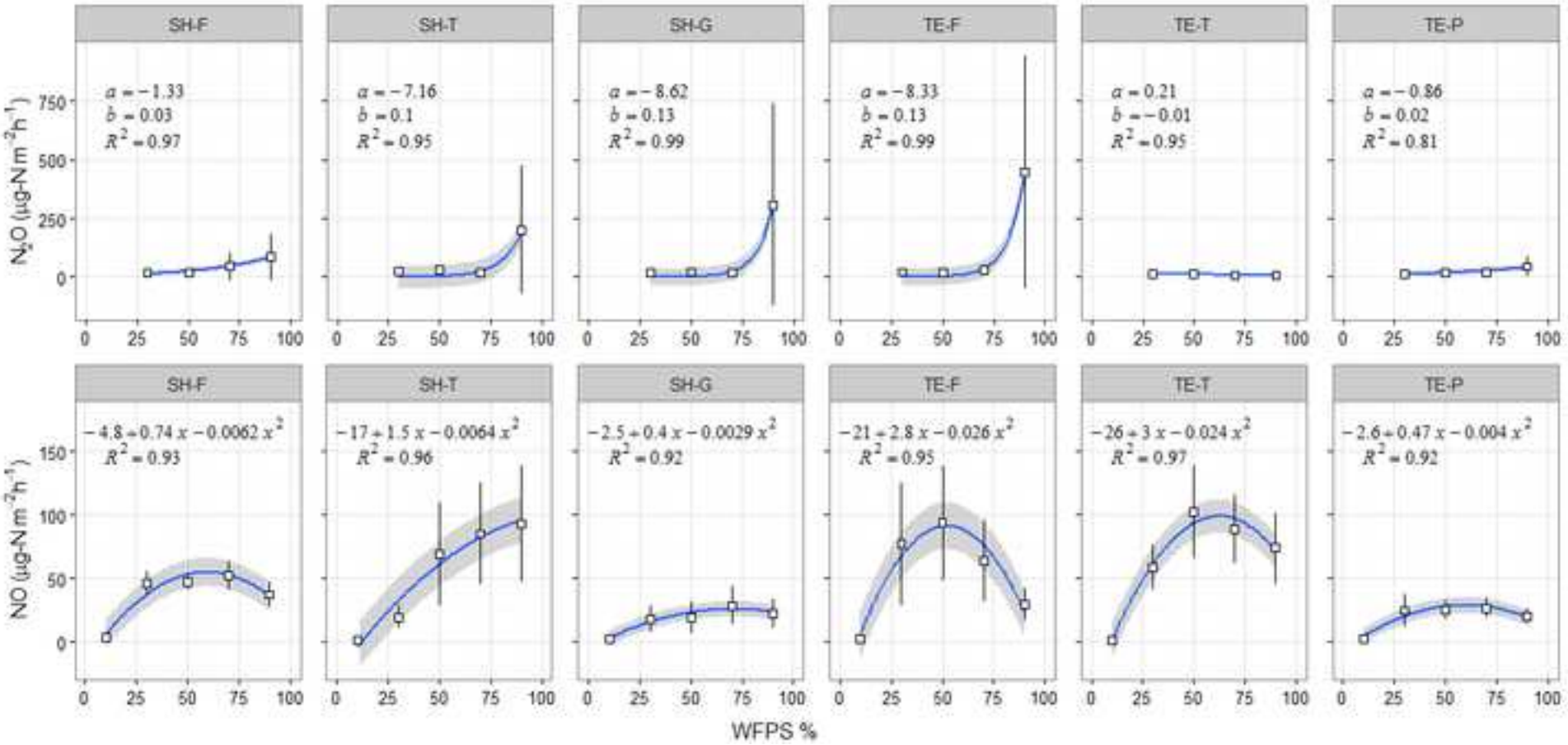

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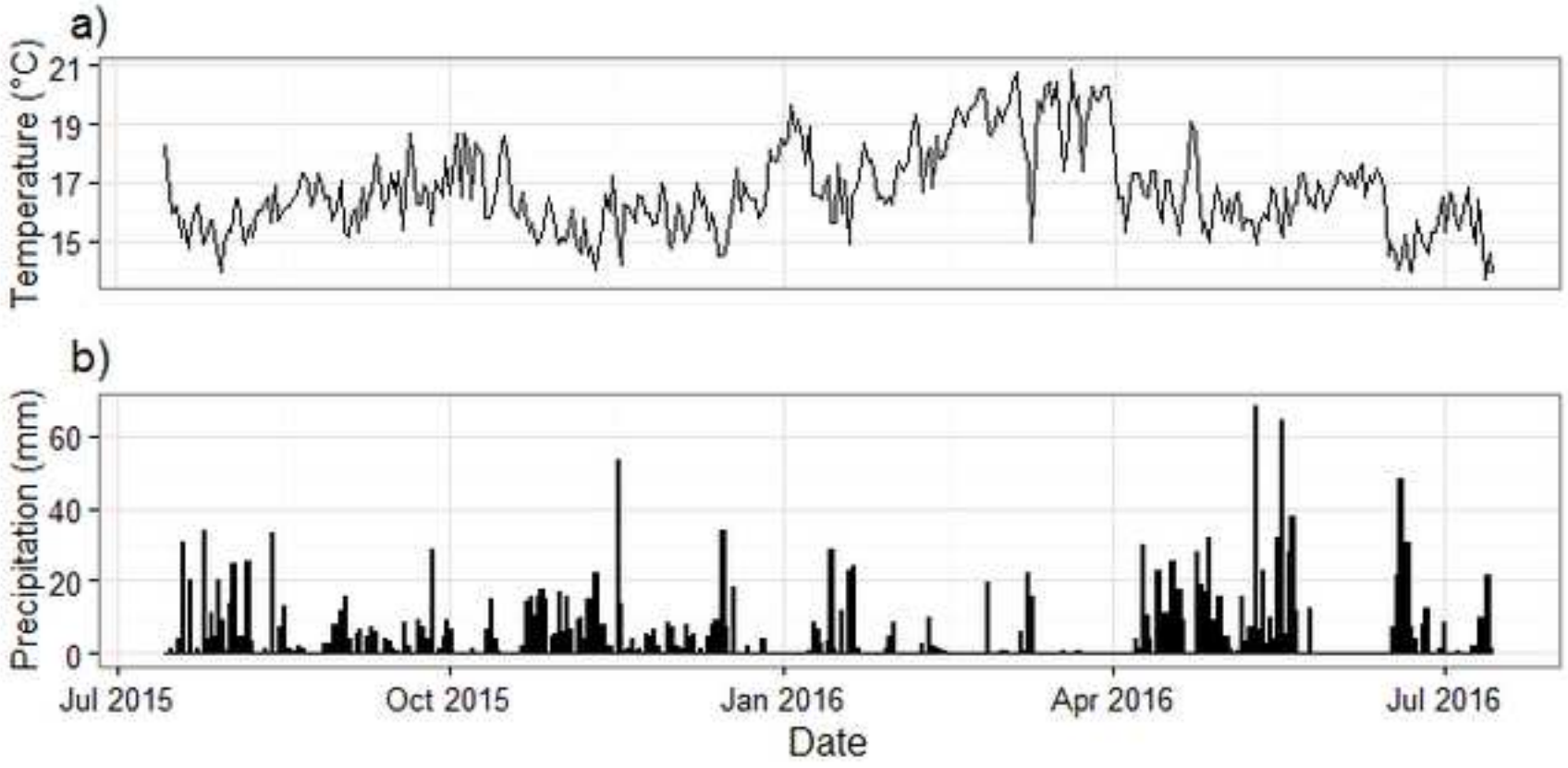

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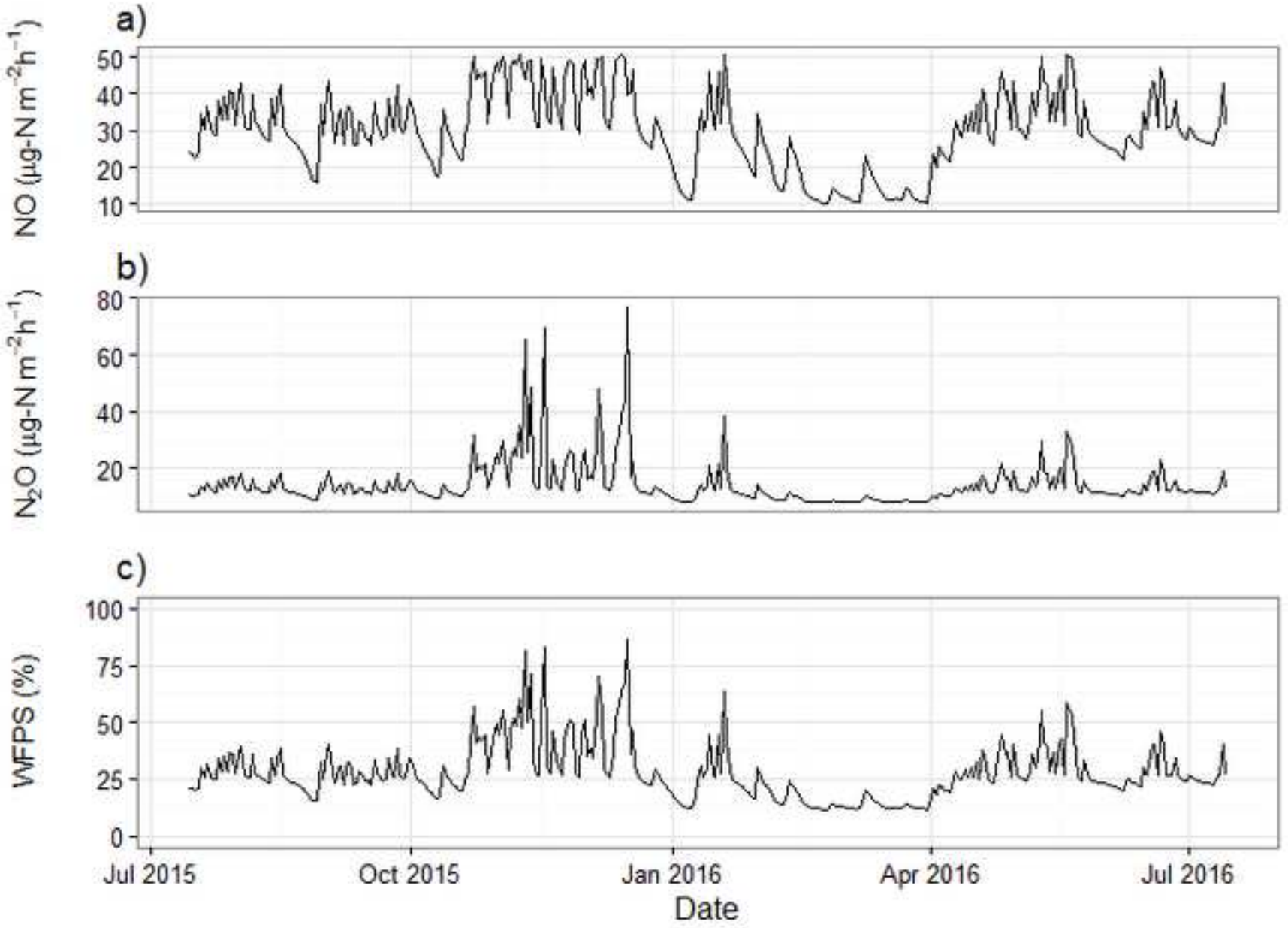

Table 1

Area	Land use	Site	Sand (g kg^{-1})	Silt (g kg^{-1})	Clay (g kg^{-1})	Bulk density (g cm^{-3})	Soil organic carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	C:N	pH
Smallholder	Forest	SH-F	74±19a	333±51ab	593±35ab	0.66±0.05b	79±7a	8.1±0.8a	9.7±0.4cd	6.0±0.2a
Tea Estates	Forest	TE-F	77±33a	257±21b	667±29a	0.63±0.05b	63±4b	6.6±0.3b	9.6±0.4d	4.9±0.4b
Smallholders	Tea	SH-T	50±9a	380±42ab	570±37ab	0.83±0.11a	62±8b	5.8±0.4b	10.5±0.6bc	5.0±0.6b
Tea Estates	Tea	TE-T	56±10a	363±63ab	581±54ab	0.63±0.11b	66±14ab	5.7±1.2b	11.6±0.3a	4.2±0.2c
Smallholders	Grazing	SH-G	50±6a	474±187a	574±22b	0.83±0.05a	66±1ab	6.3±0.3b	10.6±0.4b	5.4±0.0b
Tea Estates	Plantation	TE-P	63±9a	372±43ab	565±40ab	0.83±0.05a	58±4b	5.6±0.5b	10.4±0.4bc	5.1±0.3b

Table 2

Area	Land Use	Site	Soil nitrous oxide flux (μg N_2O-N m^{-2} h^{-1})				
			WFPS				
			30%	50%	70%	90%	**Mean**
Tea Estate	Forest	TE-F	22 ± 16	19 ± 9	33 ± 21	447 ± 497	**130 ± 300**
Small-Holders	Forest	SH-F	21 ± 10	20 ± 11	45 ± 57	86 ± 98	**43 ± 60**
Tea Estate	Tea	TE-T	13 ± 8	11 ± 6	9 ± 6	9 ± 9	**11 ±6**
Small-Holders	Tea	SH-T	24 ± 18	28 ± 19	17 ± 10	201 ± 273	**68 ± 156**
Tea Estate	Tree Plantation	TE-P	15 ± 8	21 ± 12	19 ± 15	44 ± 44	**25 ± 25**
Small-Holders	Grazing	TE-G	19 ± 1	22 ± 11	21 ± 13	309 ± 432	**93 ± 248**
Mean across sites			**19 ± 9b**	**21 ± 8b**	**24 ± 27b**	**183 ± 320a**	

Table 3

Area	Land Use	Site	Soil nitric oxide flux (µg NO-N m^{-2} h^{-1})				
			WFPS				
			30%	50%	70%	90%	**Mean**
Tea Estate	Forest	TE-F	87 ± 62	93 ± 57	61 ± 42	29 ± 16	**68 ± 42^{A}**
Small-Holders	Forest	SH-F	46 ± 27	37 ± 18	52 ± 34	37 ± 30	**43 ± 20^{AB}**
Tea Estate	Tea	TE-T	58 ± 32	102 ± 65	74 ± 49	88 ± 49	**81 ± 35^{A}**
Small-Holders	Tea	SH-T	19 ± 12	69 ± 57	85 ± 56	93 ± 64	**66 ± 49^{A}**
Tea Estate	Tree Plantation	TE-P	27 ± 23	25 ± 13	26 ± 14	20 ± 11	**25 ± 12^{B}**
Small-Holders	Grazing	TE-G	17 ± 16	19 ± 20	28 ± 23	22 ± 18	**22 ± 18^{B}**
Mean across sites			**43 ± 34^{a}**	**58 ± 45^{a}**	**55 ± 35^{a}**	**48 ± 40^{a}**	

Table 4

Area	Land Use	Site	Soil carbon dioxide flux (mg CO_2-C m^{-2} h^{-1})				
			WFPS				
			30%	50%	70%	90%	**Mean**
Tea Estate	Forest	TE-F	24 ± 15	40 ± 30	69 ± 37	85 ± 44	**55 ± 30^{A}**
Small-Holders	Forest	SH-F	10 ± 11	30 ± 25	65 ± 34	64 ± 34	**43 ± 28^{A}**
Tea Estate	Tea	TE-T	17 ± 12	28 ± 14	29 ± 15	34 ± 17	**28 ± 8^{B}**
Small-Holders	Tea	SH-T	3 ± 2	29 ± 17	52 ± 29	51 ± 27	**34 ± 23^{B}**
Tea Estate	Tree Plantation	TE-P	10 ± 8	29 ± 14	43 ± 23	45 ± 31	**32 ± 19^{B}**
Small-Holders	Grazing	TE-G	5 ± 4	25 ± 24	44 ± 37	63 ± 44	**34 ± 34^{B}**
Mean across sites			**12 ± 10^{c}**	**31 ± 17^{b}**	**51 ± 21^{a}**	**57 ± 25^{a}**	

Table 5

Area	Land Use	Site	Nitrous oxide (Kg N ha^{-1} a^{-1})	Nitric oxide (kg N ha^{-1} a^{-1})	N_2O+ NO (kg N ha^{-1} a^{-1})	% N_2O	% NO
Tea Estate	Forest	TE-F	0.3 (0.2)	5.2 (0.7)	5.5	5.4	94.6
Smallholders	Forest	SH-F	1.3 (0.2)	2.6 (0.7)	3.9	32.3	67.7
Tea Estate	Tea	TE-T	0.9 (0.3)	4.3 (0.2)	5.2	17.3	82.7
Smallholders	Tea	SH-T	0.1 (0.2)	2.1 (1.0)	2.2	3.9	96.1
Tea Estate	Tree Plantation	TE-P	1.0 (0.02)	1.5 (1.1)	2.5	40.6	59.4
Smallholders	Grazing	SH-G	0.1 (0.3)	1.1 (0.3)	1.1	5.8	94.2

Running head

Soil trace gas fluxes in tropical montane land uses

Article type

General research

Title

Quantifying the contribution of land use to N_2O, NO and CO_2 fluxes in a montane forest ecosystem of Kenya.

Authors

Arias-Navarro, C[1, 2, 3]; Díaz-Pinés, E[1, 4,*]; Zuazo, P[1]; Rufino, M C[5]; Verchot, L V[6]; Butterbach-Bahl, K [1, 3]

Affiliations

[1] Institute of Meteorology and Climate Research, Atmospheric Environmental Research (IMK-IFU), Karlsruhe Institute of Technology (KIT), Kreuzeckbahnstr. 19, 82467 Garmisch-Partenkirchen, Germany.

[2] Center for International Forestry Research (CIFOR), P.O. Box 30677, Nairobi 00100, Kenya

[3] Mazingira Centre, Environmental Research and Educational Facility, International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi 00100, Kenya

[4] Present address: Institute of Soil Research, University of Natural Resources and Life Sciences (BOKU), Peter-Jordan-Strasse 82, 1190, Vienna, Austria.

[5] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, United Kingdom

[6] International Center for Tropical Agriculture (CIAT), Km 17, Recta Cali–Palmira CP 763537, Apartado Aéreo 6713, Cali, Colombia

* Corresponding author:

eugenio.diaz-pines@boku.ac.at

Institute of Soil Research, University of Natural Resources and Life Sciences (BOKU)

Peter-Jordan-Strasse 82, 1190 – Vienna, Austria

Telephone: +43 1 47654-91120

Fax +41 1 47654-91109

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Keywords

Carbon dioxide; land use change; nitric oxide; nitrous oxide; soils; tropical forests

Abstract

Increasing demand for food and fibre by the growing human population is driving significant land use (LU) change from forest into intensively managed land systems in tropical areas. But empirical evidence on the extent to which ~~extent~~ such changes affect the soil-atmosphere exchange of trace gases is still scarce, especially in Africa. We investigated the effect of LU on soil trace gas production in the Mau Forest Complex region, Kenya. Intact soil cores were taken from natural forest, commercial and smallholder tea plantations, eucalyptus plantations and grazing lands, and were incubated in the lab under different soil moisture conditions. Soil fluxes of nitrous oxide (N_2O), nitric oxide (NO) and carbon dioxide (CO_2) were quantified, and we approximated annual estimates of soil N_2O and NO fluxes using soil moisture values measured *in situ*.

Forest and eucalyptus plantations yielded annual fluxes of 0.3-1.3 kg N_2O-N ha^{-1} a^{-1} and 1.5-5.2 kg NO-N ha^{-1} a^{-1}. Soils of commercial tea plantations, which are highly fertilized, showed higher fluxes (0.9 kg N_2O-N ha^{-1} a^{-1} and 4.3 kg NO-N ha^{-1} a^{-1}) than smallholder tea plantations (0.1 kg N_2O-N ha^{-1} a^{-1} and 2.1 kg NO-N ha^{-1} a^{-1}) or grazing land (0.1 kg N_2O-N ha^{-1} a^{-1} and 1.1 kg NO-N ha^{-1} a^{-1}). High soil NO fluxes were probably the consequence of long-term N fertilization and associated soil acidification, likely promoting chemodenitrification. Our experimental approach can be implemented in understudied regions, with the potential to increase the amount of information on production and consumption of trace gases from soils.

1. Introduction

Nitrous oxide (N_2O) and carbon dioxide (CO_2) are important greenhouse gases (GHG) contributing directly to climate change (IPCC 2014), while nitric oxide (NO) is a key substance involved in the tropospheric production of ozone, which is also a potent GHG (Chameides et al. 1992). Soils are the dominating source of atmospheric CO_2 (Raich et al. 2002) and N_2O (Butterbach-Bahl et al. 2013) and contribute considerably to the atmospheric budget of NO (Conrad 1996; Butterbach-Bahl et al. 2009). The exchange of these gases between soil and atmosphere is influenced by several factors such as land use (LU) and land use change (LUC), temperature and precipitation, N input and soil properties (Butterbach-Bahl et al. 2013).

Land use change from natural forest ecosystems into other more intensively managed LU is driven by the increasing demand for food and fibre, especially in tropical regions, where population growth fosters agriculture encroachment in forested areas (IPCC 2007). In East Africa, where agriculture is the primary LU, tropical montane forests are particularly endangered because they are located in areas highly suitable for agricultural production; thus, the expansion of cropland, grazing areas, and forest plantations at the expense of natural forests and other natural ecosystems is expected to continue in the future (Potting and Bakkes 2004). For Kenya's montane forests, deforestation was approximately 50,000 ha for the 2000-2010 period, with the encroachment of tea cultivation areas being an important driver for LUC (UNEP 2012; Mutugi and Kiiru 2015).

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Kenya is the third largest tea producer and the first black tea exporter worldwide (Monroy et al. 2013). The tea sector is divided in two production systems: the large-scale monoculture tea plantations (also called tea estates) and the local-scale smallholders, who traditionally cultivate tea to supplement subsistence agriculture. The commercial tea plantations have usually higher yields compared to those managed by smallholder producers, as more fertilizers are used and management is optimised (Kenya Human Rights Commision 2008). Following increased tea

demand, the area devoted for tea production in Kenya has grown in the past decades, primary by the smallholders (Monroy et al. 2013).

Both land use and land management play a significant role in the C and N cycling, potentially influencing the exchange rates of trace gases between the soil and the atmosphere. For example, N fertilization usually promotes both N_2O and NO production in the soil due to enhanced substrate availability for microbial utilization (Stehfest and Bouwman 2006). Changes in micro-climate (moisture, temperate) and soil bulk density, porosity, mineral N content and pH following LUC (Farquharson and Baldock 2008) influence the consumption and production of trace gases in the soil (Davidson et al. 2000b; Saiz et al. 2006; Tang et al. 2006a). However, studies of the soil-atmosphere trace gas exchange in tropical ecosystems are still scarce, especially in Africa ~~(Kim et al. 2016)~~(Kim et al. 2016). The lack of evidence translates into considerable uncertainty on the impact of LUC on soil trace gas emissions (van Lent et al. 2015).

Understanding land use effects on soil GHG fluxes remains difficult due to high spatio-temporal variations of fluxes. ~~Therefore, the design of experiments and sampling strategies for evaluating land use change effects on soil GHG fluxes is crucial (Arias-Navarro et al. submitted).~~Therefore, the design of experiments and sampling strategies for evaluating land use change effects on soil GHG fluxes is crucial (Arias-Navarro et al. 2017). Unfortunately, *in situ* measurements with high spatial and temporal resolution in remote tropical ecosystems are constrained by limited infrastructure and therefore high costs. To overcome these constrains, soil samples can be taken to the laboratory for targeted incubation experiments, e.g. for studying spatial and temporal variability of fluxes in dependence of changes of environmental conditions. Fluxes from soil cores determined in the laboratory usually agree well with fluxes determined via field chambers (Otter et al. 1999; Ludwig et al. 2001; Gut et al. 2002; van Dijk 2002; Yao et al. 2010).

In this study, we quantified soil CO_2, N_2O, and NO fluxes of representative land uses of the Mau Forest Complex in Kenya. We used a fully-automated laboratory incubation and monitoring system to study fluxes from intact soil cores at different soil moisture contents. The objectives of this study were:

(1) To evaluate the effect of LU on the soil N_2O, NO and CO_2 fluxes

(2) To analyse effects of tea plantation management (commercial versus smallholder) on the soil N_2O, NO and CO_2 fluxes.

(3) To quantify the importance of soil moisture as a driver of soil trace gas emissions.

We hypothesised that tea plantations would emit higher amounts of N-trace gases than other LUs and that N fluxes from soils taken from commercial tea plantations are higher than those from soils of smallholder farmers. Moreover, we hypothesized that soil water content could be used for approximating the seasonality of soil trace gas emissions and for calculating annual fluxes.

2. Material and Methods

2.1 Study Area

The Mau Forest Complex in Kenya is the largest indigenous Afromontane forest of East Africa covering an area of about 417,000 ha. The study site lies in the Southwest Mau part, east of Kericho town (-0° 22' 3" S, 35° 16' 59" E) (Figure 1) at approximately 2500 m a.s.l. The climate is cool and humid tropical with a mean annual precipitation between 1800 and 1950 mm (1979-2009) (Omumbo et al. 2011). The region has a bi-modal rainfall pattern, with the “long rains” falling between April and August and “short rains” between October and December, while January and February are generally the driest months. The mean annual temperature ranges from 15.7 to 18°C (1979-2009) (Omumbo et al. 2011) with modest (approx. 7°C) seasonal variations. The geology substrate is formed by Tertiary lavas from the mid-Miocene ~~(Blackie and Edwards 1981).~~(Blackie and Edwards 1979). The soils are well drained, deeply weathered,

dark reddish-brown, clayey, and with an acidic humic topsoil (Krhoda 1988; Jaetzold et al. 2010). Soils are classified as Andic Humic Nitisols (IUSS Working Group WRB 2015).

In the last decades the Mau Forest region has experienced a loss of forest cover of about 25 % at the ~~extent~~expense of other LUs (Government of Kenya 2010). In the region, tea is produced in both large estates and smallholder farms. Smallholder farms are typically less than half a hectare, with most of the land planted with tea and only 20 % of the land reserved for food crops and grazing (Milder et al. 2015). Commercial tea estates grow eucalyptus woodlots in addition to tea, as source of firewood for the tea factories.

2.2 Experimental Design

In this experiment, two contrasting tea-growing areas were investigated. The first area is located in Kapkorech Estate, a large tea estate (hereafter, TE) owned by a private company. Nearly 120 ha of commercial plantations of tea (*Camelia sinensis var. sinensis* L.) were established more than 60 years ago after clearance of the native forest. Approximately 20 ha were designated to grow eucalyptus (*Eucalyptus grandis* L.). Aerial application of NPK 26:5:5 fertilizer to the tea fields is conducted 2-3 times a year (300-400 kg N ha^{-1} a^{-1}; personal communication). The second tea-growing area is managed by smallholder farmers (hereafter, SH). Smallholder tea plantations are fertilized with NPK 26:5:5 at an approximate rate of 150 kg N ha^{-1} a^{-1} (personal communication). In addition to tea cultivation, a significant share of the land is devoted to livestock grazing.

At the SH area, we monitored plots under tea (T), grazing (G) and the contiguous natural forest (F) (Figure 1a). At the TE area, we investigated plots under tea (T), eucalyptus plantations (P) and the adjacent natural forest (F) (Figure 1b). Therefore, the experimental design comprised six experimental sites (SH-T, SH-G, SH-F, TE-T, TE-P and TE-F) each of them replicated three times, making a total of 18 experimental plots, with an area of 0.25 ha each, approximately.

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2.3 Soil sampling

We used intact soil cores to minimize the disturbance of the soil structure. Five soil cores were collected at three random locations within each plot. At each location, the uppermost layer of litter with visible undecomposed material (leaves, twigs, etc) was removed before PVC tubes (5 cm inner diameter; 10 cm height) were driven into the soil with the help of a wooden block and rubber hammer. The filled PVC cores were carefully removed and immediately air-dried at 25 °C for three days ~~before being~~. After this period, the soils had a water filled pore space (WFPS) of approximately 10-15 %. Subsequently, the soils were transported to the laboratory at IMK-IFU (Garmisch-Partenkirchen, Germany~~).~~) and stored at ambient temperature until laboratory incubations were conducted, approximately four months after sampling.

2.4 Soil incubation

The soil cores were incubated using a custom-built, temperature-regulated automatic gas sampling system encompassing 18 incubation chambers. Each chamber consists of a poly-methylmethacrylate cylinder (126 mm inner diameter; 240 mm height) acting as a steady-state dynamic chamber (Pumpanen et al. 2004; Pihlatie et al. 2013). A cylindrical piece of 7 cm height was placed at the bottom of each chamber, on top of which we placed three intact soil cores, one from each of replicate within a plot. The air volume between the cores was filled with quartz sand up to the upper edge of the soil cores. The sand was covered with a metallic sheet (2 mm thick), so that only the soil surface of the cores was directly exposed to the headspace. This design allowed small chamber headspace (374 cm^3) without dead volumes. The chamber was then closed with a gas-tight lid equipped with an inlet and an outlet.

During the incubation, background air was continuously supplied through the inlet to all the chambers, allowing for a permanent equilibrium state of the headspace (Pape et al. 2009)(Figure 2a). The sampling from the incubation chambers and the background air was controlled through electromechanically operated solenoid valves (Bürkert GmbH & Co. KG, Ingelfingen,

Germany) in 180-minute long cycles in which a measure of the concentration at the outlet of each incubation chamber was gained. Further details on the custom-build system can be found in Zuazo (2016).

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Nitrous oxide and CO_2 concentrations were determined using cavity ring-down spectroscopy (G2508, Picarro, Santa Clara, CA, USA). The gas analyser was calibrated every measuring cycle using a gas blend containing defined concentrations of N_2O (408 ppbv) and CO_2 (406 ppmv) in synthetic air (Air Liquide GmbH, Düsseldorf, Germany). Nitric oxide concentrations were quantified by a chemiluminescence detector (CLD88p, Eco Physics AG, Duernten, Switzerland) calibrated daily with four different NO concentrations in synthetic air: 0, 50, 200 and 500 ppbv NO. These blends were prepared by mixing a stable concentrated preparation (4 ppm NO in N_2; Air Liquide GmbH, Germany) with synthetic air (20 % O_2 + 80 % N_2) using a multi-gas calibration system (series 6100; Environics Inc., Tolland, CT, USA).

The soil-headspace ~~gaseous~~ exchange rate of each trace gas was calculated from the mass balance ~~of~~between the inlet and outlet concentrations assuming mass flow equilibrium conditions (Pape et al. 2009).

$$F_{chamb} = \frac{Q}{A} * \rho(\mu_{chamb-}\mu_{amb})$$

where F_{chamb} stands for the trace gas flux (nmol m^2 s^{-1}); A denotes the soil surface of the three soil cores (m^2); Q is the headspace air flow rate (m^3 s^{-1}). μ_{cham} and μ_{amb} are the trace gas mixing ratios (nmol mol^{-1}) of the inflowing ambient air and of the outflowing chamber air, respectively; and ρ is the molar density of dry air molecules (mol m^{-3}).

Because seasonal fluctuations of mean daily air temperatures at the study area are < 7 °C, our experiment focused on the effects of soil moisture changes on the trace gas fluxes. The incubation temperature throughout the experiment was set to the average annual temperature in the study area (i.e. 18 °C). We chose an air relative humidity of 70 % to avoid excessive drying of the soil during the measuring cycle. Soil water content of the cores was determined

gravimetrically prior to the experiment using a replicate intact soil core. Bulk density (BD) was determined gravimetrically using oven-dry (105 °C) soil weight divided by the core volume. BD was used to calculate the pore volume and consequently the amount of water required to reach 20, 30, 50, 70 and 90 % ~~water-filled pore space (~~WFPS~~)~~ using the equation:

$$WFPS\ (\%) = \frac{W_{vol}}{\left(\frac{1 - BD}{2.65}\right)}$$

where W_{vol} is the volumetric water content (g cm^{-3}), *BD* is soil bulk density (g cm^{-3}) and 2.65 is the soil particle density (g cm^{-3}).

Incubations at different WFPS levels were performed with independent soil cores to avoid potential bias associated with substrate depletion due to sequential incubation. Targeted WFPS was achieved by adding a standard rain solution (Breuer et al. 2002).

Each incubation run (30, 50, 70 and 90 % WFPS, respectively), was divided in three periods (Figure 2b) as follows:

Dry conditions (2 days): Trace gas fluxes from the soil cores were measured prior to soil re-wetting.

20 % WFPS (3 days): Re-wetting of dry soils usually leads to a short-lasting, over-proportionate increase of emissions (initial pulse, Figure 2b), with the magnitude of this response being dependent on moisture soil conditions prior to re-wetting (Borken and Matzner 2009; Liang et al. 2015). For this reason, we adjusted the initial moisture content to 20 % WFPS to purposely homogenize the soil moisture status before setting the soils to the final targeted WFPS % treatment. Trace gas fluxes were measured for three consecutive days, after which we observed that soil trace gas fluxes stabilized.

Targeted WFPS % (10 days): Water was added to the soil surface until the targeted WFPS % was achieved and the soil trace gas fluxes were measured for 10 consecutive days. To avoid the short-term interference typically observed as a consequence of the pulse of NO, N_2O and CO_2 occurring after rewetting of soil we excluded the data of the four days after rewetting (second

pulse, Figure 2b) to analyse the effects of soil moisture on trace gas fluxes for the different land uses (post-pulse area, Figure 2b).

Because the incubation system allowed to obtain flux measurements for each chamber every 3 hours and the incubation run for 15 days in total, approximately 120 individual flux measurements were generated per incubation chamber (N=18). Cumulative N_2O, NO and CO_2 emissions were calculated by linear interpolation between two consecutive sampling events.

2.5 Determination of soil properties

At the end of the incubation, the soils were air-dried and sieved (2 mm). We mixed 20 g from each replicate soil core to obtain a composite sample, which was sent to a commercial laboratory (Landwirtschaftliches Labor Dr. Janssen GmbH, Gillersheim, Germany) for analysis. Total nitrogen (TN) content was determined by dry combustion (DIN ISO 13878). Carbonates were removed beforehand by acid application, and the organic C content was determined by dry combustion (DIN ISO 10694). Soil pH was determined in water (10 g soil + 25 ml solution) as detailed in the VDLUFA (1991, section A, 5.1, 1). Soil texture was determined according to DIN ISO 18123.

2.6 Monitoring of environmental parameters on site.

Environmental data were collected from July 2015 to July 2016 from a near weather station located at the Kenya Forest Service Kericho forest station (-0° 21' 5" S, 35° 21' 5" E, 2184 m a.s.l.). Moisture and temperature in 10 cm soil depth were measured using a combined water potential and temperature sensor (Decagon 5TM, Decagon Devices, Inc., Pullman, WA, USA). Rainfall was measured with a rain gauge (Decagon ECRN-50, Decagon Devices, Inc.). Data were logged in 10 min intervals on a digital data logger and downloaded periodically (Decagon Em50 series, Decagon Devices, Inc.). Daily average soil volumetric water content (W_{vol})

monitored at the weather station was used to calculate the daily average WFPS of each experimental site using the respective BD values (Table 1) assuming that W_{vol} did not vary across sites.

Because soil temperature does not vary greatly in many tropical forests, soil water content is often found to be a more significant factor affecting temporal variation of soil trace gases (Butterbach-Bahl et al. 2004). Thus, estimated daily values of WFPS were used for approximating the seasonality of soil trace gas emissions and for calculating annual fluxes on basis of the regression curves describing the relationship between soil moisture and the trace gas fluxes observed in our laboratory experiments (see 3.3).

2.7 Data processing, data analysis

All statistical analyses and plotting were carried out using R 3.1.3 (R Core Team 2016). Downloading and formatting Google Maps images was done with the ggmap package (Kahle and Wickham 2013). One-way ANOVA was used to test differences in soil properties across experimental sites. Trace gas fluxes among different sites and soil moisture levels were compared using a two-way ANOVA. We used the Fisher LSD method to compare individual means. Descriptive statistics are reported as the mean of the three soil core replicates along with the standard deviation. The contribution of soil parameters to the variance of N_2O, NO and CO_2 gas fluxes was studied using regression analysis. Uncertainty of the curve fitting for linear models was calculated using the function "predict.lm" (R package stats), which produces predicted values, obtained by evaluating the regression function and calculates the standard errors of the predictions. For exponential models we used the function "predictNLS" (R package propagate, ~~Spiess 2014))~~Spiess 2014) which propagates the error using Monte Carlo simulation. Significance level was established at $p \leq 0.05$.

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3. Results

300 3.1 Soil parameters

301 All topsoils had a clayey texture, with clay contents in the range of 56-67% (Table 1). Topsoil
302 bulk density (BD) values were below 1 g cm^{-3}, with the lowest values observed for soils sampled
303 at the natural forests (SH-F and TE-F) and at the commercial tea plantations (TE-T). Soil
304 organic carbon (SOC) contents were in the range of 58-79 g kg^{-1}. Soils taken from SH-F had
305 the highest SOC content although differences are not significant from TE-T and SH-G. Nitrogen
306 contents ranged between 5.6 and 8.1 g kg^{-1}. Soils taken from SH-F had as well the highest total
307 nitrogen (TN) content. Soils were acidic (4.2-6.0), with the lowest soil pH value observed for
308 soils from TE-T (Table 1).

309

310 3.2 Soil trace gas fluxes

311 *Soil N_2O fluxes*

312 We measured a very low N_2O uptake from dried soil cores (average: -9 ± 31 µg N m^{-2} h^{-1}).
313 Initial re-wetting of soil cores to 20% WFPS did not cause N_2O emission pulses, regardless of
314 the site. After setting the soil cores to the targeted WFPS, substantial N_2O fluxes (> 50 µg N_2O-
315 N m^{-2} h^{-1}) were measured only when WFPS was finally set to 90%. The peak maximum for
316 these cores was observed after two to four days, depending on the experimental sites (Figure
317 3). The only significant differences in soil N_2O fluxes were found between 90% WFPS and the
318 other soil moisture levels. With an average post-pulse N_2O flux of 131 ± 61 µg N_2O-N m^{-2} h^{-1},
319 TE-F tended to emit more N_2O than soils from other sites, although strong variation precluded
320 significant differences (Table 2).

321 Pulse fluxes contributed to more than half (initial pulse: 22 ± 9 %, second pulse: 34 ± 16 %) of
322 the total time-weighted average cumulative soil N_2O rates during the 15 day-incubation period
323 for the different sites and levels of WFPS (Figure 4a). Cumulative soil N_2O fluxes during the
324 second pulse were significantly higher for soils moistened to 90% WFPS (159 ± 258 g N_2O-N
325 ha^{-1}) compared to soils adjusted to other moisture levels (70% WFPS: 27 ± 28 g N_2O-N ha^{-1};

50% WFPS: 27 ± 9 g N_2O-N ha^{-1}; 30 % WFPS: 27 ± 11 g N_2O-N ha^{-1}). The second pulse was not significantly different between sites.

Soil NO fluxes

Dry-soil NO fluxes were very low for all sites (average 5 ± 7 µg NO-N m^{-2} h^{-1}). Large NO pulses (average 99 ± 100 µg NO-N m^{-2} h^{-1}) occurred already following the first re-wetting event, when soil moisture was adjusted to 20 % WFPS (Figure 5). The highest peak fluxes following the first initial re-wetting were observed for soils from commercial tea plantations, while for soils from smallholder grazing plots this first pulse was negligible (TE-T: 259 ± 111 µg NO-N m^{-2} h^{-1}; SH-G: 23 ± 13 µg NO-N m^{-2} h^{-1}). Soil NO fluxes after the second soil re-wetting to the target moisture were not significantly different across soil moisture levels (Table 3). Nitric oxide emissions were significantly higher for soils from tea plantations (TE-T and SH-T) and natural forests (TE-F and SH-F) compared to those taken from ~~Eucalyptus~~eucalyptus plantations (TE-P) or grazing land (SH-G) (Table 3).

Pulse fluxes contributed to more than 80 % of the total time-weighted average cumulative soil NO rates during the 15 day-incubation period for the different sites and levels of % WFPS (initial pulse: 31 ± 14%, second pulse: 51 ± 9 %) (Figure 4b). The highest time-weighted average cumulative NO fluxes during the initial pulse (20 % WFPS) were those for soils from TE-T (196 ± 78 g NO-N ha^{-1}) followed by TE-F sites (127 ± 65 g NO-N ha^{-1}). The initial pulse was not significantly different for the rest of the sites (TE-P: 47 ± 19 g NO-N ha^{-1}; SH-T: 44 ± 21 g NO-N ha^{-1}; SH-G: 18 ± 19 g NO-N ha^{-1}; SH-F: 13 ± 9 g NO-N ha^{-1}). After setting the soil cores to the final targeted WFPS, average cumulative NO fluxes during the second pulse followed the same pattern, i.e., significant highest emissions were found for soils from TE-T and TE-F (201 ± 46 g NO-N ha^{-1} and 200 ± 68 g NO-N ha^{-1} respectively) although in this case, fluxes were not significant different from SH-T (147 ± 99 g NO-N ha^{-1}). The second pulse at SH-T and at the rest of the sites were no significantly different (TE-P: 78 ± 31 g NO-N ha^{-1};

SH-F: 67 ± 19 g NO-N ha^{-1}; SH-G: 65 ± 55 g NO-N ha^{-1}). Moisture content did not have a significant effect on the magnitude of the second pulse.

Soil CO_2 fluxes

The CO_2 emissions during the dry-incubation period were on average 2 ± 1 mg C m^{-2} h^{-1} ~~and, thus not significantly different from zero.~~. Following the initial re-wetting of soils to 20% WFPS small (< 40 mg C m^{-2} h^{-1}) and short-lived pulse fluxes of CO_2 were observed, which returned to pre-incubation levels in less than 24 hours (Figure 6). Following rewetting to target moisture levels, the soil CO_2 fluxes gradually increased with increasing WFPS for all sites. No significant differences in CO_2 fluxes were found between 70 and 90% WFPS. Soils taken from forests had significantly higher CO_2 fluxes than the rest of the soils (Table 4). The contribution to the total time-weighted average cumulative soil CO_2 rates of the 15 day-incubation period of soil CO_2 fluxes from the initial pulse event after re-wetting of soils was 12 ± 10 % (Figure 4c) and no significant differences were found among sites. The second pulse event, on the other hand, contributed with 58 ± 7% (Figure 4c) and was significantly higher for soils set to 90 and 70% WFPS (92 ± 40 kg CO_2-C ha^{-1} and 79 ± 40 kg CO_2-C ha^{-1} respectively) compared to the pulse obtained when the soils were set to 50 and 30% WFPS (45 ± 24 kg CO_2-C ha^{-1} and 23 ± 16 kg CO_2-C ha^{-1} respectively). The second pulse event was significantly higher for soils from forests (SH-F: 81 ± 53 kg CO_2-C ha^{-1}; TE-F: 69 ± 32 kg CO_2-C ha^{-1}) compared to TE-T (34 ± 11 kg CO_2-C ha^{-1}). No significant differences were found between forests and the rest of the soils (SH-G: 64 ± 60 kg CO_2-C ha^{-1} SH-T: 57 ± 37 kg CO_2-C ha^{-1}, TE-P: 53 ± 29 kg CO_2-C ha^{-1}).

3.3 Relationship between soil properties and trace gas fluxes.

Average post-pulse soil N_2O and CO_2 fluxes were significantly and positively correlated (R^2=0.4). There were no correlations between N_2O or CO_2 soil fluxes with soil properties.

Average post-pulse soil NO emissions were negatively correlated with pH (R^2=0.3), BD (R^2=0.3) and silt content (R^2=0.2) and positively correlated with clay content (R^2=0.2).

3.3 Estimates of annual emissions

In this incubation study, N_2O emissions increased exponentially with WFPS, while the relationship between WFPS and NO followed a 2th degree polynomial (Figure 7). In the case of TE-T, no satisfactory model was found for N_2O; therefore, the mean N_2O flux across WFPS levels was used for calculating the annual soil N_2O fluxes.

Measured rainfall at our weather station in the Mau region showed a bi-modal pattern, with rains falling between April-June and between October-December. The total annual rainfall for the period July 2015-July 2016 was 1956 mm and the mean air temperature was 16.7 °C (min=13.7 °C, max=20.9 °C) (Figure 8).

Soil moisture at 10 cm soil depth ranged from 10.5 to 78.5 % WFPS with an average value of 25.3 % for SH-F, TE-F, TE-T sites and between 11.6 to 86.6 % with a mean value of 28 % for SH-T, SH-G and TE-P sites. The daily values of WFPS were finally used to calculate annual emissions on basis of the relationships between WFPS and trace gas fluxes (Figure 9 shows SH-F as an example).

N_2O fluxes through the year were generally below 50 µg N_2O-N m^{-2} h^{-1} (mean value: 11.2 µg N_2O-N m^{-2} h^{-1}) with peak emissions only when the soil moisture content was above 60 % WFPS. Daily soil NO emissions were on average 31.8 µg NO-N m^{-2} h^{-1}, decreasing when the WFPS levels were below 25 %. Daily estimated soil NO and N_2O fluxes and WFPS% at the SH-F site is shown in figure 9.

Table 5 summarizes calculated annual flux rates for the different sites. Across sites, NO fluxes contributed 60-95% to the total N-oxide losses (N_2O + NO losses). Highest annual fluxes of N-oxides were observed for the TE-F and TE-T sites, with emissions of 5.5 ± 0.9 kg N ha^{-1} a^{-1} and

5.2 ± 0.5 kg N ha^{-1} a^{-1} respectively. At the smallholder site the estimated annual soil N emissions for SH-F and SH-T were 3.9 ± 0.9 kg N ha^{-1} a^{-1} and 2.2 ± 0.3 kg N ha^{-1} a^{-1}, respectively.

In general, lowest annual N_2O emissions were estimated at the smallholder tea plantations and grazing sites (SH-T: 0.1 ± 0.2 kg N_2O-N ha^{-1} a^{-1}; SH-G: 0.1 ± 0.3 kg N_2O-N ha^{-1} a^{-1}). Soil from the grazing sites showed also the lowest annual NO emissions (SH-G: 1.1 ± 0.3 kg NO-N ha^{-1} a^{-1}). Highest annual NO emissions were estimated from the natural forest sites and tea plantations in the TE area (TE-F: 5.2 ± 0.7 kg N ha^{-1} a^{-1}; TE-T: 4.3 ± 0.2 kg NO-N ha^{-1} a^{-1}).

4. Discussion

Soil moisture is a key governing parameter in the production and consumption of N oxides in the soil as it controls both soil gas diffusion and oxygen (O_2) availability for microbial use (Davidson et al. 2000a). Whereas both NO and N_2O may be produced through the same processes (i.e. nitrification and denitrification), the ratios of the two products may vary strongly depending on O_2 availability ~~(Pilegaard 2013)~~(Pilegaard 2013). Soil NO production during nitrification requires O_2 as electron acceptor, while N_2O is more commonly produced by reductive processes –i.e. under O_2-limiting environmental conditions- such as denitrification or nitrifier-denitrification (Butterbach-Bahl et al. 2013). Therefore, highest emission rates for NO have been frequently observed at soil moisture contents below field capacity, which for many soils is about 60 % WFPS. With regard to N_2O, maximum emission rates are reported at values between 50 and 90 % WFPS, depending on soil properties (Davidson et al. 1991; Breuer et al. 2002; Werner et al. 2007) and at WFPS > 90 % dominant soil anaerobiosis favours complete denitrification, yielding N_2 as product.

In our study, N_2O fluxes increased exponentially if soil cores were wetted at WFPS higher than 70 %, but were relatively low at 30–50 % WFPS. These findings are in agreement with many other studies which have reported that N_2O production and emission increase exponentially with soil water content (Garcia-Montiel et al. 2001; Arai et al. 2008). We further found high

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soil CO_2 rates, and a significant correlation between N_2O fluxes and soil respiration rates at 90 % WFPS. This correlation has also been described in field studies conducted by Castaldi et al. (2012) at a rainforest site in Ghana and by Werner et al. (2007) for a lowland rain forest in Kenya. While soil moisture contents close to water saturation may favour the development of anaerobiosis and therefore reduce organic matter decomposition and the soil CO_2 efflux (e.g. Smith 1990), it seems that our soils were predominantly aerobic even at 90 % WFPS due to the low bulk density of the topsoils (< 1 g cm^{-3}). Similarly, in Chinese montane, subtropical rainforests Zhou et al. (2013) found that soil CO_2 fluxes significantly increase with soil moisture at constant temperatures due to improved substrate availability for microbial respiration.

In our study, optimum soil moisture content for NO emission for different sites and land uses varied, which was probably an effect not only of land use but also of varying soil properties. ~~Many field and laboratory studies do as well report maximum NO fluxes at soil moisture levels in the range of 32-66% WFPS for various land uses (Chameides et al. 1992; Martin et al. 1998; Verchot et al. 1999; Akiyama et al. 2004; Akiyama et al. 2006; Li et al. 2007). The effect of WFPS on soil NO emissions was markedly lower~~The effect of WFPS on soil NO emissions was markedly lower than on N_2O fluxes, leading to a decrease of the NO: N_2O ratio with increasing WFPS, suggesting that denitrification or nitrifier-denitrification processes took over nitrification as source process for N trace gases with increasing soil moisture levels (Breuer et al. 2002; Butterbach-Bahl et al. 2004; Werner et al. 2007).

For the forest sites, our approach yielded flux rate estimates of 0.3 ± 0.2 kg N_2O-N ha^{-1} a^{-1} and 1.3 ± 0.2 kg N_2O-N ha^{-1} a^{-1} for TE-F and SH-F, respectively. ~~This is similar to what has been measured *in situ* at other tropical montane forests (0.3 – 0.8 kg N_2O-N ha^{-1} a^{-1}) (Riley et al. 1995; Ishizuka et al. 2002; Purbopuspito et al. 2006), but lower than most of the studies performed in tropical lowland forests (1.9 – 6.1 kg N_2O-N ha^{-1} a^{-1})~~This is similar to what has been measured *in situ* at other tropical montane forests (0.3 – 1.3 kg N_2O-N ha^{-1} a^{-1}) (Riley et al. 1995; Ishizuka et al. 2002; Purbopuspito et al. 2006; Koehler et al. 2009; Müller et al. 2015),

but lower than most of the studies performed in tropical lowland forests, which range between 1.9 and 6.1 kg N_2O-N ha^{-1} a^{-1} ~~(Keller et al. 1993; Serca et al. 1994; Keller and Reiners 1994; Verchot et al. 1999; Breuer et al. 2000; Melillo et al. 2001; Garcia-Montiel et al. 2003; Werner et al. 2007; Castaldi et al. 2013).~~(Keller et al. 1993; Serca et al. 1994; Keller and Reiners 1994; Verchot et al. 1999; Breuer et al. 2000; Melillo et al. 2001; Garcia-Montiel et al. 2003; Werner et al. 2007; Castaldi et al. 2013), with some studies showing exceptionally lower N_2O fluxes (e.g. Wieder et al. 2011, ca. 0.75 kg N_2O-N ha^{-1} a^{-1}). Some evidence suggests that in contrast to tropical lowland forests, tropical montane forests may be N limited, as they e.g. show low net N mineralization rates (Purbopuspito et al. 2006; Nottingham et al. 2015; Gütlein et al. 2016). ~~However,~~Our results ~~from our experiment are probably~~may be a conservative estimate since we removed the litter layer, which may have contributed to additional N_2O losses (e.g. Wang et al. 2014) and we only incubated the top 10 cm of soil, while N_2O fluxes of tropical soils have been shown to have their N_2O production optima at 5-20 cm soil depth (Nobre et al. 2001). On the contrary, no effective plant N uptake in the soil cores may have led to higher microbial N availability and thus, to a modest overestimate of N_2O fluxes compared to field conditions (Brumme 1995).

Fluxes of NO from soils of tropical montane forests have so far only rarely been reported, but the annual flux which we estimated (3.9 ± 1.8 kg NO-N ha^{-1} a^{-1}) seems to be higher than in previous studies in montane (0.03 – 0.4 kg NO-N ha^{-1} a^{-1}~~)(Johansson et al. 1988; Riley et al. 1995; Davidson and Kingerlee 1997; Purbopuspito et al. 2006))~~ (Johansson et al. 1988; Riley et al. 1995; Davidson and Kingerlee 1997; Purbopuspito et al. 2006; Koehler et al. 2009) and in lowland tropical forests (0.7 – 1.5 kg NO-N ha^{-1} a^{-1}) ~~(Keller et al. 1993; Serca et al. 1994; Keller and Reiners 1994; Verchot et al. 1999)~~(Keller et al. 1993; Serca et al. 1994; Keller and Reiners 1994; Verchot et al. 1999). However, Butterbach-Bahl et al. (2004) found that soils of a tropical lowland rainforest in Australia emitted approx. 3.0 kg NO-N ha^{-1} within a three-month period following the rewetting of soils after a drought period, ~~and, our~~suggesting that

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the emission of NO from tropical rain forest soils may still be underestimated. Our estimate is however very similar to the 3.0 kg NO-N ha^{-1} a^{-1} model-estimated by Gharahi Ghehi et al.(2014) for a tropical montane forest in Rwanda. ~~The high NO fluxes might be related to e.g. the high clay content, high SOC and low soil pH value in both studies.~~The high NO fluxes we found might be related to the high clay content, the high SOC and the low soil pH. Soil organic C content has been found to be positively correlated with NO emissions (Bouwman et al., 2002), and rates of microbial transformation of N are expected to be higher in soils with high SOC content (Matson et al. 1990; Li et al. 2005). Further, chemodenitrification (abiotic reduction of nitrite) may have contributed to some NO production due to the low soil pH found in our sites, as this process has been observed to occur under strongly acidic conditions, both in temperate (Venterea et al. 2003) and tropical environments (Serca et al. 1994, Gharahi Ghehi et al. 2014).

Annual estimates of soil N_2O emissions from eucalyptus plantations (TE-P) were, with ~~approximately 1~~1 ± 0.02 kg N_2O-N ha^{-1} a^{-1}, similar, though towards the lower end, to those in other studies covering measurements of N_2O fluxes from tropical rainforest forest soils.

Forest conversion to pasture influences strongly soil N cycling (mineralization, nitrification, and denitrification) and therefore also soil N_2O and NO fluxes (Davidson et al. 2000b). In our study, soils from smallholder grazing sites (SH-G) showed relative low N_2O (except at 90% WFPS) and low NO fluxes, in line with previous studies in tropical ecosystems (Keller et al. 1993; Veldkamp et al. 1999; Davidson and Verchot 2000; Garcia-Montiel et al. 2001; Melillo et al. 2001).

Background N_2O and NO emissions occurring in non-fertilized control areas are crucial for developing robust national emission inventories of nitrogenous gases and corresponding emission factors (Zheng et al. 2004); nevertheless, direct measurements of background emissions in tea plantations, especially measurements covering an entire year, have been rarely reported (Akiyama et al. 2006). The few studies available from commercial tea plantations are from Asia where large amounts of N fertilizers are applied (up to 2600 kg ha^{-1} a^{-1} ~~Fu et al.~~

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2012; Han et al. 2013; Li et al. 2013)Fu et al. 2012; Han et al. 2013; Li et al. 2013), while at the commercial tea plantation in our study 300 kg N ha^{-1} a^{-1} was applied. The calculated annual N_2O emission rate for commercial tea plantations in our study (0.9 ± 0.3 kg N_2O-N ha^{-1} a^{-1}) is relatively low compared to the 4-7 kg N_2O-N ha^{-1} a^{-1} estimated from "zero N-control" tea plantations in China and Japan (Akiyama et al. 2006; Fu et al. 2012; Yao et al. 2015) although those flux estimates were likely highly affected by the previous application of large amounts of N fertilizer (average of 553 kg N ha^{-1} a^{-1}). Regarding smallholder tea plantations, our annual N_2O estimations (0.1 ± 0.2 kg N_2O-N ha^{-1} a^{-1}-) are somewhat lower than those estimates from Rosenstock et al. (2015) who reported annual N_2O fluxes of 0.38 and4 ± 0.752 kg N_2O-N ha^{-1} $a^{-1}$and 0.7 ± 0.5 kg N_2O-N ha^{-1} a^{-1} kg N ha^{-1} a^{-1} in similar systems in Kenya and Tanzania., respectively. They further suggestsuggested that the emission factor from N application in smallholder tea systems would be below 1 % of N applied. Beyond N_2O, our work shows that tea plantations are a major source for NO. The estimated annual NO flux of 4.3 ± 0.7 Kg ha^{-1} a^{-1} and 2.1 ± 1.1 kg ha^{-1} a^{-1} for TE-T and SH-T, respectively is higher than the annual NO fluxes reported by Yao et al. (2015) for a tea plantation in China (1.6 ± 0.4 kg NO-N ha^{-1} a^{-1}, no N fertilizer application).

The higher N_2O and NO emissions (N_2O + NO) from soils of the commercial tea plantation (4.35.2 ± 0.5 kg N ha^{-1} a^{-1}) compared to soils of tea plantations from smallholder farmers (2.2 ± 0.3 kg N ha^{-1} a^{-1}) are very likely due to the long-term N fertilization and the subsequent soil acidification (Tokuda and Hayatsu 2004; Yamamoto et al. 2014). Soil from the commercial tea plantation (TE-T) in our study showed a mean pH value of 4.2, which was significantly lower than soils from tea plantations of smallholders (SH-T, mean: 5.0). SoilEnhanced soil acidity is an important factor affecting biotic and abiotic processes and consequently promoting N losses, by e.g. inducing chemo-denitrificationchemodenitrification and therefore NO losses but also N_2O (Ventera et al. 2003; Kesik et al. 2006; Medinets et al. 2015; Yao et al. 2015). Chemo-denitrification (process involving abiotic reduction of nitrite)(Venterea et al. 2003; Kesik et al.

2006; Medinets et al. 2015; Yao et al. 2015). Chemodenitrification has been suspected to be an important source of NO emissions from soils after drying and wetting of soil, and in excessively fertilized soils, as it is the case in the soils from the commercial tea plantations, where nitrite can accumulate ~~(Davidson 1992; Neff et al. 1995; Verchot et al. 1999)~~(Davidson 1992; Neff et al. 1995; Verchot et al. 1999). ~~Positive correlations of NO emissions with clay content and negative correlations with soil pH in our study give further supporting evidence that chemo-denitrification might play a significant role (Nelson and Bremner 1970). This reasoning agrees with conclusions by Serca et al. (1994) who found that chemo-denitrification in acidic forest soils is a potentially important cause of N oxide gases production. This is also in line with results from Gharahi Ghehi et al. (2014) who suggested that the acidic soils of the Nyungwe tropical montane forest in Rwanda in combination with high free iron contents could favour chemo-denitrification~~Thus, while relevant chemodenitrification may already occur in the forest and~~or~~ in the small holder tea areas, high rates of fertilization in combination with very low soil pH values may have further increased the contribution of chemodenitrification to the total NO efflux in the commercial tea plantations.

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~~In the tea estate area, it seems that the low pH effect was observed beyond the area cultivated with tea. The native forest soil had also significant lower pH values than the soils at the smallholder areas, having similar parental material and topographic conditions. This strongly suggests that airborne fertilization in nearby tea plantations has led to unintended N fertilization of the forest, dramatically increasing atmospheric N deposition and driving soil acidification.~~

5. Conclusions

Large uncertainties still exist with regard to the magnitude of soil NO and N_2O emissions from tropical African terrestrial ecosystems. Our observations contribute to a growing body of empirical evidence on soil trace gas emissions from different land uses in the African tropics

and their governing parameters. Temporal upscaling solely based on soil moisture carries additional uncertainty, since we were not able to include spatial variations in soil C and N availability for microbial processes. We present a conservative upscaling of flux rates which do not include the effect of consecutive watering-drying cycles. For a robust understanding of the trace gas exchange processes in tropical ecosystems, long-term observations at multiple sites are strongly required. Our results reveal aspects of control of N_2O, NO and CO_2 emissions that may assist to the development of baseline information required to develop land use and agricultural practices and management approaches aiming to ensure sustainable increases in productivity while reducing the contribution of agriculture to climate change.

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Field Code Changed

Table caption list

Table 1 Topsoil (0-10 cm) properties of individual sites (mean ± 1 standard deviation, n=3). Values followed by different letters indicate significant differences ($p \leq 0.05$) within columns.

Table 2 Average post-pulse fluxes (mean ± 1 standard deviation, n=3) of N_2O at different soil moisture levels (WFPS: water-filled pore space). Same letters indicate no significant differences ($p > 0.05$) between sites (uppercase) and between % WFPS (lowercase)

Table 3 Average post-pulse fluxes (mean ± 1 standard deviation, n=3) of NO at different soil moisture levels (WFPS: water-filled pore space). Same letters indicate no significant differences ($p > 0.05$) between sites (uppercase) and between % WFPS (lowercase)

Table 4 Average post-pulse fluxes (mean ± 1 standard deviation, n=3) of CO_2 at different soil moisture levels (WFPS: water-filled pore space). Same letters indicate no significant differences ($p > 0.05$) between sites (uppercase) and between % WFPS (lowercase)

Table 5 Estimated annual emission (± standard error of the estimate) without pulse emission contribution for different sites (SH: smallholder, TE: tea estate; F: forest, T: tea, G: grazing and P: eucalyptus plantations)

Figure caption list

Fig 1 Location of the experimental plots at a) the smallholders area and b) at the tea estate area. Different symbols denote different land uses

Fig 2 Schematic overview of the experiment. a) Incubation chamber. b) Incubation setup. Dots represent flux measurements for a given gas for an incubation chamber. Blue arrows represent watering events. Temperature (T) and relative humidity (RH) were kept constant at 18°C and 70% .c) Soil analysis. The incubation procedure outlined here was replicated four times for separate soil cores (once for each of the four soil moisture levels, WFPS: water-filled pore space)

Fig 3 Temporal evolution of the soil fluxes of nitrous oxide (N_2O) for different soil moisture levels (WFPS %) and different sites (SH: smallholder, TE: tea estate; F: forest, T: tea, G: grazing and P: eucalyptus plantations). Vertical bars indicate standard deviations of the three spatial replicates. Water was applied at day 2 and at day 5 to reach 20 % WFPS and targeted WFPS, respectively. Grey area indicates measurements used to calculate the mean post-pulse fluxes (day 9 to 12 of the incubation run)

Fig 4 Time-weighted average cumulative soil a) N_2O, b) NO and c) CO_2 emission rates during the different incubation periods and different levels of water filled pore space (WFPS) for different land uses (SH: smallholder, TE: tea estate; F: forest, T: tea, G: grazing and P: eucalyptus plantations)

Fig 5 Dynamics of soil fluxes of nitric oxide (NO) at different water-filled pore space (% WFPS) and for different sites (SH: smallholder, TE: tea Estate; F: forest, T: tea, G: grazing and P: eucalyptus plantation). Vertical bars indicate standard deviations of three spatial replicates. Water was applied at day 2 and at day 5 to reach 20 % WFPS and targeted WFPS, respectively. Grey area indicates measurements at the post-pulse period (day 9 to 12 of the incubation cycle)

Fig 6 Dynamics of soil fluxes of carbon dioxide (CO_2) at different water-filled pore space (% WFPS) and for different sites (SH: smallholder, TE: tea estate sites; F: forest, T: tea, G: grazing and P: eucalyptus plantation). Vertical bars indicate standard deviations of three spatial replicates. Water was applied at day 2 and at day 5 to reach 20 % WFPS and targeted WFPS, respectively. Grey area indicates measurements at the post-pulse period (day 9 to 12 of the incubation cycle)

Fig 7 Relationships between soil WFPS and N_2O (upper panel) and NO (lower panel) fluxes determined in laboratory experiments. The curve fits were used for calculating annual flux estimates for different sites (SH: smallholder, TE: tea estate; F: forest, T: tea, G: grazing and P: eucalyptus plantations) using observed *in situ* daily WFPS values at our meteorological observation site. The grey areas indicate the 95 % confidence intervals for the individual curve fits. Vertical bars indicate standard errors of three spatial replicates

Fig 8 Daily a) mean air temperature and b) cumulative rainfall from July 215 to July 2016

Fig 9 Daily values of a) soil NO, b) soil N_2O flux estimations and c) % WFPS at the natural forest site in the smallholder area (SH-F) from July 2015 to July 2016

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