Spatial variability of greenhouse gas (GHG) fluxes in tropical systems of East Africa: Effects of land use and topography

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Thesis submitted in partial fulfilment of the requirements of
the degree Doctor rer. nat of the Faculty of Environment and Natural Resources,
Albert-Ludwigs-Universität Freiburg im Breisgau, Germany

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Garmisch-Partenkirchen

2017

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I dedicate this thesis to my family,
for their constant support and unconditional love.
I love you all.

Summary

Studies of the soil-atmosphere trace gas exchange in tropical ecosystems are still scarce, especially for Africa. The lack of evidence-based data translates into considerable uncertainty about the direction and extent of the impact of land use change on soil trace gas emissions. The high uncertainty is increased by the high temporal and spatial variability of soil greenhouse gas (GHG) fluxes, which are known to vary due to micro site-specific soil properties and soil environmental conditions. Thus, quantifying and understanding the small-scale variability of soil GHG emissions is essential for improving the reporting of GHG budgets from ecosystems, to regional and national scale.

In my study, I evaluated the spatial variability of soil GHG fluxes at different spatiotemporal scales in tropical systems in Kenya. For that, first I developed a robust measurement technique -named "gas pooling"- to overcome the spatial variability associated to soil GHG fluxes. The technique was further applied in soil laboratory incubation experiments aiming at assessing and quantifying the spatial variability of soil nitrous oxide (N_2O) and carbon dioxide (CO_2) emission and their relation to topography and soil properties in a tropical montane forest. Finally I evaluated the differences in soil N_2O, nitric oxide (NO) and CO_2 fluxes for different land uses and management practices using intact soil cores taken from natural forest, commercial and small-holder tea plantations, eucalyptus plantations and grazing land in the Mau region, Kenya, and incubated them in the laboratory under controlled temperature and soil moisture conditions.

My data show that in tropical mountain forest ecosystems topography has a major influence on soil N_2O emissions, with soils at mid-slope position emitting significantly less N_2O than at ridge tops and valley bottom positions. Soil N_2O and CO_2 fluxes were not spatially correlated, but showed a high spatial variability with "hot spots" strongly contributing to the total soil N_2O and CO_2 emissions of the investigated plots. The high spatial variation was further increased by changes in vegetation and soil properties resulting from human disturbance of the tropical mountain forests associated with charcoal production. Further, my results show that it remains difficult to reduce uncertainty in emission estimates by running a manageable number of flux measurements by the chamber technique.

Nevertheless, specifically the gas pooling protocol for static chamber measurements provides guidelines and recommendations on how to consider spatial variability while reducing number of gas samples. With differences of only 2-8 % for CO_2 and 3-4 % for N_2O, means of individual chamber fluxes compared very well with fluxes pooled over several chambers, showing that the gas pooling technique is an acceptable approach to integrate spatial heterogeneity.

Incubation of soil cores under controlled conditions in the laboratory allowed for the estimation of soil N_2O and NO annual fluxes from tropical montane ecosystems by using *in-situ* measured soil moisture values. For forest and eucalyptus sites my approach yielded annual flux estimations of 0.3-1.3 kg N_2O-N ha^{-1} yr^{-1} and 2.1-5.2 kg NO-N ha^{-1} yr^{-1}. Highly fertilized soils of commercial tea plantations showed significantly higher emissions (0.9 kg N_2O-N ha^{-1} yr^{-1} and 4.3 kg NO-N ha^{-1} yr^{-1}) compared to small-holder tea plantations (0.1 kg N_2O-N ha^{-1} yr^{-1} and 2.1 kg NO-N ha^{-1} yr^{-1}) and grazing land (0.1 kg N_2O-N ha^{-1} yr^{-1} and 1.1 kg NO-N ha^{-1} yr^{-1}). High soil N-oxide emissions from the soils of tea plantations were probably the consequence of long-term N fertilization and associated soil acidification, which likely promoted NO, but possibly also N_2O production by chemodenitrification. However, a comparison between annual flux estimates derived by laboratory measurements versus those from field measurements is still missing.

The results of my thesis highlight that soil N and C trace gas fluxes in tropical montane systems are highly variable and affected by land use, management and soil environmental conditions. The developed laboratory approach for estimating annual fluxes may however help to identify hotspots of soil GHG fluxes in understudied regions. My thesis substantially contributes to a growing body of empirical evidence on soil trace gas emissions from different land uses in the African tropics and their governing parameters.

Zusammenfassung

Studien zum Boden-Atmosphäre-Austausch von atmosphärischen Spurengasen tropischer Ökosysteme sind bisher nur spärlich verfügbar, insbesondere im Hinblick auf terrestrische Systeme in Afrika. Der Mangel an verfügbaren Daten hat zur Konsequenz das erhebliche Unsicherheiten bezüglich der Richtung und des Ausmaßes von Auswirkungen von Landnutzungswandel auf den Boden-Atmosphäre Austausch von Spurengasen und hier insbesondere der Treibhausgase N_2O, CH_4 und CO_2, besteht. Diese schon bestehende hohe Unsicherheit wird noch weiter dadurch verstärkt, dass Boden-Treibhausgasflüsse eine hohe zeitliche und räumliche Variabilität aufweisen, da die Produktion, Konsumption und Emission von Bodenspurengasen von der kleinräumigen Variabilität von Bodeneigenschaften und regionalen Unterschieden in klimatischen Bedingungen abhängen. Für eine bessere Quantifizierung von Treibhausgasbudgets terrestrischer Systeme von der Ökosystemskala bis zur nationalen Skala ist es daher nötig die kleinräumige Variabilität des Boden-Spurengasaustausches zu verstehen und treibende Faktoren zu identifizieren.

In meiner Arbeit habe ich die zeit-räumliche Variabilität von Boden-Treibhausgasflüssen in tropischen Ökosystemen von Kenia untersucht. Hierzu habe ich zuerst eine neue Messmethodik etabliert, die sogenannte „gas pooling“ Technik. Diese Technik erlaubt es die kleinräumliche Variabilität von Bodenspurengasflüssen unter deutlicher Reduktion des analytischen Aufwandes zu adressieren. Die „gas pooling“ Technik wurde auch in Laboruntersuchungen an intakten Bodenkernen eingesetzt, welche zum Ziel hatte die räumliche Variabilität von Boden-Lachgas (N_2O) und Kohlendioxid (CO_2) Flüssen in tropischen, montanen Waldökosystemen in Beziehung zu topographischen Gegebenheiten und Bodenparametern zu setzen. Der abschließende Teil meiner Arbeit hat sich damit auseinandergesetzt wie Landnutzung in der Mau Region in Kenia die Boden-Spurengasflüsse von N_2O, Stickstoffmonoxid (NO) und CO_2 beeinflusst hat. Hierzu habe ich intakte Bodenkerne von Flächen in der Mau Region, Kenia, gewonnen, die entweder als tropischer, montaner Regenwald, Eukalyptus-Plantage, kommerzielle oder kleinbäuerliche Teeplantage oder Weideland genutzt wurden. Diese Bodenkerne wurden im Labor am IMK-IFU unter kontrollierten Temperatur- und Bodenfeuchtebedingungen inkubiert und die Spurengasflüsse erfasst.

Meine Resultate zeigen das in montanen, tropischen Regenwäldern die Topographie einen entscheidenden Einfluss auf die Höhe der Boden-N_2O-Flüssen haben. Die höchsten Boden-N_2O Flüsse wurden dabei für Kamm- und Tallagen festgestellt, während die Boden-N_2O-Flüsse in Hanglagen deutlich niedriger waren. Die Boden-N_2O und CO_2 Flüsse waren kleinräumlich auf einer Skala von 1 m nicht korreliert. Allerdings zeigten meine Untersuchungen auch, dass eine hohe räumliche Variabilität mit ausgeprägten „hotspots" der Emissionen existieren. Diese „hotspots" waren teilweise durch Störungen von Bodeneigenschaften- und Vegetationsbedeckung aufgrund anthropogener Nutzung des Waldes zur Produktion von Holzkohle hervorgerufen. Desweiteren zeigen meine Untersuchungen, dass es schwierig bleiben wird die kleinräumliche Variabilität von Bodenspurengasemissionen in tropischen, montanen Regenwaldökosystemen mit einer limitierten Anzahl von herkömmlichen Kammermessungen adäquat zu adressieren.

Eine Lösung dieses Problem bietet hier die „gas pooling" Technik, da diese es erlaubt kleinräumige Variabilität zu adressieren, bei gleichzeitiger Reduktion der Anzahl der zu analysierenden Gasproben. Die mittleren Differenzen zwischen Gasflüssen verschiedener Kammern, die entweder mittels der „gas pooling" Technik ermittelt oder aus den Ergebnissen von Messungen an einzelnen Kammern errechnet wurden, unterschied sich nur um 2-8 % für CO_2 Flüsse und 3-4 % für N_2O Flüsse. Dies zeigt, dass die „gas pooling" Technik sehr gut dazu geeignet die kleinräumliche Variabilität von Bodengasflüsse unter deutlicher Verringerung des Messaufwandes zu integrieren.

Unter Einbeziehung der im Feld beobachteten zeitlichen Variabilität der Bodenfeuchte konnte ich die Ergebnisse des Labor-Parametrisierungsversuches mit Bodenkernen dazu nutzen den Einfluss von Landnutzung auf die jährlichen Bodenspurengasflüsse abzuschätzen. Für Böden von montanen Regenwäldern und Eukalyptusplantagen in der Mau Region ergaben meine Berechnungen jährliche Emissionsraten von 0.3-1.3 kg N_2O-N ha^{-1} yr^{-1} and 2.1-5.2 kg NO-N ha^{-1} yr^{-1}. Die intensiv gedüngten Böden kommerzieller Teeplantagen emittieren danach wesentlich höhere Mengen an N-Spurengasen (0.9 kg N_2O-N ha^{-1} yr^{-1} und 4.3 kg NO-N ha^{-1} yr^{-1}) als Böden von kleinbäuerlichen Teeplantagen (0.1 kg N_2O-N ha^{-1} yr^{-1} and 2.1 kg NO-N ha^{-1} yr^{-1}) oder Weideböden (0.1 kg N_2O-N ha^{-1} yr^{-1} and 1.1 kg NO-N ha^{-1} yr^{-1}). Die hohen N-Spurengasemissionen von Böden kommerzieller Teeplantagen ist wahrscheinlich eine Konsequenz der jahrzehntelangen hohen Stickstoff-Düngung und der damit einhergehenden,

beobachteten Versauerung der Böden, welche insbesondere zu höheren Boden-NO aber auch höheren Boden-N_2O-Emissionen durch Chemo-Denitrifikation führt.

Inwieweit meine auf Laborinkubation und Feld-Bodenfeuchtemessungen ermittelten jährlichen N_2O und NO Bodenemissionen jedoch in-situ Verhältnisse widerspiegeln wird letztendlich nur ein Vergleich mit Feldmessungen zeigen.

Die im Rahmen meiner Doktorarbeit erzielten Ergebnisse bestätigen das die vermutete hohe Variabilität von Boden- N und –C Spurengasflüsse in der Mau-Region, Kenia, existiert und das diese in hohem Maße durch Landnutzung, Landmanagement und Bodenumweltbedingungen beeinflusst wird. Der entwickelte Labor-Parametrisierungsansatz zur Abschätzung von Bodenspurengasemissionen eröffnet die Möglichkeit zur Gewinnung eines besseren Verständnisses von Treibern der Bodenspurengasemissionen in bisher nur marginal untersuchten Regionen Afrikas und anderswo. Mit der Entwicklung innovativer Versuchs- und Extrapolationsansätzen hat meine Arbeit damit neue Informationen zu Bodenspurengasemissionen für das tropische Afrika aufgezeigt und einen wesentlichen Beitrag zum wachsenden Kenntnisstand zu Auswirkungen des Landnutzungswandel auf Bodenspurengasemissionen bereitgestellt.

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1 Introduction

1.1 Motivation

The gases nitrous oxide (N_2O) and carbon dioxide (CO_2) are important greenhouse gases (GHG) directly contributing to climate change (IPCC, 2014), while nitric oxide (NO) is a key chemical substance involved in the tropospheric production of ozone, which is also a potent GHG and due to its oxidative capacity a thread to ecosystem and human health (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 (Butterbach-Bahl et al., 2009; Conrad, 1996). The contribution of total annual soil emissions to their respective atmospheric budgets are estimated to be 35 % for CO_2, 53 % for N_2O and 21 % for NO (IPCC, 2007). As these estimates are still highly uncertain there is an urgent need to properly assess soil GHG-sources and to develop a better understanding on how soil-atmosphere fluxes are affected by land use and environmental changes.

Soil production of CO_2 is the result of root respiration and microbial decomposition of soil organic matter, which returns to the atmosphere some of the carbon (C) fixed in the first step through photosynthesis (Paterson et al., 2009). Soil N_2O and NO releases are driven by the microbial processes of nitrification (oxidation of NH_4^+ to NO_3^- via NO_2^-) and denitrification (reduction of NO_3^- to N_2O and N_2) (Butterbach-Bahl et al., 2013). Soil physical, chemical and biological factors as well as environmental conditions such as temperature and moisture control these processes, which vary both in space and time. As a result, the magnitude of soil N_2O, NO and CO_2 emissions vary considerably across spatial and temporal scales.

Understanding the factors that affect spatial and temporal variability of soil GHG emissions is an important area of ongoing research. While both soil temperature and water content have been shown to be key factors responsible for the variation in soil GHG fluxes, a different picture is emerging for tropical soils. Soil water content regulates the activity of microorganisms and the availability of oxygen for respiratory processes in the soil (Butterbach-Bahl et al., 2013). As soil temperature does not vary greatly in many tropical regions, it is often found that variations in soil moisture are a more significant factor affecting temporal and spatial variations of soil trace gas fluxes as compared to temperature (Butterbach-Bahl et al., 2004; Davidson et al., 2000).

Land use change involves major transformations of the soil-plant-atmosphere continuum. As a result of land-clearing, fires, mechanical ploughing and compaction,

vegetation change, fertilization, etc., the soil system is highly altered from its previous state. Specifically land use change provokes significant changes in micro-climate and soil environmental properties such as bulk density, porosity, moisture, water-filled pore space, temperature, mineral N content and pH (Farquharson and Baldock, 2008). All these factors are known to influence the consumption and production of trace gases in the soil and therefore the trace gas exchange at the soil-atmosphere interface (Davidson and Verchot, 2000; Saiz et al., 2006; Tang et al., 2006).

Understanding land use effects on soil GHG fluxes remains difficult due to the 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., 2017a). Unfortunately, *in-situ* high-resolution soil GHG measurements in remote tropical ecosystems are usually constrained by limited logistics and available infrastructure. To overcome these constraints, soil samples can be taken to the laboratory for targeted incubation experiments, e.g. for studying spatial and temporal variability of fluxes in relation to changes in environmental conditions. However, the nature of incubation experiments (i.e., the need for replicated, high frequency sampling and climate controlled storage) severely limits the number of soil samples that can be handled in such experiments. On the other hand, when investigating GHG fluxes in landscapes with a mosaic of land uses, the total number of samples needed to provide a reliable flux estimate quickly exceeds analytical capacities (Arias-Navarro et al., 2013). Therefore, practical methods are needed to quantify soil GHG fluxes in order to better understand magnitudes, spatial and temporal variability of soil-atmosphere trace-gas exchange. Specifically, in view of the importance of terrestrial ecosystems as sources and sinks for atmospheric GHGs (IPCC, 2007) and the need to mitigate climate change there is an urgent need to develop improved management practices aiming towards lower soil GHG emissions.

1.2 Objectives

Studies of the soil-atmosphere trace gas exchange in tropical ecosystems are still scarce, especially in Africa (Kim et al., 2015; van Lent et al., 2015). The lack of evidence-based data translates into considerable uncertainty about the direction and extent of the impact of land use change on soil trace gas emissions. Land use change from natural forest ecosystems into other more intensively managed land uses 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 land use, tropical montane forests are particularly endangered because they are located in areas highly suitable for agricultural production; thus, according to different scenarios, the expansion of cropland, grazing areas, and forest plantations at the extent of natural forests is expected to continue (Potting and Bakkes, 2004).

In my work I aimed to quantify the contribution of land use to soil trace gas fluxes in a tropical montane forest ecosystem of Kenya, the Mau Forest region. The pressure on Kenya's Mau Forest and the land use changes occurring in and around it are among the most pressing environmental issues the country is facing today, raising questions about the future of the forest—and of the millions of people who depend on the services and products it provides.

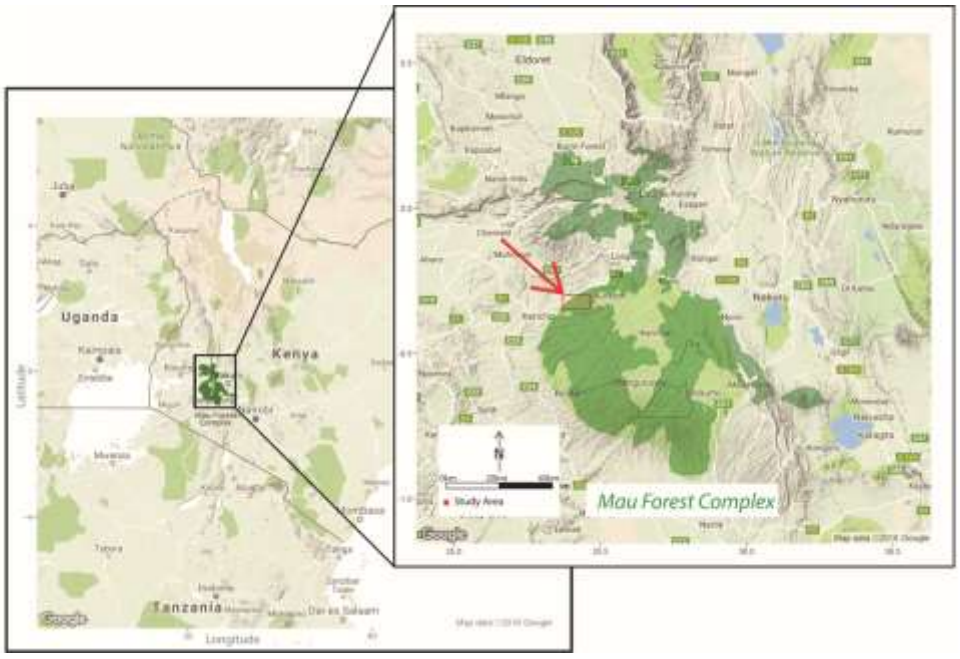

Figure 1 Localization of the study area.

My study sites were located in the Southwest Mau Forest, east of Kericho town (-0° 22' 3 S, 35° 16' 59 E) in Kenya (Figure 1). The 135000 hectares South-West Mau Forest is part of the largest indigenous Afromontane forest in East Africa. It is also the largest closed-canopy forest system in Kenya.

The climate is a cool and humid tropical climate with a mean annual precipitation between 1800 and 1950 mm (1979-2009) (Omumbo et al., 2011). The area has a bi-modal rainfall pattern, with the "long rains" falling between April and August and "short rains" between October and December, respectively, while January and February are generally the driest months. The mean annual temperature ranges from 15.7 to 17.5 °C (1979-2009) (Omumbo et al., 2011) with slight variations over the year. The geology is dominated by Tertiary lavas from the mid-Miocene epoch (Blackie and Edwards, 1979). The soils are well drained, very deep, dark reddish-brown, clayey, and with an acidic humic topsoil (Jaetzold et al., 2010; Krhoda, 1988) and classified as Andic Humic Nitisols (IUSS Working Group WRB, 2015).

In the last decades the Mau Forest region has experienced a substantial loss of forest land at the extent of other land uses, mainly tea, leading to a reduction of over 25 % of the forest area (Government of Kenya, 2010). The land use in the area is a mixture of commercial tea and tree plantations, natural forest and small-holder agriculture. Both large estates and small-holder farms produce tea. Small-holder farms are typically less than half a hectare, with most of the land planted with tea and usually no more than 20 % of land reserved for food crops and grazing (Milder et al., 2015). Commercial tea-estates grow tree woodlots as source of firewood for the tea factories. The commercial tea-estate plantations have usually higher yields compared to those managed by small-holder producers, as more fertilizers are used and plantation management such as pruning and harvest is professionalized (Kenya Human Rights Commision, 2008). Whereas the border of the natural forest is relatively stable close to commercial tea plantations, there is a lot of encroachment and forest degradation through cattle grazing, collection of firewood, illegal logging and charcoal burning at sites where the forest borders small-holder farms.

The main goal of my thesis was to evaluate the spatial variability of soil GHG fluxes at different scales in tropical systems in Kenya. This study was designed to:

- Develop and test a less-expensive measurement methodology to overcome spatial variability of soil N_2O and CO_2 fluxes.
- Assess and quantify the spatial variability of soil N_2O and CO_2 emission potential and soil properties at varying topographic positions in tropical montane forest in Kenya to optimize sampling strategies.
- Evaluate differences in soil N_2O, NO and CO_2 fluxes for different land uses in the Mau region, Kenya.

- Analyse the effects of tea plantation management (commercial versus small-holder) on the soil N_2O, NO and CO_2 fluxes.

I hypothesized that:

i) Pooling of gas samples, analogously to soil sample pooling, is an acceptable approach for chamber-based GHG flux measurements to overcome spatial heterogeneity of soil N_2O and CO_2 fluxes.

ii) Small-scale spatial variability of soil GHG fluxes is affected by topography (valley bottom, mid-slope, and ridge top).

iii) Soil nitrogen (N) trace gas emissions increase with increasing land use intensity and such being highest for tea plantations as compared to natural forest, tree plantations and low N input agricultural systems.

iv) That higher N fertilizer use and longer tea growing history result in higher soil N trace gas fluxes from soils taken from commercial tea-estates plantations as compared to those taken from small-holder farms.

2 Sampling technique to overcome spatial heterogeneity of soil GHG fluxes

Static chambers are the most widely used technique for measuring soil greenhouse gas (GHG) fluxes (Butterbach-Bahl et al., 2016a) due to relatively low costs, simple operation, and portability (Butterbach-Bahl et al., 2011; Denmead, 2008).
The basic principle of static chamber measurements is that a number of gas samples (three to six) are taken over a period of time from the headspace of a gas-tight chamber enclosing the soil surface. Greenhouse gas fluxes are calculated from the rate of change in the headspace gas concentration over time.

Soil fluxes of GHGs vary significantly over space and time driven by microbiological processes, environmental conditions, heterogeneity of soil properties as well as spatial variation in available nutrients and root distribution (Butterbach-Bahl et al., 2011; Davidson and Verchot, 2000; Verchot et al., 1999). Due to the presence of "hot spots" in soils, e.g. due to the incorporation of crop residues or dying of plant roots, fluxes may differ by 100 %, even within a few centimetres (Parkin and Venterea, 2010). Thus, having too few sampling locations could seriously undermine the quality of an estimate of soil GHG fluxes at ecosystem scale and, thus, also could hamper the calculation of GHG budgets for a specific land use/ecosystem (Arias-Navarro et al., 2017a). Multiple chamber measurements are therefore required to overcome such small-scale variability and capture flux dynamics, to finally conclude on a representative flux rate at plot and ecosystem scale. When multiplying the number of samples needed per chamber for calculating soil GHG fluxes, the number of replicate chambers per site or treatment to be representative for a site/treatment, and the instrument analytical time (three to six min per gas sample analysis), the resource demand in terms of labour and laboratory time of the chamber methodology becomes apparent. The consequence is that our understanding of the diversity and heterogeneity of flux rates across different land uses, landscapes, or management practices aiming at mitigating soil GHG fluxes is extremely limited, especially in parts of the world where advanced laboratory facilities are scarce, as for example in Africa.

To reduce the time and analytical costs of monitoring soil GHG fluxes in complex landscapes, I developed a novel sampling method –called "gas pooling". Pooling of samples is a widely accepted method for obtaining a representative soil sample, but the same principle has not been applied to soil emission measurements before (Arias-Navarro et al., 2013).

To evaluate if pooling of gas samples can also be used while measuring soil GHG fluxes with the chamber method I conducted an experiment to determine if it was necessary to analyse each gas sample separately, or if I could combine gas samples from several chambers before analysing them, thus reducing numbers of gas samples. For evaluation, I measured N_2O and CO_2 soil-atmosphere fluxes at three contrasting sites –forest, cropland and grassland– with five chambers each over a four-week period and applied the gas pooling technique as outlined in Figure 2. Details about the sites, the measurement campaign and chamber specifications are described in Arias-Navarro et al. (2013).

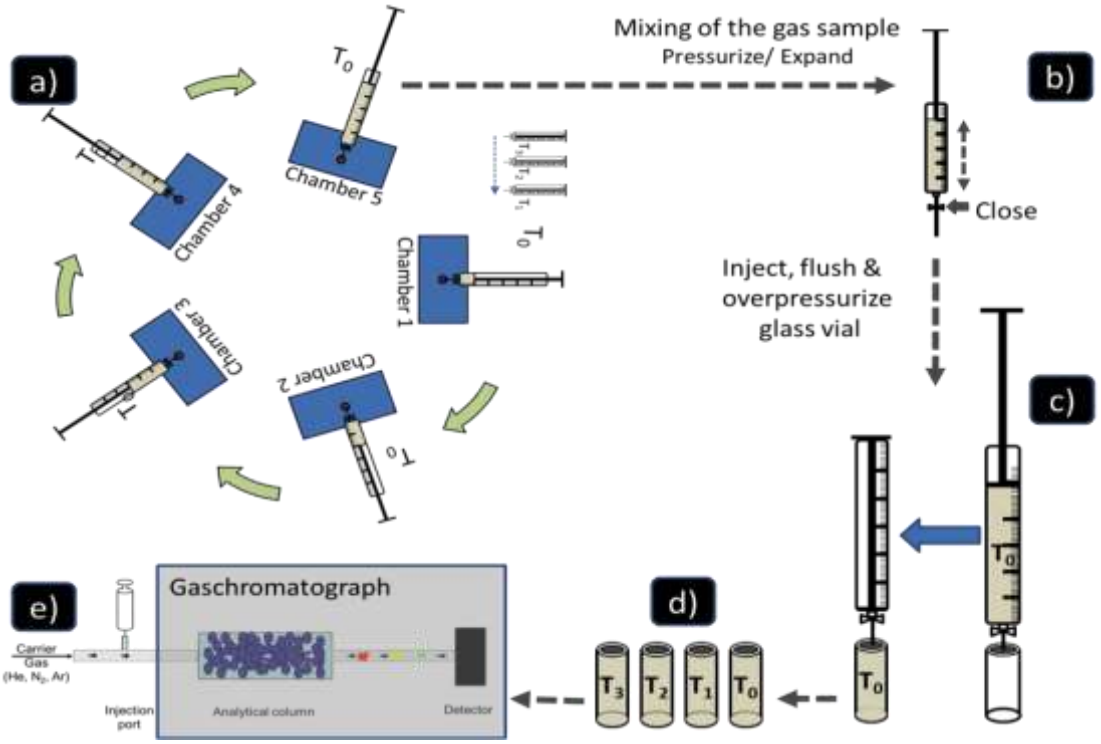

Figure 2 The concept of gas pooling. a) Gas pooling across chambers for a given sampling time, b) gas sample mixing within the syringe, c) transfer of the gas sample to a vial, d) four vials for four sampling times and five chambers, e) air sample analysis via gas chromatography (Modified from Arias-Navarro et al., 2013; Butterbach-Bahl et al., 2016a).

In short: for comparing the conventional gas sampling approach with the gas pooling technique I collected a 50 ml gas sample from each individual chamber headspace with a gas tight syringe through a stopcock valve at 10-minute intervals (T_0=0, T_1=10, T_2=20, T_3=30, and T_4=40 min after chamber closure). For the sample pooling technique I subsequently took a 10 ml gas sample from each of the same five chambers with the same syringe at each time interval equalling 50 ml in total (Figure 2, Modified from Arias-Navarro et al., 2013; Butterbach-Bahl 2016a). The 50 ml gas samples from the individual and pooled chambers were then immediately transferred into 10 ml glass vials for both methods. Samples were analysed by gas

chromatography within the 1-2 days (8610C; SRI Instruments, Torrance, CA, USA) with a ^{63}Ni-Electron capture detector for N_2O and flame ionization detector equipped with a methanizer for CO_2. Flux rates of N_2O and CO_2 were calculated from the linear change in gas concentrations in the chamber headspace with time. Further details on the analytical procedure can be found in Rosenstock et al. (2015).

Following gas analyses and calculation of fluxes, I compared fluxes obtained with the pooling technique with the mean flux rates obtained by sampling the individual chambers following the traditional method. The comparison showed that mean values across the entire observation period calculated for single chambers and the gas sample pooling method differed by 3-4 % for N_2O. Linear regression between gas pooling and conventional sampling revealed no systematic bias ($y = -2 \pm 0.99\ x$; $R^2 = 0.99$) (Figure 3a). Comparable results were obtained for soil CO_2 fluxes, i.e., fluxes calculated on basis of the gas pooling technique differed by 2-8 % when compared to individual chamber means without a systematic bias ($y = 7.1 \pm 0.94\ x$, $R^2 = 0.98$) (Figure 3b).

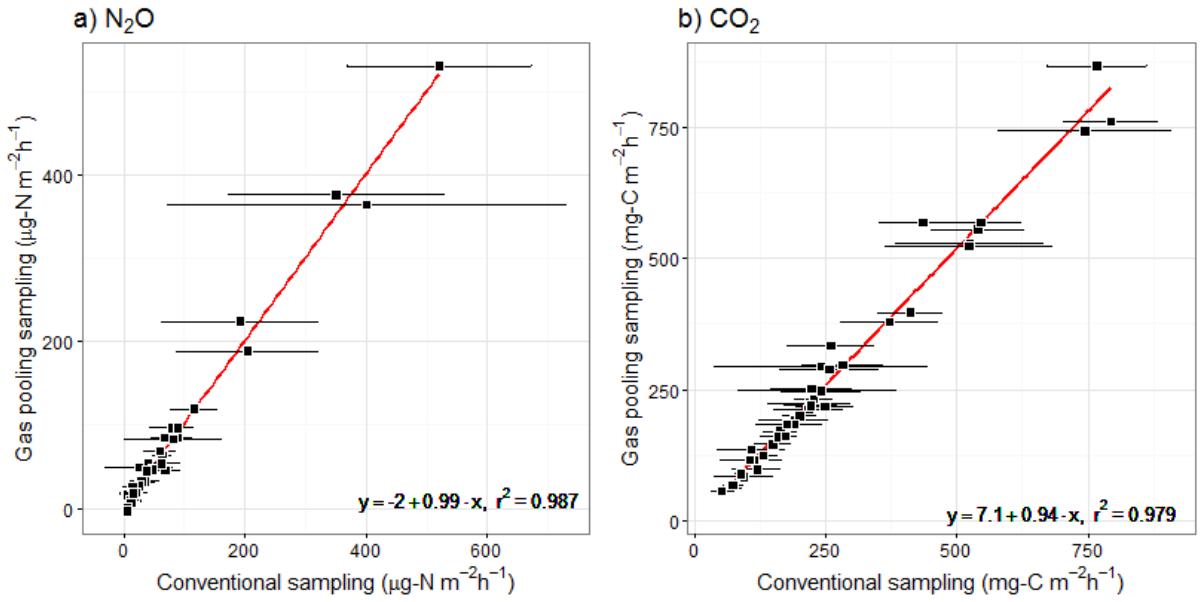

Figure 3 Pooled versus mean individual measurements for a) N_2O and b) CO_2 fluxes.

Comparison of soil CO_2 and N_2O fluxes obtained by the gas pooling technique and by the conventional sampling shows the reliability of the gas pooling technique. Thus, gas pooling for flux measurements compares well with results obtained when soil and water samples are pooled in ecosystem studies (Patil, 2006; Pennock et al., 2006; Wilde, 2006). Gas pooling has the potential to overcome small-scale variability of soil GHG fluxes and, thus, is a valuable

technique to increase the amount of information available on exchange of GHGs between the biosphere and atmosphere.

3 Spatial variability of soil GHG in mountain tropical landscapes of Kenya

3.1 Influence of topography and soil parameters on soil N_2O and CO_2 fluxes in mountain tropical forests

3.1.1 Field sites and experimental design

In this subchapter I aimed to identify controls of the small scale variability of soil greenhouse gas (GHG) fluxes. The experimental work was carried out at tropical montane forest sites in the South West Mau forest region (Figure 1). I used a stratified sampling approach to select sampling plots based on topographic information. For that I segmented the study area (4 km x 3 km; figure 4a, Arias-Navarro et al., 2017a) into three topographic classes representing ridge top, mid-slope and valley bottom positions. Three transects across the forest served as spatial replicates. Along these transects, I selected a one-ha plot for each topographic position, resulting in nine plots (figure 4a, Arias-Navarro et al., 2017a). Further details about the landscape segmentation and the selection criteria for plot identification can be found in Arias-Navarro et al. (2017a). Within each one-ha plot, 90 sampling locations were randomly placed on the nodes of a 5 m x 5 m grid (figure 4b, Arias-Navarro et al., 2017a) yielding a total of 810 sampling locations. At each sampling location seven intact soil cores were collected from the uppermost 5 cm of the mineral soil profile using sharpened-edge PVC cylinders (5 cm inner diameter, 5 cm height). The cylinders were carefully taken out of the soil, sealed with Parafilm® (Bemis NA, Neenah WI, USA) and transported to the laboratory at Mazingira Centre, International Livestock Research Institute (Nairobi, Kenya) for analysis. The intact soil cores were air-dried and stored at ambient temperature until analyses were conducted. Four intact soil cores were used for GHG sampling and the other three were used for soil analysis.

Some of the plots showed signs of anthropogenic disturbance such as charcoal burning and illegal logging. To capture these disturbance effects, at each sampling location I recorded: i) percentage of canopy cover using a Crown Mirror–Densiometer (Grube KG Forstgerätestelle, Bispingen, Germany), ii) shortest distance to a charcoal kiln. Further I recorded number of charcoal kilns within each one-ha plot.

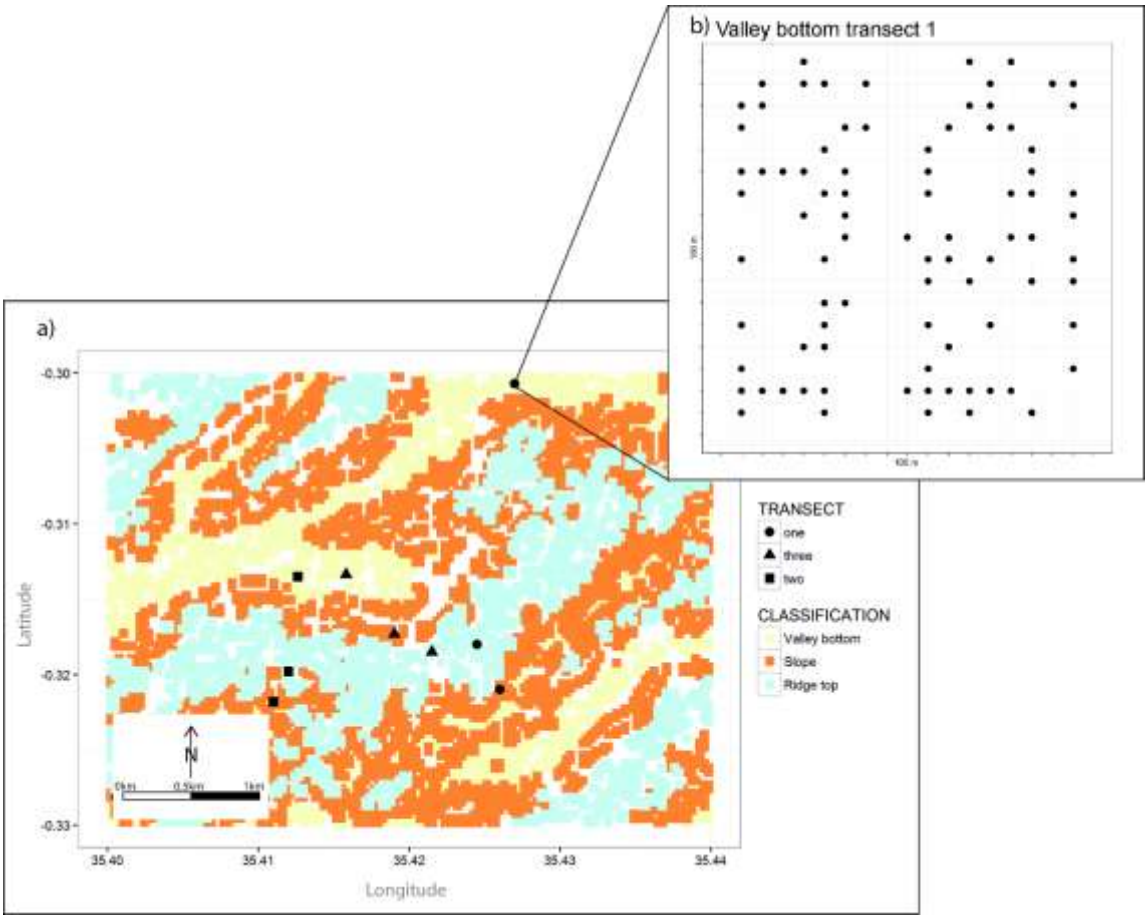

Figure 4 Sampling approach. a) Topographic classification of the study area and localization of the sampling plots, b) diagram of the sampling scheme in the Valley bottom transect 1 plot showing the 90 random sampling locations on a grid of 5 m x 5 m (source: Arias-Navarro et al., 2017a).

Soil properties

Total soil organic carbon (SOC), total nitrogen (TN), pH and bulk density (BD) were measured using three spare soil cores for each sampling location. Total SOC and TN were analysed with an Elemental Combustion Analyser (ECS 4010, Costech Analytical Technologies, Inc., Milano, Italy). Soil pH was measured in deionized water suspension (water: soil weight ratio 2.5:1) using a glass electrode. Bulk density (BD) was calculated as the mass of the oven-dry soil (105 °C) divided by the core volume.

Experiment 1: Potential N_2O and CO_2 flux measurements

I measured potential soil N_2O and CO_2 emissions from air-dried and rewetted soil samples. For soil rewetting, distilled water equivalent to a rainfall of 10 mm, was added to the surface of the soil cores. Water addition increased the soil moisture to approx. 50 % water-holding capacity (WHC) which was close to the moisture content of the soil during soil sample collection. Following rewetting, the soil was allowed to equilibrate for 24 hours to the new moisture condition before measurements of soil N_2O and CO_2 emissions started. Air

temperature during the incubation was kept constant at 17 °C as this represents the annual mean temperature in the Mau Forest.

Soil cores were incubated using adapted gas-tight Kilner jars (volume 640 cm^3) fitted with rubber septum in the lid for headspace gas sampling by a syringe (Figure 5). I used four soil core replicates and pooled the samples as per Arias-Navarro et al. (2013) for each flux calculation, i.e. 10 ml gas sample was taken from each jar headspace at 15 min intervals (T_0=0, T_1=15, T_2=30 and T_3=45 min after chamber closure) with the same syringe at each time interval resulting in a 40 ml composite sample (Figure 5). The first 20 ml of the sample was used to flush a 10 ml glass vial, which was filled with the remaining 20 ml creating over-pressure to minimize the risk of contamination with ambient air.

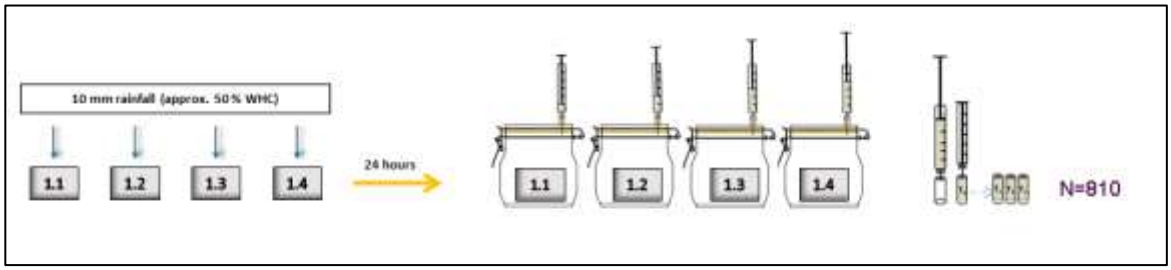

Figure 5 Schematic overview of the incubation set up in experiment 1. Gas pooling was applied across soil core replicates (1.1 -1.4 refers to replicated intact soil cores taken from one sampling location). The incubation outlined in this figure was performed for the 810 sampling locations.

The gas pooling technique allowed to i) integrate centimeter-scale variability within sampling locations and ii) to obtain enough gas sample to fill the glass vial without creating a significant pressure change -associated with the removal of an air sample- inside the Kilner jar (pressure difference between samples < 1.5 %).

Concentrations of CO_2 and N_2O in the gas samples were analysed within 2-3 days by gas chromatography (8610C; SRI Instruments, Torrance, CA, USA) with a ^{63}Ni-Electron capture detector for N_2O and flame ionization detector equipped with a methanizer for CO_2. I calculated soil CO_2 and N_2O fluxes from the linear change of the gas concentrations over time, considering the headspace volume and referred the fluxes to the soil surface of the core. Further details on the analytical procedure can be found in Rosenstock et al. (2015).

Experiment 2: Effect of soil water content on soil N_2O and CO_2 fluxes at different topographic positions

I conducted a second incubation study using, after air drying, the same soil cores as in experiment 1. The aim was to examine the effect of defined soil moisture contents on soil N_2O

and CO_2 fluxes at the different topographic positions. Due to the analytical capacity of the laboratory I used a subset of cores from transect 3. Based on results from experiment 1, the soil cores from each topographic position were classified in three classes: high potential N_2O emission (upper ⅓ of all cores previously investigated), medium potential N_2O emission (⅓–⅔ of all cores previously investigated) and low potential N_2O emission (lowest ⅓ of all cores previously investigated) (Figure 6a). From each emission potential class, 20 sampling locations were randomly selected and gas pooling was applied in groups of four samples (Figure 6c). To avoid a bias of follow-up measurements, all soil moisture levels were analysed with independent cores adjusted to defined soil moisture contents of 20, 50, 70 and 90 % WHC adding distilled water to the soil surface (Figure 6b).

Gas fluxes from soil cores were measured 4, 24, 48 and 72 hours after the initial rewetting. Determination of GHG concentrations and calculation of GHG fluxes was done similarly to Experiment 1. Cumulative N_2O and CO_2 emissions over the three-day incubation period were calculated from the integrated individual fluxes, assuming a constant flux rate between gas sampling times.

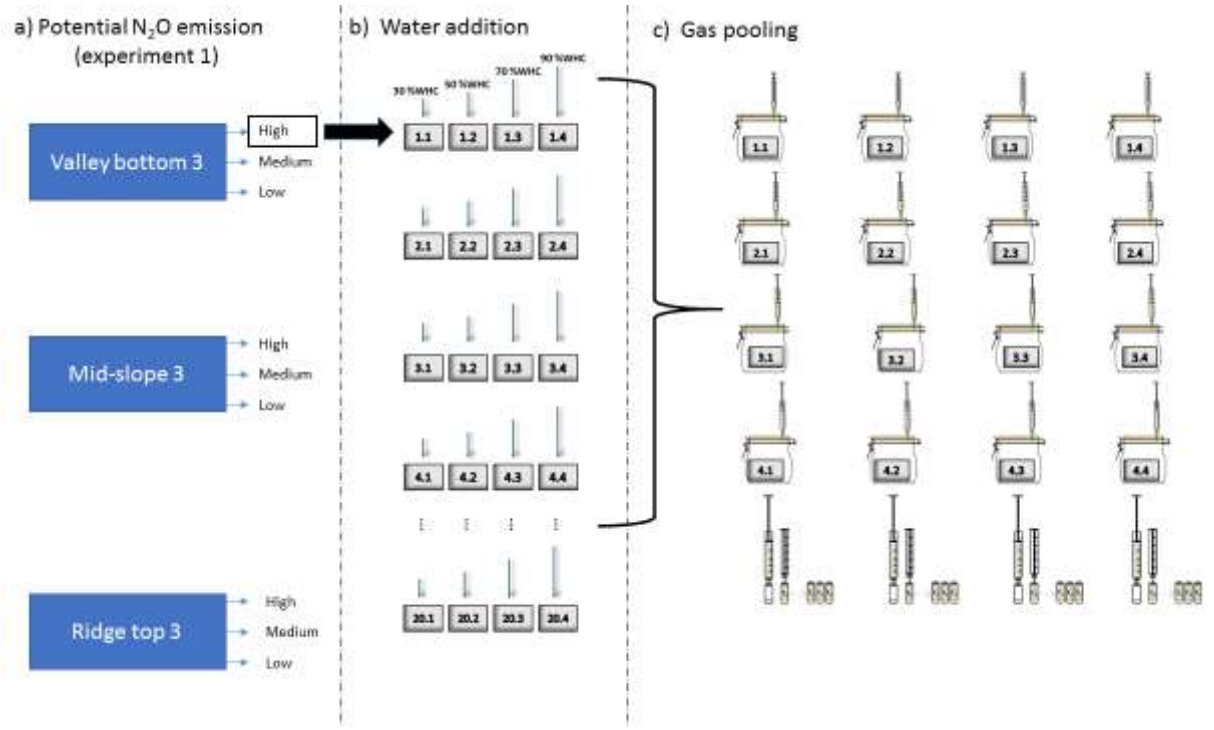

Figure 6 Schematic of the experimental design used in experiment 2. a) Classification of soil cores based on results from experiment 1: high, medium and low potential N_2O emission. b) Water addition to the 20 randomly selected sampling locations for each class and topographic position (1.1 -1.4 refers to replicated intact soil cores taken from one sampling location). Each core replicate was adjusted to a specific % water-holding capacity (WHC). c) Gas pooling was applied across groups of 4 sampling locations.

Analysis of small-scale spatial variability at the plot level

In this study, spatial variability was analysed using geostatistics. The degree of spatial dependence was measured using the semivariogram i.e. plotting of the semivariance and the distance between measurement points.

On the other hand, for each landscape plot, i.e. ridge top, mid-slope or valley bottom position (three times replicated), z-scores of GHG fluxes were computed using the formula:

$$Z_x = \frac{x - \mu_x}{\sigma_x}$$

The z-score (Z_x) thereby represents the difference between the flux observed at a point (x) minus the plot mean (μ_x) and divided by the standard deviation of the plot (σ_x). The z-score analysis allowed to identify "hot spots", with hot spots being defined as GHG fluxes being in the top 10 % (or 90th percentile) of all observed fluxes for all points of a landscape plot.

Further, I calculated the minimum number of point flux measurements needed to represent the observed spatial variability in soil N_2O and CO_2 potential fluxes across all 90 measuring points (true mean). For that, I calculated the distance between the true mean and the mean values with sample sizes K (K=1, 2… 89), hereafter M_K. The M_K values are computed using a large number of sample subsets of size K from the set of 90 points to obtain ranges of means. For each K, I analysed the range of the means and identified the minimum K being within 10 % to 30 % of the true mean of various confidence levels (80-95 %).

3.1.2 Spatial variability of N_2O and CO_2 fluxes

Effect of topography

Experiment 1 showed that topographic position significantly influenced potential N_2O fluxes. Mean N_2O fluxes from mid-slope plots (105.0 ± 9.4 µg-N m^{-2} h^{-1}) were significantly lower than the fluxes observed from soil cores taken from ridge top (135.2 ± 9.9 µg-N m^{-2} h^{-1}) and valley bottom plots (156.3 ± 12.0 µg-N m^{-2} h^{-1}). Soil N_2O fluxes correlated positively with % WHC at all topographic positions (20 % WHC: 11.1 µg N m^{-2} h^{-1}, 50 % WHC: 122.0 µg N m^{-2} h^{-1}, 70 % WHC: 452.9 µg N m^{-2} h^{-1}, 90 % WHC: 899.6 µg N m^{-2} h^{-1}). Experiment 2 corroborated the results from experiment 1, with a decrease in soil N_2O emissions in cores taken from the mid-slop position for all moisture contents. This result indicates that variation in topography exerts a primary control on N_2O emissions, thereby confirming my hypothesis (ii).

Analysis of small-scale variability in topography of N_2O emission is essential for an understanding of N_2O emission from tropical forest soils. This has been demonstrated by a number of studies in temperate ecosystems (Corre et al., 1996; Negassa et al., 2015; Pennock et al., 1992; Vilain et al., 2010) but in the tropics the spatial variability in GHG efflux has not been extensively studied (Reiners et al., 1998). According to these authors, soil N_2O emissions are greater in valley bottom than in ridge top or mid-slope positions. In these studies differences in moisture content expressed by the water-filled pore space (WFPS) between the different positions along the slope generally explain well the observed variability in N_2O fluxes with the highest soil N_2O fluxes found in valley bottom positions closely correlated with the highest WFPS in these positions. However, in my study, position-driven variations of soil N_2O emission were observed without significant changes in soil moisture as all soil cores were air dried for weeks before the start of the experiment. Soil N_2O fluxes followed a gradient such that less N_2O was emitted with increased inclination of the terrain (mid-slope plots) when compared with flat plots (valley bottom and ridge top plots). Significant effects of topographic slope and landscape position on multiple aspects of the N cycle have been shown as well by Weintraub et al. (2014). These authors linked the lower N availability in steep parts of the landscape compared to a relatively flat terrain to a less open N cycle, supporting my findings.

Potential soil CO_2 mean fluxes (experiment 1) decreased along the topographic gradient with ridge top (50.4 ± 1.0 mg C m^{-2} h^{-1}) having significantly higher fluxes than the valley bottom (44.8 ± 1.3 mg C m^{-2} h^{-1}) and mid-slope positions (46.3 ± 1.3 mg C m^{-2} h^{-1}). These results were not further supported by experiment 2, since no significant differences could be found for soil CO_2 fluxes from cores from different positions, at any WHC level. Several authors point out that the spatial variation in soil CO_2 fluxes can be linked to topographic characteristic (Brito et al., 2009; Martin and Bolstad, 2009; Riveros-Iregui and McGlynn, 2009) but in my study topographic position did not have a significant effect on soil CO_2 fluxes. High tree diversity in tropical forest system is known to lead to heterogeneity in chemical, structural and functional plant traits and thus, of plant litter, which finally affect biogeochemical C and N ecosystem turnover processes (Hättenschwiler et al., 2008; Townsend et al., 2008) as well as the spatial heterogeneity of soil respiration (Katayama et al., 2009; Matvienko et al., 2014). Large diversity of tree species along with the decreased susceptibility to microbial SOC degradation caused by the likely presence of allophane minerals (McBride, 1994; Parfitt, 2009) and the disturbance caused by charcoal making in my plots may have influenced the spatial variability of the soil CO_2 efflux (see further), thereby overriding possible topographic effects in my study.

Multiple comparisons of the effect of topographic positions on soil parameters showed significant effects. E.g. total SOC content, which was very high in all plots (118.6-171.9 g kg^{-1}), was significantly higher in soils taken from mid-slope positions compared to soils taken from ridge top and valley bottom. Total nitrogen (TN) ranged from 12.5 to 15.3 g kg^{-1}.Soils at mid-slope position showed as well the highest TN content although not significantly different from valley bottom position. Soils were generally slightly acidic with soils from ridge top position showing the lowest pH values (5.8 ± 0.5). Soil pH values increased significantly towards mid-slope (6.0 ± 0.6) to bottom (6.2 ± 0.4). However, the observed changes in soil properties in my study could not explain the differences in soil N_2O and CO_2 emissions along the topographic gradient.

Small-scale spatial variability

Soil properties in this study showed no or only weak correlations with N_2O and CO_2 fluxes. Surprisingly, soil CO_2 fluxes were negative correlated with SOC concentrations (R=-0.26), possibly because areas with high SOC were associated with higher levels of black carbon due to the presence of charcoal making. Distance to charcoal kiln was negatively correlated with CO_2 (R= -0.26) whereas high numbers of charcoal kilns in a plot were associated with higher CO_2 fluxes. The increase in soil CO_2 emissions in the vicinity to the charcoal kilns could have been driven by a priming effect prompted by increased production of extracellular enzymes due to the added substrate which "co-metabolize" soil organic matter (Wang et al., 2012; Zimmerman et al., 2011). Similar effects have been reported in many types of laboratory and field studies after biochar addition to soil (Kuzyakov et al., 2014). On the other hand, other studies suggest that biochar may cause a decrease in soil N_2O emissions (Cavigelli and Robertson, 2001). This would explain my observation that N_2O emissions from soil cores taken from inside the zone of influence of a charcoal kiln (i.e. within 5 m) were lower as compared to soil core N_2O emissions elsewhere.

Photo 1 Charcoal kilns in the Mau Forest (photos: Cristina Arias-Navarro).

Charcoal production implies that whole or parts of trees are felled and wood carbonization is made in traditional kilns (Photo 1). Charcoal production impacts the soil at two different levels. Firstly, impact occurs at the kiln site as a result of the extreme heat generated during the carbonization process and the digging to make a pit and to cover the wood pile. Secondly impacts the area surrounding the kiln where the wood is harvested. As the rate of forest regeneration on kiln sites following charcoal production is different from that of surrounding areas (Chidumayo and Gumbo, 2013), this could have also affected soil N_2O and CO_2 fluxes. Charcoal burning delays forest recovery for many decades and during this period the secondary vegetation on kiln sites is dominated by herbaceous plants, especially grasses (Boutette and Karch, 1984) in agreement with the positive correlation between distance to charcoal kiln and canopy cover (R=0.24) observed in this study. Canopy openness is related to light availability, which influences seedling establishment in the forest, especially pioneer and light-demanding species (Schnitzer and Carson, 2001). All this may have affected soil N_2O and CO_2 fluxes as well as their respective spatial patterns. However, the interaction between N_2O and CO_2 emissions and the presence of charcoal kilns needs further investigations, addressing both the short and long-term effects on CO_2 and N_2O fluxes during vegetation succession (Arias-Navarro et al., 2017a).

Spatial variability in this study was high, especially of soil N_2O fluxes, as already observed in other studies in tropical forest ecosystems (Breuer et al., 2000; Verchot et al., 2006, 1999; Vitousek et al., 1989). Soil N_2O and CO_2 fluxes showed a large variability among sampling locations within each plot (an example is provided in Figure 7). Coefficients of variation were between 115 and 143 % for soil N_2O fluxes and between 20 and 50 % for soil CO_2 fluxes for individual plots of my landscape transects.

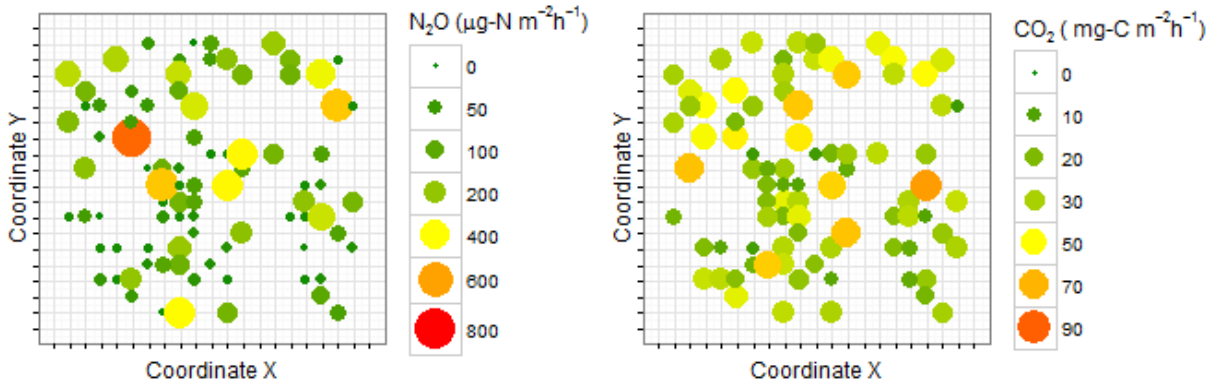

Figure 7 Potential soil N_2O (left panel) and CO_2 (right panel) fluxes 24 hours after a 10 mm rainfall simulation for soil cores taken from a 1-ha plot. Symbol size and colours denote the magnitude of individual fluxes. The x-axis is the coordinate x (in 5 m intervals) and the y-axis is the coordinate y (in 5 m intervals) of the sampling points (modified from Arias-Navarro et al 2017a).

Semivariogram calculation for N_2O and CO_2 fluxes showed that the relationship between semivariance and lag distance was characterized by a pure nugget effect, i.e., no increase in variance with distance. This indicates that N_2O and CO_2 fluxes had no spatial autocorrelation, at least for the range of distances investigated here (5-90 m). Earlier studies found that due to the presence of "hot spots" in soil, trace gas production and emission can vary substantially on a scale of less than one meter (Ambus and Christensen, 1994; Folorunso and Rolston, 1984; Parkin, 1993). Since I chose 5 m as the shortest distance between measurement points, I cannot exclude the existence of a small-scale (distances smaller than 5-m) spatial pattern of soil N_2O and CO_2 emission rates in my plots. "Hot spots" contributed substantially to the mean soil N_2O and CO_2 fluxes. The contribution of the samples above the 90^{th} percentile to the total soil N_2O and CO_2 fluxes was 73 % and 50 % respectively. Out of the total 810 sampling locations, 84 were identified as "hot spots" for N_2O fluxes (mean value: 545.1 µg-N m^{-2} h^{-1}), while for CO_2 fluxes, this number was 73 (mean value: 54.66 mg C m^{-2} h^{-1}). Excluding the "hot spot" data, the overall potential soil N_2O mean emission rate decreased from 132.3 µg-N m^{-2} h^{-1} to 83.7 µg-N m^{-2} h^{-1} while the average soil respiration rate decreased only from 47.2 mg C m^{-2} h^{-1} to 46.4 mg C m^{-2} h^{-1}. The observation that "hot spots" of N_2O fluxes contributed much stronger to the overall mean flux at the plot level as compared to soil CO_2 fluxes has also consequences for the design of flux measurements. For example, to obtain an estimate within 10 % of the true mean at a 95 % confidence level 78 ± 3 and 30 ± 11 sample locations should be sampled for estimating the plot N_2O and CO_2 mean fluxes, respectively.

The inherent spatial variability of the N_2O and CO_2 emissions and their controlling factors makes it difficult to reduce uncertainty in emission estimates by taking a manageable number of flux measurements with chamber investigations (Arias-Navarro et al., 2017a). In this

study, omitting "hot spots" could have led to an underestimation of the site-specific mean emission rates of roughly 2 % for CO_2 fluxes, but of 37 % for N_2O fluxes. This shows, that with a less ambitious sampling design a systematic bias in the estimate of plot mean soil CO_2 and N_2O fluxes from a tropical montane rain forest would be introduced. This strongly underlines the importance of carefully addressing spatial heterogeneity when designing field samplings (Arias-Navarro et al., 2017a). My studies also show, that additional information regarding the sources of "hot spots" and their sensitivity to environmental condition such as e.g. human disturbances needs to be considered for reliably estimating ecosystem trace gas fluxes and annual budgets for tropical forests.

3.2 Effect of land use in the Mau Forest region of Kenya on soil CO_2, N_2O and NO fluxes

3.2.1 Field sites and experimental design

In this experiment, I investigated two contrasting tea-growing areas with adjacent land uses (Figure 8). The first area is a large tea-estate (hereafter, TE) owned by a private company (approximately 120 ha). Plantations of tea (*Camelia sinensis var. sinensis* L.) have been established more than 60 years ago, directly following clearance of native forests. Within the tea-estate, approximately 20 ha are designated to grow Eucalyptus plantations (*Eucalyptus grandis* L.) for fuelwood production for the tea factories. Airborne fertilization (NPK 26:5:5) over the tea fields (approx. 10 ha) is conducted 2-3 times a year at a rate of 300-400 kg N ha^{-1} yr^{-1} (personal communication). The second tea-growing area is managed by small-holder farmers (hereafter, SH). Tea plantations, with tea fields being approx. 25 m x 25 m in size, are fertilized manually with NPK 26:5:5 at an approximate rate of 150 kg N ha^{-1} yr^{-1} (personal communication). In addition to tea cultivation, small-holder farmers manage grazing plots for livestock.

At the SH area, I monitored plots under tea (T), grazing (G) and the contiguous natural forest (F). At the TE area, I investigated plots under tea (T), eucalyptus plantations (P) and the adjacent natural forest (F). 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 (18 experimental plots in total). Five intact soil cores (5 cm inner diameter; 10 cm height) were collected at three random locations within each plot (Figure 8). Soils were immediately air-dried for three days

before being transported to the laboratory at IMK-IFU (Garmisch-Partenkirchen, Germany) for analysis.

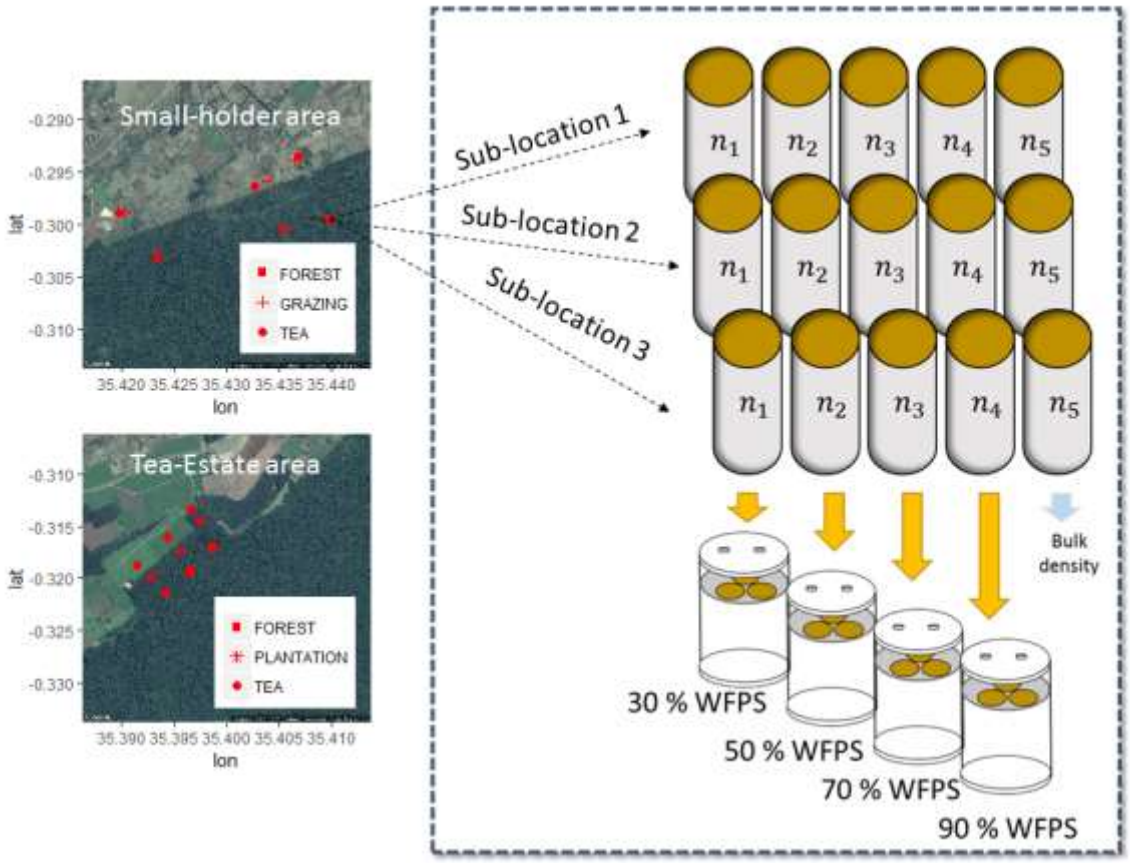

Figure 8 Localization of the 18 experimental plots (the x axis is the longitude and the y axis is the latitude). Different symbols denote different land uses (forest, grazing and tea plots at the small-holder area; forest, tree plantation and tea plots at the tea-estate area). Within each experimental plot, 5 replicated intact soil cores (n_1-n_5) were collected at 3 randomly selected sub-locations. One soil core from each sub-location was used to calculate the bulk density of the soil. The remaining soil cores were place inside the incubation chambers (one replicate for each of the four soil moisture treatments, WFPS: water-filled pore space).

In this experiment, I used a custom-built fully automated laboratory monitoring and incubation system to study fluxes from intact soil cores at different moisture conditions. The system had capacity for 18 steady-state dynamic incubation chambers (126 mm inner diameter; 240 mm height). A cylindrical piece of 7 cm height was placed at the bottom of each incubation chamber, on top of which I placed three intact soil cores from the same experimental plot to account for the variability within plot (Figure 8, Figure 9a; Arias-Navarro et al., 2017b). 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 for air supply (Figure 9a; Arias-Navarro et al., 2017b).

The sampling from the incubation chambers and the background air was automatically controlled through electromechanically operated solenoid valves in 180-minute long cycles in which a measure of the concentration at the outlet of each incubation chamber was gained once. Further details on the custom-build system can be found in Zuazo (2016).

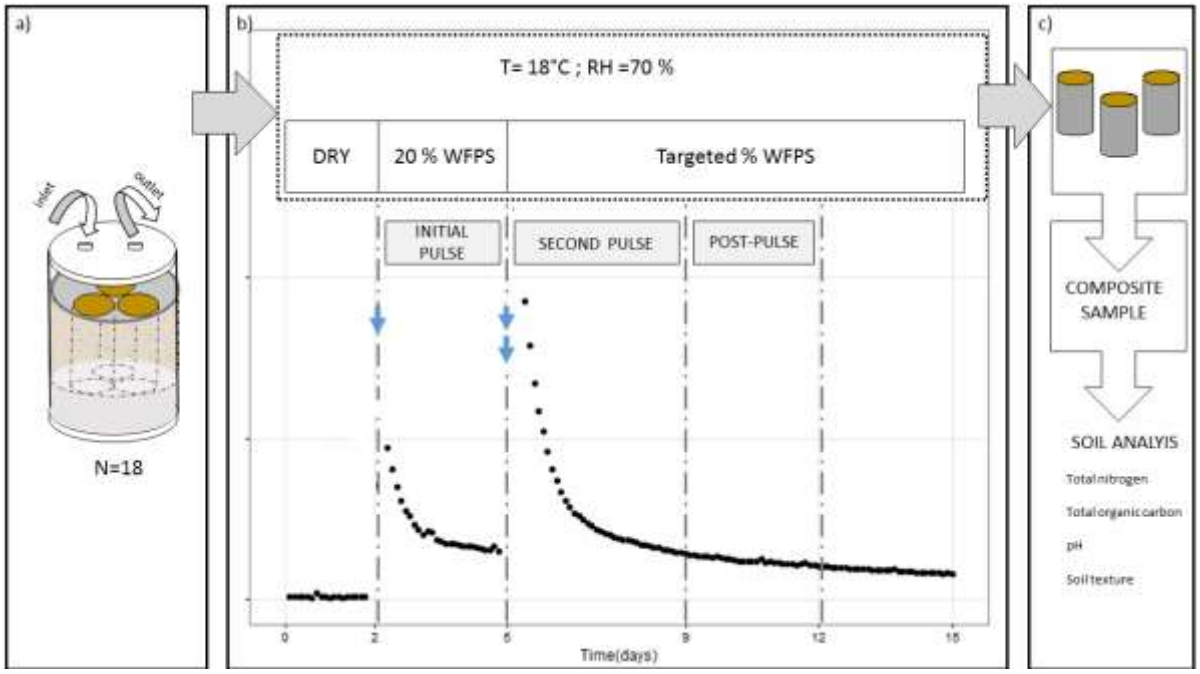

Figure 9 Schematic overview of the experiment. a) Incubation chamber. b) Incubation setup. Dots represent flux measurements for a given gas for an incubation chamber. Blue arrows represent watering events. Temperature (T) and relative humidity (RH) were kept constant at 18°C and 70 % .c) Soil analysis. The incubation outlined in this figure was replicated four times for separate soil cores (once for each of the four soil moisture levels, WFPS: water-filled pore space) (Source: Arias-Navarro et al., 2017b).

A schematic overview of the incubation is shown in figure 9b. Trace gas fluxes from the soil cores were measured for 2 days prior to soil re-wetting. Then, initial moisture content was adjusted to 20 % WFPS to purposely generalize the soil moisture status and soil trace gas fluxes were monitored over 3 days. Finally, simulated rain was added to the soil surface until the targeted WFPS % was reached and trace gas fluxes were again measured for 10 consecutive days. To avoid that the pulse emissions usually occurring after rewetting of soil affect my interpretation of soil moisture and land use effects on soil N and C trace gas fluxes, I excluded the data of the first four days after rewetting (Second pulse: Figure 9b; Arias-Navarro et al., 2017b), i.e. I used results of flux measurements observed for days 9 to 12 of the incubation experiment. These flux measurement are hereafter named post-pulse emissions (Figure 9b; Arias-Navarro et al., 2017b).

Nitrous oxide and CO_2 concentrations were determined using cavity ring-down spectroscopy (G2508, Picarro, Santa Clara, CA, USA). The gas analyser was calibrated once per 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. The soil-headspace gaseous exchange rate was calculated from the mass balance of the inlet and outlet concentrations and mass flow assuming equilibrium conditions (Pape et al., 2009). For further details about the analytical procedure see Arias-Navarro et al. (2017b).

Environmental data in the study region were collected from July 2015 to July 2016 by a weather station located at the Kenya Forest Service forest station (-0° 21' 5" S, 35° 21' 5" E, 2,184 m a.s.l.), approximately 2 km apart from the sites of soil core extraction. Soil moisture and temperature in 10 cm soil depth were measured using combined water potential and temperature sensor (Decagon 5TM, Decagon Devices, Inc., Pullman, WA, USA) while rainfall was measured with a rain gauge (Decagon ECRN-50, Decagon Devices, Inc.).

In the study region, measured rainfall showed a bi-modal pattern, with rains falling between April-June and October-December. The total annual rainfall for the studied period was 1956 mm. Mean air temperature was 16.7 °C (min=13.7 °C, max=20.9 °C). Further information on the seasonality of the measured environmental data can be found in Arias-Navarro et al. (2017b). Daily average soil volumetric water content (W_{vol}) as monitored at the weather station was used to calculate the daily average % WFPS of each experimental site using the respective bulk density information of each site (see Table 1) assuming that W_{vol} did not vary across sites. Soil moisture in 10 cm soil depth ranged from 10.5 % to 78.5 % WFPS with an average value of 25.3 % for SH-F, TE-F and 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. Daily soil temperature in 10 cm soil depth for the period did not vary much across seasons (min = 16.8 °C; max= 22.4 °C) so that, in agreement with Butterbach-Bahl et al. (2004) in a study carried out in tropical rainforests of Australia, it was assumed that seasonal changes in soil water content are a more significant factor affecting temporal variation of soil trace gases than soil temperature. Hence, calculated daily values of % WFPS were used for approximating the seasonality of soil trace gas emissions and for calculating annual fluxes on basis of regression curves describing the relationship between soil moisture and trace gas fluxes as observed in my laboratory experiment (see 3.2.4).

3.2.2 Soil properties

At the end of the incubation experiment at IMK-IFU the soils were sent to an external laboratory (Landwirtschaftliches Labor Dr. Janssen GmbH, Gillersheim, Germany) for soil analysis (Figure 9c; Arias-Navarro et al., 2017b) .Table 1 shows soil properties for the different sites. Bulk density (BD) values were calculated as the mass of the oven-dry soil (105 °C) divided by the core volume. Topsoil bulk density values were below 1 g cm^{-3}, with the lowest values observed for soils sampled at the natural forest sites (SH-F and TE-F) and at the commercial tea plantations (TE-T). All topsoils had a clayey texture, with clay contents being in the range of 56-67 % according to DIN ISO 18123. Total soil organic carbon (SOC) and total nitrogen (TN) were determined by dry combustion. Soil organic carbon contents were in the range of 58-79 g kg^{-1}. Soils taken from SH-F had the highest total SOC content. Nitrogen contents ranged between 5.6 and 8.1 g kg^{-1}. Soils taken from SH-F had as well the highest TN content although differences were not significant from TE-T and SH-G. Soils were acidic (4.2-6.0; water: soil weight ratio 2.5:1), with the lowest soil pH value observed for soils taken from TE-T.

Table 1 Topsoil (0-10 cm) properties of individual sites (mean and standard deviation, n=3). Values followed by different letters in the same row indicate significant differences ($p \leq 0.05$) (Modified from Arias-Navarro et al., 2017b).

	SH-F	TE-F	SH-T	TE-T	SH-G	TE-P
Sand (g kg^{-1})	74 (19)^{a}	77 (33)^{a}	50 (9)^{a}	56 (10)^{a}	50 (6) ^{a}	63 (9)^{a}
Silt (g kg^{-1})	333 (51)^{ab}	257 (21)^{b}	380 (42)^{ab}	363 (63)^{ab}	474 (187)^{a}	372 (43)^{ab}
Clay (g kg^{-1})	593 (35)^{ab}	667 (29)^{a}	570 (37)^{ab}	581(54)^{ab}	574 (22)^{b}	565 (40)^{ab}
BD (g cm^{-3})	0.66 (0.05)^{b}	0.63 (0.05)^{b}	0.83 (0.11)^{a}	0.63 (0.11)^{b}	0.83 (0.05)^{a}	0.83 (0.05)^{a}
SOC (g kg^{-1})	79.0 (7)^{a}	63.0 (0.3)^{b}	61.9 (8.5)^{b}	66.2 (142)^{ab}	66.2 (0.8)^{ab}	58.2 (4.4)^{b}
TN (g kg^{-1})	8.1 (0.8)^{a}	6.6 (0.3)^{b}	5.8 (0.4)^{b}	5.7 (0.2)^{b}	6.3 (0.3)^{b}	5.6 (0.5)^{b}
C:N ratio	9.7 (0.4)^{cd}	9.6 (0.4)^{d}	10.5 (0.6)^{bc}	11.6 (0.3) ^{a}	10.6 (0.4)^{b}	10.4 (0.4)^{bc}
pH	6.0 (0.2)^{a}	4.9 (0.4) ^{b}	5.0 (0.6) ^{b}	4.2 (0.2) ^{c}	5.4 (0.0)^{b}	5.1 (0.3) ^{b}

3.2.3 Soil CO_2 fluxes

The soil CO_2 baseline emissions of air-dried soil cores 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 emissions of CO_2 were observed, with fluxes returning to pre-incubation levels in less than 24 hours. The second pulse event, following further wetting of soil cores to the target WFPS, on the other hand, was significant higher for soils set to 90 and 70 % WFPS compared to the pulse event observed for soils being wetted to 50 and 30 % WFPS. Overall, soil CO_2 fluxes gradually increased from 30 to 90 %

WFPS across all sites. However, no significant differences in soil CO_2 fluxes were found between soil cores wetted to 70 and 90 % WFPS (30 % WFPS: 12 ± 10 mg C m^{-2} h^{-1}; 50 % WFPS: 31 ± 17 mg C m^{-2} h^{-1}; 70 % WFPS: 51 ± 21 mg C m^{-2} h^{-1}; 90 % WFPS: 57 ± 25 mg C m^{-2} h^{-1}) (Figure 10). Although many field studies fail to find relationships between soil moisture and soil CO_2 emissions, results in this experiment agree with other studies where, at constant temperature, wetter soils emitted more CO_2, most likely due to improved substrate availability for microbial respiration (Zhou et al., 2013). It should be noted that this observation holds true only until the point of water saturation when soil CO_2 fluxes tend to decrease, since soil moisture contents close to water saturation may favour the development of anaerobiosis, thereby reducing organic matter decomposition and CO_2 diffusion to the atmosphere (Knowles et al., 2015; Smith, 1990). As soils in this study were characterized by low bulk densities (<1 g cm^{-3}) it seems that even at 90 % WFPS soil were still predominantly aerobic.

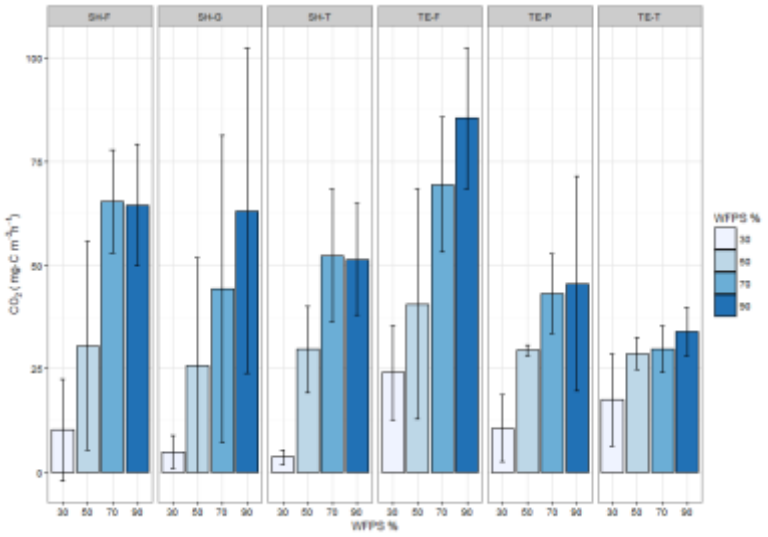

Figure 10 Average post-pulse soil CO_2 fluxes at different soil moisture levels (WFPS: water-filled pore space) and for different sites (SH: small-holder, TE: tea-estate sites; F: forest, T: tea, G: grazing and P: eucalyptus plantation). Vertical bars indicate standard deviations of three spatial replicates.

Soils taken from forest sites had significantly higher average soil CO_2 fluxes (TE-F: 55 ± 30 mg C m^{-2} h^{-1}; SH-F: 43 ± 28 mg C m^{-2} h^{-1}) than the rest of soils taken from other land uses (SH-G: 34 ± 34 mg C m^{-2} h^{-1}; SH-T: 34± 23 mg C m^{-2} h^{-1}; TE-P: 32± 19 mg C m^{-2} h^{-1}; TE-T: 27 ± 9 mg C m^{-2} h^{-1}) (Figure 10). Paterson et al. (2009) points out that for *in-situ* measured soil CO_2 fluxes around half of soil respiration is derived from plant root respiration (autotrophic respiration), while the remaining respiration is associated with the decomposition of organic matter by the microbial community (heterotrophic respiration). In this study the soil cores were removed from the field cutting any existing roots, air-dried and stored for some weeks before further analyses. Therefore, the observed soil fluxes only represent heterotrophic

respiration (mainly derived from soil microbial activity). The rate of heterotrophic respiration is largely a function of microbial community composition and organic matter quality, with organic matter quality being regulated by plant inputs (Bardgett et al., 2008; Wardle, 2004). Therefore, lower soil CO_2 fluxes after land use change in this study could be associated with reductions in microbial biomass and changes in its community composition (i.e. bacteria and fungi).

3.2.4 Soil N_2O and NO fluxes

Very low uptake or zero N_2O fluxes was measured from dried soil cores (average: -9 ± 31 µg N m^{-2} h^{-1}). First re-wetting of soil cores to 20 % WFPS did not provoke N_2O emission pulses, regardless of the site. After setting the soil cores to the final WFPS %, significant N_2O emissions (> 50 µg N_2O-N m^{-2} h^{-1}) were observed only for those soil cores for which the WFPS was finally adjusted to 90 %. Differences in soil post-pulse N_2O fluxes were found only between 90 % WFPS and the other soil moisture levels (30 % WFPS: 19 ± 9 µg N_2O-N m^{-2} h^{-1}; 50 % WFPS: 21 ± 8 µg N_2O-N m^{-2} h^{-1}; 70 % WFPS: 24 ± 30 µg N_2O-N m^{-2} h^{-1}; 90 % WFPS: 183 ± 320 µg N_2O-N m^{-2} h^{-1}).

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 (SH-G: 93 ± 248 µg N_2O-N m^{-2} h^{-1}; SH-T: 68 ± 156 µg N_2O-N m^{-2} h^{-1}; SH-F: 43 ± 61 µg N_2O-N m^{-2} h^{-1}; T E-P: 25 ± 25 µg N_2O-N m^{-2} h^{-1}; TE-T: 11 ± 6 µg N_2O-N m^{-2} h^{-1}) although strong variation precluded significant differences (Figure 11a). A significant correlation between N_2O soil fluxes with soil properties was not found.

Dry-soil NO emissions were very low for all sites (average 5 ± 7 µg NO-N m^{-2} h^{-1}). Large NO emission pulses (average 99 ± 100 µg NO-N m^{-2} h^{-1}) occurred already following the first re-wetting, when soil moisture was adjusted to 20 % WFPS. Highest peak emissions following the first initial re-wetting were observed for soils taken at commercial tea plantation plots, while for soils from small-holder 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 emissions after the second soil re-wetting were not significantly different between soil moisture levels (30 % WFPS: 43 ± 34 µg NO-N m^{-2} h^{-1}; 50 % WFPS: 58 ± 42 µg NO-N m^{-2} h^{-1}; 70 % WFPS: 55 ± 35 µg NO-N m^{-2} h^{-1}; 90 %WFPS: 48 ±40 µg NO-N m^{-2} h^{-1}). Nitric oxide emissions were significantly higher for soils taken from tea plantations (TE-T: 81 ± 35 µg NO-N m^{-2} h^{-1}; SH-T: 67 ±49 µg NO-N m^{-2} h^{-1}) and natural forest sites in the tea-estate area (TE-F: 68 ± 42 µg NO-N m^{-2} h^{-1}) compared to those taken from eucalyptus plantations (TE-P: 25 ± 12µg NO-N m^{-2} h^{-1}) or grazing land (SH-G: 22 ± 18 µg NO-N m^{-2} h^{-1}). No significant differences were found between

natural forest in the small-holder area (SH-F: 43 ± 20 µg NO-N m^{-2} h^{-1}) and the rest of the sites (Figure 11b).

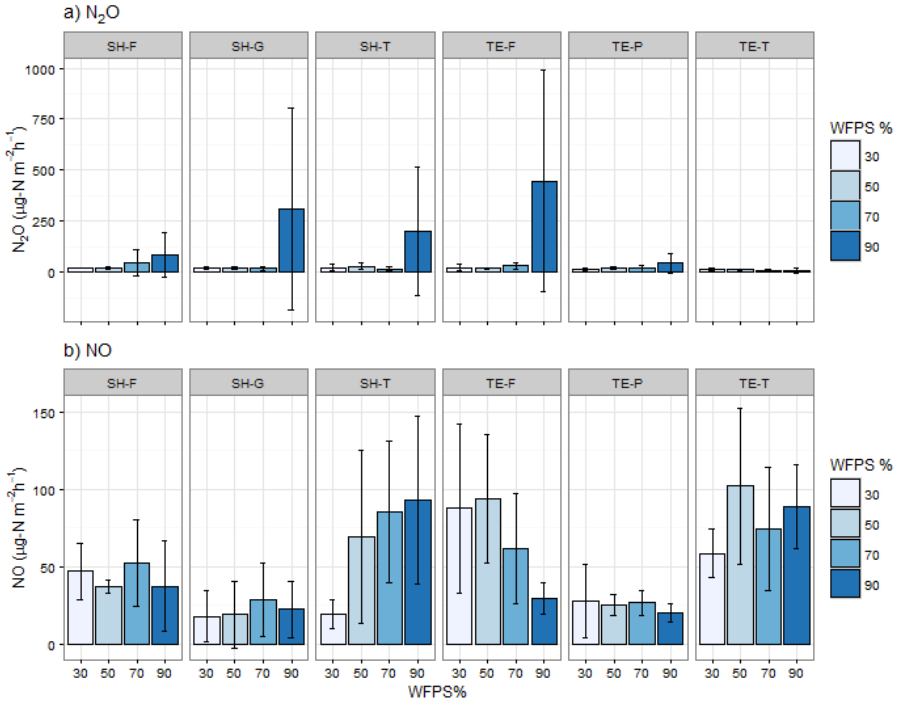

Figure 11 Average post-pulse soil a) N_2O and b) NO fluxes at different soil moisture levels (WFPS: water-filled pore space) and for different sites (SH: small-holder , TE: tea-estate sites; F: forest, T: tea, G: grazing and P: eucalyptus plantation). Vertical bars indicate standard deviations of three spatial replicates.

Soil moisture is a key governing parameter in the production and consumption of N oxides in the soil as it controls soil gas diffusion, oxygen (O_2) availability for microbial use (Davidson et al., 2000), but also substrate availability for microbial communities. Whereas both NO and N_2O are produced during the same processes (i.e. nitrification and denitrification), the ratios of the two products strongly vary depending on O_2 availability (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). Thus, NO dominates the emissions from soils below field capacity (usually around 60 % WFPS), N_2O becomes dominant at intermediate WFPS % values (50 and 90 % depending on soil properties; Davidson et al., 1991; Breuer et al., 2002; Werner et al., 2007) and at WFPS > 90%, N_2 dominates the gas flux. In my study, soil N_2O fluxes increased exponentially if soil cores were wetted at WFPS higher than 70 %, but were relatively low at 30–50 % WFPS. This huge differences in soil N_2O fluxes between rather dry and very wet soils is in line with other studies which have reported a non-linear (exponential) increase in N_2O emission rate with increasing soil water content (Arai et al., 2008; Garcia-Montiel et al., 2001). Regarding soil NO emissions,

in my study optimum soil moisture for different sites and land uses varied, which was probably an effect not only of land use but also of different soil properties. Average post-pulse soil NO emissions were negatively correlated with pH (R= -0.5), bulk density (R= -0.5) and positively correlated with soil clay content (R=0.5). Although soil NO emissions were not significant different between the four applied % WFPS levels, a decrease of the NO: N_2O ratio with increasing WFPS was observed. This indicates that denitrification or nitrifier-denitrification processes was possibly the main pathway of N_2O gas production rather than nitrification. This assumption is in good agreement with previous observations in rain forest ecosystems (Breuer et al., 2002; Butterbach-Bahl et al., 2004; Werner et al., 2007) and the general assumption that at N_2O:NO ratios higher than unity denitrifiers drive N trace gas emissions, while at values lower than unity N trace gas emissions are dominated by the nitrification process and nitrifiers (e.g., Anderson and Levine, 1986; Skiba et al., 1992).

Estimate of soil N_2O and NO annual emissions

In this study, NO fluxes were found to be significantly correlated with % WFPS in a quadratic manner (NO = % WFPS + I (% $WFPS^2$); R> 0.9) while N_2O emissions increased exponentially with WFPS (N_2O = exp (a + b * % WFPS); R> 0.8). Figure 12 shows the fitted regression equations for the different sites.

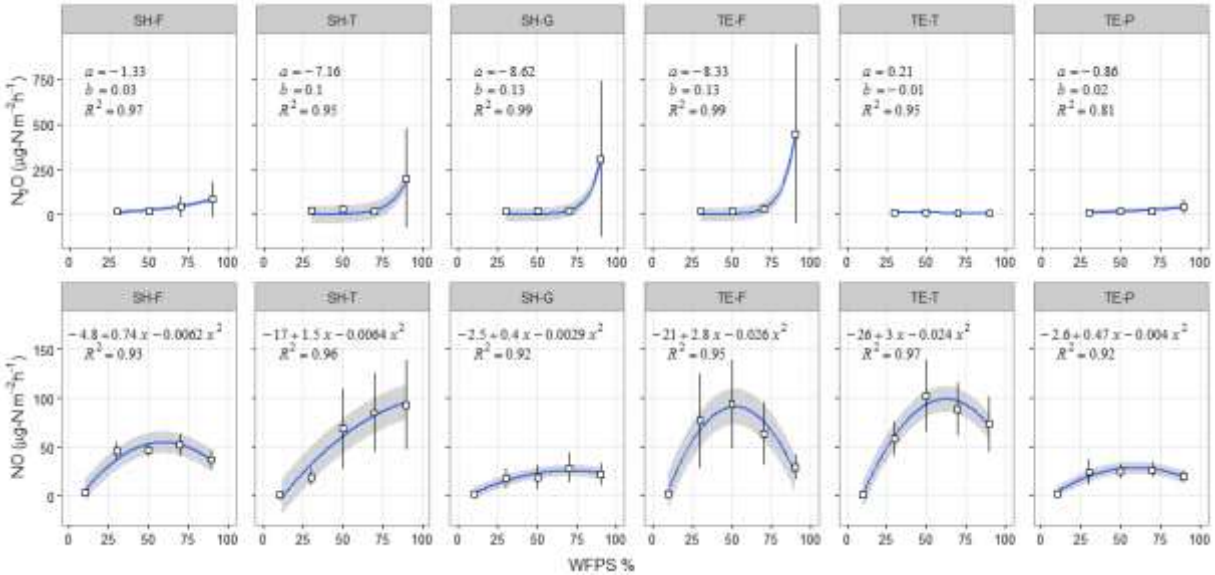

Figure 12 Relationship between soil water-filled pore space (WFPS) and N_2O (upper panels) and NO (lower panels) fluxes determined in laboratory experiments for different sites (SH: small-holder, TE: tea estate; F: forest, T: tea, G: grazing and P: eucalyptus plantations). The grey areas indicate the 95 % confidence intervals for the individual curve fits. Vertical bars indicate standard errors of three spatial replicates (source: Arias-Navarro et al., 2017b).

Due to the strong correlation between NO and N_2O fluxes and soil % WFPS, NO and N_2O fluxes can be predicted on the basis of those equations using available moisture data.

Hence, I used the observed in-situ daily volumetric water content values measured at the meteorological weather station located close to the field sites in Kenya and the measured soil bulk density values to calculate dynamics of daily WFPS % changes at each site. Further I used those WFPS values as driver for calculating possible seasonal changes of soil N trace gas emissions using the correlations I found in the laboratory experiment. Finally I accumulated daily estimated fluxes to annual fluxes. Figure 13 summarizes the calculated annual flux rates for the different sites.

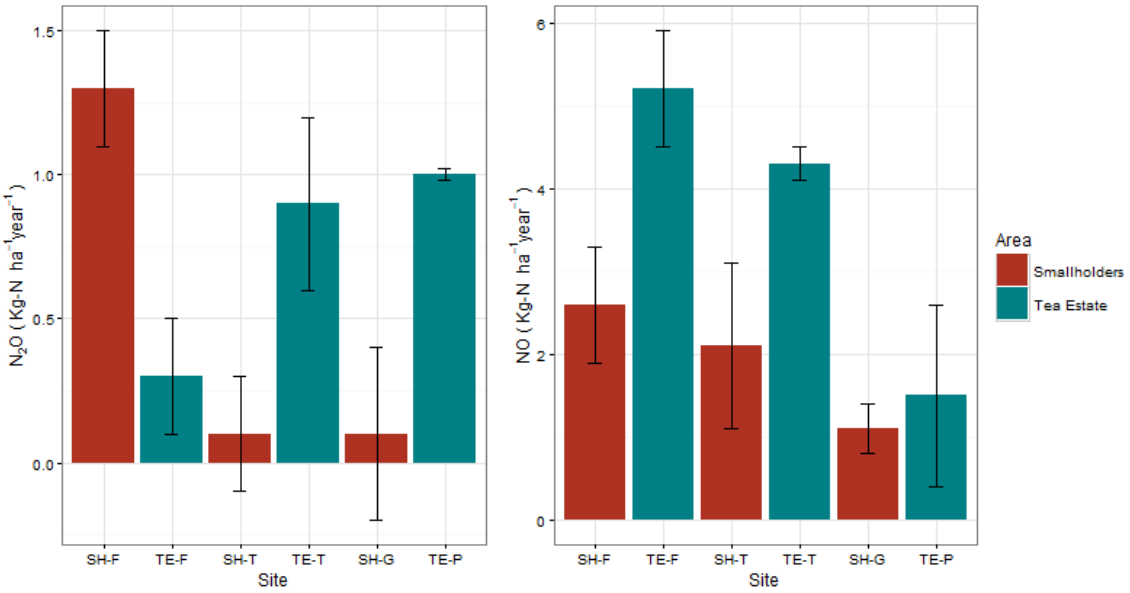

Figure 13 Estimated annual N_2O (left panel) and NO (right panel) emission rates without pulse emission contribution for different sites (SH: small-holder, TE: tea-estate; F: forest, T: tea, G: grazing and P: eucalyptus plantation). Vertical bars indicate the standard error of the estimate.

There is limited knowledge about N_2O and NO fluxes from tropical forest soils, especially for Africa (van Lent et al., 2015). Table 2 shows an overview of the range of published data. The up scaling approach presented here yielded annual flux estimates of 0.3 ± 0.2 kg N_2O-N ha^{-1} yr^{-1} and 1.3 ± 0.2 kg N_2O-N ha^{-1} yr^{-1} for TE-F and SH-F, respectively. This is in good agreement with estimates based on *in-situ* measurements of soil N_2O fluxes at other tropical montane forest sites (0.3 - 0.8 kg N_2O-N ha^{-1} yr^{-1}) but lower than most of the studies performed in tropical lowland forests (1.9 - 7.4 kg N_2O-N ha^{-1} yr^{-1}) (see also van Lent et al., 2015). The difference between lowland and montane tropical rainforests has been ascribed to lower net mineralization rates in tropical montane forest suggesting that those forests are possibly N limited (Gütlein et al., 2016; Koehler et al., 2009; Nottingham et al., 2015; Purbopuspito et al., 2006).

Table 2 Reported annual nitrous oxide (N_2O) and nitric oxide (NO) emissions from tropical forest.

Reference	Country	Site		kg N_2O-N ha^{-1} yr^{-1}	kg NO-N ha^{-1} yr^{-1}
Arias-Navarro et., at 2017b	Kenya	Mau Forest ,tea estates	Tropical montane Forest	0,3	5,2
Arias-Navarro et., at 2017b	Kenya	Mau Forest, smallholders		1,3	2,6
Gharahi Ghehi et.,al 2014	Rwanda	Nyungwe		4	3
Ishizuka et al., 2005	Indonesia	Sumatra		0,26	-
Riley and Vitousek 1995	Hawai	Kauai		0,79	0,27
Purbosito et al., 2006	Indonesia	Cental Sulawesi		0,3	no measurable
Johansson et al., 1988	Venezuela	Altos de Pipe		-	0,4
Breuer et al.,2000	Australia	Kauri Creek	Tropical lowland Forest	5,36	-
Breuer et al., 2000	Australia	Lake Eacham		1,15	-
Breuer et al.,2000	Australia	Massey Creek		3,75	-
Castaldi et al., 2013	Ghana	Ankasa National Park		2,33	-
Garcia-Montiel et., al 2003	Brazil	Rondônia		3,21	2,38
Davidson et al., 2004	Brazil	Tapajos National Forest		1,4	
Keller and Rainers 1994	Costa Rica	La Selva		5,86	0,9
Kellers and Rainers 1994	Costa Rica	La Selva		3,74	-
Keller et al., 1993	Costa Rica	La Selva		6	-
Keller et al., 2005	Brazil	Tapajos Forest Ultisol		1,4	-
Keller et al., 2005	Brazil	Tapajos Oxisol		6,5	-
Kiese and Butterbach-Bahl 2002	Australia	Kauri Creek		4,36	-
Kiese and Butterbach-Bahl 2002	Australia	Bellender Ker		7,45	-
Kiese and Butterbach-Bahl 2002	Australia	Pin Gin		6,89	-
Kiese et al., 2003	Australia	Bellender Ker		0,97	-
Koehler et al., 2009	Panama	Gigante lowland		1,16	-
Luizao et al., 1989	Brazil	Terra Firme		1,9	-
Maddock et al., 2001	Brazil	Tingu`a biol.		3,14	-
Melillo et al., 2001	Brazil	Rondônia		1,94	-
Nepstad et al., 2002	Brazil	Tapajos		2,3	1,7
Serca et al., 1994	Congo	Mayombe region		2,9	0,7
Sousa Neto et al., 2011	Brazil	Picinguaba		2,2	-
Verchot et al., 1999	Brazil	East Amazon primary		2,43	1,5
Verchot et al., 1999	Brazil	East Amazon secondary		0,94	0,3
Verchot et al., 2006	Indonesia	Sumatra, forest		1,2	-
Verchot et al., 2006	Indonesia	Sumatra, wet forest		1,3	-
Yan et al., 2008	China	Xishuangbanna		2,7	-
Werner et al., 2007	Kenya	Kakamega forest		2,26	-

*The listing is not meant to be complete but to give an overview of the range of published data

To date, data about NO emissions from tropical forest soils are scarce, with available *in-situ* measurements being around 10 studies. Annual emission estimates in this study (3.9 ± 1.8 kg NO-N ha^{-1} yr^{-1}) were at the upper end of those reported for tropical forests (mean:1.7 ± 0.48 kg N-NO ha^{-1} yr^{-1}; van Lent et al., 2015). However, Butterbach-Bahl et al. (2004) found remarkably large soil NO emissions with a total amount of approx. 3.0 kg NO-N ha^{-1} within a three month period following the rewetting of soils after a drought period. Furthermore, my estimate was in line with the 3.0 kg NO-N ha^{-1} yr^{-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, high SOC and low pH of soils in both studies.

Annual estimates of soil N_2O emissions from soils taken at eucalyptus plantations (TE-P) were, with approx. 1 kg N_2O-N ha^{-1} yr^{-1}, similar, though towards the lower end, to those in

other studies covering measurements of N_2O fluxes from tropical rainforest forest soils. In tendency, lowest annual total N_2O emissions were observed from soils taken at the small-holder tea plantation and grazing sites (SH-T: 0.1 ± 0.2 kg N_2O-N ha^{-1} yr^{-1}; SH-G: 0.1 ± 0.3 kg N_2O-N ha^{-1} yr^{-1}). In accordance with N_2O fluxes, I also found that estimated annual soil total NO emissions were with 1.1 ± 0.3 kg NO-N ha^{-1} yr^{-1} lowest for soils taken at the grazing sites. This observation agrees with findings of other studies in the tropics which reported that tropical pasture soils tend to emit less N_2O and NO than tropical forest soils (Davidson and Verchot, 2000; Garcia-Montiel et al., 2001; Keller et al., 1993; Melillo et al., 2001; Veldkamp et al., 1999). Those studies argue that forest conversion to pasture slows down soil N cycling (mineralization, nitrification, and denitrification) and therefore also limits soil N_2O and NO fluxes (Davidson et al., 2000).

Annual total soil emissions were estimated to be 0.9 kg N_2O-N ha^{-1} yr^{-1} at the commercial tea site. This is relatively low compared to the estimate of 4-7 kg N_2O-N ha^{-1} yr^{-1} soil emissions from "zero N-control" tea plantations in China and Japan (Akiyama et al., 2006; Fu et al., 2012; Yao et al., 2015). However, one can assume that those flux estimates were likely highly affected by the application of large amounts of N fertilizer (average of 553 kg N ha^{-1} yr^{-1}) in preceding years. On the other hand, Rosenstock et al. (2015) reported annual N_2O fluxes from small-holder farmers tea plantations in Kenya and Tanzania of 0.38 and 0.75 kg N_2O-N ha^{-1} yr^{-1} respectively, in line with the annual soil N_2O estimate for small-holder tea cultivation in this study (0.1 ± 0.2 kg N_2O-N ha^{-1} yr^{-1}). Their work, which included fertilizer-induced and background fluxes, suggests that the N_2O emission factor for fertilizer N applications in small-holder tea systems would be below 1% of applied N. In conclusion, the study conducted by Rosenstock et al. (2015) as well as my own study suggests that the IPCC N_2O emission factor of 1 % for N fertilizer application to soils (IPCC, 2006) is by far too high and does not reflect the current soil N_2O emission situation for small-holder tea gardens in East Africa. Beyond N_2O, my work shows that tea plantations are also a major source for NO. The estimated annual soil NO flux of 4.3 ± 0.7 kg ha-^{1} yr^{-1} and 2.1 ± 1.1 kg ha^{-1} yr^{-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 without N fertilizer application (1.6 ± 0.4 kg NO-N ha^{-1} yr^{-1}).

The higher N_2O and NO emissions (N_2O + NO) from soils of the commercial tea plantation (4.3 kg N ha^{-1} yr^{-1}) compared to soils of tea plantations from small-holder farms (2.2 kg N ha^{-1} yr^{-1}) are very likely due to long-term high rates of N fertilization, which among others resulted in soil acidification (Tokuda and Hayatsu, 2004; Yamamoto et al., 2014). Soil of the commercial tea plantation (TE-T) in my study showed a mean pH of 4.2, which was

significantly lower than soils from the small-holder tea sites (SH-T, mean: 5.0). Soil acidity is an important factor affecting biotic and abiotic N turnover and conversion processes such as e.g. chemo-denitrification which can result in significant environmental N losses including emissions of NO and N_2O (Kesik et al., 2006; Medinets et al., 2015; Ventera et al., 2003; Yao et al., 2015). Chemo-denitrification (process involving abiotic reduction of nitrite) has been suspected to be an important source of soil NO emissions after wetting of dry soil and in excessively fertilized soils where nitrite can accumulate (Davidson, 1992; Neff et al., 1995; Verchot et al., 1999) as is the case in the tea soils from the tea-estates.

On the other hand, atmospheric N deposition has been found to contribute significantly to forest soil acidification in Europe (Velthof et al., 2011). Forest soil acidification due to atmospheric deposition might have also affected soil N trace gas fluxes in my study as soils taken from native forests neighbouring the tea-estate showed a significant lower pH as compared to native forest soils at the small-holder area. I assume that the airborne fertilization of nearby tea plantations has led to unintended N fertilization of the forest, which finally resulted in the observed soil acidification. This has not happened in the small-holder farmer area as small-holder farmers apply fertilizer by hand, thereby avoiding unintentional fertilization of the neighbouring forest.

4 Conclusion and outlook

The observations of my study highlight the complexity of drivers of C and N trace gas emissions from soils of tropical ecosystems and the need to carefully address and assess those in targeted field and laboratory experiments to finally come up with improved estimates of soil GHG fluxes at ecosystem to landscape scale.

In my study, the high spatial variation of soil N_2O and CO_2 fluxes observed at montane tropical forest sites was further increased by changes in vegetation and soil properties induced by human activity (e.g. via charcoal production or selective cutting). However, at the plot scale a distinct pattern of soil N_2O and CO_2 fluxes could not be demonstrated. Nevertheless, I was able to show that at the ecosystem scale, "hot spots" of biogeochemical cycling were responsible for the majority of the total N_2O and CO_2 flux. This implies that having too few sampling locations or discarding the observations of high fluxes, could lead to a systematic bias in soil GHG flux estimates of tropical montane forests, strongly underlining the importance of carefully addressing spatial heterogeneity when designing field sampling. Further, I calculated the minimum number of samples required to account for the spatial variability of biogeochemical cycling in this type of forest and provided guidelines for sample size needed to capture the variability, especially important for N_2O which has higher spatial variability than CO_2. Although the soil physical properties were not significant predictors of either the soil N_2O or CO_2 fluxes, topography was shown to affect the N_2O fluxes of the soils incubated in the laboratory under ideal and variable levels of soil moisture. My work therefore shows that soil GHG fluxes from targeted ecosystems must be estimated from an ensemble of measurements and measurement sites that is sufficient in size and representative for the topography of the site.

Despite the inherent spatial variability of soil GHG emissions and their controlling factors makes it difficult to reduce the uncertainty of emission estimates by taking a manageable number of flux measurements, the "gas pooling" method developed in the frame of this Ph.D. thesis provides an opportunity to overcome the heterogeneity of soil GHG fluxes and, thus, has a great potential for improving estimates of soil GHG fluxes in complex landscapes or large regions.

On the other hand, the innovative laboratory study with trace gas flux measurements at high temporal resolution under controlled moisture and temperature conditions I conducted, shows how soil parameterization experiments can be used to assess effects of land use change on soil-atmosphere trace gas exchange for understudied regions such as the Mau Forest region

in Kenya. Although results from this study are based on short-term laboratory incubations, those observations contribute to a growing body of information concerning N and C trace gas emissions from different land uses in the tropics and their governing parameters. In conclusion, my study suggests that land use intensification of agriculture in montane tropical landscapes may significantly increase the biosphere to atmosphere flux of the environmentally important N trace gases N_2O and NO. Temporal up scaling only based on soil moisture may have led to additional uncertainty, since this approach excluded e.g. effects of plants and plant litter on soil C and N dynamics and microbial processes. However, even though the approach I used might be associated with significant uncertainties, it clearly shows that past and on-going land use changes are significantly altering soil GHG fluxes in the tropics and turn these systems towards significant sources of N trace gases. Nevertheless, further improved understanding of trace gas exchange processes in tropical ecosystems will require that the number of studies is substantially increased, including long-term observations of soil-atmosphere GHG exchange at multiple sites.

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Acknowledgments

Undertaking this PhD has been a truly life-changing experience for me and it would not have been possible to do without the support and guidance that I received from many people.

First, I would like to express my sincere gratitude to Prof Dr Klaus Butterbach-Bahl for the continuous support of my Ph.D. study and related research, motivation, and immense knowledge, during both the three years I spent undertaking my field work in Kenya and also the time I spent at the Institute of Meteorology and Climate Research, Atmospheric Environmental Research (IMK-IFU), in Garmisch-Partenkirchen, Germany. I could not have imagined having a better supervisor and mentor for my Ph.D. study.

Besides my supervisor, I would like to thank PD Dr Ralf Kiese and Prof Dr Heinz Rennenberg for serving on my dissertation committee. My sincere thanks also goes to Dr Eugenio Diaz-Pines, who provided me an opportunity to join IMK-IFU team as intern, for enlighten me the first glance of research. Without his precious support and patience, this Ph.D. would not have been achievable.

I gratefully acknowledge the funding received towards my Ph.D. from the Center for International Forestry Research (CIFOR) fellowship. I would especially like to thank to Dr Louis Verchot for his supervisory role and constant feedback. Also thanks to Dr Mariana Rufino for her valuable input.

I am also grateful to the funding received through the Climate, Food and Farming (CLIFF) Network, an initiative of the CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS). A very special thank you to Dr Todd Rosenstock for his invaluable advice and for always being so supportive of my work.

My thanks also go out to the technical support received by the Mazingira Centre, Environmental Research and Educational facility. I thank to my Mazingira fellow lab mates for all the good times we had during the long hours we were working together, although we also ended up arguing sometimes.

I would like to thank the Kenya Forest Service (KFS) for access to the sites and field assistance, especially to the ranger Sylvester Maara. My deep appreciation goes out to the local field research team members. Their excellent work during data collection has made an invaluable contribution towards my Ph.D. “Asante sana” to them and their families for their

friendship and the warmth they extended to me during my time in the villages and for always making me feel so welcome.

Many thanks to Flavia Della Rosa, Vivalda Poggiali, Giulia Polidori and Olivia Freeman. Your love, laughter and company have kept me smiling and inspired thought my three years in Kenya.

I would also like to say a heartfelt thank you to Pablo García, for always believing in me; for always believing in us.

Last but not the least, I would like to thank my family: my parents, my brother and sister and my nephew, for supporting me spiritually throughout writing this thesis and my life in general.

Publications

Publication I

Arias-Navarro, C., Díaz-Pinés, E., Kiese, R., Rosenstock, T.S., Rufino, M.C., Stern, D., Neufeldt, H., Verchot, L.V., Butterbach-Bahl, K. (2013)

Gas pooling: A sampling technique to overcome spatial heterogeneity of soil carbon dioxide and nitrous oxide fluxes.

Soil Biology and Biochemistry 67, 20–23

doi:10.1016/j.soilbio.2013.08.011

Publication II

Arias-Navarro, C., Díaz-Pinés, E., Klatt, S., Brandt, P., Rufino, M.C., Butterbach-Bahl, K., Verchot, L.V. (2017a)

Spatial variability of soil N_2O and CO_2 fluxes in different topographic positions in a tropical montane forest in Kenya.

Journal of Geophysical Research: Biogeosciences, accepted article published online

doi:10.1002/2016JG003667

Publication III

Arias-Navarro, C., Díaz-Pinés E., Zuazo P., Rufino M. C., Verchot L.V., and Butterbach-Bahl K. (2017b)

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

Biogeochemistry, submitted for publication.

Soil Biology & Biochemistry 67 (2013) 20–23

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Short communication

Gas pooling: A sampling technique to overcome spatial heterogeneity of soil carbon dioxide and nitrous oxide fluxes

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ARTICLE INFO

Article history:
Received 22 February 2013
Received in revised form
14 August 2013
Accepted 16 August 2013
Available online 28 August 2013

Keywords:
Composite sample
Pooling technique
Soil CO_2 and N_2O fluxes
Spatial variability
Static chamber

ABSTRACT

Small-scale spatial variability in soil carbon dioxide (CO_2) and nitrous oxide (N_2O) fluxes poses serious challenges to the experimental design, and number of gas samples needed to provide a reliable estimate of flux usually exceeds analytical capacities. We pooled gas samples –analogously to soil pooling – to overcome this challenge. Our sample pooling technique collects a composite gas sample from several chambers instead of the conventional practise of analyzing samples from chambers individually, thus reducing numbers of gas samples. The method was verified to be reasonably accurate in forest, grassland and agricultural fields over a four week measurement campaign. Pooling technique results differed by 2–8% for CO_2 and by 3–4% for N_2O when compared to individual chamber means. That shows pooling of gas samples across individual static chambers is an acceptable approach to integrate spatial heterogeneity.

Practical methods are needed to quantify soil CO_2 and N_2O fluxes in order to better understand magnitudes, spatial and temporal variability of soil-atmosphere CO_2 and N_2O exchange. This information is needed to develop improved management practices aiming towards lower CO_2 and N_2O emissions. Static chambers are the most commonly used approach for measuring soil greenhouse gas (GHG) fluxes (Grahammer et al., 1991; Livingston and Hutchinson, 1995; Smith et al., 1995) because relatively low cost, simple operation, and portability (Butterbach-Bahl et al., 2011; Denmead, 2008). Furthermore, the simple technique and deployment protocol can be adapted to a wide range of ecosystems and experimental designs (Rochette, 2011).

The basic principle of static chambers measurements is that a number of gas samples (three to six) are taken over a period of time from the headspace of a gas-tight chamber enclosing the soil surface. GHG fluxes are calculated from the rate of change in the headspace gas concentration over time.

Soil fluxes of CO_2 and N_2O vary significantly over space and time driven by microbiological processes, environmental conditions, heterogeneity of soil properties and spatial variation in available nutrients and root distribution (Butterbach-Bahl et al., 2011; Davidson et al., 2000; Verchot et al., 1999). Specifically, small-scale spatial variability – within a few meters – commonly exceeds 100% (Parkin and Venterea, 2010). Thus, replicated chamber measurements on sites investigated is required to achieve robust representative emission rates. Furthermore, when investigating CO_2 and N_2O fluxes in landscapes with a mosaic of land uses and land covers, the total number of samples needed to provide a reliable flux estimate quickly exceeds analytical capacities.

Pooling is accepted and widely used for soil sampling, but it was so far not tested as a method to overcome the limitations imposed by time-consuming (and hence cost intensive) analytics

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http://dx.doi.org/10.1016/j.soilbio.2013.08.011

and procedures in CO_2 and N_2O measurements. Here we propose gas sample pooling to reduce the number of gas samples required while maintaining the reliability of the estimated CO_2 and N_2O flux.

We selected three different experimental sites for measuring N_2O and CO_2 soil-atmosphere fluxes with five chambers each over a four-week period: a forest (a 30-years old *Eucalyptus* plantation) a non-grazed grassland and a kale (*Brassica oleracea* L.) cropland. Each site had at least an area of 0.25 ha. We compared the emission rates obtained with the gas sample pooling technique (Fig. 1) with the mean rates of each observation date calculated from sampling the five individual chambers following the traditional method. The number of chambers was chosen in order to allow one operator to conduct the gas sampling within chamber closure period (40 min).

The study sites were located on the Maseno University Campus (Kenya) (0°, 34° 36′E). The experiment was conducted from 29 October to 29 November in 2012. Site characteristics are presented in Table 1. At each site, five 35 by 25 cm^2 PVC frames (collars) were inserted prior to the first measurement and remained in place throughout the experimental period. For CO_2 and N_2O measurements, a PVC chamber (12 cm high), equipped with a fan, a non-forced vent and a sampling port was affixed to the frame by metal clamps and a rubber sealing between frame and chamber to assure air-tight seal.

For individual chamber measurements 50 ml gas sample was taken from the chamber headspace with a gas tight syringe through a stopcock valve at 10 min intervals (0, 10, 20, 30, and 40 min after chamber closure). If the use of a fan is not possible, an extra syringe should be used to carefully pump several times before taking the gas sample to obtain homogenous mixing of the headspace air.

For the gas pooling technique, a 10 ml sample was collected from each of the five chambers with the same syringe at each time interval equaling 50 ml in total (Fig. 1a). The 50 ml gas samples were then immediately transferred into 10 ml sealed glass vials (Fig. 1b) and analyzed by gas chromatography (^{63}Ni-Electron capture detector for N_2O and Flame ionization detector equipped with a methanizer for CO_2). Detailed information about the analytical procedure can be found in Gauder et al. (2012) Flux rates of N_2O and CO_2 were calculated from the linear change in gas concentrations in the chamber headspace with time.

It was our aim to test the applicability of the gas pooling technique across a range of N_2O and CO_2 emission rates. Therefore, on November 5th, cropland and grassland experimental sites were fertilized with granular urea dissolved in water at a rate of 100 kg N ha^{-1} simulating a 10 mm rainfall event.

Results from individual chamber measurements and gas sample pooling technique highly agreed for CO_2 measurements, capturing temporal variations of fluxes over the observation period (Fig. 2).

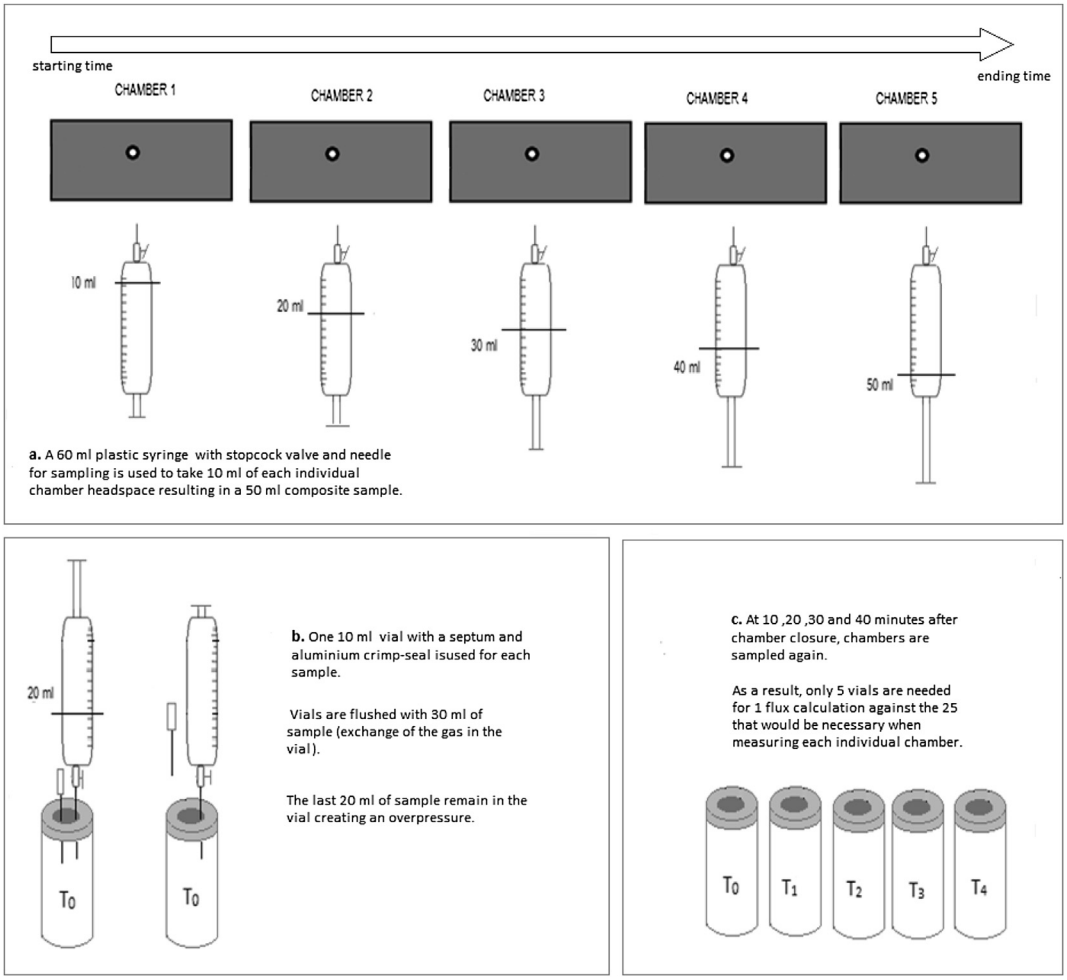

Fig. 1. Concept of gas sample pooling.

Table 1
Soil properties of the sites.

Site	Depth (cm)	C (g kg^{-1})	N (g kg^{-1})	pH	Sand (%)	Silt (%)	Clay (%)
Forest	0–5	28.6 ± 6.8	2.14 ± 0.39	5.0 ± 0.6	18.7 ± 1.7	29.2 ± 1.6	52.1 ± 3.1
	5–20	9.0 ± 0.5	0.93 ± 0.05	4.8 ± 0.1	10.2 ± 2.6	21.8 ± 2.0	68.0 ± 3.5
Grassland	0–5	26.1 ± 4.0	2.47 ± 0.38	6.1 ± 0.1	17.5 ± 1.6	28.3±1.9	54.2 ± 3.0
	5–20	20.4 ± 1.9	1.96 ± 0.17	6.0 ± 0.1	16.5 ± 0.6	23.8 ± 1.6	59.7 ± 2.2
Cropland	0–5	15.6 ± 1.1	1.44 ± 0.12	5.2 ± 0.2	20.6 ± 5.3	20.1 ± 2.0	59.3 ± 6.1
	5–20	13.9 ± 1.9	1.28 ± 0.14	4.8 ± 0.1	16.5 ± 5.2	17.4 ± 1.6	66.1 ± 4.8

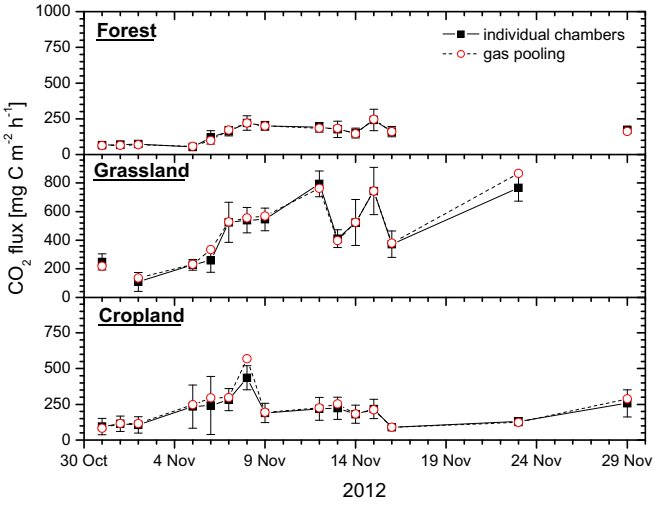

Fig. 2. Soil CO_2 Fluxes in the three experimental sites over the observation period. Vertical bars denote one standard error of the mean of the individual chambers.

CO_2 fluxes ranged between 50 and greater than 700 mg CO_2–C m^{-2} h^{-1} (Table 2) and absolute differences in single chamber means and gas pooling values for individual measuring days and CO_2 fluxes were in average 21, 22 and 6 mg CO_2–C m^{-2} h^{-1} for cropland, grassland and forest, respectively. The mean values across the entire observation period calculated for single chambers and the gas sample pooling method differed by 8.3, 2.5 and 1.6% (Table 2). Linear regression between conventional sampling and gas pooling revealed no systematic bias (f(x) = 1.043 ± 0.024 x; $R^2 = 0.98$).

Comparable results were obtained for N_2O fluxes (Fig. 3) ranging between 0 and 400 μg N_2O–N m^{-2} h^{-1} (Table 2): Mean fluxes for individual observation days differed by 14, 7, 5 μg N_2O–N m^{-2} h^{-1}, representing a difference between single chambers and gas sample pooling between 3 and 4% for the whole observation period (Table 2). Linear regression results for N_2O fluxes comparing conventional sampling and gas sample pooling were: f(x) = 0.996 ± 0.018 x; $R^2 = 0.99$.

Based on these results we conclude that the gas sample pooling method provides an opportunity for an optimized targeted sampling design to overcome heterogeneity of soil CO_2 and N_2O exchange. The method has advantages over ultra large chambers (e.g. Galle et al., 1994), which are more expensive, difficult to handle and can cover the spatial variability up to only a few square meters. Though our results show good agreement between the conventional and the gas pooling method, a careful application is required since reliable CO_2 and N_2O flux estimates still highly depend on a scientific sound experimental design. The gas sample pooling method can be used in, mainly, two ways: 1) gas samples from several chambers can be pooled from one plot, obtaining a single spatial representative flux; or 2) several sets of chambers can be placed within a plot, and each of the sets can be pooled separately. The former will dramatically decrease the analytical capacities needed, while losing the information of the spatial variability of the fluxes at the plot. With the same analytical capacity as when analyzing individual chambers, the latter will increase both the area effectively sampled and the human resources needed on the field. Thus, the design of the gas pooling technique needs to be specifically adjusted, based on the characteristics of the environment under investigation and the research questions of each specific experiment. To spatially locate the chambers in representative spots, we recommend to measure the soil CO_2 and N_2O fluxes from individual chambers prior to the start of the experiment in order to assess the spatial variability of the site and eventually replace chambers, since subsequent removal of outliers upon results (Crill et al., 2000) is not feasible. Furthermore, the spatial variability should be characterized at regular intervals by applying the standard method of single chamber based measurements, since the spatial variability may seasonally vary.

The approach of sample pooling has been widely applied for ecosystem studies e.g. plant and soil material and water (Patty, 2013; Pennock et al., 2006; Wilde, 2006). Our results demonstrate that pooling is a sensible, low cost and easy to deploy technique for monitoring soil CO_2 and N_2O fluxes with potential to radically increase the amount of information on the biosphere–atmosphere exchange of CO_2 and N_2O.

Table 2
Summary of soil CO_2 and N_2O fluxes, soil temperature and soil moisture during the measurement campaign.

Site	CO_2 flux pooling	CO_2 flux individual chambers	CV CO_2 flux individual chambers	N_2O flux pooling	N_2O flux individual chambers	CV N_2O flux individual chambers	Soil temperature	Soil moisture
	mg CO_2–C m^{-2} h^{-1}		%	μg N_2O–N m^{-2} h^{-1}		%	°C	m^3 m^{-3}
Forest	144.2 (57.4–247.1)	146.7 (51.7–241.7)	25 (8–40)	21.2 (−3.5–54.4)	20.5 (2.4–47.3)	63 (24–123)	20.7 (19.9–21.7)	0.40 (0.24–0.49)
Grassland	480.3 (135.2–867.2)	466.6 (108.8–793.7)	52 (12–97)	39.8 (13.7–96.3)	38.2 (3.1–88.5)	90 (26–326)	24.3 (22.0–27.7)	0.43 (0.37–0.48)
Cropland	219.2 (83.5–568.4)	201.1 (89.5–435.6)	25 (14–84)	150.9 (12.2–530.6)	145.5 (8.5–520.5)	72 (28–220)	23.3 (20.2–26.9)	0.36 (0.24–0.44)

Mean (minimum–maximum) daily values for the three sites under investigation along the measurement campaign. CV denotes coefficient of variation.

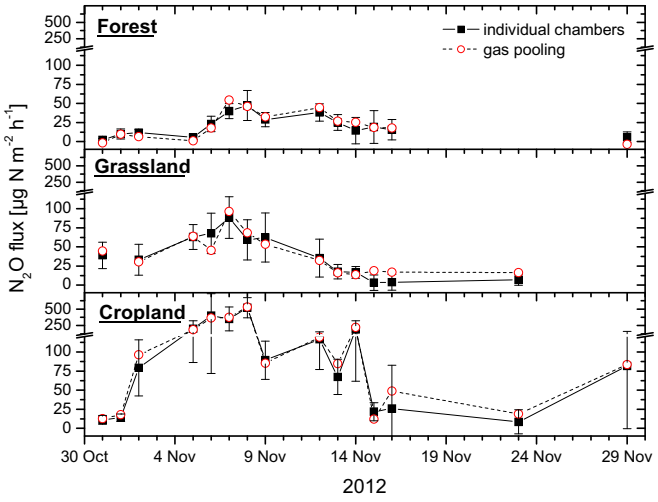

Fig. 3. Soil N_2O Fluxes in the three experimental sites over the observation period. Vertical bars denote one standard error of the mean of the individual chambers.

Acknowledgments

This work was funded by the SAMPLES project under the CCAFS program of the Consultative Group on International Agricultural Research, the German Science Foundation under the contract BU 1173/12-1 and the EU funded IA project InGOS. CAN acknowledges financial support by CLIFFS, CCAFS. Funding for RK and KBB has been partly provided by DFG in the frame of the research group ICON (FOR 1701).

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Spatial variability of soil N_2O and CO_2 fluxes in different topographic positions in a tropical montane forest in Kenya.

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Key Points:

- We evaluated the spatial variability of soil CO_2 and N_2O emissions and their relation to topography in a tropical montane forest.
- Soils at mid-slope position emitted significantly less N_2O than at ridge top and valley bottom while no effect of topography on the soil CO_2 fluxes was found.
- Soil N_2O and CO_2 fluxes show no spatial pattern at plot level, with "hot spots" strongly contributing to the total emissions.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/2016JG003667

Abstract

Quantifying and understanding the small-scale variability of nitrous oxide (N_2O) and carbon dioxide (CO_2) emission is essential for reporting accurate ecosystem greenhouse gas budgets. The objective of this study was to evaluate the spatial pattern of soil CO_2 and N_2O emissions and their relation to topography in a tropical montane forest. We measured fluxes of N_2O and CO_2 from 810 sampling locations across valley bottom, mid-slope and ridge top positions under controlled laboratory conditions. We further calculated the minimum number of samples necessary to provide best estimates of soil N_2O and CO_2 fluxes at the plot level. Topography exhibited a major influence on N_2O emissions, with soils at mid-slope position emitting significantly less than at ridge tops and valley bottoms, but no consistent effect of topography on soil CO_2 emissions was found. The high spatial variation of N_2O and CO_2 fluxes was further increased by changes in vegetation and soil properties resulting from human disturbance associated with charcoal production. Soil N_2O and CO_2 fluxes showed no spatial pattern at the plot level, with "hot spots" strongly contributing to the total emissions (10 % of the soil cores represented 73 and 50 % of the total N_2O and CO_2 emissions, respectively). Thus, a large number of samples are needed to obtain robust estimates of N_2O and CO_2 fluxes. Our results highlight the complex biogeochemical cycling in tropical montane forests, and the need to carefully address it in research experiments to robustly estimate soil CO_2 and N_2O fluxes at the ecosystem scale.

1 Introduction

Soils are dominant sources of atmospheric carbon dioxide (CO_2) and nitrous oxide (N_2O), important greenhouse gases (GHG) contributing to global warming [*IPCC*, 2014]. Globally, soil respiration releases about 100 Pg C yr^{-1} to the atmosphere [*Bond-Lamberty and Thomson*, 2010] and typically accounts for between 30 and 80 % of total ecosystem respiration [*Davidson et al.*, 2006]. Tropical forests contain about 40 % of the total terrestrial biomass C stock [*Field et al.*, 1998; *Phillips et al.*, 1998] and contribute approximately 2/3 of the global annual total soil C flux [*Bond-Lamberty and Thomson*, 2010]. Therefore, small variations in the soil CO_2 efflux can have a strong influence on the ecosystem C balance. Tropical forests also play a key role in the global atmospheric balance of N_2O [*Werner et al.*, 2007; *van Lent et al.*, 2015], contributing an annual release of 4.4 Tg N (14 to 23 % of the total N_2O source strength) [*IPCC*, 2007]. This estimate is associated with a high uncertainty due to both high temporal [*Meixner et al.*, 1997; *Werner et al.*, 2007; *Lin et al.*, 2010] and spatial variability [*Breuer et al.*, 2000; *Allen et al.*, 2010] of soil N_2O fluxes at different scales. In addition, the scarcity of reliable estimates in the peer-reviewed literature hampers drawing general conclusions and upscaling at larger spatial scales, especially in African ecosystems, which remain highly underrepresented [*van Lent et al.*, 2015].

Understanding the factors that affect spatial and temporal variability of GHG emissions is an important area of ongoing research. While both soil temperature and water content have been shown to be key factors responsible for the variation in soil CO_2 efflux at scales ranging from global to the plot [*Davidson et al.*, 2000; *Bond-Lamberty and Thomson*, 2010], a different picture is emerging for tropical soils. 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 and spatial variation of soil respiration [*Davidson et al.*, 2000].

Soil production of CO_2 is the result of root respiration and heterotrophic decomposition of soil organic matter, which returns to the atmosphere some of the C fixed in the first step through photosynthesis. The microbial processes of nitrification and denitrification are the dominant sources of N_2O from the soil [*Butterbach-Bahl et al.*, 2013]. Soil physical, chemical and biological factors and their interactions control these processes, which vary in both space and time. As a result, N_2O and CO_2 fluxes emitted from soils usually show a high degree of spatial and temporal variability [*Folorunso and Rolston*, 1984; *Parkin*, 1993]. In turn, the parameters controlling the soil GHG fluxes are affected by topography, either directly through dynamics of surface and subsurface water, nutrients and dissolved organic matter [*Fang et al.*, 2009], or indirectly, via soil texture and vegetation [*Luizão et al.*, 2004].

Analysis of the combined effect of small-scale spatial variability and topography on soil N_2O and CO_2 emissions has been thoroughly investigated in temperate ecosystems [e.g. *Corre et al.*, 1996; *Ambus*, 1998; *Jungkunst et al.*, 2008; *Yu et al.*, 2008]. However, in the tropics this issue has been scarcely studied [*Reiners et al.*, 1998; *Breuer et al.*, 2000; *Fang et al.*, 2009], despite the fact that this information is essential to scale up processes measured at small scales (plots) to larger scales (ecosystem), which is of high relevance in ecological research [*Butterbach-Bahl et al.*, 2013].

To obtain robust estimations at the ecosystem level, large sample sizes are needed, but labor and time constraints limit the number of measurements in soil GHG fluxes studies [*Adachi et al.*, 2005]. Yet, laboratory incubations usually allow for measuring trace gas emissions from a larger number of samples than is often possible in field studies, which can be a better approach to addressing spatial variability of soil GHG fluxes more effectively.

Given the few studies investigating N_2O and CO_2 fluxes from African tropical montane forest soils and the limited knowledge on both their spatial variability and controlling factors, we collected intact topsoil cores (0-5 cm) from 810 spatially-explicit

locations at three topographic positions (valley bottom, mid-slope, and ridge top) in the Southwest Mau Forest Complex in Kenya and estimated potential soil N_2O and CO_2 fluxes through laboratory incubations under standardized conditions.

This study was designed to:

1. Provide estimates of potential soil-atmosphere N_2O and CO_2 exchange rates from a tropical montane forest in Kenya.
2. Assess and quantify the spatial variability of soil N_2O and CO_2 emission potential and soil properties at varying topographic positions.
3. Determine the minimum number of samples necessary to provide best estimates of soil N_2O and CO_2 fluxes at plot level.

We hypothesized i) topographic position (valley bottom, mid-slope, and ridge top) would influence the soil emission rates of N_2O and CO_2 and ii) that soil physico-chemical properties would be useful parameters for explaining spatial variability of soil N_2O and CO_2 fluxes at the plot level.

2 Study Site

The Mau Forest Complex in Kenya is the largest closed canopy forest in the country as well as indigenous Afromontane forest in East Africa covering an area of about 417000 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 a cool, humid-tropical with a mean annual precipitation between 1800 and 1950 mm (1979-2009) [*Omumbo et al.,* 2011]. The area has a bi-modal rainfall pattern, with the "long rains" falling between April and August and "short rains" between October and December, respectively, while January and February are generally the driest months. The mean annual temperature ranges from 15.7 to 17.5 °C (1979-2009) [*Omumbo et al.,* 2011] with slight variations over the year. The geology is

dominated by Tertiary lavas from the mid-Miocene epoch [*Blackie and Edwards*, 1981]. The soils are well drained, very deep, dark reddish-brown, clayey, and with an acidic humic topsoil [*Krhoda*, 1988; *Jaetzold et al.*, 2010] and classified as Andic Humic Nitisols [*IUSS Working Group WRB*, 2015].

The Mau Forest has undergone large scale deforestation and degradation since the mid-1990s due to forest excisions and illegal logging, encroachments, and charcoal burning [*UNEP*, 2009; *Were et al.*, 2013]. Especially in the Southwest part of the Mau, due to the proximity to smallholder farms, the use of temporary charcoal kilns for production of charcoal on-site in the forest is still common.

3 Materials and Methods

3.1 Experimental design

We used a stratified sampling approach to select sampling plots based on topographic information. Gridded elevation data was obtained from the Advanced Space Borne Thermal Emission and Reflection Radiometer (ASTER, v. 2) in a 30 m x 30 m grid cell resolution. We calculated the standardized topographic position index (TPI) for each grid cell segmenting the study area (4 km x 3 km, Figure 2a) into three topographic classes that represent ridge top, mid-slope and valley bottom positions [*Wilson and Gallant*, 2000] using ArcGis 10.1 and the Land Facet Corridor Designer (v. 1.2.884). We further restricted the three TPI classes to level and gentle slopes (< 7 %) for ridge top and valley bottom positions, and steep slopes (>= 7 %) for mid-slope positions, to avoid potential mis-positioning of sampling plots due to vertical errors inherent to ASTER data [*ASTER GDEM Validation Team*, 2011]. Three transects across the forest served as spatial replicates. One 1-ha plot for each topographic position was selected along these transects, resulting in nine sampling plots (Figure 2a).

Within each sampling plot, 90 sampling locations were randomly placed on the nodes of a grid of 5 m x 5 m (Figure 2b), yielding a total of 810 sampling locations.

3.2 Soil samples collection

Soil core sampling was conducted in February and March 2014, under stable meteorological conditions without rainfall. To minimize disturbance we used intact soil cores in our study [*Reichstein et al.*, 2005]. Prior to the core collection, the forest floor was removed. At each sampling location seven soil cores (n =5670) were collected from the uppermost 5 cm of the mineral soil profile using sharpened-edge PVC cylinders (5 cm inner diameter, 5 cm height). The cylinders were gently hammered into the soil with help of a wooden block, to keep soil compaction to a minimum. The cylinders were carefully taken out of the soil, sealed with Parafilm® (Bemis NA, Neenah WI, USA) and transported to the laboratory at Mazingira Centre, International Livestock Research Institute (Nairobi, Kenya) for analysis. The intact soil cores were air-dried and stored at ambient temperature until analyses were conducted, on average 4 weeks after sampling. Four intact soil cores were used for GHG sampling and the other three for soil analysis.

There were indications of anthropogenic disturbance on most of the plots, such as charcoal burning and illegal logging. To capture these disturbance effects, we recorded at each sampling location: i) percentage of canopy cover using a Crown Mirror – Densiometer (Grube KG Forstgerätestelle, Bispingen, Germany), ii) shortest distance to a charcoal kiln. Further we recorded number of charcoal kilns within each 1 ha plot.

3.3 Soil laboratory incubations

3.3.1 Potential N_2O and CO_2 flux measurements: Experiment 1

We measured potential N_2O and CO_2 emissions from air-dried and rewetted soil samples equivalent to a rainfall of 10 mm. The soil was allowed to equilibrate for 24 hours to the new moisture condition before starting measurements for N_2O and CO_2 production. The water content in this study was roughly 50 % water-filled pore space (WFPS) which is sufficient to induce a peak for microbial activity [*Liebig et al.*, 1996; *Appel*, 1998; *Haney and Haney*, 2010] and it was close to the moisture content of the soil during sample collection. Air temperature during the incubation was kept constant at 17 °C as this represents the annual mean temperature. Soil cores were incubated using adapted gas-tight Kilner jars (volume 640 cm^3) fitted with rubber septum in the lid for headspace gas sampling by a syringe. We homogenized the jar headspace by pumping with an extra empty syringe before sampling. No ambient air was injected to correct for the change in pressure associated with the removal of air sample. We used four soil core replicates and pooled the samples as per *Arias-Navarro et al.* [2013] for each flux calculation, i.e. a 10 ml gas sample was taken from each individual jar headspace at 15 min intervals (0, 15, 30 and 45 min after chamber closure) with the same syringe at each time interval resulting in a 40 ml composite sample. The first 20 ml of the sample was used to flush a 10 ml-glass vial, which was filled with the remaining 20 ml creating over-pressure to minimize the risk of contamination by ambient air. Concentrations of CO_2 and N_2O in the gas samples were analyzed within 2-3 days by gas chromatography (8610C; SRI Instruments, Torrance, CA, USA) with a ^{63}Ni-Electron capture detector for N_2O and Flame ionization detector equipped with a methanizer for CO_2. Further details on the analytical procedure can be found in *Rosenstock et al.* [2015]. We calculated CO_2 and N_2O fluxes from the linear change of the gas concentrations over time, considering the headspace

volume and referred the fluxes to the soil surface of the core. No saturation effect in the headspace was observed over the entire incubation period.

3.3.2 Effect of soil moisture: Experiment 2

A second incubation study was conducted to examine the effect of soil water content on soil N_2O and CO_2 fluxes at the different topographic positions. We used a subset of samples from transect 3. Potential N_2O emissions (from experiment 1) were sorted from smallest to largest and then divided in groups by terciles (< 33 %: low emission potential group, 34-66 %: medium emission potential group, > 67 %: high emission potential group). We randomly selected 20 sampling locations from each group to represent each tercile. Maximum water-holding capacity (WHC) was determined on one spare soil core by placing the soil wrapped in absorbent filter paper into a glass jar and saturating it with water. The soil core was weighed after 4 hours, dried at 105 °C to constant weight and weighed again. Water holding capacity was calculated as g water per g dry weight of soil based on the difference. During the incubation, gas pooling was applied [*Arias-Navarro et al.*, 2013], yielding 5 replicates of 4 sampling locations each. The air-dried soil cores were measured at each incubation to record the baseline emission. Afterwards, the soil cores were adjusted to soil moisture contents of 20, 40, 70 and 90 % WHC each, by adding water before the flux measurements started. Soil cores were incubated and measured 4, 24, 48 and 72 hours after the initial rewetting. Determination of GHG concentrations and calculation of GHG fluxes were performed similarly to Experiment 1. Cumulative N_2O and CO_2 emissions were calculated from the integrated individual fluxes, assuming a constant flux rate between gas sampling times.

3.4 Soil properties

We measured total soil organic carbon (SOC), total nitrogen (TN), pH and bulk density (BD) for each of the 810 sampling locations, using three spare soil cores. Total SOC and TN were analyzed with an Elemental Combustion Analyzer (ECS 4010, Costech Analytical Technologies, Inc., Milano, Italy). Soil pH was measured in deionized water suspension (water:soil weight ratio 2.5:1), using a glass electrode. Bulk density (BD) was calculated as the mass of the oven-dry soil (105 °C) divided by the core volume. Stones or coarse fragments were absent so no correction was needed.

3.5 Sample size required for accurate estimates

In order to estimate the minimum number of samples needed to reliably represent the heterogeneity observed by measuring at 90 points we compared the mean soil CO_2 and N_2O fluxes and soil properties of various smaller sample sizes with those considering the 90 samples (true mean). That is, we calculated the distance between the true mean and the mean values with sample sizes K (K=1, 2…, 89), hereafter M_K .The M_K values are computed using a large number of sample subsets of size K from the set of 90 points to obtain ranges of means. Because of the enormous number of possible subsets for most K, we randomly drew 100,000 distinct sample subsets without replacements for each M_K [*Jackson and Somers*, 1989]. To produce all combinations with equal probabilities we used a uniform random generation algorithm. For each K we analysed the range of the means and identified the minimum K being within 10 % to 30 % of the true mean of various confidence levels (80 % - 95 %).

3.6 Statistical analysis

Pearson's correlation coefficient was used to identify significant correlations between measured variables. One-way analysis of variance (ANOVA) - with transects as blocking

factor - was used to test differences in soil N_2O and CO_2 fluxes, canopy cover and soil properties across topographic positions. Two-way ANOVA was used to test differences between measured N_2O and CO_2 fluxes at the various % WHC and topographic positions. The data were tested for normality and homogeneity of variances and a log transformation was used for both N_2O and CO_2 fluxes. If the ANOVA results showed significant difference, a Post-hoc Student-Newman-Keuls (SNK) test method was used to identify the difference. The contribution of soil parameters and disturbance variables to the N_2O and CO_2 gas fluxes was calculated using step-wise multiple regression analysis. The final models were chosen based on the lowest Akaike's Information Criterion (AIC). All statistical analyses and plotting were carried out using R 3.3.2 [*R Core Team*, 2016]. Downloading and formatting Google Maps images was done with the ggmap package [*Kahle and Wickham*, 2013]. We have used the functions aov {stats} to fit ANOVA models and lm {stats} to carry out regressions. Statistical models were interpreted using effect displays for a particular term in the model (R Package effects; [*Fox et al.*, 2003]). Spatial analyses were carried out using the R package for geostatistical analysis gstat [*Pebesma*, 2004] based on variography and interpolation techniques. We computed z-scores (differences between each value and the mean divided by standard deviation) using the R package outliers [*Komsta*, 2011] and defined “hot spots” as observations that exceededthe 90^{th} percentile based on a given score. Statistical significance is given at the 95% confidence level ($p \leq 0.05$). Significance of statistical tests is noted as follows: *** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$; n.s. = $p > 0.05$.

4 Results

4.1 Soil properties

Soil properties for each topographic position are shown in Table 1. Soil organic carbon was very high (118.6-171.9 g kg^{-1}) in all plots and showed significantly higher values in the mid-slope position compared to the ridge top and valley bottom. Total nitrogen (TN) ranged from 12.5 to 15.3 g kg^{-1}.Soils at mid-slope position showed as well the highest TN content although not significantly different from valley bottom position. The lowest soil pH values were found at the ridge top positions and increased along the topographic gradient. No significant differences among topographic positions were found for BD and canopy cover. Presence of charcoal kilns in our study were higher in mid-slope plots.

4.2 Experiment 1

4.2.1 Potential N_2O and CO_2 fluxes

The water addition increased water content in the soil cores to approximately 50 % WHC. Soil N_2O and CO_2 fluxes showed a large variability among sampling locations within each plot (results from Valley bottom transect 1 are provided in Figure 3 as an example; the rest of the plots are included in the supplementary material file). Potential soil N_2O emissions rates ranged from 1.3 to 980.0 µg N m^{-2} h^{-1} for valley bottom, 0.05 to 1184.6 µg N m^{-2} h^{-1} for mid-slope, and 0.2 to 1356.3 µg N m^{-2} h^{-1} for ridge top plots. Coefficients of variation were between 115 and 143 % for each plot. Our analyses show that topographic position significantly influenced N_2O flux emission potentials (Table 1). Mean N_2O fluxes from mid-slope plots (105.0 ± 9.4 µg-N $m^{-2}h^{-1}$) were significantly lower than the fluxes from ridge top (135.2 ± 9.9 µg-N $m^{-2}h^{-1}$) and valley bottom plots (156.3 ± 12.0 µg-N $m^{-2}h^{-1}$).

Potential soil CO_2 fluxes rates ranged from 3.0 to 104.0 mg C m^{-2} h^{-1} for the valley bottom, from 4.4 to 145.0 mg C m^{-2} h^{-1} for the mid-slope, and from 1.7 to 98.6 mg C m^{-2} h^{-1}

for the ridge top plots. Coefficients of variation were between 20 and 50 %. Soil CO_2 mean fluxes decreased along the topographic gradient with ridge top (50.4 ± 1.0 mg C m^{-2} h^{-1}) having significantly higher fluxes than the valley bottom (44.8 ± 1.3 mg C m^{-2} h^{-1}) and the mid-slope (46.3 ± 1.3 mg C m^{-2} h^{-1}).

4.2.2 Spatial Variability

The multiple linear regression models for N_2O and CO_2 fluxes using measured soil properties and canopy cover had very low explanatory power, with an adjusted R^2 of 0.04 and 0.20, respectively. Soil N_2O and CO_2 fluxes were weakly but significantly correlated ($R=0.19$, $p \leq 0.001$). Soil N_2O fluxes were positively correlated with TN ($R=0.19$, $p \leq 0.001$) and SOC ($R=0.15$, $p \leq 0.001$), and negatively correlated with BD ($R= -0.15$, $p \leq 0.001$). No correlation was found between the soil C:N ratio and flux data. The soil CO_2 flux showed a negative correlation with pH ($R= - 0.24$, $p \leq 0.001$), BD ($R= -0.12$, $p \leq 0.001$), SOC ($R= -0.10$, $p \leq 0.05$) and soil C:N ratio ($R= -0.13$, $p \leq 0.001$). Bulk density was negatively correlated with TN and SOC ($R = -0.57$ and -0.59, respectively, $p \leq 0.001$). The presence of charcoal kilns affected soil N_2O and CO_2 fluxes and soil properties. Inside the zone of influence of a charcoal kiln (i.e. within 5 m) the soils emitted less N_2O ($p \leq 0.05$). Distance to charcoal kiln was negatively correlated with CO_2 ($R=-0.26$, $p \leq 0.001$). Higher numbers of charcoal kilns in a plot were associated with higher CO_2 fluxes ($R=0.12$, $p \leq 0.001$) and BD values ($R=0.17$, $p \leq 0.001$). Canopy cover showed positive correlations ($R = 0.18$, $p \leq 0.001$) with the soil C:N ratio, pH ($R = 0.11$, $p \leq 0.001$), and distance to charcoal kiln ($R = 0.24$, $p \leq 0.001$).

Occasional locations with particularly high rates (i.e. “hot spots”) contributed substantially to the mean N_2O and CO_2 fluxes, as it is visualized in the empirical cumulative density functions (Figure 4). The contribution of the samples above the 90^{th} percentile to the total N_2O and CO_2 fluxes was 73 % and 50 % respectively. For N_2O, 84 sampling locations

were identified as "hot spots" (mean value: 545.1 µg-N $m^{-2}h^{-1}$) and 73 for CO_2 (mean value: 54.66 mg C m^{-2} h^{-1}). When the "hot spot" data are excluded, the overall N_2O mean emission rate decreased from 132.3 µg-N $m^{-2}h^{-1}$ to 83.7 µg-N $m^{-2}h^{-1}$ while the average soil respiration rate decreased from 47.2 mg C m^{-2} h^{-1} to 46.4 mg C m^{-2} h^{-1}.

The geostatistical analyses indicated that N_2O and CO_2 fluxes and soil properties had no spatial autocorrelation, at least for the range of distances investigated here (5-90 m).. Semivariograms for CO_2 and N_2O fluxes and soil properties for the 9 sampling plots are presented in the supplementary information file. Due to the lack of an identifiable semivariance structure, kriging interpolation for the plot level is not appropriate. This result enabled us to consider our measurement locations as independent samples for inferential statistics.

4.2.3 Minimum sample size for accurate estimates

Our results indicate that measurements at 37 ± 5 locations at each plot are needed to obtain an estimate within 30% of the true mean of N_2O at a confidence level of 95%. For 20 % and 10 % precision, 55 ± 5 and 78 ± 5 locations are needed, respectively. Measurements at6 ± 2, 11 ± 5 and 30 ± 11 locations are needed to obtain an estimate within 30 %, 20 % and 10 % of the true mean for CO_2. These numbers are average values for all 9 sampling plots. Variability among plots was small as can be noted in the small standard deviation in the number of samples needed to reach a desired precision. Three samples are needed for estimating soil properties within 20 % of variation with respect to the true mean, except for BD, where 9 samples are necessary (Table 2).

4.3 Effect of soil moisture: Experiment 2

Detectable N_2O emissions were observed four hours after watering the cores. At 30 and 50 % WHC, soils reached maximum emission rates immediately after water application; in the case of 70 and 90 % WHC treatments, N_2O production steadily increased until 48 hours after the initial wetting, when the highest emission rates were observed. The N_2O pulse emissions correlated positively with % WHC at all topographic positions (20 % WHC: 11.1 µg N m^{-2} h^{-1}, 50 % WHC: 122.0 µg N m^{-2} h^{-1}, 70 % WHC: 452.9 µg N m^{-2} h^{-1}, 90 % WHC: 899.6 µg N m^{-2} h^{-1}). Cores from the mid-slope position tended to emit less N_2O compared with ridge top or valley bottom positions although there was no significant difference between topographic positions at 20 % and 50 % WHC. At 70 % and 90 % WHC, the mid-slope N_2O flux was significantly lower than the valley bottom but not the ridge top (Figure 5a).

Increased CO_2 emissions were detected 4 hours after watering the cores with CO_2 fluxes of 19.0, 24.7, 27.4 and 52.4 mg C m^{-2} h^{-1} for soils at 20, 50, 70 and 90 % WHC respectively. Emissions decreased the following day for 20, 50 and 90 % WHC, while soils at 70 % WHC showed a second pulse (40.3 mg C m^{-2} h^{-1}) after 2 days. Soil CO_2 flux rates leveled off after 3 days for all topographic positions and water contents. Soil CO_2 fluxes from the three topographic positions increased at 70 and 90 % WHC compared with low moisture contents (20 and 50 % WHC treatments). No significant differences among topographic positions were observed at any WHC level (Figure 5b).

5 Discussion

Soil properties showed no or weak correlations with N_2O fluxes, as observed in other studies examining the factors governing spatial variability of N_2O emission at different levels [e.g. *van den Heuvel et al*., 2009]. Positive correlation of N_2O emissions with TN is in agreement to observations from *Booth et al.* [2005] from a wide range of ecosystems. Surprisingly, soil CO_2 fluxes were negatively correlated with SOC, possibly because areas with high SOC were associated with higher levels of black C i.e. a highly stable C form, due to charcoal making. Soils in our study had low BD, were very porous, and had high organic matter content and high water storage capacity. Bulk density was strongly related to SOC as has been found in numerous other studies [*Tamminen and Starr*, 1994]. The low BD values found here are in good agreement to other studies in tropical montane forest, and have been associated to the high organic matter content in the topsoil [*Hafkenscheid*, 2000; *Jeyanny et al*., 2014].

The importance of landscape position on N_2O emissions has been documented by other studies [*Pennock et al.*, 1992; *Corre et al.*, 1996], which found greater N_2O emissions in the valley bottom than in the ridge top or mid-slope positions. Differences in moisture content along the slope usually explain the N_2O flux variability [*Vilain et al.*, 2011; *Negassa et al.*, 2015], with higher fluxes in the lower positions closely correlated with the higher WFPS. However, our approach involved standardized soil moisture levels across the treatments, and we still observed position-driven variations of N_2O emission with lower N_2O emitted at the steeply sloping plots when compared with flat plots (ridge top and valley bottom plots). Experiment 2 corroborated the results from experiment 1, with a lower N_2O emissions in cores taken from the mid-slope position for all moisture contents. This result indicates that topography is an important factor influencing N_2O emissions, in line with our

first hypothesis. Significant effects of topographic position on multiple aspects of the N cycle have been shown by *Weintraub et al.* [2014] indicating lower N availability and a less open N cycle in steep parts of the landscape compared to a relatively flat terrain, supporting our findings.

Soil water content (expressed as % WHC) in our study was a determinant parameter for the N_2O flux rates in all topographic positions. Microbial denitrification, which yields N_2O, is mainly driven by redox potential, substrate availability, and oxygen diffusion, which strongly depend on the water availability and the water-free pore space in soil [*Dobbie and Smith*, 2003; *Bateman and Baggs*, 2005]. Higher N_2O fluxes at high soil water contents have been reported from laboratory [*Hergoualc'h et al.*, 2007; *Schaufler et al.*, 2010; *Werner et al.*, 2014] and field studies [*Pennock et al.*, 1992; *van Kessel et al.*, 1993; *Corre et al.*, 1996; *Pennock and Corre*, 2001] and have been associated to increasing denitrifying activity due to reduced O_2 diffusion into the soil [*Ruser et al.*, 2006; *Yanai et al.*, 2007].

Soil moisture also played a major role in the soil CO_2 fluxes, which is consistent with other studies where, at constant temperature, wetter soils emitted more CO_2 due to better conditions for microbial respiration [*Zhou et al.*, 2013]. This observation is only true until the point of water saturation when CO_2 fluxes tend to decrease, as those conditions favor the development of anaerobiosis, slowing down the decomposition of organic matter and reducing CO_2 diffusion to the atmosphere [*Smith*, 1990; *Knowles et al.*, 2015] but our soils were predominantly aerobic even at 90 % WHC due to the low BD of the topsoils (< 1 g cm^{-3}). Contrary to previous studies [*Brito et al.*, 2009; *Martin and Bolstad*, 2009; *Riveros-Iregui and McGlynn*, 2009] soil CO_2 fluxes were not related to topographic position. The decreased susceptibility to microbial SOC degradation caused by the presence of allophane minerals in our plots [*McBride*, 1994; *Parfitt*, 2009] and the high tree diversity may have influenced to some extent the spatial pattern of the soil CO_2 efflux [*Katayama et al.*, 2009; *Matvienko et*

al., 2014]. Large diversity of tree species in tropical forests lead to heterogeneity in chemical, structural and functional traits that affect biogeochemical processes [*Hättenschwiler et al.*, 2008; *Townsend et al.*, 2008] contributing to the spatial heterogeneity of soil respiration.

Furthermore, the disturbance caused by charcoal making also affected soil CO_2 fluxes. Charcoal production implies felling of trees or parts of them and wood carbonization in traditional kilns, affecting the soil at two different levels. Firstly, soil is disturbed at the kiln site due to the extreme heat generated during the carbonization process and the digging to make a pit or to use the soil to cover the wood pile. Secondly, charcoal production impacts the area surrounding the kiln where the wood is harvested [*Chidumayo and Gumbo*, 2013]. We suggest that the increase in soil CO_2 emissions in the vicinity of the charcoal kilns could have been driven by a priming effect triggered by increased production of extracellular enzymes due to the added substrate which "co-metabolize" soil organic matter [*Zimmerman et al.*, 2011], but indirect mechanisms are also possible, such as the stimulation of microbial activity through nitrogen or other nutrient additions [*Kuzyakov et al.*, 2009; *Smith et al.*, 2010; *Wang et al.*, 2012], similar to the effects of biochar addition to soil on CO_2 evolution reported in many types of laboratory and field studies [*Kuzyakov et al.*, 2014]. A review by *Glaser et al.*[2002] on the effects of charcoal in tropical soils confirmed that carbonization results in higher nutrient retention and availability on the kiln sites, supporting our findings. In our study, the highest SOC content was found in the mid-slope plot with highest number of charcoal kilns (six), suggesting that charcoal production in the area affects SOC dynamics. In addition, charcoal, as well as biochar, may increase the N_2O-reducing activity of denitrifying communities, which might cause a decrease in N_2O emissions [*Cavigelli and Robertson*, 2001] in agreement with the reduction of N_2O observed inside the zone of influence of a charcoal kiln.

Forest regeneration on kiln sites following charcoal production is different from that of surrounding areas [*Chidumayo and Gumbo*, 2013], with forest recovery being delayed due to the intense impact at the kiln site [*Boutette and Karch*, 1984]. Therefore, the secondary vegetation on kiln sites is usually dominated by herbaceous plants for several decades, in agreement with the observed positive correlation between distance to the charcoal kiln and canopy cover. Canopy openness favours the establishment of pioneer and light-demanding species in the forest [*Schnitzer and Carson*, 2001]; therefore, shifts in tree species composition might cause a difference of soil CO_2 fluxes [*Katayama et al.*, 2009; *Díaz-Pinés et al.*, 2014]. Furthermore, vegetation structure and species composition affect C allocation patterns [*Wang et al.*, 2006] and modify microclimate and substrate availability [*Raich and Tufekcioglu*, 2000; *Butler et al.*, 2012], all of which may have affected soil N_2O and CO_2 fluxes as well as their respective spatial patterns. However, the interaction between N_2O and CO_2 emissions and the presence of charcoal kilns needs further investigation, which should address both the short- and long-term effects on CO_2 and N_2O fluxes during vegetation succession.

We are confident that the large number of sampling locations per plot yielded a low uncertainty in the mean estimate of soil N_2O and CO_2 fluxes and soil properties, capturing spatial heterogeneity adequately [*Savage and Davidson*, 2003]. Spatial variability in our study, especially of soil N_2O fluxes was high, as expected in tropical forest soils [*Vitousek et al.*, 1989; *Verchot et al.*, 1999, 2006; *Breuer et al.*, 2000]. Because of the presence of "hot spots" in soil, trace gas production and emission can vary substantially on a scale of less than one meter [*Folorunso and Rolston*, 1984; *Parkin*, 1993; *Ambus and Christensen*, 1994]. Since we chose 5 m as the shortest distance between measurement points, we cannot exclude the existence of a small-scale (distances smaller than 5-m) spatial pattern of soil N_2O and CO_2

emission rates in our plots. The lack of spatial dependence of N_2O and CO_2 emissions, as it was observed in our study, has been reported by other studies [e.g *Nishina et al.*, 2009].

Production of N_2O was localized in "hot spots" to a greater extent than CO_2, and therefore, a larger number of samples is required to achieve the same level of precision. For example, 78±3 and 30±11 samples are needed for N_2O and CO_2, respectively, to obtain an estimate within 10 % of the true mean at 95 % confidence level. Our results for CO_2 are comparable with *Yim et al.* [2003] who calculated that between 27 to 33 samples were needed for estimating the soil respiration rates in a larch plantation for the same error and probability level. *Davidson et al.* [2002] reported 41 for a temperate mixed hardwood forest and *Adachi et al.* [2005] reported 50 for a tropical forest. For N_2O emissions rates, *Turner et al.*[2008] estimated 181 as the number of measurements required for the sample mean to be within 10 % of the true mean for a 90 m x 90 m pasture field using the mean and standard deviation from a large sampling size [*Folorunso and Rolston*, 1984]. While we assumed that our 90 sample points represent the true population mean of our plots, they used a more conservative approach.

The inherent spatial variability of the N_2O and CO_2 emissions and their controlling factors makes it difficult to reduce uncertainty in emission estimates by taking a manageable number of flux measurements with chamber investigations. The compositing procedure that we developed previously and that we used here should help overcome this problem [*Arias-Navarro et al.*, 2013]. It should however be noted that we used soil cores covering few square centimeters so that the observed variability is likely larger compared to chamber measurements, as the latter would overcome centimeter-scale variability.

Although the cause for the substantial spatial variability could not be linked to the parameters measured, we attribute this observation to the presence of small-scale

denitrification hotspots in the soil (e.g. due to the scattered availability of organic material in anaerobic microsites), and to the presence of charcoal kilns (which affects SOC dynamics).

In our case, omitting "hot spots" could have led to an underestimation of the site-specific mean emission rates of roughly 2 % for CO_2 and 37 % for N_2O indicating that having too few sampling locations or discarding the observations of high fluxes, could lead to a systematic bias in CO_2 and N_2O budgets from tropical forests, strongly underlining the importance of carefully addressing spatial heterogeneity when designing field sampling.

6 Conclusions

This study provides evidence of the complexity of the mechanisms controlling the spatial variability of soil N_2O and CO_2 fluxes in tropical montane forest ecosystems. The intrinsically high spatial variation of soil N_2O and CO_2 fluxes at our study sites was probably further increased by changes in vegetation and soil properties resulting from charcoal production and associated disturbances. Because of the large spatial dispersion of soil N_2O and CO_2 emissions, it remains a challenge for future measurements and modeling to adequately reproduce the spatial variability both between and within sites.

Although the soil physical properties were not significant predictors of either the N_2O or CO_2 flux, topography was shown to affect the N_2O flux —but not the CO_2 flux. Plots with lower N_2O emissions coincided with steeper slopes, regardless of soil moisture levels. There was no spatial correlations of the soil N_2O and CO_2 fluxes with measured soil parameters, for distances equal or larger than 5 meters. We conclude that N_2O and CO_2 fluxes from tropical montane forest soils must be estimated from an ensemble that is a) sufficient in size and b) representative for the topography of the site. Further investigations at both the plot and landscape level are strongly needed to evaluate the seasonal and annual fluctuations of emission rates to reliably estimate CO_2 and N_2O budgets from African tropical forest soils.

Acknowledgments

This work was funded by the CGIAR program on Climate Change, Agriculture, and Food Security (CCAFS). The authors acknowledge financial support by The Climate Food and Farming Research Network (CLIFF). EDP and KBB received additional funding from a BMBF-Project (Förderzeichnen 01DG13012). Authors are grateful to the technical support received by the Mazingira Centre, Environmental Research and Educational facility (https://mazingira.ilri.org). We greatly thank the Kenya Forest Service (KFS) for access to the sites and field assistance. All data are archived on CIFOR's Dataverse Web site and available for free upon request [*Arias-Navarro et al.*, 2017]. Thanks to the two reviewers and the editor for their constructive comments and helpful suggestions on an earlier version of the manuscript.

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Tables

Table 1. Summary (mean ± standard deviation) of soil properties and disturbance variables of each topographic position and results of the analysis of variance ANOVA of log-normally transformed soil N_2O and CO_2 fluxes, soil properties and canopy cover for each topographic position. Values within the same row followed by the same letter indicate no statistical difference among topographic positions ($p > 0.05$). Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	F value	Pr > F	Valley bottom	Mid-slope	Ridge top
log N_2O	7.5	0.000568 ***	4.2 ± 1.3^{a}	3.8 ± 1.4^{b}	4.2 ± 1.4^{a}
log CO_2	12.1	6.34e-06 ***	3.6 ± 0.6^{b}	3.7 ± 0.6^{b}	3.8 ± 0.4^{a}
Total carbon (g kg^{-1})	6.3	0.00194 **	152.5 ± 49.3^{b}	166.5 ± 25.9^{a}	154.9 ± 20.4^{b}
Total nitrogen (g kg^{-1})	3.7	0.025 *	13.6 ±3.9^{ab}	14.2 ± 4.0^{a}	13.3 ± 3.6^{b}
pH	33.2	1.43e-14 ***	6.2 ± 0.4^{a}	6.0 ± 0.6^{b}	5.8 ± 0.5^{c}
Bulk density (g cm^{-3})	0.5	0.584	0.5 ± 0.2^{a}	0.5 ± 0.2^{a}	0.5 ± 0.2^{a}
Canopy cover (%)	2.5	0.0854 .	53.4 ± 35.6^{a}	49.7 ± 36.1^{a}	46.8 ± 35^{a}
No. charcoal kilns ha^{-1}	-	-	1.3 ± 1.2	4 ± 2.1	2.6 ± 1.2

Table 2. Number of samples required for various levels of precision (± 10 %, ± 20 % and ± 30 % of the true mean) for a confidence level of 95 % based on a population of 90 measurements (1 ha). Values are mean (standard deviation) of the nine plots.

	No. of samples		
	± 10 %	± 20 %	± 30 %
N_2O	78 (± 3)	55 (± 4)	37 (± 6)
CO_2	30 (± 11)	11 (± 5)	6 (± 2)
Total organic carbon	3 (± 0)	3 (± 0)	3 (± 0)
Total nitrogen	3 (± 0)	3 (± 0)	3 (± 0)
pH	3 (± 0)	3 (± 0)	3 (± 0)
Bulk density	25 (± 8)	9 (± 3)	5 (± 1)

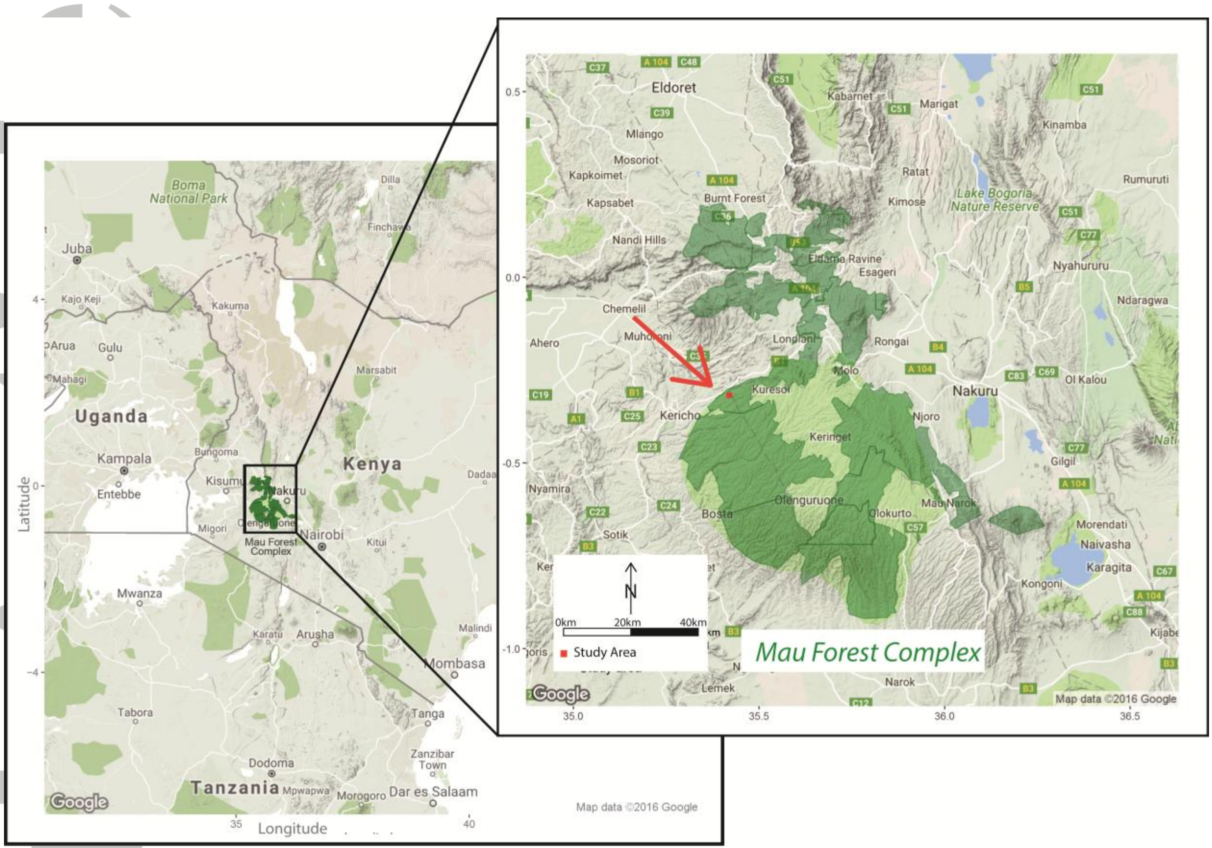

Figure 1. Localization of the study area.

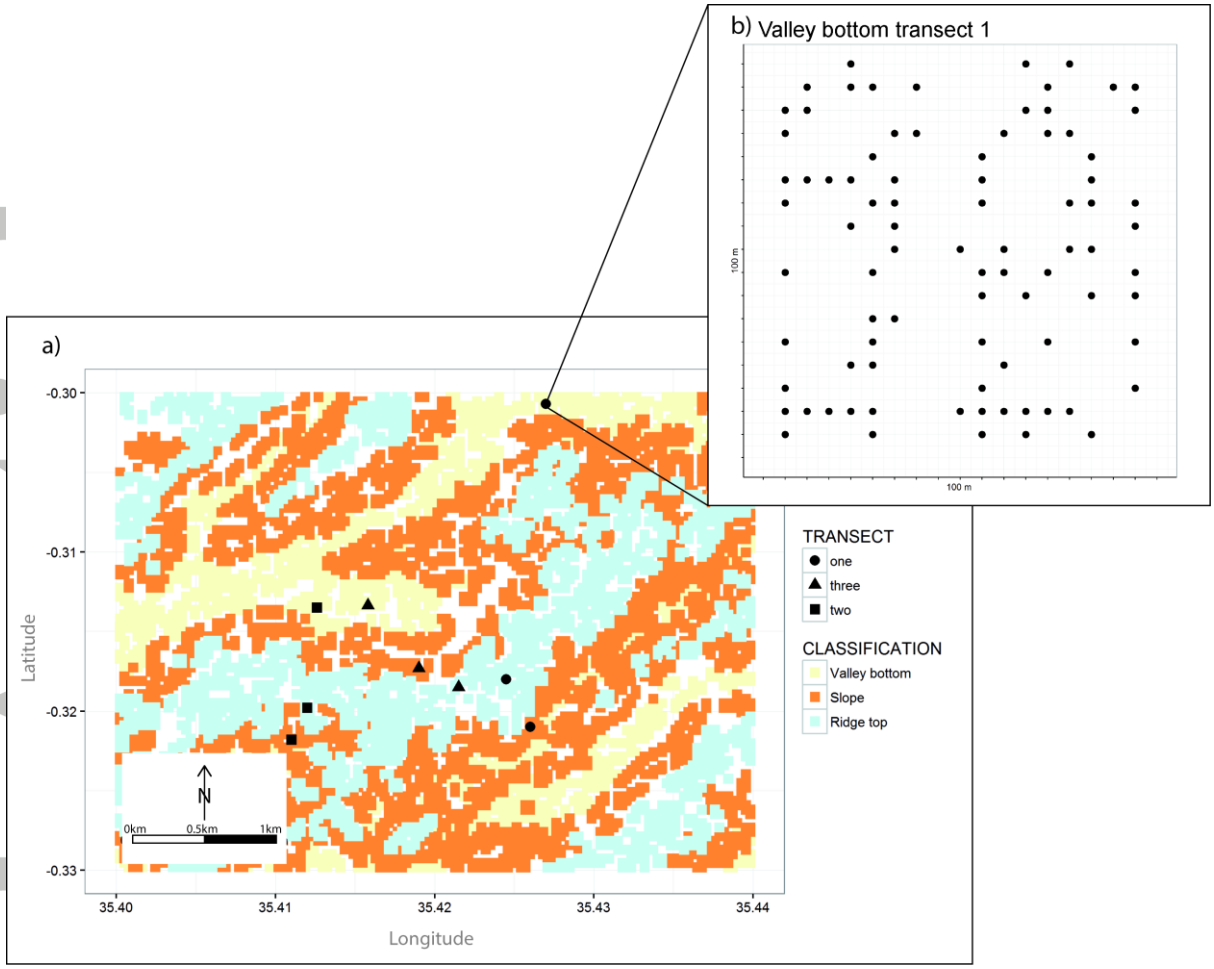

Figure 2. a) Topographic classification of the study area and localization of the sampling plots. b) Diagram of the sampling scheme in the Valley bottom transect 1 showing the 90 random sampling locations on a grid of 5m x 5m.

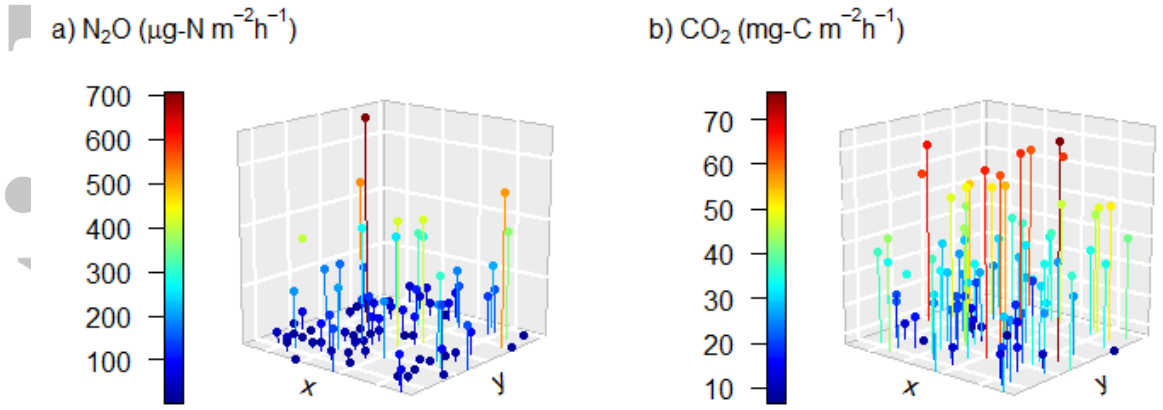

Figure 3. Potential soil a) N_2O and b) CO_2 fluxes 24 hours after a 10 mm rainfall simulation (experiment 1) in Valley bottom transect 1. Symbols denote individual fluxes. The x axis is the coordinate x and the y axis is the coordinate y of the sampling points.

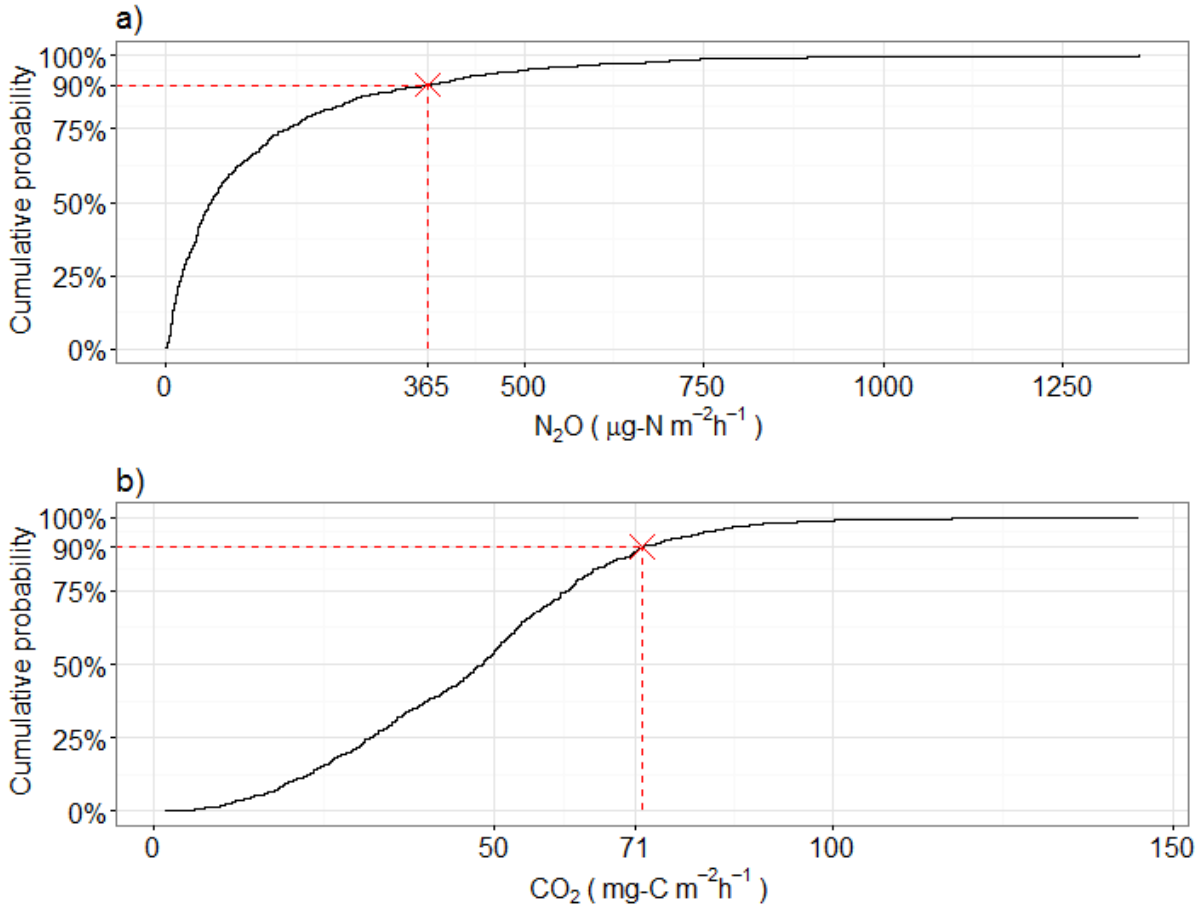

Figure 4. Empirical cumulative density function for a) N_2O and b) CO_2 fluxes. Red crosses represent the 90th percentile.

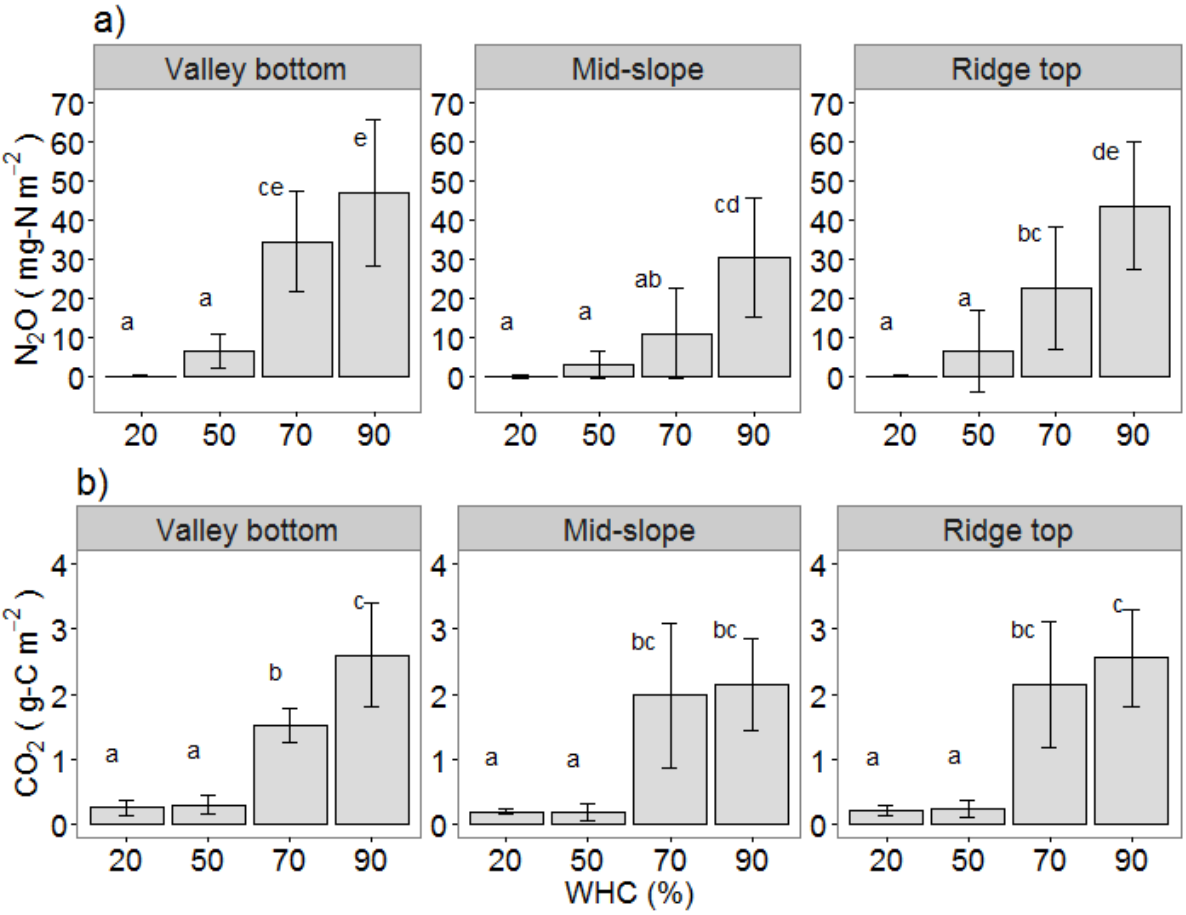

Figure 5. Time-weighted average cumulative soil N_2O (upper panels) and CO_2 (lower panels) emission rates during a three-day incubation for different positions and levels of water holding capacity (WHC). Vertical bars denote standard deviation. Columns with the same letters indicate no significant differences ($p>0.05$) between positions and WHC levels in soil N_2O or CO_2 fluxes.

Journal of Geophysical Research Biogesciences

Supporting Information for

Spatial variability of soil N2O and CO2 fluxes in different topographic positions in a tropical montane forest, Kenya.

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Figures S1 to S19

Introduction

This supporting information provides the same figures as figure 3 (S1 to S9) in the main article for all sampling plots, Variograms for CO2 and N2O fluxes (log data) and soil properties (S10 to S18) and the range of means for all sample sizes for CO2 and N2O fluxes (S19).

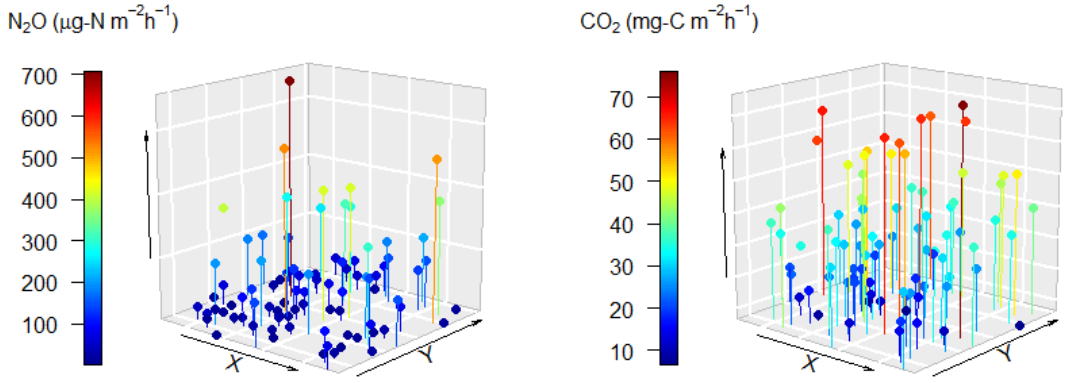


Figure S1. Potential soil N_2O (left panel) and CO_2 (right panel) fluxes 24 hours after a 10 mm rainfall simulation (experiment 1) in Valley bottom transect 1. Symbols denote individual fluxes. The x axis is the coordinate x and the y axis is the coordinate y of the sampling points.

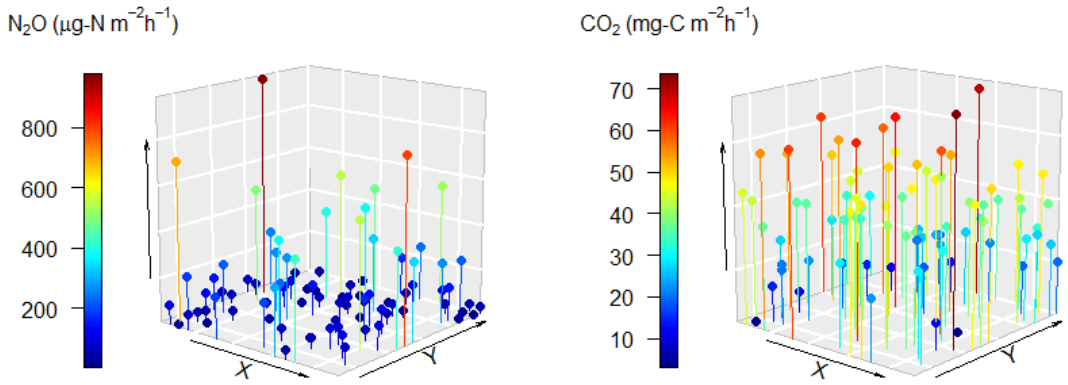


Figure S2. Potential soil N_2O (left panel) and CO_2 (right panel) fluxes 24 hours after a 10 mm rainfall simulation (experiment 1) in Valley bottom transect 2. Symbols denote individual fluxes. The x axis is the coordinate x and the y axis is the coordinate y of the sampling points.

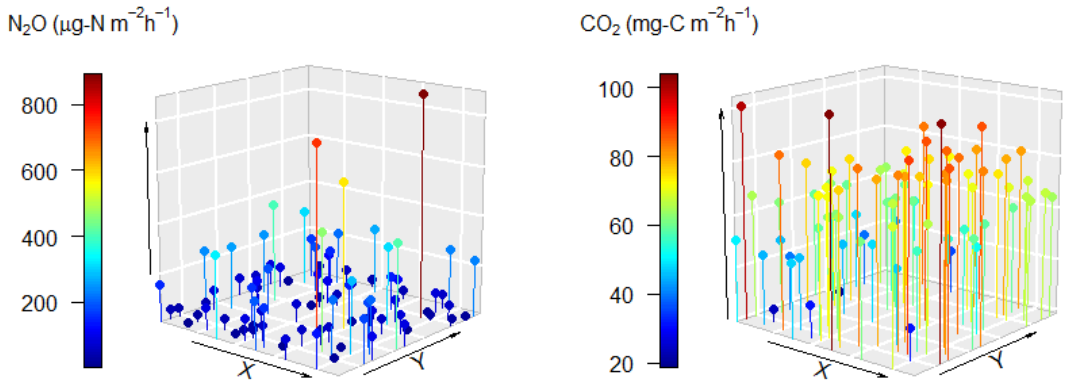


Figure S3. Potential soil N_2O (left panel) and CO_2 (right panel) fluxes 24 hours after a 10 mm rainfall simulation (experiment 1) in Valley bottom transect 3. Symbols denote individual fluxes. The x axis is the coordinate x and the y axis is the coordinate y of the sampling points.

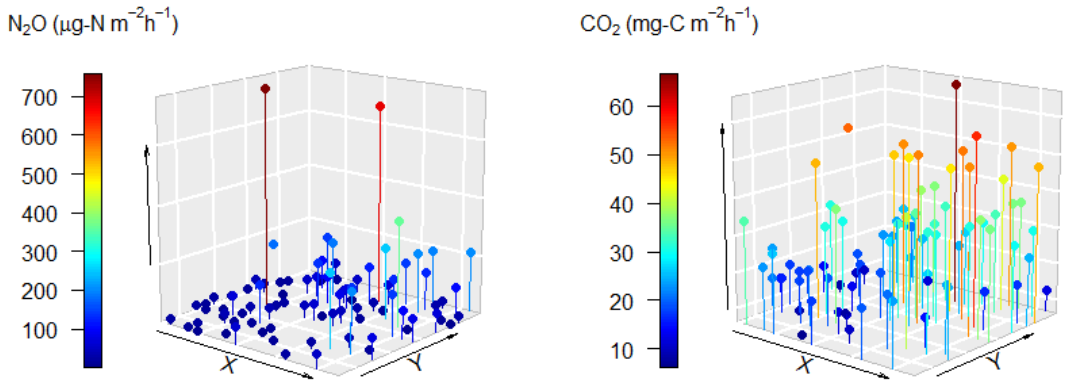


Figure S4. Potential soil N_2O (left panel) and CO_2 (right panel) fluxes 24 hours after a 10 mm rainfall simulation (experiment 1) in Mid-slope transect 1. Symbols denote individual fluxes. The x axis is the coordinate x and the y axis is the coordinate y of the sampling points.

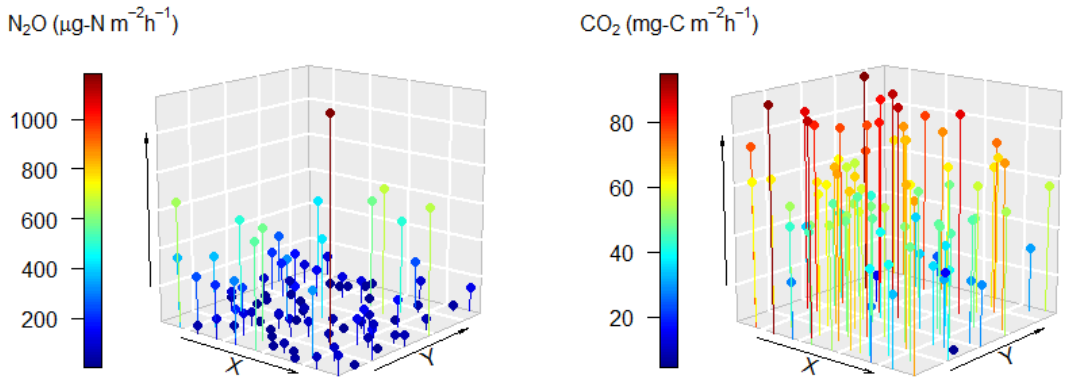


Figure S5. Potential soil N_2O (left panel) and CO_2 (right panel) fluxes 24 hours after a 10 mm rainfall simulation (experiment 1) in Mid-slope transect 2. Symbols denote individual fluxes. The x axis is the coordinate x and the y axis is the coordinate y of the sampling points.

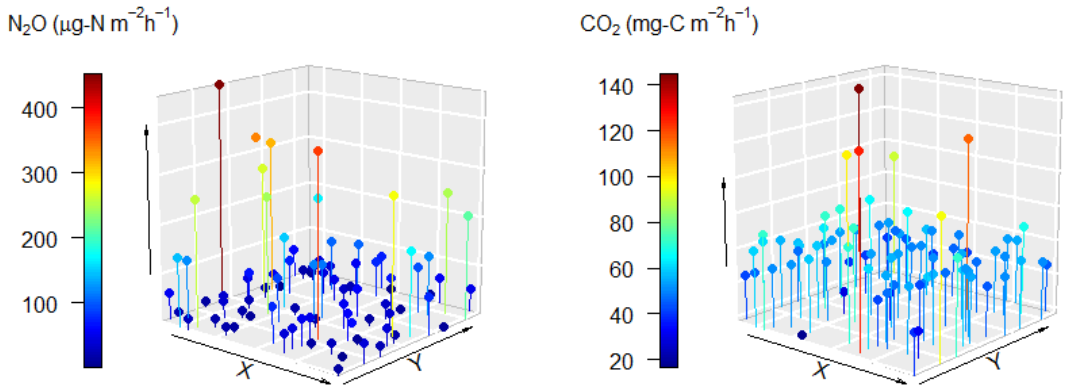


Figure S6. Potential soil N_2O (left panel) and CO_2 (right panel) fluxes 24 hours after a 10 mm rainfall simulation (experiment 1) in Mid-slope transect 3. Symbols denote individual fluxes. The x axis is the coordinate x and the y axis is the coordinate y of the sampling points.

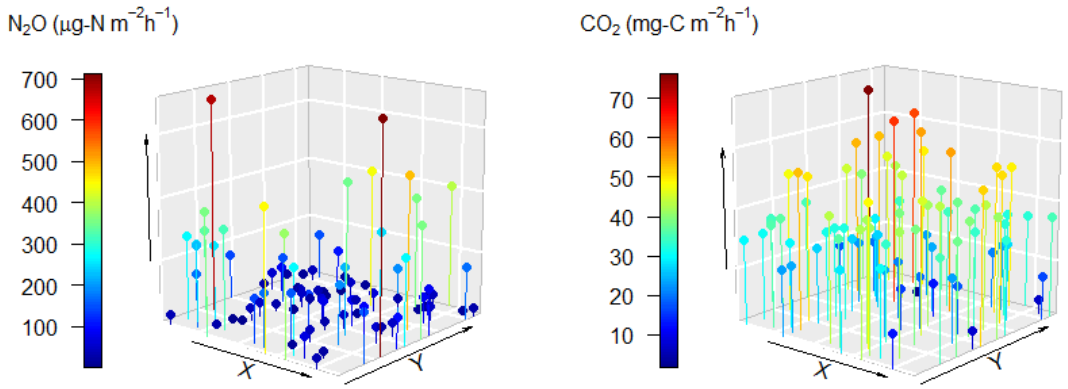


Figure S7. Potential soil N_2O (left panel) and CO_2 (right panel) fluxes 24 hours after a 10 mm rainfall simulation (experiment 1) in Ridge top transect 1. Symbols denote individual fluxes. The x axis is the coordinate x and the y axis is the coordinate y of the sampling points.

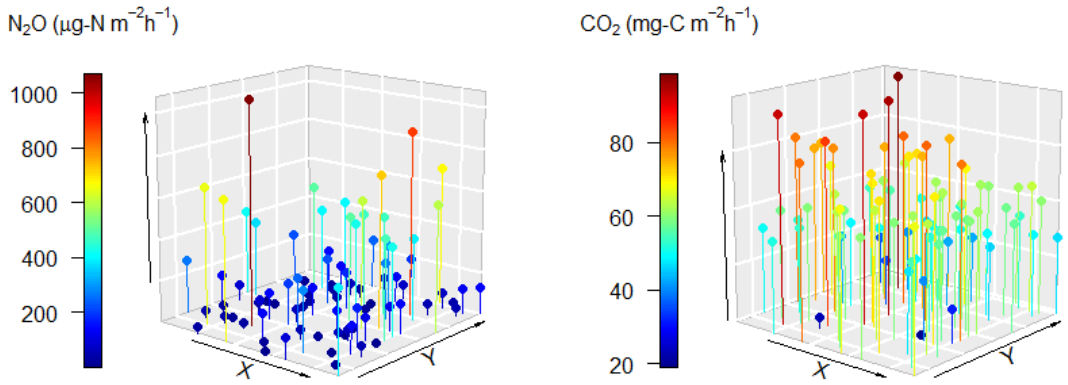


Figure S8. Potential soil N_2O (left panel) and CO_2 (right panel) fluxes 24 hours after a 10 mm rainfall simulation (experiment 1) in Ridge top transect 2. Symbols denote individual fluxes. The x axis is the coordinate x and the y axis is the coordinate y of the sampling points.

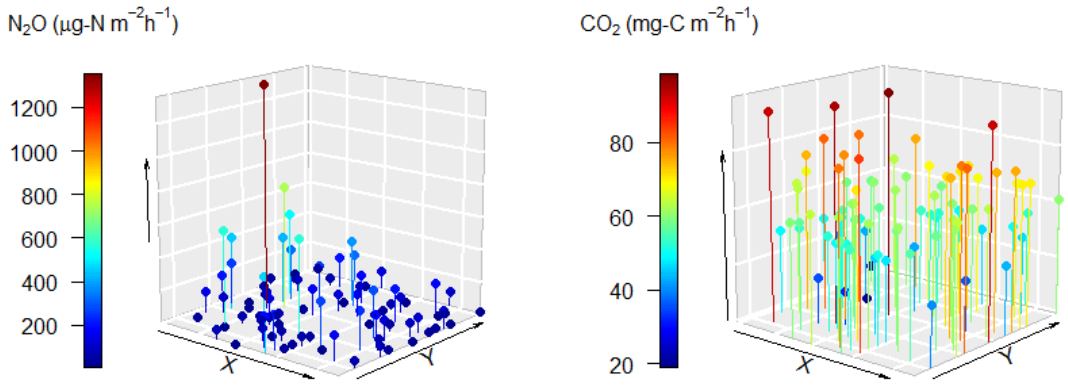


Figure S9. Potential soil N_2O (left panel) and CO_2 (right panel) fluxes 24 hours after a 10 mm rainfall simulation (experiment 1) in Ridge top transect 3. Symbols denote individual fluxes. The x axis is the coordinate x and the y axis is the coordinate y of the sampling points.

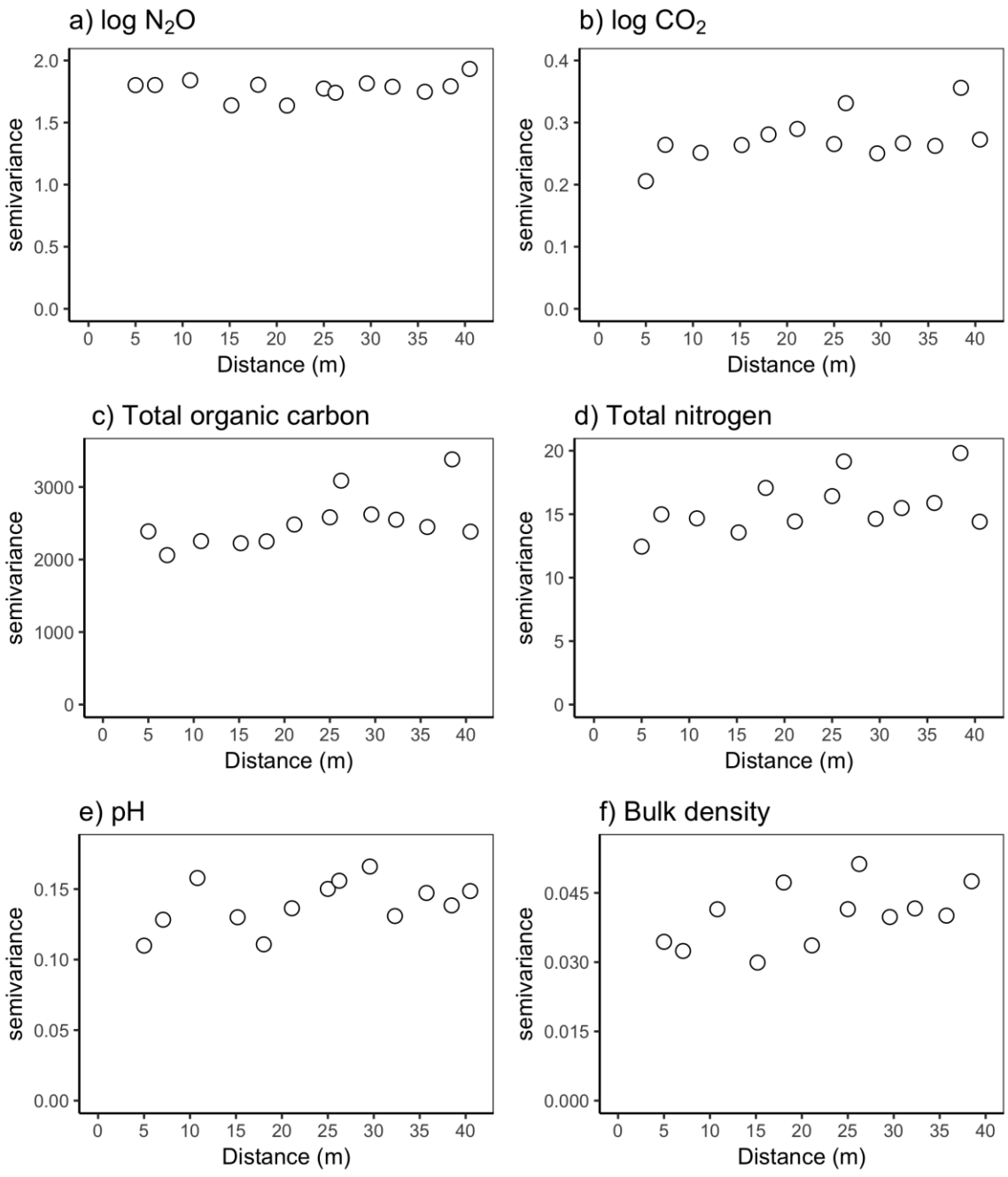
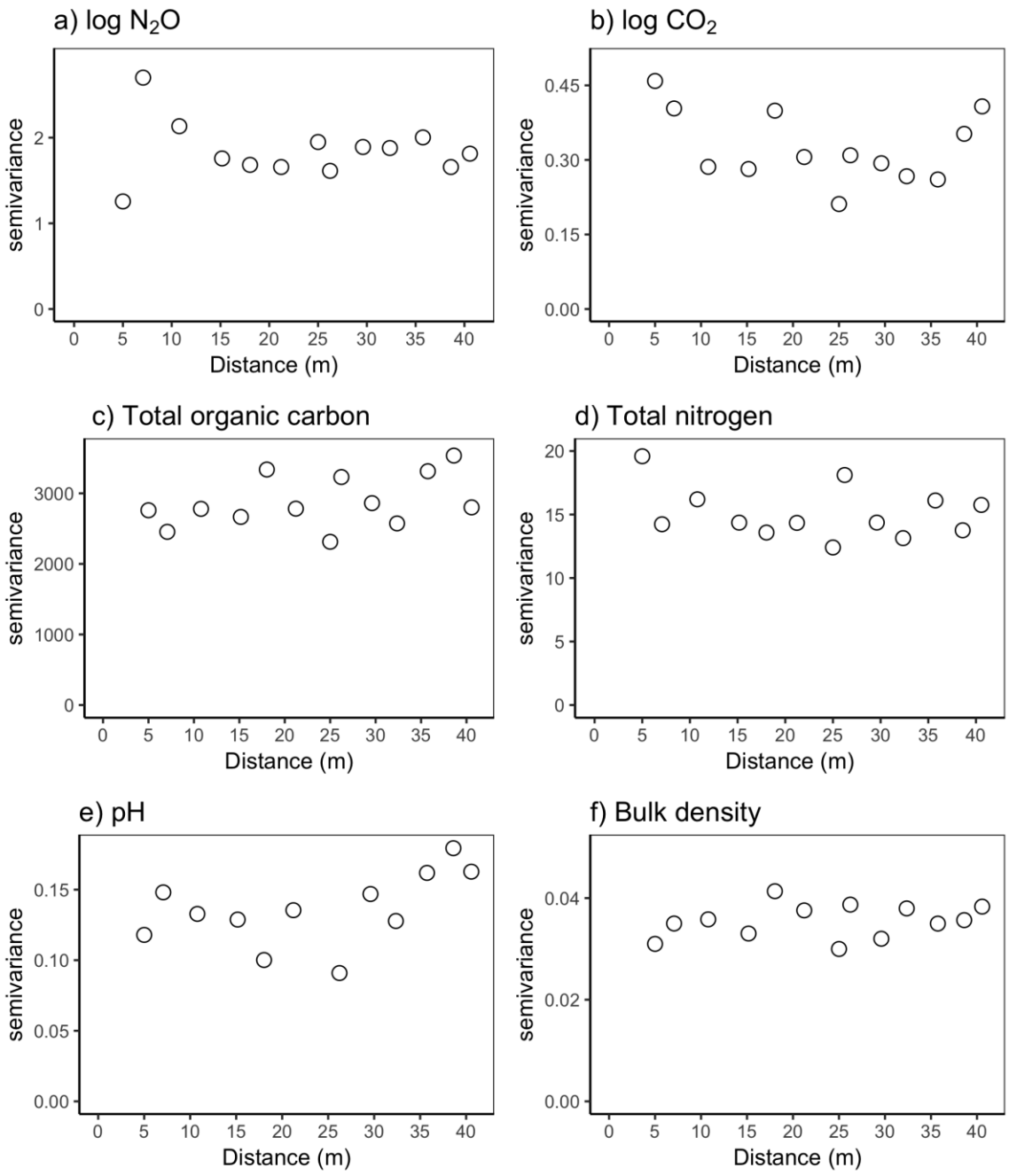


Figure S10. Variograms for CO_2 and N_2O fluxes (log data) and soil properties in Valley bottom transect 1. The x axis is the distance between measurements locations in meters and the y axis is the semivariance in the 90 respective measurements made in Valley bottom transect 1.



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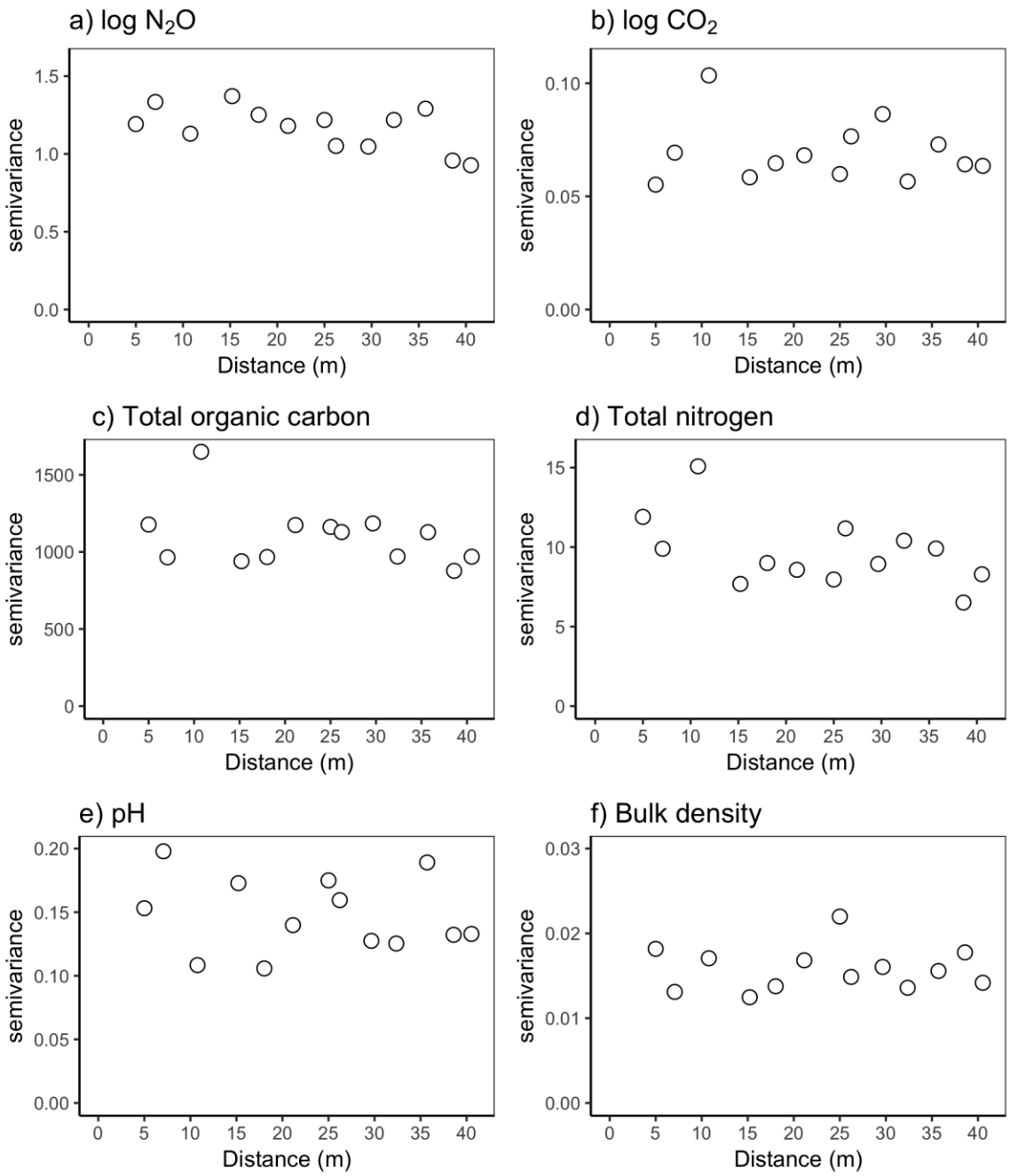
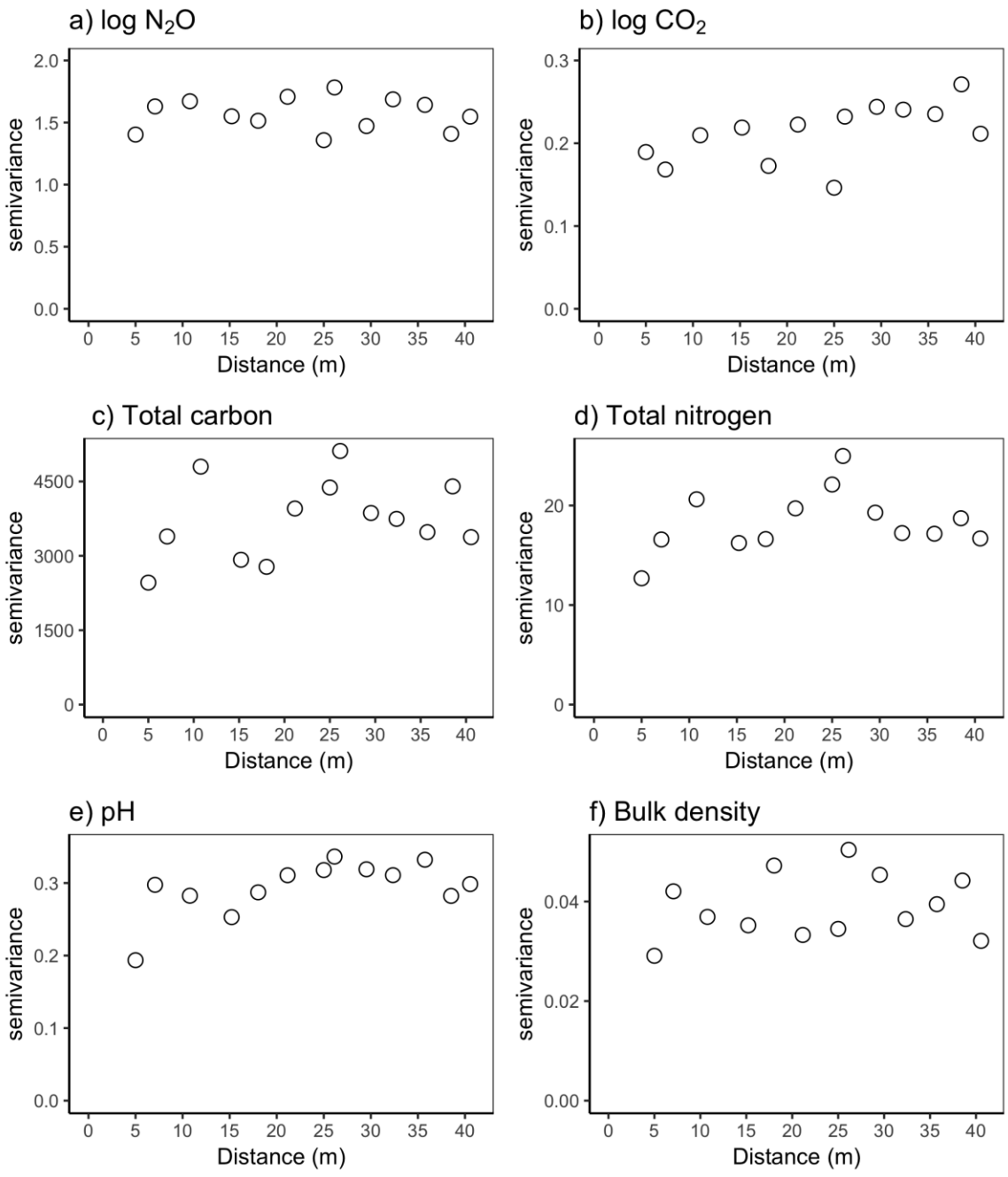


Figure S12. Variograms for CO_2 and N_2O fluxes (log data) and soil properties in Valley bottom transect 1. The x axis is the distance between measurements locations in meters and the y axis is the semivariance in the 90 respective measurements made in Valley bottom transect 3.



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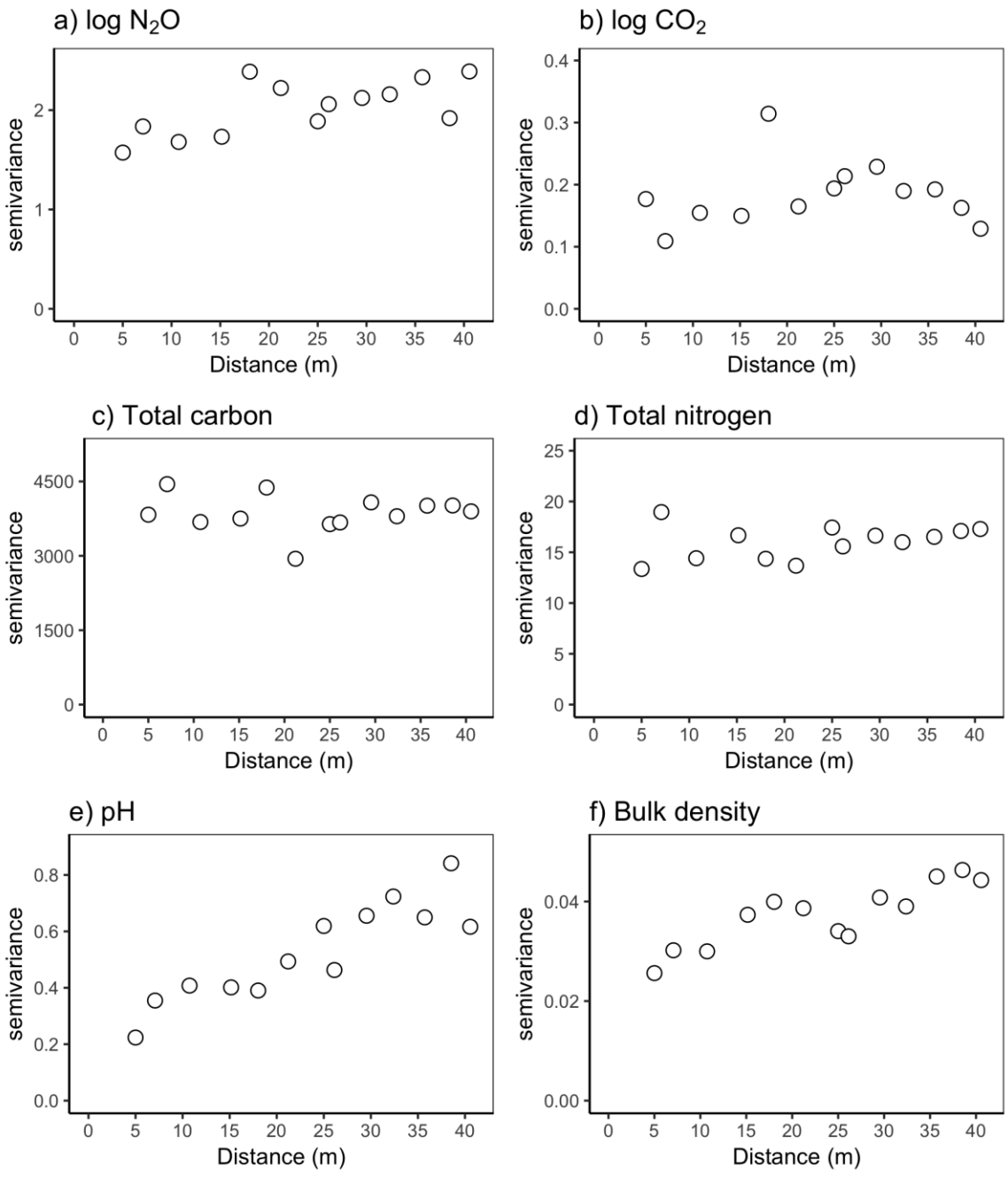

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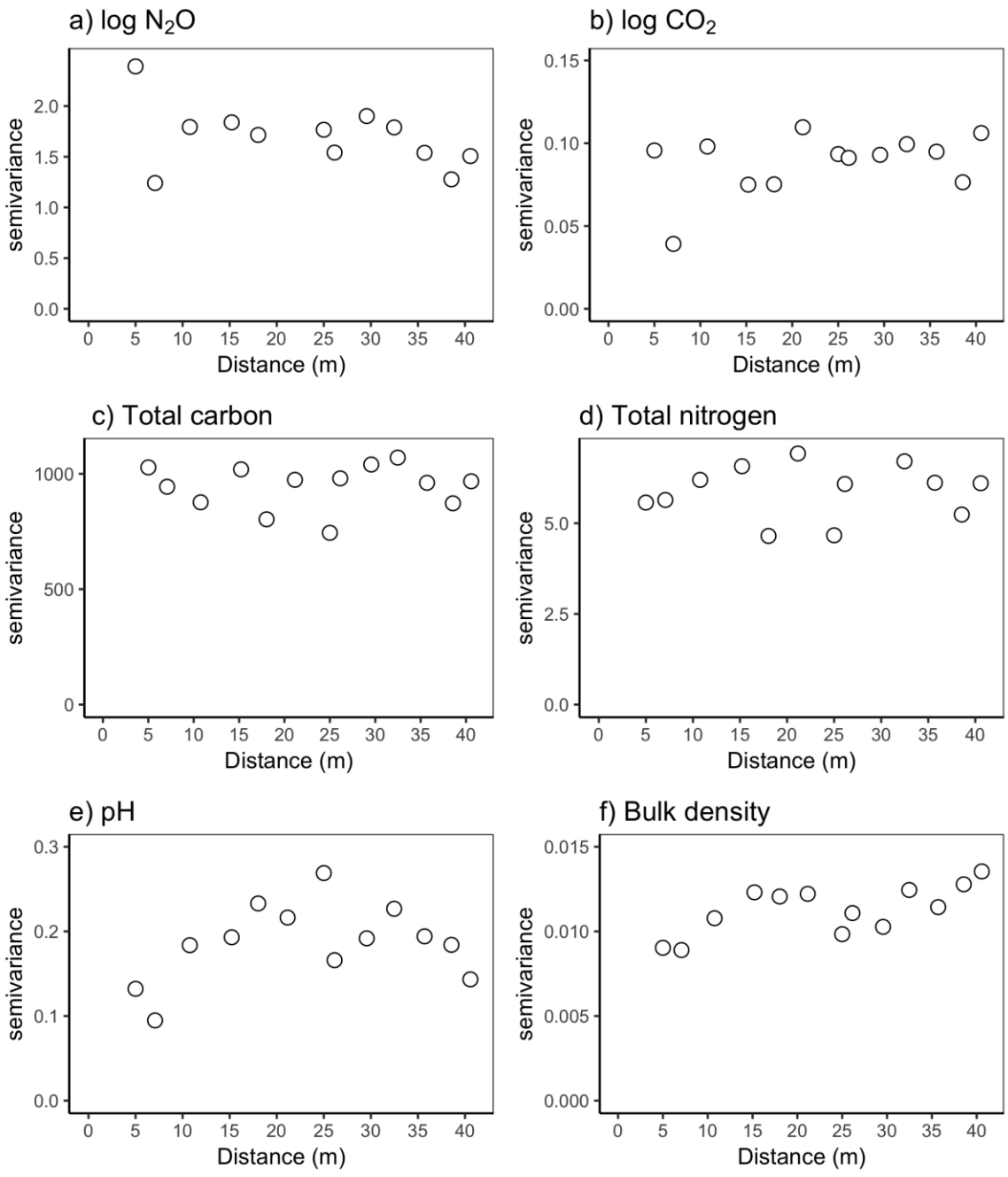


Figure S15. Variograms for CO_2 and N_2O fluxes (log data) and soil properties in Valley bottom transect 1. The x axis is the distance between measurements locations in meters and the y axis is the semivariance in the 90 respective measurements made in Mid-slope transect 3.

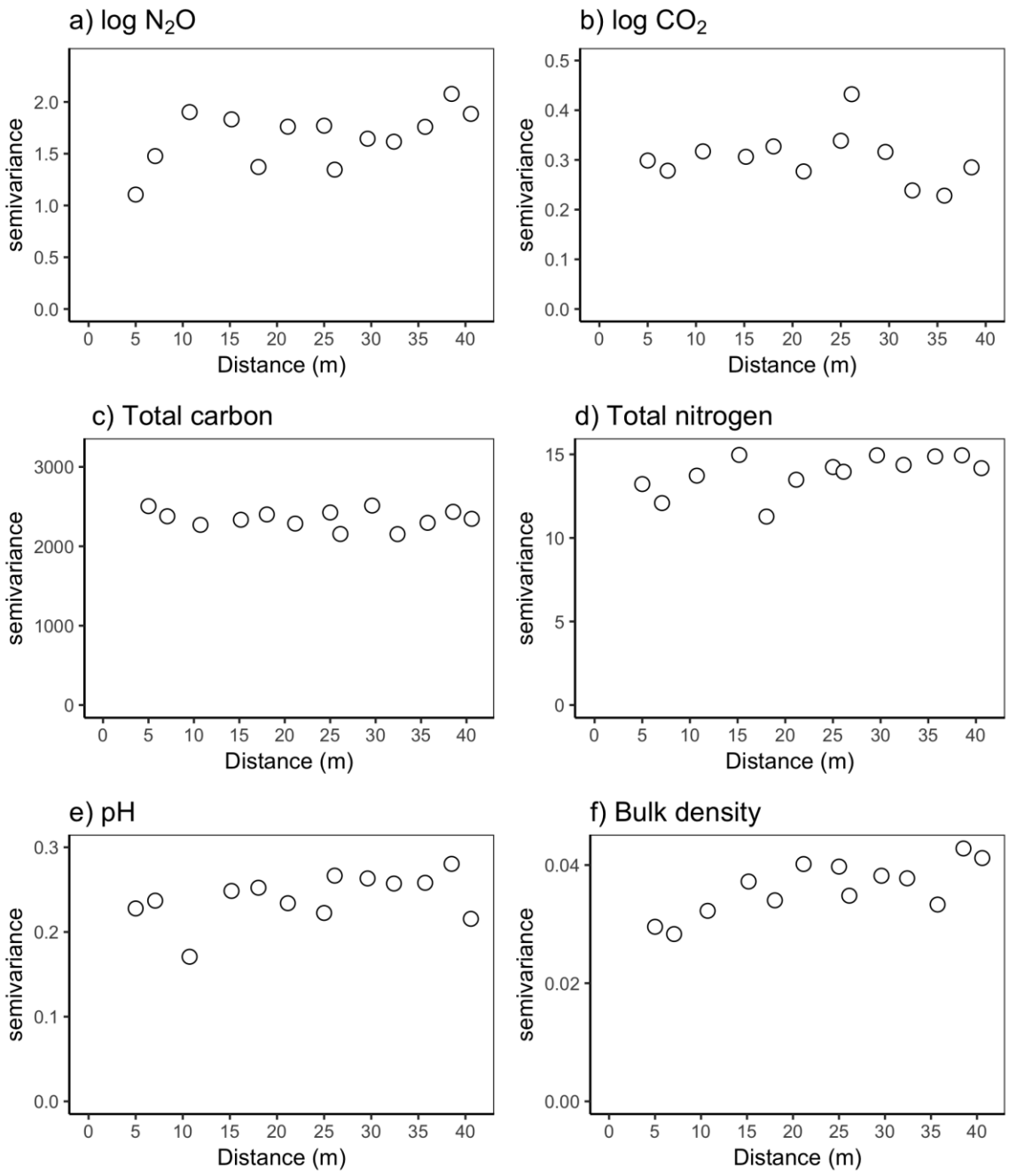
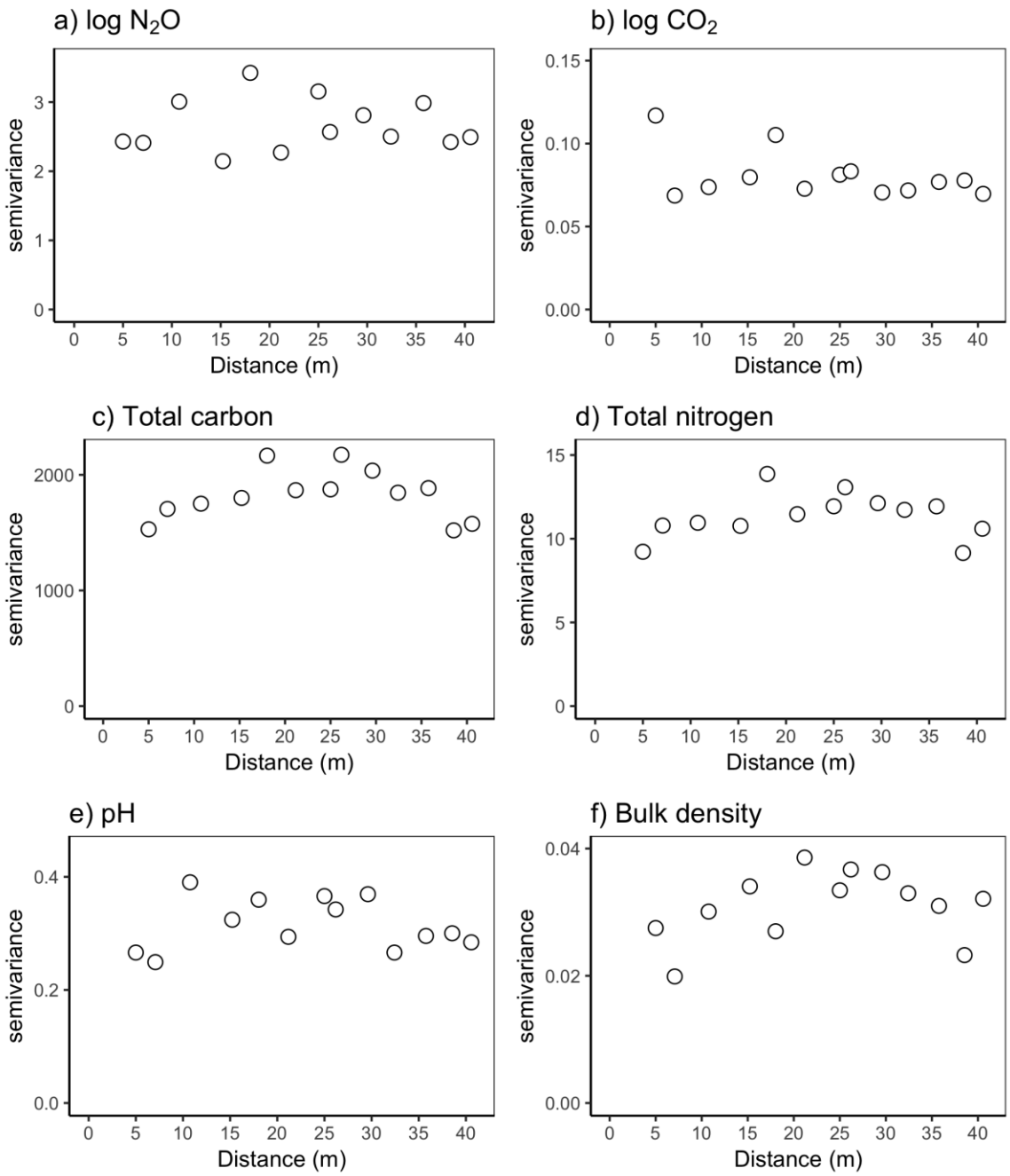


Figure S16. Variograms for CO_2 and N_2O fluxes (log data) and soil properties in Valley bottom transect 1. The x axis is the distance between measurements locations in meters and the y axis is the semivariance in the 90 respective measurements made in Ridge top transect 1.



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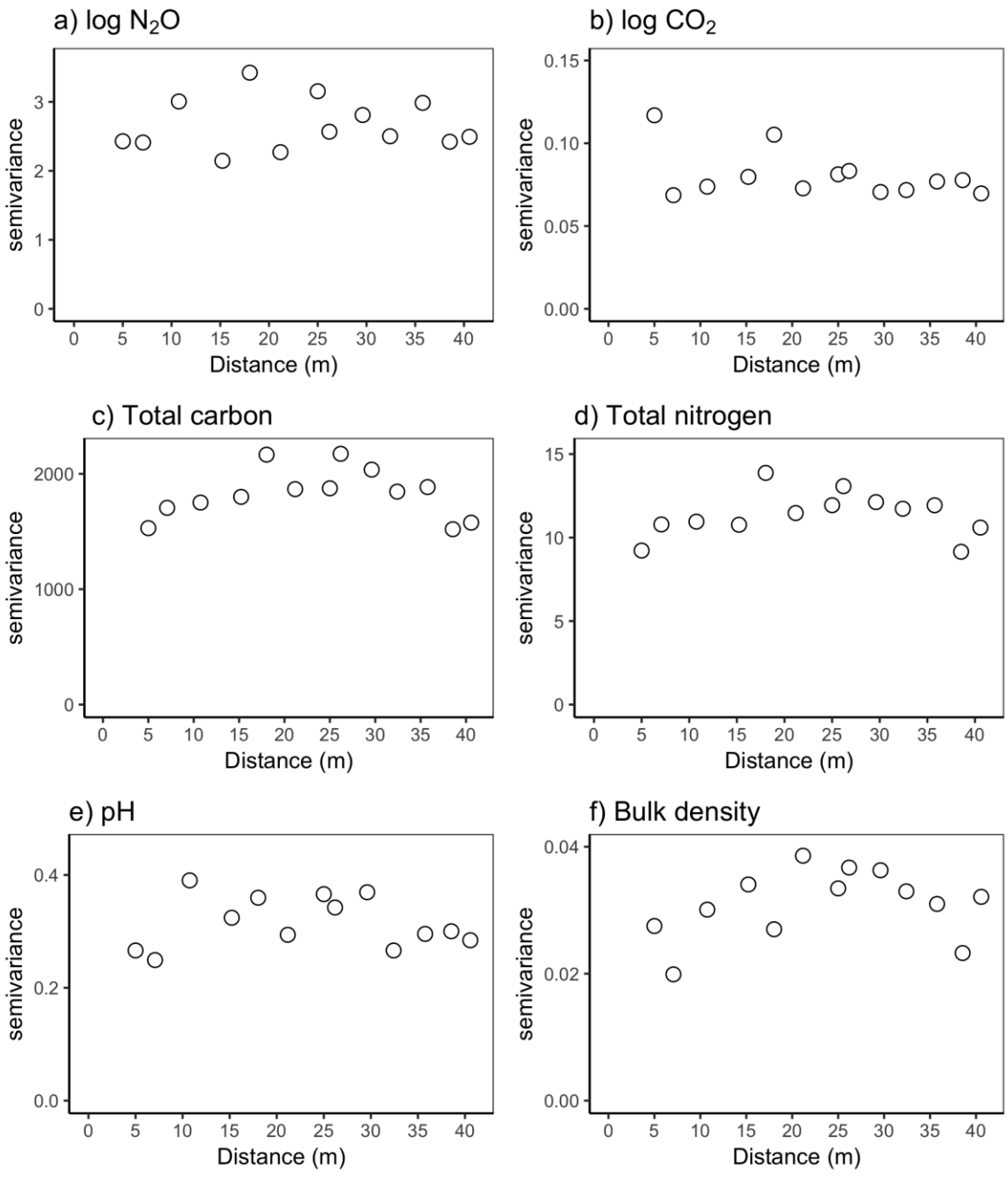

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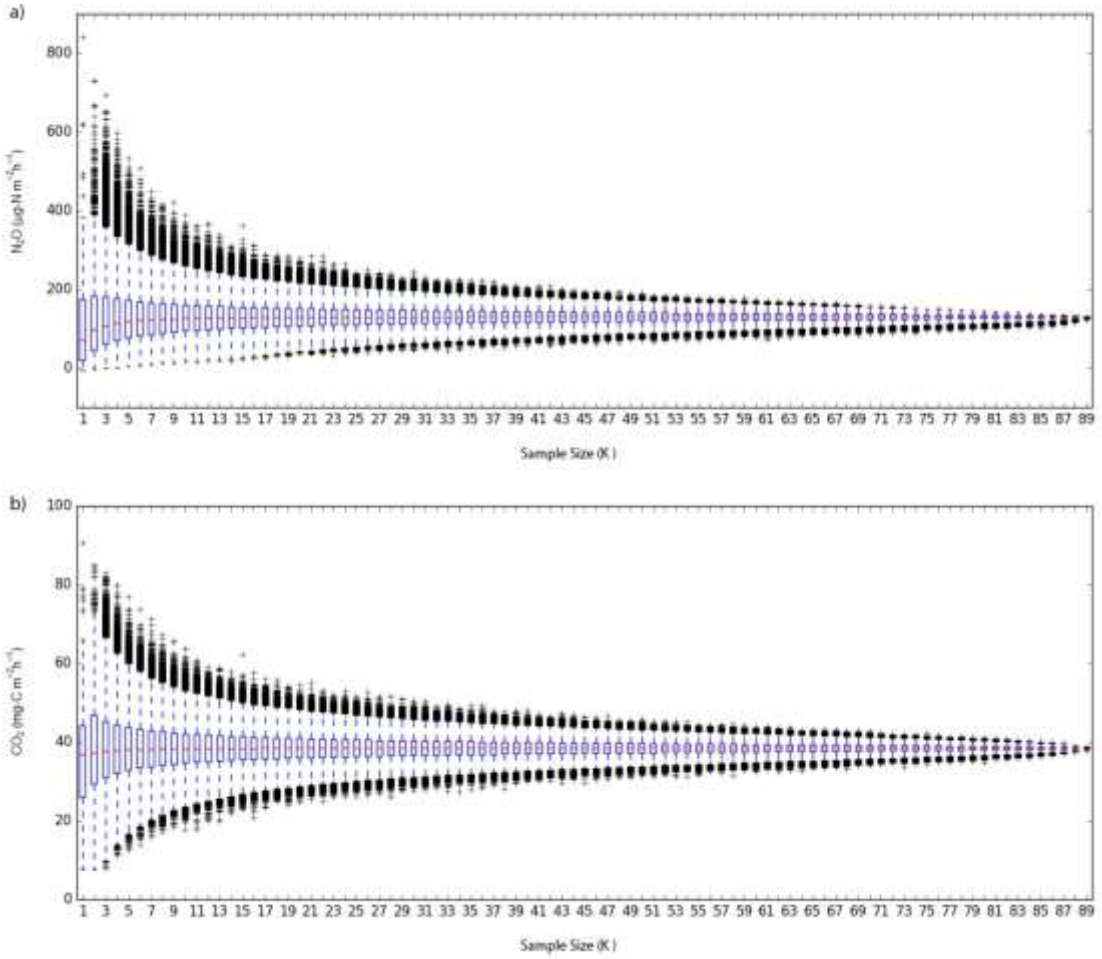

Figure S19. Boxplots of means for all sample sizes (K=1, 2…89) for 100,000 randomly drawn sample subsets for a) N2O and b) CO2 fluxes.

Biogeochemistry

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

--Manuscript Draft--

Manuscript Number:	
Full Title:	Quantifying the contribution of land use to N2O, NO and CO2 fluxes in a montane forest ecosystem of Kenya
Article Type:	Manuscript
Keywords:	Carbon dioxide; land use change; nitric oxide; nitrous oxide; soils; tropical forests
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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 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 (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.
Suggested Reviewers:	Clemens Scheer Queensland University of Technology clemens.scheer@qut.edu.au Dr. Scheer has a profound knowledge on soil trave gas fluxes from a variety of ecosystems
	Pascal Boeckx Universiteit Gent pascal.boeckx@ugent.be Prof. Dr. Boecks is an expert on biogeochemical cycles with long expertise on tropical ecosystems
	Simona Castaldi simona.castaldi@cmcc.it Dr. Castaldi has an extense experience in greenhouse gas exchange in various ecosystems, including tropical areas

Dear editor,

Hereby we submit a manuscript for publication consideration in Biogeochemistry. The manuscript aims to quantify the contribution of land use to soil N_2O, NO and CO_2 fluxes in a montane forest ecosystem of Kenya.

We consider that the work is highly novel and substantially contributes to a growing body of empirical evidence on soil trace gas emissions from different land uses in the African tropics and their governing parameters. We applied an innovative laboratory approach with high temporal resolution under controlled conditions for estimating annual fluxes which can be further applied to enhance our knowledge on the effects of land use on soil-atmosphere trace gas exchange in understudied regions.

Our study highlights that land use changes in montane tropical landscapes are significantly altering soil trace gas fluxes and turn these systems towards significant sources of N trace gases. In summary, we consider that our work is highly relevant, contributing to an improved mechanistic understanding of drivers and constrains of soil trace gas emissions. We hope it will receive full consideration for publication.

Yours sincerely,

Dr. Eugenio Díaz-Pinés on behalf of all co-authors

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

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Acknowledgments

This work was funded by the Consultative Group on International Agricultural Research (CGIAR) Research program on Climate Change, Agriculture, and Food Security (CCAFS), which is carried out with support from CGIAR Fund Donors and through bilateral funding agreements. CAN acknowledges financial support by The Climate Food and Farming Research Network (CLIFF) and by The Helmholtz Research School MICMoR. EDP and KBB received additional funding from the German Federal Ministry of Education and Research (Förderzeichnen 01DG13012). Authors are grateful to the technical support received by the Mazingira Centre, Environmental Research and Educational facility (https://mazingira.ilri.org). We greatly thank the Kenya Forest Service (KFS) for access to the sites and field assistance.

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 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).

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. submitted). 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). 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 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 for three days before being transported to the laboratory at IMK-IFU (Garmisch-Partenkirchen, Germany).

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 gaseous exchange rate was calculated from the mass balance of 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. 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

299 nitrogen (TN) content. Soils were acidic (4.2-6.0), with the lowest soil pH value observed for
300 soils from TE-T (Table 1).
301
302 3.2 Soil trace gas fluxes
303 *Soil N_2O fluxes*
304 We measured a very low N_2O uptake from dried soil cores (average: -9 ± 31 µg N m^{-2} h^{-1}).
305 Initial re-wetting of soil cores to 20% WFPS did not cause N_2O emission pulses, regardless of
306 the site. After setting the soil cores to the targeted WFPS, substantial N_2O fluxes (> 50 µg N_2O-
307 N m^{-2} h^{-1}) were measured only when WFPS was finally set to 90%. The peak maximum for
308 these cores was observed after two to four days, depending on the experimental sites (Figure
309 3). The only significant differences in soil N_2O fluxes were found between 90% WFPS and the
310 other soil moisture levels. With an average post-pulse N_2O flux of 131 ± 61 µg N_2O-N m^{-2} h^{-1},
311 TE-F tended to emit more N_2O than soils from other sites, although strong variation precluded
312 significant differences (Table 2).
313 Pulse fluxes contributed to more than half (initial pulse: 22 ± 9 %, second pulse: 34 ± 16 %) of
314 the total time-weighted average cumulative soil N_2O rates during the 15 day-incubation period
315 for the different sites and levels of WFPS (Figure 4a). Cumulative soil N_2O fluxes during the
316 second pulse were significantly higher for soils moistened to 90% WFPS (159 ± 258 g N_2O-N
317 ha^{-1}) compared to soils adjusted to other moisture levels (70% WFPS: 27 ± 28 g N_2O-N ha^{-1};
318 50% WFPS: 27 ± 9 g N_2O-N ha^{-1}; 30 % WFPS: 27 ± 11 g N_2O-N ha^{-1}). The second pulse was
319 not significantly different between sites.
320
321 *Soil NO fluxes*
322 Dry-soil NO fluxes were very low for all sites (average 5 ± 7 µg NO-N m^{-2} h^{-1}). Large NO
323 pulses (average 99 ± 100 µg NO-N m^{-2} h^{-1}) occurred already following the first re-wetting
324 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} 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 2^{th} 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 and 5.2 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 and 2.2 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; 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). 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 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 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, 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}) (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). 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, results from our experiment are probably 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).

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) 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, and, our estimate is 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.

Annual estimates of soil N_2O emissions from eucalyptus plantations (TE-P) were, with approximately 1 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

481 N_2O-N ha^{-1} a^{-1} estimated from "zero N-control" tea plantations in China and Japan (Akiyama
482 et al. 2006; Fu et al. 2012; Yao et al. 2015) although those flux estimates were likely highly
483 affected by the previous application of large amounts of N fertilizer (average of 553 kg N ha^{-1}
484 a^{-1}). Regarding smallholder tea plantations, our annual N_2O estimations (0.1 ± 0.2 kg N_2O-N
485 ha^{-1} a^{-1}) are lower than those estimates from Rosenstock et al. (2015) who reported annual N_2O
486 fluxes of 0.38 and 0.75 kg N ha^{-1} a^{-1} in similar systems in Kenya and Tanzania. They further
487 suggest that the emission factor from N application in smallholder tea systems would be below
488 1 % of N applied. Beyond N_2O, our work shows that tea plantations are a major source for NO.
489 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
490 SH-T, respectively is higher than the annual NO fluxes reported by Yao et al. (2015) for a tea
491 plantation in China (1.6 ± 0.4 kg NO-N ha^{-1} a^{-1}, no N fertilizer application).
492 The higher N_2O and NO emissions (N_2O + NO) from soils of the commercial tea plantation
493 (4.3 kg N ha^{-1} a^{-1}) compared to soils of tea plantations from smallholder farmers (2.2 kg N ha^{-1}
494 a^{-1}) are very likely due to the long-term N fertilization and the subsequent soil acidification
495 (Tokuda and Hayatsu 2004; Yamamoto et al. 2014). Soil from the commercial tea plantation
496 (TE-T) in our study showed a mean pH value of 4.2, which was significantly lower than soils
497 from tea plantations of smallholders (SH-T, mean: 5.0). Soil acidity is an important factor
498 affecting biotic and abiotic processes and consequently promoting N losses, by e.g. inducing
499 chemo-denitrification and therefore NO losses but also N_2O (Ventera et al. 2003; Kesik et al.
500 2006; Medinets et al. 2015; Yao et al. 2015). Chemo-denitrification (process involving abiotic
501 reduction of nitrite) has been suspected to be an important source of NO emissions from soils
502 after drying and wetting of soil, and in excessively fertilized soils, where nitrite can accumulate
503 (Davidson 1992; Neff et al. 1995; Verchot et al. 1999). Positive correlations of NO emissions
504 with clay content and negative correlations with soil pH in our study give further supporting
505 evidence that chemo-denitrification might play a significant role (Nelson and Bremner 1970).
506 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.

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.

Acknowledgments

This work was funded by the Consultative Group on International Agricultural Research (CGIAR) Research program on Climate Change, Agriculture, and Food Security (CCAFS), which is carried out with support from CGIAR Fund Donors and through bilateral funding agreements. CAN acknowledges financial support by The Climate Food and Farming Research Network (CLIFF) and by The Helmholtz Research School MICMoR. EDP and KBB received additional funding from the German Federal Ministry of Education and Research (Förderzeichnen 01DG13012). Authors are grateful to the technical support received by the Mazingira Centre, Environmental Research and Educational facility (https://mazingira.ilri.org). We greatly thank the Kenya Forest Service (KFS) for access to the sites and field assistance.

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)

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

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Figure 1

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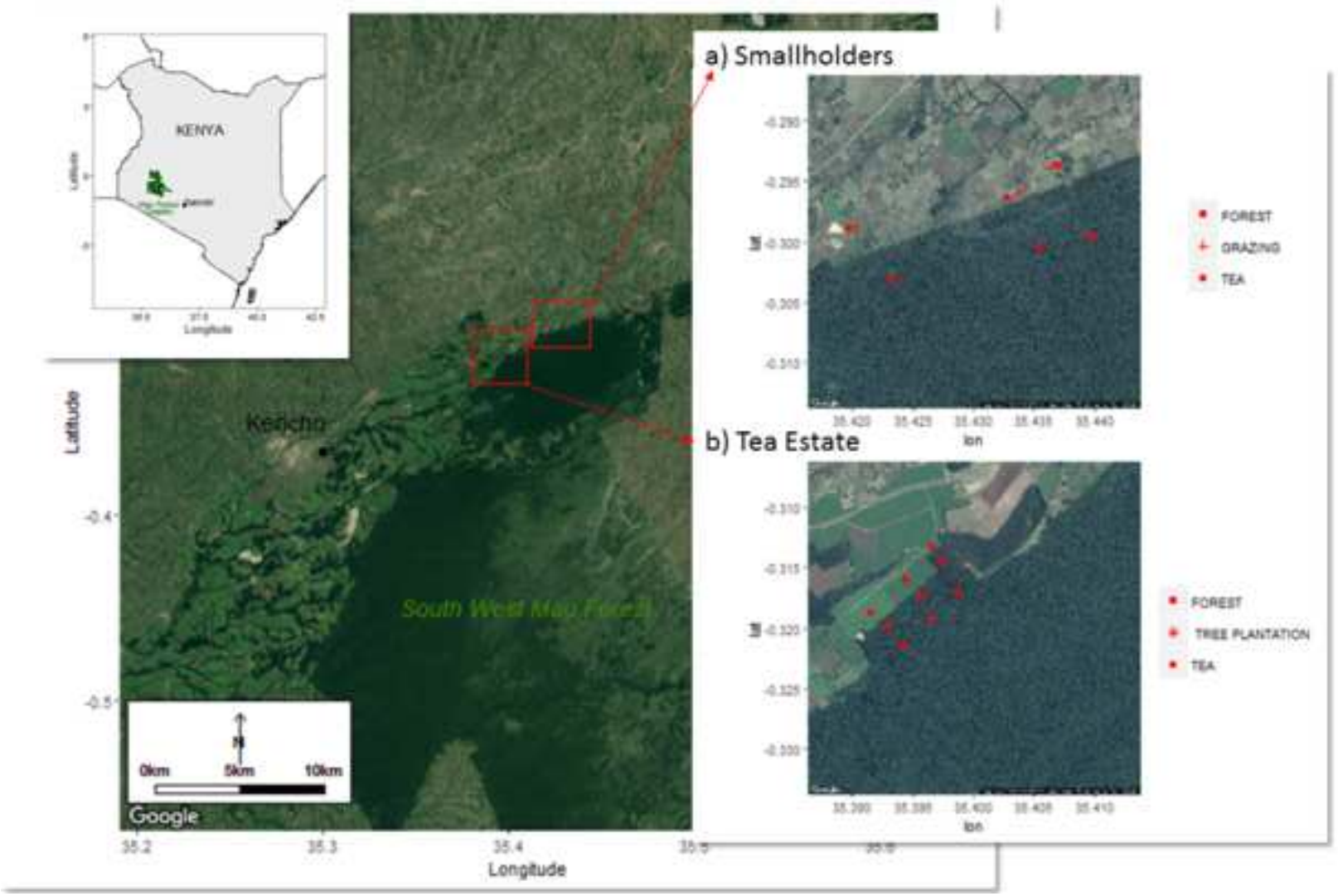

Figure 2

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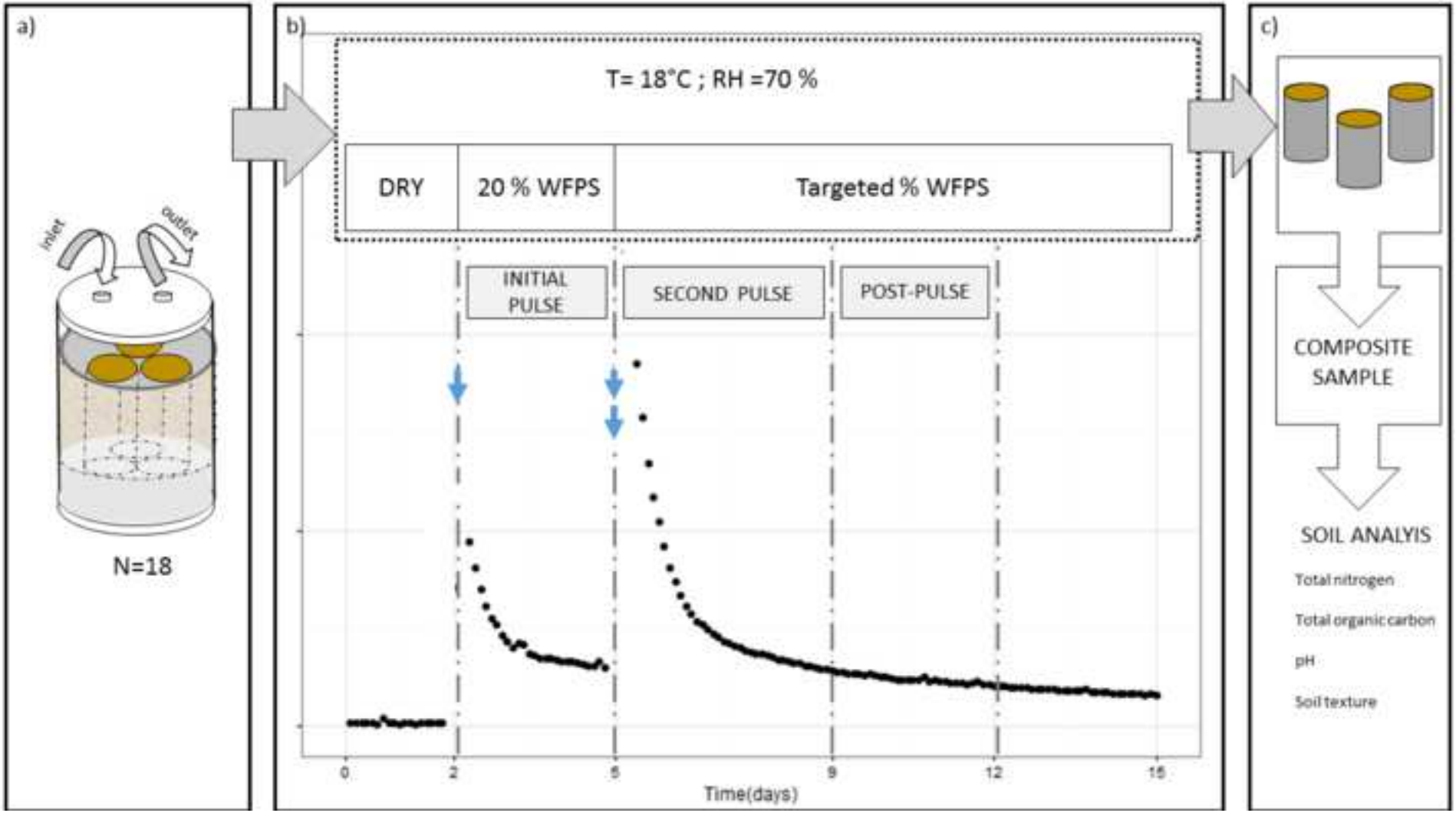

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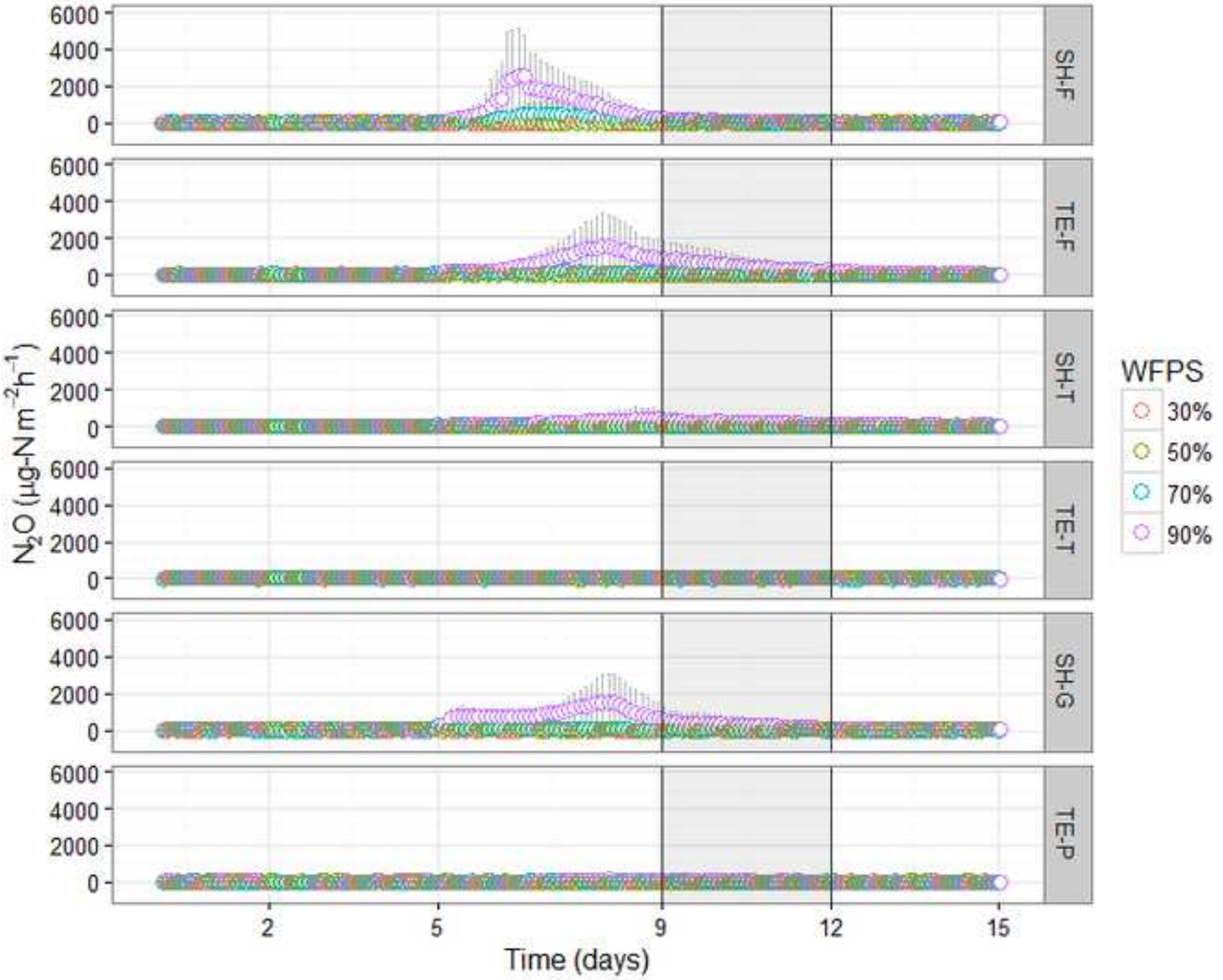

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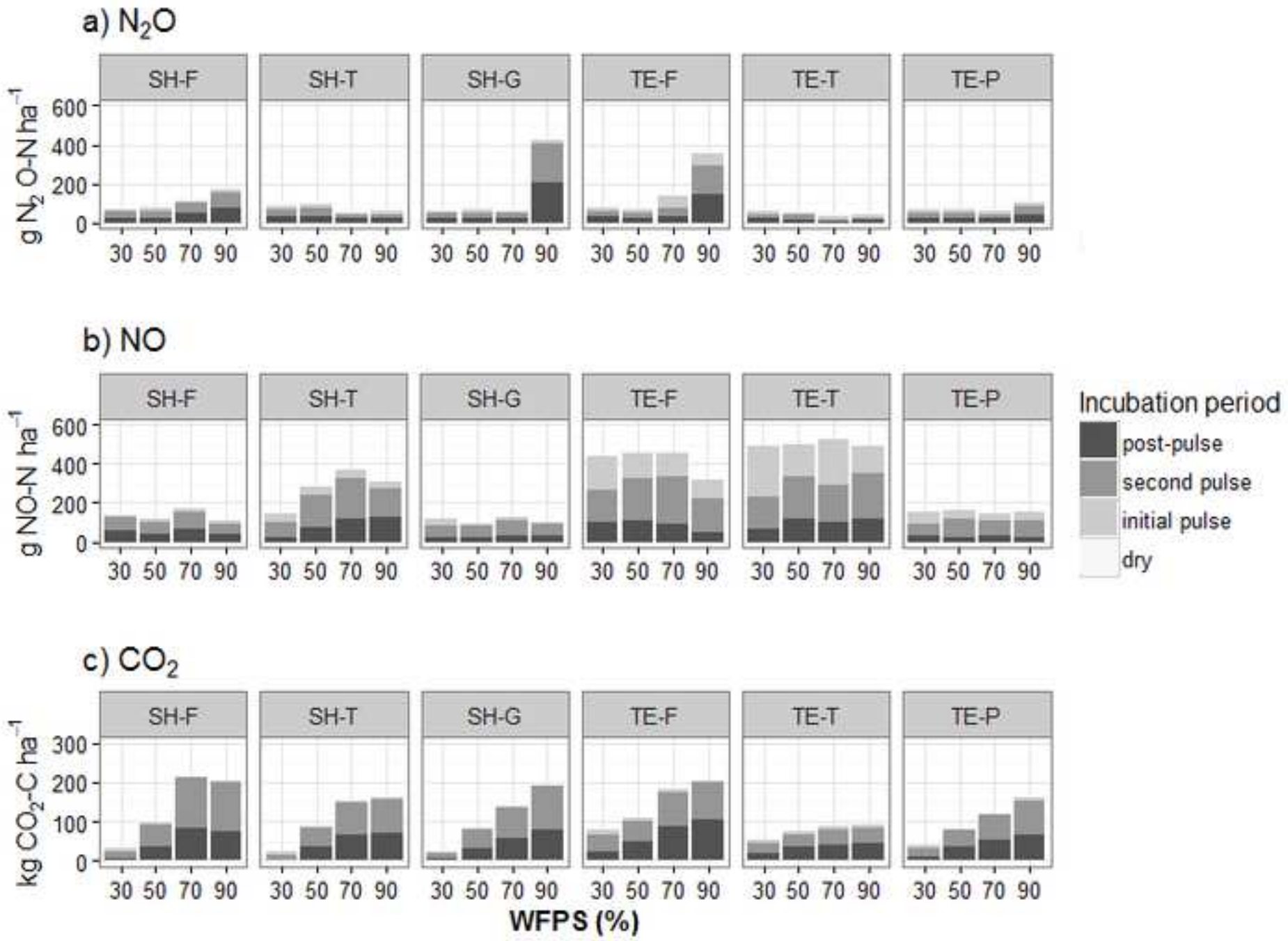

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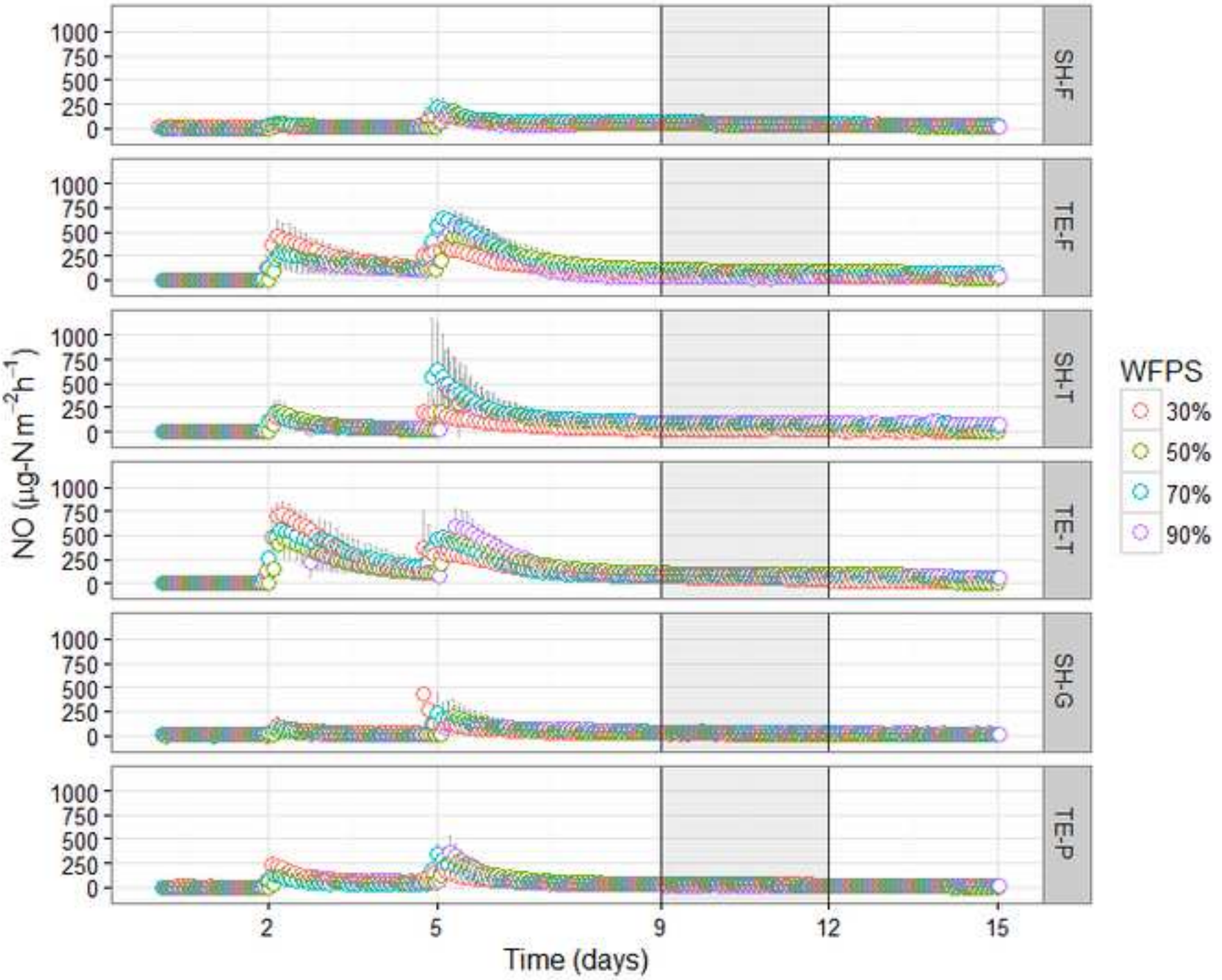

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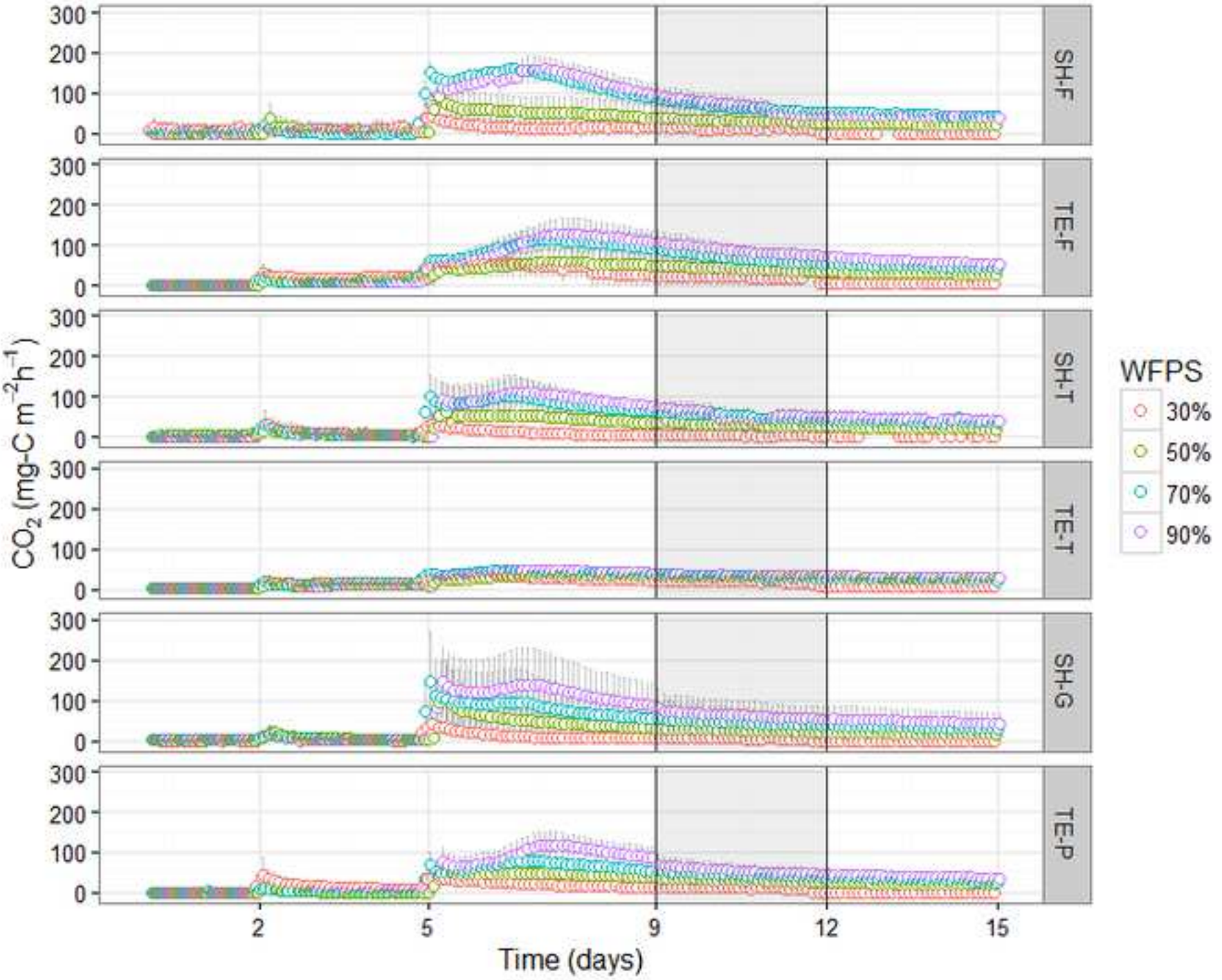

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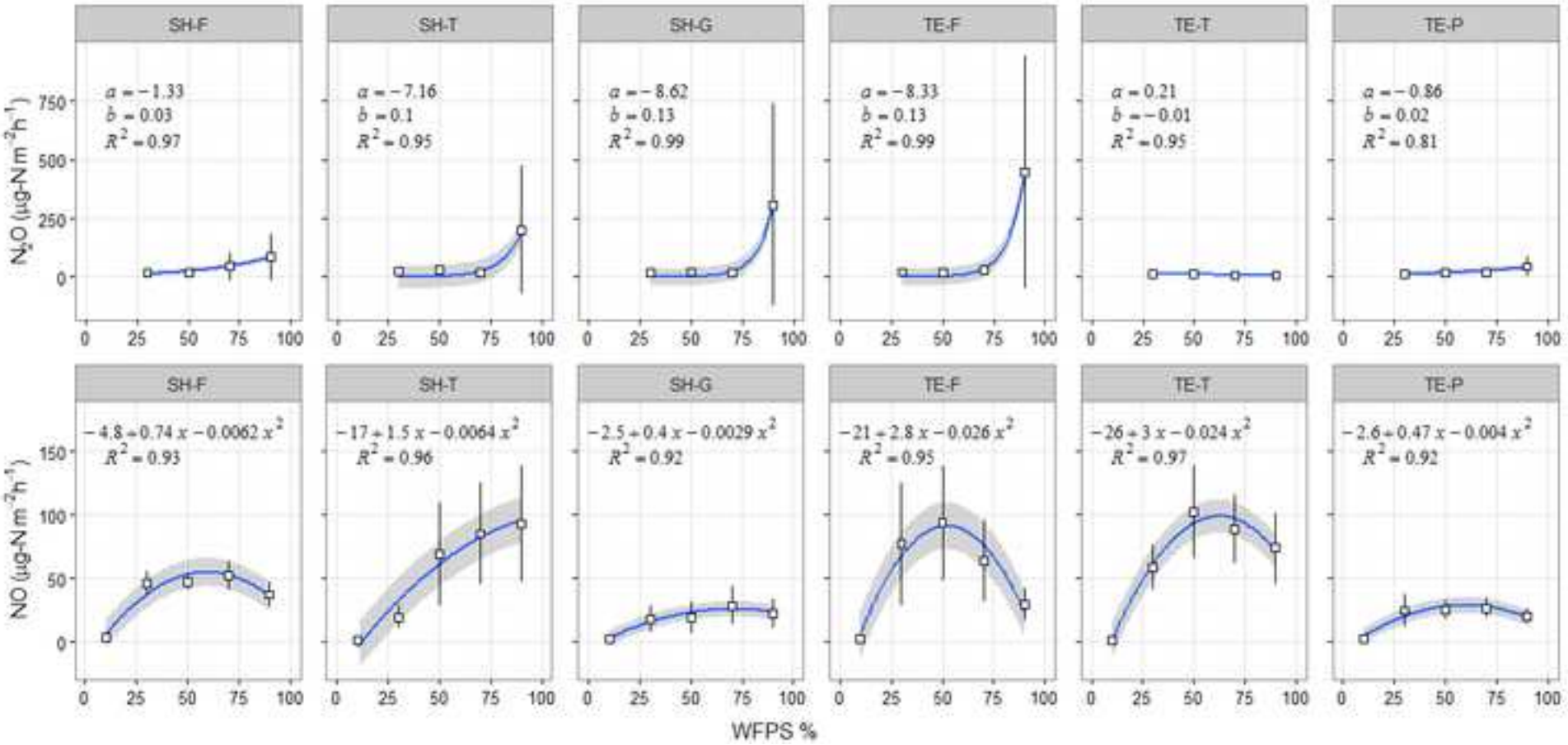

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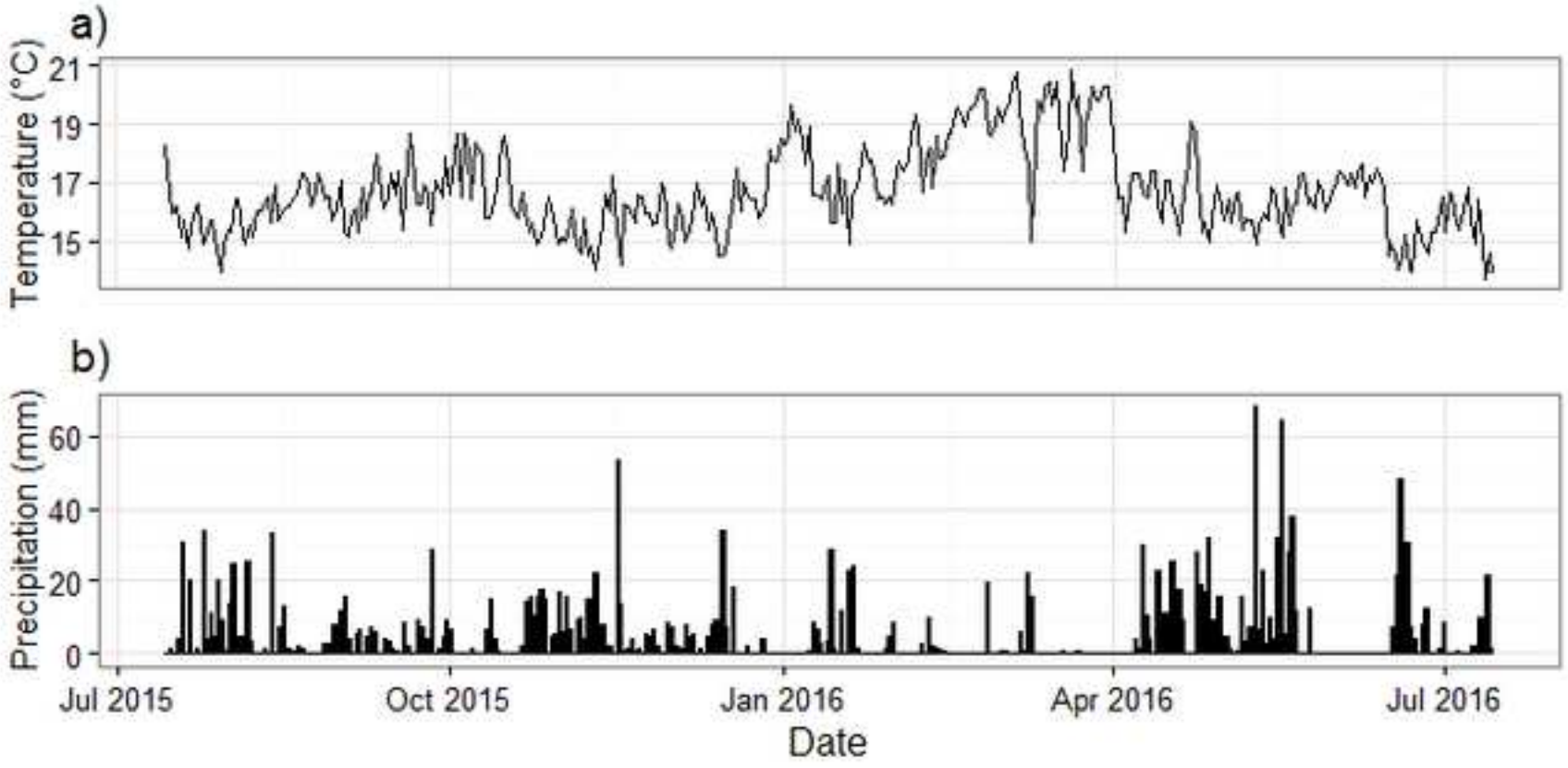

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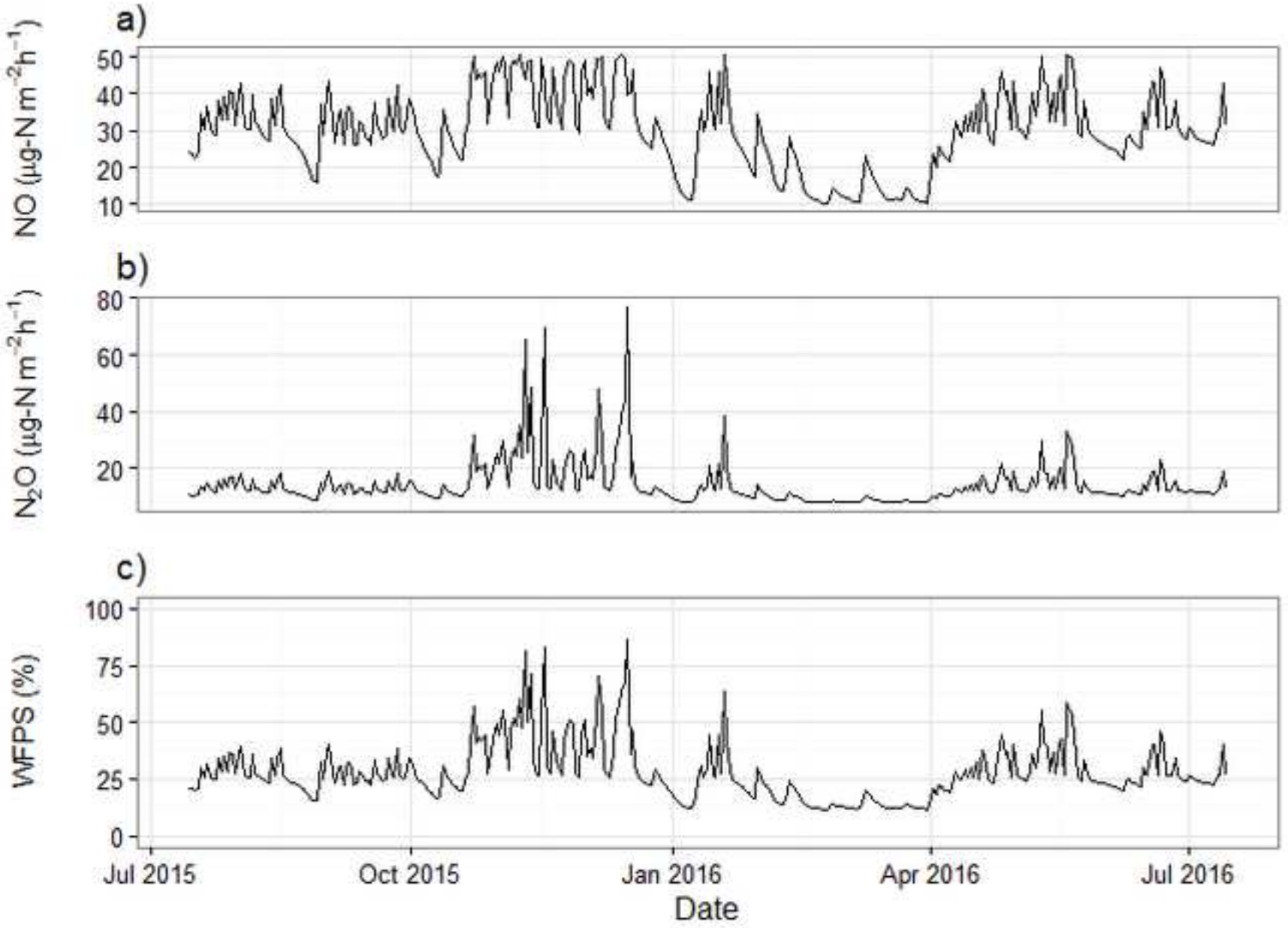

Table 1

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

Biogeochemistry

Supplementary material

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

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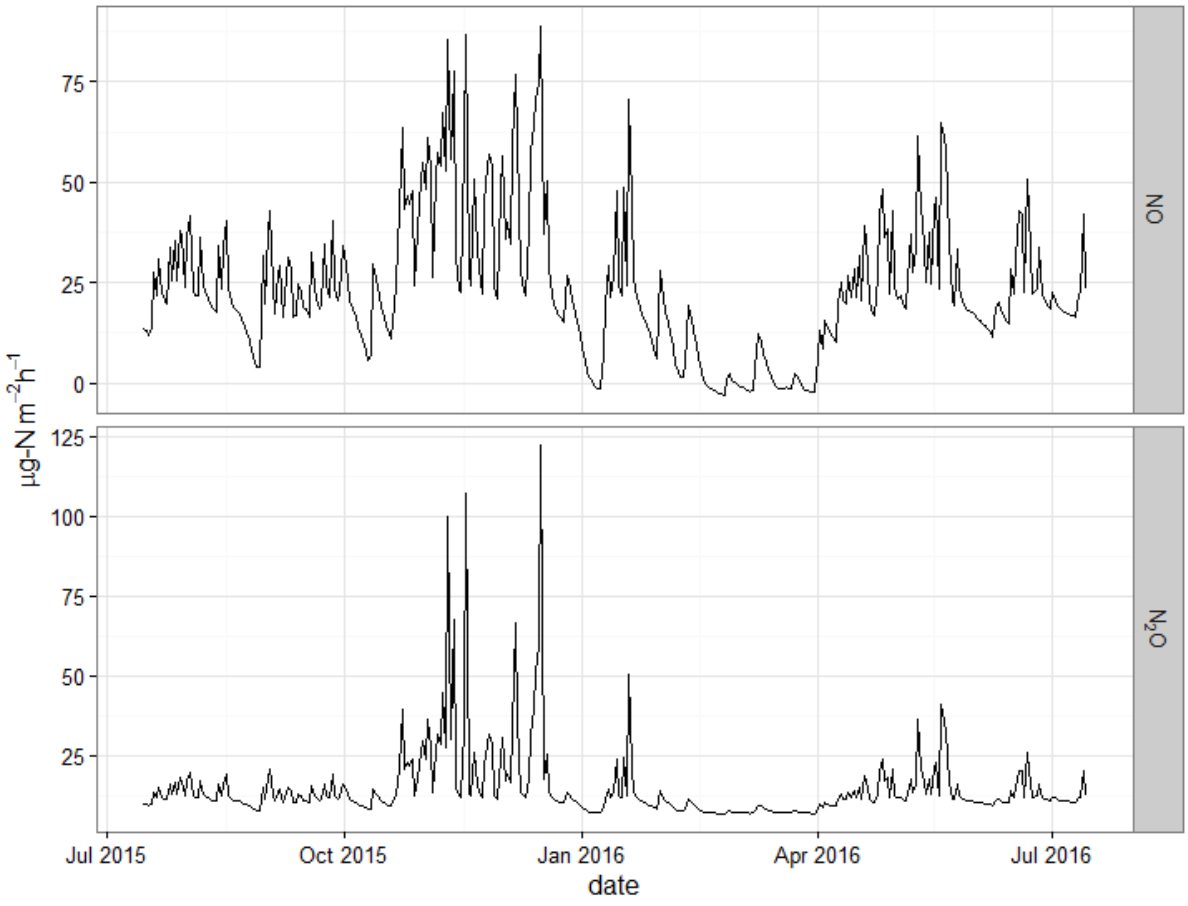

Figure S1. **Seasonal variation of soil NO (upper panel) and N_2O (lower panel) flux estimations from tea plantation site in the smallholder area (SH-T) for the period July 2015 to July 2016.**

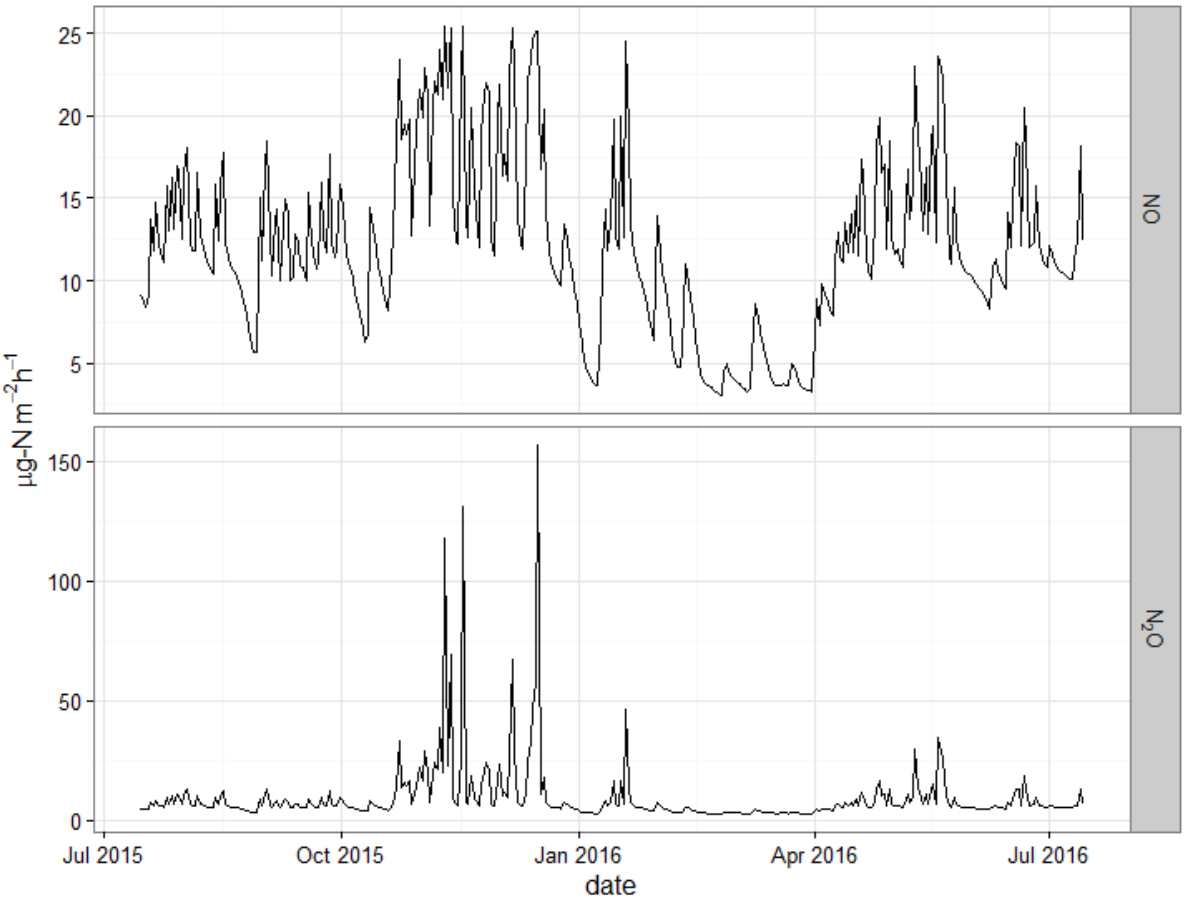

Figure S2. **Seasonal variation of soil NO (upper panel) and N_2O (lower panel) flux estimations from grazing sites in the smallholder area (SH-G) for the period July 2015 to July 2016.**

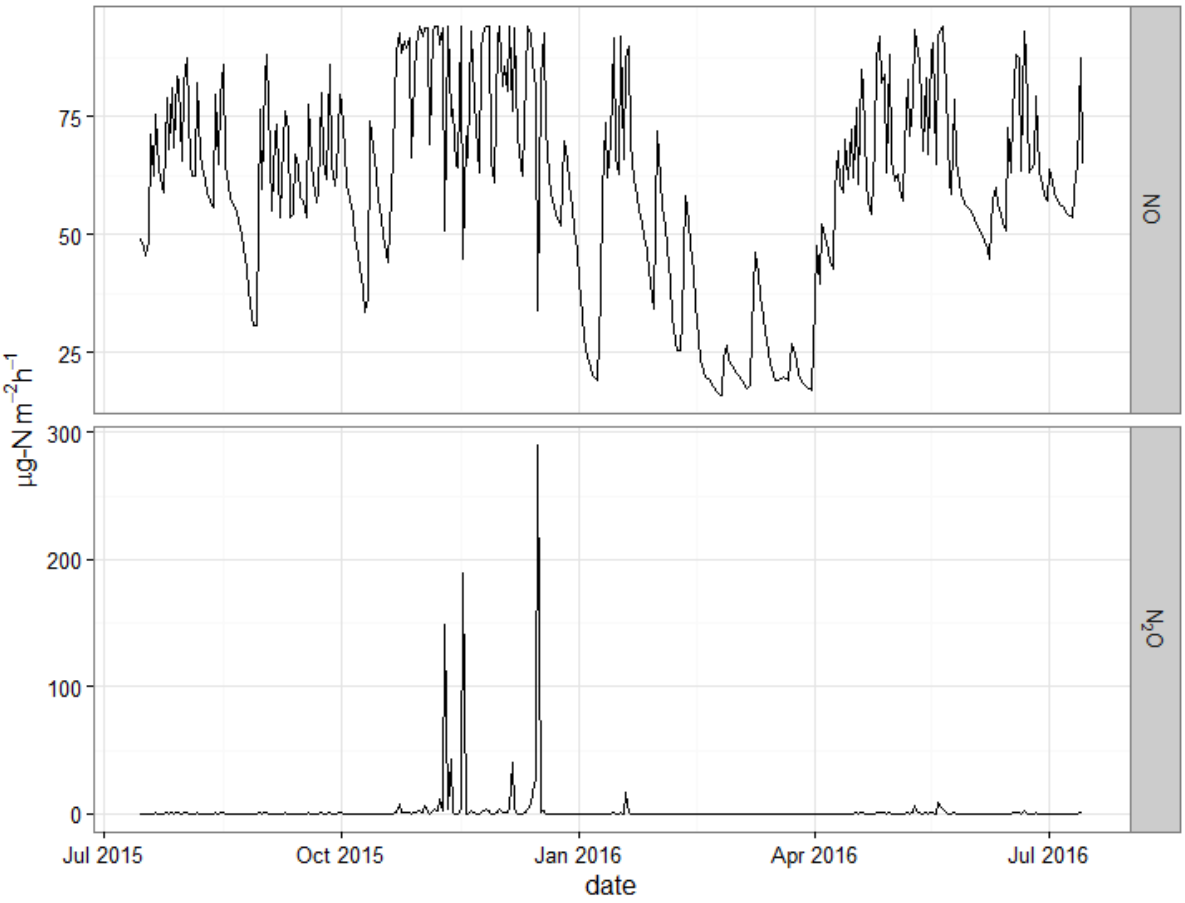

Figure S3. **Seasonal variation of soil NO (upper panel) and N_2O (lower panel) flux estimations from natural forest sites in the tea estate area (TE-F) for the period July 2015 to July 2016.**

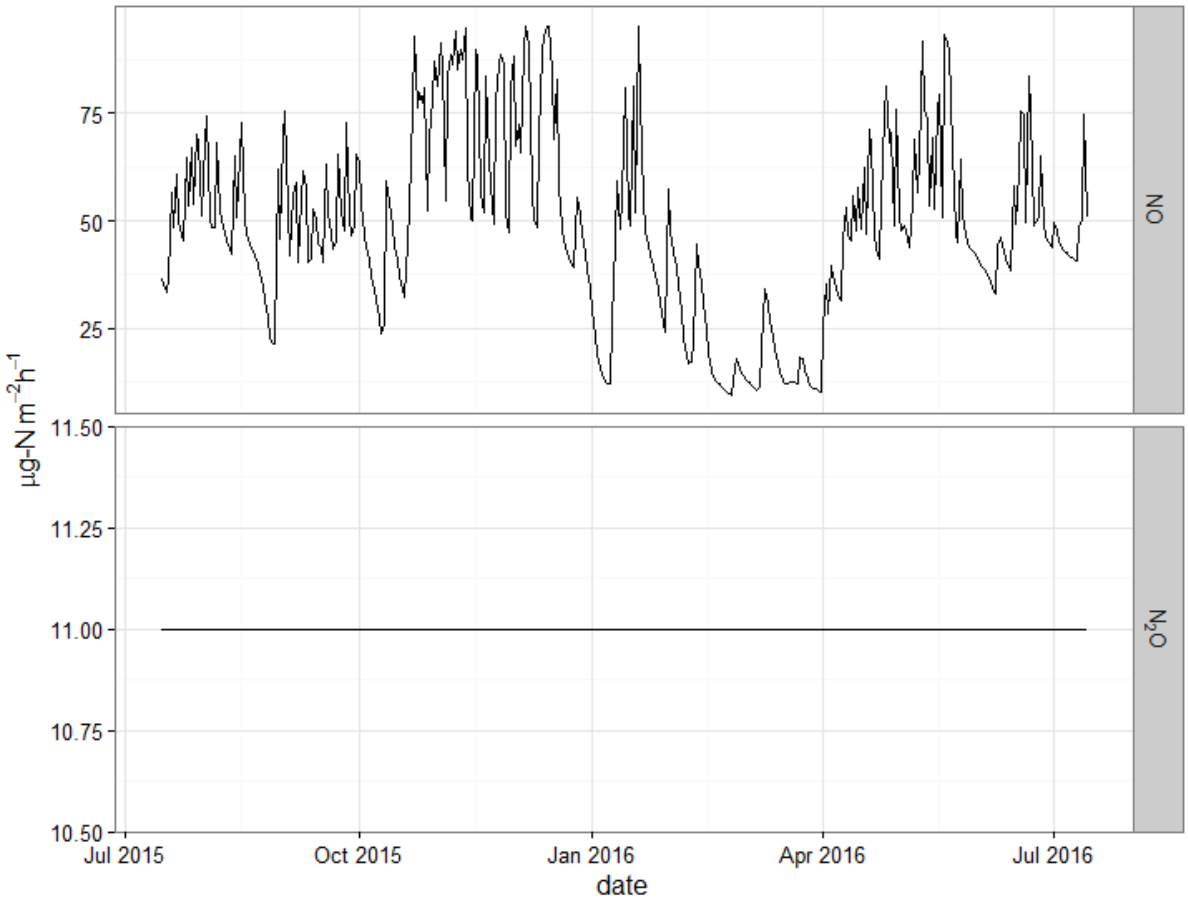

Figure S4. **Seasonal variation of soil NO (upper panel) and N_2O (lower panel) flux estimations from tea plantation in the tea estate area (TE-T) for the period July 2015 to July 2016.**

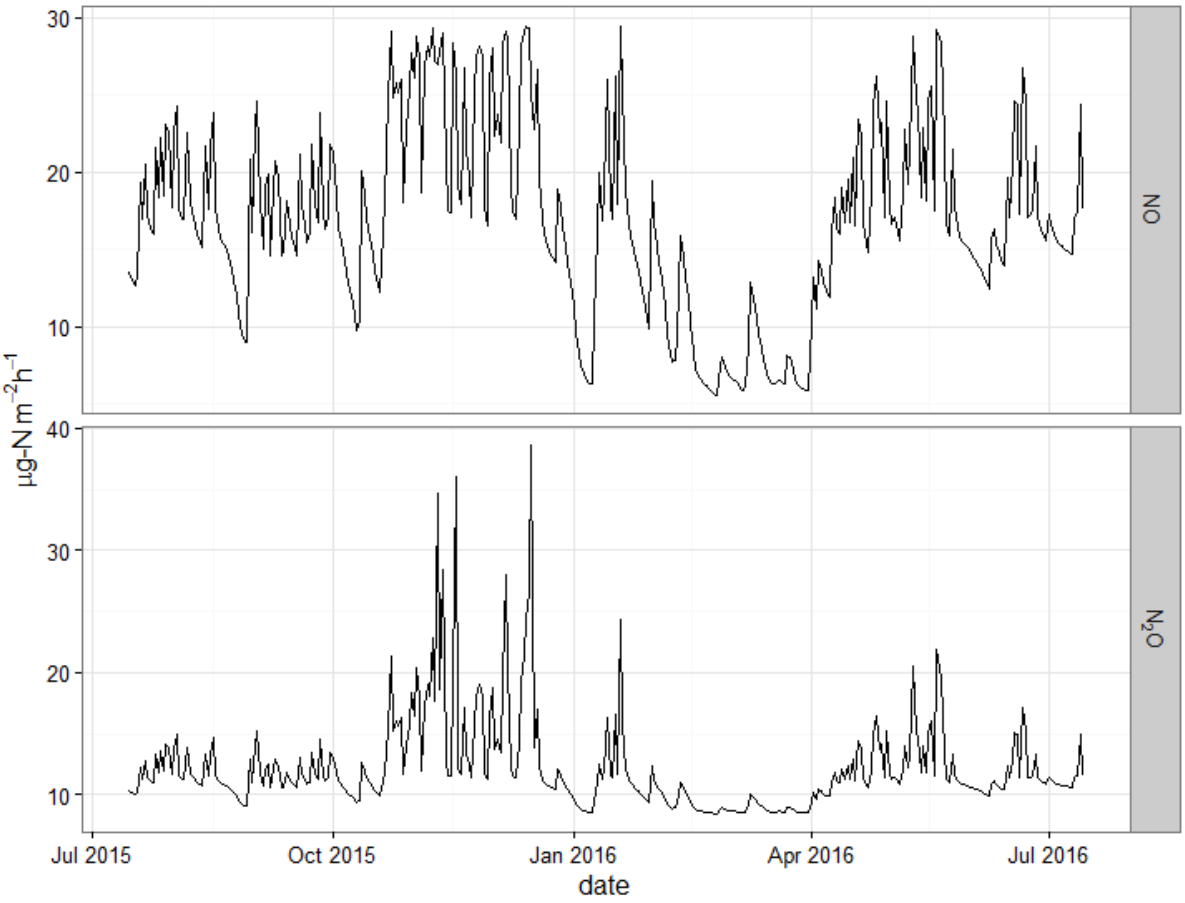

Figure S5. **Seasonal variation of soil NO (upper panel) and N_2O (lower panel) flux estimations from eucalyptus plantation sites in the tea estate area (TE-P) for the period July 2015 to July 2016.**