Management intensity controls soil N_2O fluxes in an Afromontane ecosystem

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Abstract

Studies that quantify nitrous oxide (N_2O) fluxes from African tropical forests and adjacent managed land uses are scarce. The expansion of smallholder agriculture and commercial agriculture into the Mau forest, the largest montane forest in Kenya, has caused large-scale land use change over the last decades. We measured annual soil N_2O fluxes between August 2015 and July 2016 from natural forests and compared them to the N_2O fluxes from land either managed by smallholder farmers for grazing and tea production, or commercial tea and eucalyptus plantations (n=18). Air samples from 5 pooled static chambers were collected between 8:00 am and 11:30 am and used within each plot to calculate the gas flux rates. Annual soil N_2O fluxes ranged between 0.2-2.9 kg N ha^{-1} yr^{-1} at smallholder sites and 0.6-1.7 kg N ha^{-1} yr^{-1} at the commercial agriculture sites, with no difference between land uses ($p=0.98$ and $p=0.18$, respectively). There was marked variation within land uses and, in particular, within those managed by smallholder farmers where management was also highly variable. Plots receiving fertilizer applications and those with high densities of livestock showed the highest N_2O fluxes (1.6±0.3 kg N_2O- N ha^{-1} yr^{-1}, n=7) followed by natural forests (1.1±0.1kg N_2O-N ha^{-1} yr^{-1}, n=6); although these were not significantly different ($p=0.19$). Significantly lower fluxes (0.5±0.1kg N ha^{-1} yr^{-1}, $p<0.01$, n=5) were found on plots that received little or no inputs. Daily soil N_2O flux rates were not correlated with concurrent measurements of water filled pore space (WFPS), soil temperature or inorganic nitrogen (IN) concentrations. However, IN intensity, a measure of exposure of soil microbes (in both time and magnitude) to IN concentrations was strongly correlated with annual soil N_2O fluxes.

Keywords: Tea, grazing, plantations, agricultural intensification, inorganic N intensity

1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG), estimated to contribute about 6% to anthropogenic climate forcing (Blanco et al. 2014). The atmospheric N_2O concentration has increased from 270 ppbv during the pre-industrial era to approximately 320 ppbv, mainly due to stimulated soil N_2O emissions following the use of increasing amounts of reactive N synthesized via the Haber-Bosch process for crop production (Parkin et al. 2012). While agricultural soils are considered major N_2O sources primarily due to fertilizer application, tropical forest soils are also a major natural N_2O source because of often high soil N availability and environmentally favorable conditions for N_2O production (Fowler et al. 2009; Werner et al. 2007a)

In soils, N_2O is mainly produced through two microbial, enzyme-mediated processes: nitrification (autotrophic and heterotrophic) and denitrification (Butterbach-Bahl et al. 2013; Davidson et al. 2000), although other production pathways such as nitrifier-denifrication (Kool et al. 2010) and dissimilatory nitrate reduction to ammonia (Silver et al. 2001) have also been reported. Autotrophic nitrification is enhanced by oxygen availability, moderate water content (approximately 60% water filled pore space WFPS), ammonium (NH_4^+-N) availability, temperature greater than 5°C and soil pH greater than 5. Heterotrophic nitrification requires organic carbon (C), NH_4^+-N supply and occurs in acidic soils (Wood 1990; Zaman et al. 2012). Denitrification, an anaerobic microbial process where nitrogen oxides are used as alternative terminal electron acceptors instead of O_2, is driven by high soil water content (above 60% WFPS) as this hampers O_2 diffusion and results in creation of soil anaerobiosis. Besides the availability of nitrate (NO_3^-) and nitrite (NO_2^-), denitrification also requires the availability of easily degradable C substrates. Several studies have observed a linear relationship between NO_3^--N pools and soil N_2O fluxes (Groffman et al. 2000; Schelde et al. 2012). However, at higher levels of NO_3^--N (>0.4 µg NO_3^--N g^{-1}) the N_2O flux yield by denitrification often decreases (Gelfand et al. 2016; Schelde et al. 2012) as C substrate availability might become the rate limiting factor. Both nitrification and denitrification therefore, are influenced by the size of inorganic-N pools in the soil, and these pools depend on N turnover through mineralization and soil amendments such as fertilizers and livestock excreta.

Nitrification and denitrification have been linked to N_2O fluxes through a conceptual “hole in the pipe” model (Davidson et al. 2000) that links fluxes to the “size of the pipe” (i.e. the amount of N that is nitrified and denitrified), and the “size of the holes” (i.e. the N_2O losses from each process). Typically, this model relates the hole-size to soil water content, which controls the anaerobic status of the soil through its effect on gas diffusion. However, prediction of N_2O fluxes based on simultaneously observed environmental factors and substrate concentrations (NH_4^+-N and NO_3^--N) shows very weak to no correlations in most studies (Gelfand et al. 2016; Maharjan and Venterea 2013; Veldkamp et al. 2008; Wolf et al. 2011), partly because of complex interactions between drivers and temporal variation in soil moisture. Mixed evidence has been reported with strong correlations between cumulative N_2O and cumulative NO_3^-, referred to as nitrate intensity (Burton et al. 2008), however another study found no relationship between either nitrate or ammonium intensity and annual N_2O flux but did find a strong correlation with nitrite intensity (Maharjan and Venterea 2013).

N_2O fluxes measurements from agricultural and natural ecosystems in Africa are limited (Kim et al. 2016; van Lent et al. 2015). Recently, some studies have measured soil N_2O emission datasets from African tropical forests covering lowland (Castaldi et al. 2013; Gharahi Ghehi et al. 2013; Werner et al. 2007b), and montane (Gütlein et al. 2017) tropical forests. However, these studies cover mostly a few weeks, and thus do not capture seasonal variability in fluxes (Werner et al. 2007b). Also, the focus of these studies has been on natural forests and not necessarily on the succeeding land uses. Only a few studies, (e.g. Gütlein et al. 2017, Arias-Navarro et al. 2017) have attempted to fill this data gap and have studied GHG fluxes from tropical montane forests and compared those to agricultural land uses. However, the latter study is an incubation study with intact soil cores and applied regression analysis using observed changes in soil moisture to calculate annual fluxes.

In the tropics, primarily in the Brazilian Amazon and Sumatra, conversion of natural forest to agricultural land use has been shown to elevate soil N_2O emission for a short period after which the emissions become lower or equal to the original forest (Melillo et al. 2001; van Lent et al. 2015; Verchot et al. 2006). In land uses where inorganic fertilizers and organic/manure inputs were used, soil N_2O emissions were often greater than those from the fluxes from the original forest soils (Katayanagi et al. 2008; Lin et al. 2012; Veldkamp et al. 2008).

Land use change involves changes in vegetation type and management practices that may cause changes in soil organic stocks and their quality (Metcalfe et al. 2011), soil microbial communities and microclimate modification (i.e. soil temperature and water content), all of which will influence GHG fluxes (Gates 2012). The Mau forest is the largest contiguous montane forest in Kenya (Wass 1995). Land use change in this forest has occurred rapidly since the 1960s driven by the expansion of smallholder agriculture and by commercial agriculture. While tea plantations replaced forests more than 50 years ago, smallholder agriculture, primarily for grazing or for small-scale tea plantations, continue to drive forest loss. Within large tea estates, the main land uses are either tea or eucalyptus and cypress plantations, with the wood used as fuel for the boilers to run the tea processing plants. On both the small and large-scale farms, tea fields are typically fertilized with NPK (26% N, 5% P_2O_5 and 5% K_2O) compound fertilizer once or twice a year suggesting that emissions from these fields could be higher than emissions from the natural forests.

The aim of this study therefore, was to quantify annual soil N_2O emissions from a tropical montane forest and compare these to the annual soil N_2O emissions from converted land uses: grazing land, tea in smallholder agriculture, tea in commercial plantations and eucalyptus plantations. We also examined mineral nitrogen availability, soil pH, soil temperature and soil water content to explain spatial changes in soil N_2O fluxes. We hypothesized that tea fields and grazing lands have higher soil N_2O fluxes compared to natural forest and eucalyptus plantations due to fertilizer application and animal excreta deposition. In addition, we hypothesized that natural forests would have greater soil N_2O emissions than the eucalyptus plantations.

2. Experimental methods and design

2.1 Study sites

This study was carried out in the South West (SW) Mau forest of Kenya in East Africa. The Mau forest is a tropical montane forest, with high rates of deforestation (Baldyga et al. 2008). Overall, forest cover was reduced from 520,000 ha to 340,000 ha between 1986 and 2009 (Hesslerova and Pokorny 2010), while between the 1990s and early 2000s the forest area of the SW Mau decreased from 84,000 to 60,000 ha (Kinyanjui 2009). The vegetation in the SW Mau is classified as afro-montane mixed forest with broad-leafed species such as *Polyscias fulva* (Hiern.Harms), *Prunus Africana* (Hook. f Kalkman), *Macaranga capensis* and *Tabernaemontana stapfiana* (Britten), further information on vegetation of the study area is reported by (Kinyanjui et al. 2014). This forest ranges from 2100 to 3300 m above sea level, has a mean annual rainfall of 1,988±328 mm at 2100 m elevation (Jacobs et al., 2017) in a bimodal pattern with three to five drier months, and a mean annual air temperature between 15 and 18°C, and so it is situated in a semi-humid climatic zone (Kinyanjui et al. 2014). During the study period (1 August 2015 to 31 July 2016), the study site received 2,050 mm of rainfall and the average daily air temperatures was 16.6±3.9°C. The area received rainfall throughout the year, except for a drier period between January 2016 and mid-April 2016, during which 217 mm of precipitation was recorded. Weather data were obtained from a weather station (Decagon Devices, Meter group, Pullman WA, USA) installed within a radius of 5-10 km of our study sites at elevation 2,173 m asl. A preliminary study revealed that the major land uses at adjacent to the natural forests and settlements were grazing lands, tea and eucalyptus plantations (Swart 2016).

For this study, we selected two sites (Table 1 and Figure 1) approximately 5 km apart. Chepsir is an area occupied by smallholder farms, with most of the land used for annual cropping, grazing or tea production. The second site was at Kapkatugor, where most of the land was used for commercial tea and eucalyptus production. Tea production at both sites involves fertilizer application. At the commercial tea plantations (Kapkatugor site) fields received 150-250 kg N ha^{-1} yr^{-1} as NPK fertilizer, while the application rates at the smallholder farms (Chepsir site) ranged from no fertilizer to 125 kg N ha^{-1}. The rates and timing of fertilizer applications varied between sites and between the replicates at the smallholder site and are shown in Figures 2e and 3e for the smallholder and tea estate sites, respectively. The soils at both sites are classified as humic Nitisols (Jones et al. 2013), which are well drained, very deep, dark reddish brown to dark red soils, with friable clays (FAO 2015).

2.2 Experimental design

At each site, we selected three transects crossing the land uses of interest (Table 1), in such a way that slope position, slope gradient and elevation were similar for each transect. At the tea estate site of Kapkatugor the land uses were tea plantation (TET1, TET2 and TET3), eucalyptus plantation (TEP1, TEP2 and TEP3) and natural forest (TEF1, TEF2 and TEF3), thus each land use was replicated three times (Table 1). The eucalyptus plantations were monoculture eucalyptus planted at 2500 trees ha^{-1} that received no fertilizer inputs. The tea companies restrict human access to the adjacent natural forest which results in reduced human activity and therefore limits illegal activities such as charcoal production (Arias-Navarro et al. 2017) and illegal logging. At the smallholder site of Chepsir, the three land uses we were grazing (SHG1, SHG2 and SHG3), tea (SHT1, SHT2 and SHT3) and natural forest (SHF1, SHF2 and SHF3), thus land uses were replicated three times. The natural forest site at the smallholder landscape had less control and therefore more human encroachment; charcoal production and illegal logging were more common than in the natural forest adjacent to the tea estates. Grazing management was variable, with some farmers using continuous grazing at low stocking densities (SHG3; 1.3 head ha^{-1}) and others using rotational grazing at higher stocking densities (SHG1 and SHG2; 66 and 26 heads per ha^{-1}). In the two rotational grazing paddocks, the animals were kept for approximately 12 hours per day for only 4-5 months of the year, while the continual grazing paddock (SHG3) consisted of a large area (39 ha) where 50 cattle grazed throughout the entire year.

2.3 Gas sampling and analysis

We used the static chamber method (non-flow-through, non-steady state) to estimate soil N_2O fluxes. At each sampling point five, 0.35 by 0.25 m PVC frames were inserted approximately 0.07 m deep in the soil at least 24 hours prior to the first sampling and these frames remained in place until the end of the sampling campaign. In a few cases bases were re-inserted after being removed or when broken/damaged, with gas sampling done at least 24 hours after re-insertion. The sampling was done twice per week from August to December 2015, after which we sampled once per week until the end of the campaign (31 July 2016). We increased the sampling frequency immediately after a fertilization event when we sampled every two days until fluxes returned to pre-fertilization levels.

During gas sampling, a ventilated PVC chamber fitted with a fan, a non-forced vent and a sampling port was mounted to the PVC frame by metal clamps. Rubber sealing between frame and chamber ensured air-tight sealing. We removed 10 ml of gas from each chamber immediately upon closure and then after 15, 30 and 45 min. The five gas samples from each of the five chambers were then pooled for analysis as explained by (Arias-Navarro et al. 2013). During gas sampling, soil water content at a depth of 0.05m was measured using a digital Pro-Check sensor (Decagon Devices, Inc. Pullman, WA99163, US), while soil and chamber temperatures were taken with a digital probe thermometer (TFA-Dostmann GmbH, Zum Ottersberg, Germany). Atmospheric pressure was measured using a Garmin GPS version V (Garmin International, 1200 East 151 street, Olathe, Kansas 66062, USA).

Gas samples were transported to the Mazingira Environmental Center at the International Livestock Research Institute (ILRI), Nairobi, Kenya and analyzed within a week by gas chromatography using a ^{63}Ni electron capture detector (SRI 8610C) for N_2O detection. The minimum flux detection limit was 1.3 µg N_2O-N m^{-2} h^{-1} (Parkin et al. 2012). For further details on GC analytical conditions see e.g. Breuer et al. (2000). Gas concentrations (ppb) were calculated by comparing peak areas of the samples to peak areas of standard gases with known N_2O concentrations. The N_2O fluxes were calculated from observed changes in headspace N_2O concentration during chamber deployment using linear regression after accounting for air pressure and temperature (Pelster et al. 2017). Annual cumulative fluxes were obtained by calculating the area under the flux-time curve and summing the results while assuming linear changes in measurements between time intervals.

2.4 Soil sampling and analysis

At each sampling plot, five soil samples were taken from depth 0-0.05m and 0.05-0.2m using a Eijkelkamp core sampler and rings (Eijkelkamp Agrisearch Equipment, Gies beek, The Netherlands). Soil samples were air dried at 30°C and sieved through 2mm sieve. These samples were used for soil texture, pH, and total C and N measurements. Soil samples for bulk density determination were dried at 105°C until constant weight was attained. Soil texture was analyzed by the hydrometer method (Gee and Bauder 1986). Soil pH was measured in 1:2.5 soil to deionized water slurry using a glass electrode (Jackson 1958). The sieved soil was finely ground to powder and analyzed for total C and N using the elemental combustion system (ECS 4010, Costech Instruments, Italy).

Inorganic N concentrations (NH_4^+-N and NO_3^--N) were determined every fourteen (14) days during the gas sampling campaign. At each sampling plot, a composite fresh soil sample was taken from 0-0.05m depth from at least 3 points beside the chamber frames using a sharpened-edge PVC cylinder (0.05 m height and inner diameter). Each fresh sample had the plant litter removed and was mixed thoroughly. Approximately 10 g of the fresh soil sample was placed into a plastic bottle and 50ml of 0.5M $K_2SO_4^-$ was added. The slurry was shaken for 1 hour on a reciprocating shaker and was then filtered through 110 mm Whatman^{TM} filter enhanced with a vacuum pump, further filtering was done using a 0.45 µm syringe filter (Minisart®, Sartorius Stedim Biotech Gmbh, 37079 Goettingen, Germany) to remove fine particles and filter blank corrections were applied. The extracts were frozen immediately until analysis. Analyses for NH_4^+-N and NO_3^--N were done using an Epoch™ micro-plate spectrophotometer (BioTek® Instruments, Inc., Winooski, USA). The remaining composite fresh soil sample was oven dried at 105°C until constant soil weight to determine soil water content; thereafter inorganic N (IN) was calculated on dry soil mass basis. Annual cumulative NH_4^+ and NO_3^- was calculated by integrating the area under respective curves and herein referred to as NH_4^+-N intensity and NO_3^--N intensity (Burton et al. 2008) respectively, and the total of NH_4^+-N and NO_3^--N named “Inorganic N intensity”.

2.5 Data analysis

The mixed linear model of the lmerTest in the R package (R Team 2016) was used to analyze the effect of fixed factor land use, with transect and/or sampling month as blocking (random) factors on soil N_2O fluxes and/or monthly soil N_2O means. We also compared soil N_2O fluxes from 1) natural forest to converted land uses where 2) no external inputs were added (N) and 3) those that received external inputs fertilizer or animal excreta (Y) (Table 1). Here, 'external inputs' was the fixed factor while land use was the random variable in the mixed linear model. Prior to analysis, data were tested for normality using Shapiro-Wilk test (Shapiro and Wilk 1965) and log transformed (apart from pH) when necessary. Differences of least squares means (difflsmeans) of the lmerTest in the R package (Kuznetsova et al. 2015) were used for multiple comparison of the treatments. When normality could not be achieved through data transformation, we used the Friedman non-parametric test to carry out ANOVA. Correlations between annual soil N_2O fluxes and soil variables were evaluated using the Spearman rank test. One point of the grazing land use (SHG2) was not used for correlation analysis between soil N_2O fluxes and total inorganic N after it was identified as an outlier with standardized residual 4.5 times larger than the standard deviation. To test the effect of rainfall on N_2O fluxes, we categorized dry and wet periods based on WFPS (%) rather than using the seasons. We decided to do this because the study site receives sporadic rains even during the dry seasons. For our tests, we used 40% WFPS as a threshold that divides periods from being dry to wet assuming this value to be between wilting point and field capacity (Harrison-Kirk et al. 2013).

3. Results

3.1 Soil properties

There were marked variations in soil properties among the land uses at both depths (0-0.05 and 0.05-0.20m) and at both sites (Table 2). Soil texture was generally clay except for the grazing and forest land uses in the smallholder sites, which were clay loams and loams respectively. Total C and N in both soil depths were strongly affected by land use ($p<0.01$). The highest concentrations of total soil nitrogen (TN) in the top soil was measured in the native forest soils, while lowest values were observed at the tea and grazing land at the smallholder site (Table 2). At the lower depth (0.05 – 0.20 m), the grazing land and forest land use at the smallholder site had the highest TN. Total carbon concentrations varied similarly to TN in both soil depths. The C:N ratio was highest for the tea plantations while the forest C:N ratio was lowest for both soil depths. Soil pH in the top soil ranged from 3.8 at the tea plantation to 6.6 at the smallholder forest plot, with a similar trend observed at the lower soil depth. Soil bulk density (BD) was highest under grazing land and lowest under forest at both soil depths. Intermediate BD values were observed in the rest of the land uses.

Soil water varied widely through the year in all land uses, ranging from 20 to 80% WFPS, while soil temperature remained near to 15°C for most of the land uses (mean=16.7°C), with the exception of the grazing plots where temperatures were consistently higher (mean=18.8°C) than in all other plots (Figure 2c). Soil inorganic concentrations ranged from 3.6 to 40 µg N g^{-1} soil through most of the season, but increased up to 132 µg N g^{-1} soil in the tea plantations shortly after synthetic fertilizers were applied (Figure 2e and 3e, Table 3) although the highest concentration (111 µg N g^{-1} soil) was measured in grazing lands, likely because of animal excreta deposition. Differences in IN intensities were observed only at the tea estate site where both IN intensity and NH_4^+-N intensity were higher (p= 0.016 and p<0.001, respectively) in the tea than the forest and eucalyptus land uses. However, there was marked variation within land uses especially for the tea plots at the smallholder site, where the coefficient of variation (CV%) was 89% (Table 3).

3.2 N_2O fluxes

Mean N_2O flux rates for the different land uses from 1st August 2015 to 1st August 2016 ranged between 0.87±3.5 µg N_2O-N m^{-2} hr^{-1} (on 6th October 2015) and 153.4±6.7µg N_2O-N m^{-2} hr^{-1} (on 23rd May 2016) for land uses at the tea estate site of Kapkatugor; and from -2.1±2.4 µg N_2O-N m^{-2} hr^{-1} (on 5th January 2016) to 118±123 µg N_2O-N m^{-2} hr^{-1} (on 17th September 2015) for land uses at the smallholder site of Chepsir. At both sites and all land uses, the mean daily fluxes were lower when WFPS was below 40%, but increased significantly when WFPS was above 60% (Figure 2d and 3d, Appendix Table A1). Peak soil N_2O fluxes corresponded to wetter periods, whereas soil N_2O fluxes observed during the drier periods were between half to one-third smaller (Appendix Table A1). Weekly temperatures of the top soil (0-0.05 m) were higher in the grazing land use (18.8±1.3°C) compared to the natural forest (15.2±0.8°C) and tea plots (15.7±1.1°C) at the smallholder site (Figure 2c). At the tea estate, soil temperatures were consistent among the different land uses. Despite these differences in soil temperature, there was no significant correlation between N_2O fluxes and soil temperature (Appendix Fig A1).

Peak soil N_2O fluxes corresponded to IN peak concentrations in the tea plots from Kapkatugor as well as high values for WPFS (above 60%), although the relationship between weekly N_2O fluxes and IN concentrations and WFPS was very weak across land uses ($r<0.01$, $p>0.10$). Annual N_2O fluxes were similar between the different land uses at the smallholder ($p=0.985$) and at the tea estate ($p=0.179$) sites. However, high coefficients of variation (CV) in soil N_2O fluxes were observed within similar land uses of the smallholder site; especially in the grazing lands (CV=107%) and tea fields (CV=62%).

Management of similar land uses differed largely within the smallholder site (Table 1). In grazing lands, the N_2O fluxes were highest in the plots with high stocking density (SHG2, followed by SHG1), while the lowest fluxes were measured in the plot with low stocking density (SHG3, 1.3 head per hectare). There were also large variations in N_2O emissions within the smallholder tea fields with the lowest fluxes in plot SHT3 (0.67 kg N_2O-N ha^{-1} yr^{-1}) where no fertilizer was applied, and the highest (2.34 kg N_2O-N ha^{-1} yr^{-1}) at plot SHT1 where 125 kg N ha^{-1} of fertilizer was applied (Table 1).

Annual fluxes were highest (1.6±0.3 kg N_2O-N ha^{-1} yr^{-1}) for plots receiving N inputs (SHT1, SHG1, SHT2, SHG2, TET1, TET2 and TET3), which were similar ($p=0.19$) to the annual flux of the natural forest plots (1.1±0.1 kg N_2O-N ha^{-1} yr^{-1}). Annual fluxes from the converted plots receiving no N inputs (SHT3, SHG3, TEP1, TEP2 and TEP3) were lower (0.5±0.1 kg N_2O-N ha^{-1} yr^{-1}; $p<0.01$) than both the natural forest and the managed plots receiving N inputs.

Monthly soil N_2O flux at the smallholder site followed the same trend as annual fluxes where no significant difference ($p=0.627$) between land uses was observed. However, monthly soil N_2O fluxes were significantly different among land uses at the tea estate site, where fluxes from forest soils and tea plantations were higher ($p=0.001$) than from eucalyptus plantations.

There were strong correlations between annual N_2O fluxes from all plots and IN intensity ($p<0.001$; r=0.72), ammonium intensity ($p<0.01$; r=0.57) and nitrate intensity ($p<0.05$, r=0.57) (Figure 5 and Table 4). No relationships were observed ($p>0.05$) between annual N_2O flux from all plots and other soil properties (e.g. pH, total carbon and nitrogen). The combination of converted sites with no or little external N inputs and natural forest showed positive correlations between annual N_2O fluxes and total N ($p<0.01$, r=0.74) and total C ($p<0.05$; r=0.67) concentration, while bulk density ($p<0.01$; r=0.72) and C:N ratio ($p<0.05$; r=0.47) were negatively correlated with annual N_2O fluxes (Table 4). Also, the relationship between annual N_2O and IN and NO_3^--N intensities were stronger among plots where no or little external inputs were applied (inclusive of natural forest plots).

4. Discussion

Cumulative annual N_2O fluxes from natural montane forest in this study (1.1±0.11 kg N_2O-N ha^{-1} yr^{-1}) were within the range measured in other tropical and sub-tropical montane forests; 1.2 kg N_2O-N ha^{-1} yr^{-1} in Panama (Koehler et al. 2009), 1.1-5.4 kg N_2O-N ha^{-1} yr^{-1}) for sites in Queensland, Australia (Breuer et al., 2000), 0.3–1.1 kg N_2O-N ha^{-1} yr^{-1} for sites at Mt. Kilimanjaro, Tanzania (Gütlein et al. 2017), and 0.29 -1.11 kg N_2O-N ha^{-1} yr^{-1} in Central Sulawesi, Indonesia (Purbopuspito et al. 2006). However, annual cumulative N_2O fluxes at our forest sites were at the lower end compared to earlier studies in Africa: 3.0±2.0 kg N_2O-N ha^{-1} yr^{-1} (Castaldi et al. 2013) in a tropical humid forest in Ghana, and 2.6 kg N−N_2O ha^{-1} yr^{-1} (Werner et al. 2007b) for a tropical lowland forest in Kenya. Spatial variation in N_2O fluxes from different forest sites have been attributed to thermal and hydrological variations that drive processes such as soil organic matter mineralization, nitrification and denitrification (Zhuang et al. 2012). Mean annual air temperature at the Kakamega is 20.4ºC (Werner et al. 2007b) compared to 16.6°C at our study area, difference that can be explained by elevation (1530 m Kakamega forest site, 2200 m at our study sites). Higher elevation and lower temperatures are associated with reduced net mineralization rates (Koehler et al. 2009; Liu et al. 2017) resulting in lower N availability in the soil (Arnold et al. 2009; Purbopuspito et al. 2006; Wolf et al. 2011), and with reduced rates of biological N_2 fixation at ecosystem scale (Cleveland et al., 1999).

These differences are consistent with observations that highland forests are typically N limited (Nottingham et al. 2015)

The annual N_2O fluxes from the smallholder and tea estate sites in this study (1.4±0.5 and 1.2±0.3 kg N_2O-N ha^{-1} yr^{-1}, respectively) were higher than the fluxes (0.38 and 0.75 kg N ha^{-1} yr^{-1}) reported by Rosenstock et al. (2016) for other tea producing areas in the western Kenyan highlands where farmers applied approximately 112 kg N ha^{-1} yr^{-1}. The authors attributed the relatively low rates to low sampling frequency that could have led to missing out N_2O emissions peaks after fertilizer application as discussed by Barton et al. (2015). Because we sampled every two days immediately following a fertilization event, we likely captured any N_2O emission pulses that occurred after the addition of N, resulting in a more accurate representation of cumulative N_2O fluxes from tea crops. Additionally, the soils at the western Kenyan highlands in the study by Rosenstock et al. (2016) were more porous (sandy clay loams) compared to the clay soils in our study region. Generally, relatively porous soils emit less N_2O because the development of soil anaerobic state that is required for denitrification is restricted by relatively high oxygen diffusion rates into soils (Rochette et al. 2008). At the smallholder site in our study, the high variability in annual N_2O fluxes among the tea plots could be explained by the different rates of fertilizer applications, which led to differential concentrations of inorganic N in the soil (cf. Fig. 5).

Other studies that compared N_2O fluxes from forests and converted land use found either increased, decreased or no difference fluxes between forest and converted land use depending on the time of conversion and management practices which affected soil carbon and nitrogen content (Cheng et al. 2013; Melillo et al. 2001; Veldkamp et al. 2008; Wang et al. 2006). Lack of a difference in annual N_2O fluxes between land uses was due to the high variability of management intensities within plots of a given land use. In both the smallholder tea and smallholder grazing sites, there was a wide range of management intensities. The N_2O fluxes from the grazing land use in our study was similar to those from a previous study on grazing land in western Kenyan highlands with annual flux rates of between 0.5 and 3.9 kg N_2O-N h^{-1} yr^{-1} (Rosenstock et al. 2016), where variation was attributed to management practices. Likewise, there were large variations in animal densities between the three different grazing plots. The plots with the higher stocking densities had higher annual N_2O fluxes (1.18 and 3.01 kg N ha^{-1} yr^{-1}, respectively) than the plot with low stocking densities (SHG3; 0.20 kg N ha^{-1} yr^{-1}) perhaps because there was greater transfer of nutrients from outside to inside the paddocks via animal excreta, but also likely due to more rapid cycling of N associated with pulses of high intensity. More animal excreta likely led to N_2O emissions directly from the dung and urine (Pelster et al. 2016), as well as increased N and C inputs to the soil that contributed to N_2O emissions. However, when considering converted plots where no external inputs were added, we observed a reduction in soil N_2O relative to natural forest, consistent with observations by van Lent et al. (2015) where reduced fluxes were attributed to lower N availability. This is further supported by our results where topsoil N concentrations were lower in eucalyptus and tea plots that received no inputs (Table 2).

Monthly soil N_2O fluxes from eucalyptus plantations were the lowest in our study and the annual fluxes (0.6±0.2 kg N_2O-N ha^{-1} yr^{-1}) were also on the lower end compared to the other land uses. Lower soil N_2O flux from eucalyptus plantations may be related to lower N cycling rates as reflected by lower IN intensities (Table 3). Relatively slower N mineralization has been previously reported in eucalyptus plantation soils (Bernhard-Reversat 1988). Net mineralization decreases with increased soil C:N ratio (Springob and Kirchmann 2003) and consequently reduced N_2O fluxes. In our study we also observed a strong negative correlation between C:N ratio and soil N_2O fluxes (Table 4). In addition, total N was lowest in eucalyptus plantations (Table 2). Therefore, the lower total N coupled with lower N mineralization likely caused the lower soil N_2O fluxes in eucalyptus plantations.

The environmental variables that we measured at weekly intervals and soil inorganic N concentrations did not predict soil N_2O fluxes well. This is consistent with studies by Veldkamp et al. (2008) in the humid tropical forest margins of Indonesia and of Rowlings et al. (2012) in a subtropical rainforest site in Australia who found no correlation between N_2O and inorganic N (NH_4^+ and NO_3^-) concentrations, while studies by Wolf et al. (2011) and Purbopuspito et al. (2006) also found no correlation between WFPS and soil N_2O fluxes. This could be attributed to three factors:

(i) complex interactions between drivers of soil N_2O fluxes in time and space (i.e. hot moments and hot spots: Groffman et al. 2000) in a way that mask the effect of the measured variables in our study;

(ii) gases originate from deeper soil layers for which environmental parameters were not measured (our study: 0-0.05 m). This is supported by studies by Verchot et al. (1999) in native forests and coffee plantations in Sumatra and by Wang et al. (2014) for winter-wheat and summer-maize rotation in Northern China who reported larger gas fluxes from deeper layers. Furthermore, Nobre et al. (2001) reported the highest soil N_2O production from 5 to 20 cm of soil depth. The soils in our study area are deep and well drained. Thus, deeper layers might contribute significantly to the soil N_2O fluxes at the soil-atmosphere boundary;

(iii) time lags between measurements of inorganic N concentrations and increases in soil N_2O fluxes. Such effects, which are partly related to low frequency sampling (Barton et al. 2015), can only be captured by using of automatic high-resolution temporal sampling.

Nevertheless, inorganic N intensities (NH_4^+-N, NO_3^--N and total IN intensities) correlated well with annual N_2O fluxes, which was previously observed by Burton et al. (2008). In our study the magnitude and temporal persistence of IN are likely related to the amount of substrate added through management (inorganic fertilizer, manure and urine) or the speed of N cycling in plots where no external N was added and in the natural forests.

Soil temperature did not influence N_2O fluxes in our study, the same observation was reported by Werner et al. (2007b) in Kakamega forest in Kenya, contrary to what has been observed in many other studies as summarized by Skiba and Smith (2000). In our study area, temperature within land uses did not vary much throughout the study period, as is the case in many tropical systems.

The significant positive relationship between annual N_2O fluxes and annual IN intensity shows that N_2O fluxes were closely coupled to N availability. The missing saturation effect, which finally manifests as an exponential increase in N_2O fluxes (Shcherbak et al. 2014), might be used to indicate that N_2O fluxes in this ecosystem are still N limited

(Davidson et al. 2000; Rowlings et al. 2012) and that increasing N availability, e.g. through increased fertilization applications, would result in even higher N_2O fluxes.

5. Conclusions

This study of a tropical montane forest in Kenya showed lower annual N_2O fluxes (1.1±0.1 kg N_2O-N ha^{-1} yr^{-1}) than those from lowland tropical forests, which typically have fluxes around 2.0 kg N_2O-N ha^{-1} yr^{-1} (van Lent et al., 2015). We attribute this difference in fluxes to differences in environmental conditions such as air temperature. Wide variations of annual soil N_2O fluxes within the managed land uses made it difficult to detect a land use effect; with variability of soil properties also added a confounding factor. The magnitude of annual N_2O fluxes relative to the natural forest varied considerably within a given land use depending on management intensity and this makes generalizations difficult. We found no correlation between N_2O flux rates and soil temperature, whereas peaks in flux rates tended to occur at high (>60% WFPS) moisture content. To understand emissions at annual scales and the factors that regulate these emissions, we looked at cumulative N_2O fluxes and compared them with IN intensity. We found a linear increase in annual soil N_2O fluxes with increasing IN intensity. Fertilized plots had the highest IN intensities and also the highest cumulative N_2O emissions, indicating that management of converted lands plays a larger role in determining the amount of N_2O emissions than land use in this environment.

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Figures

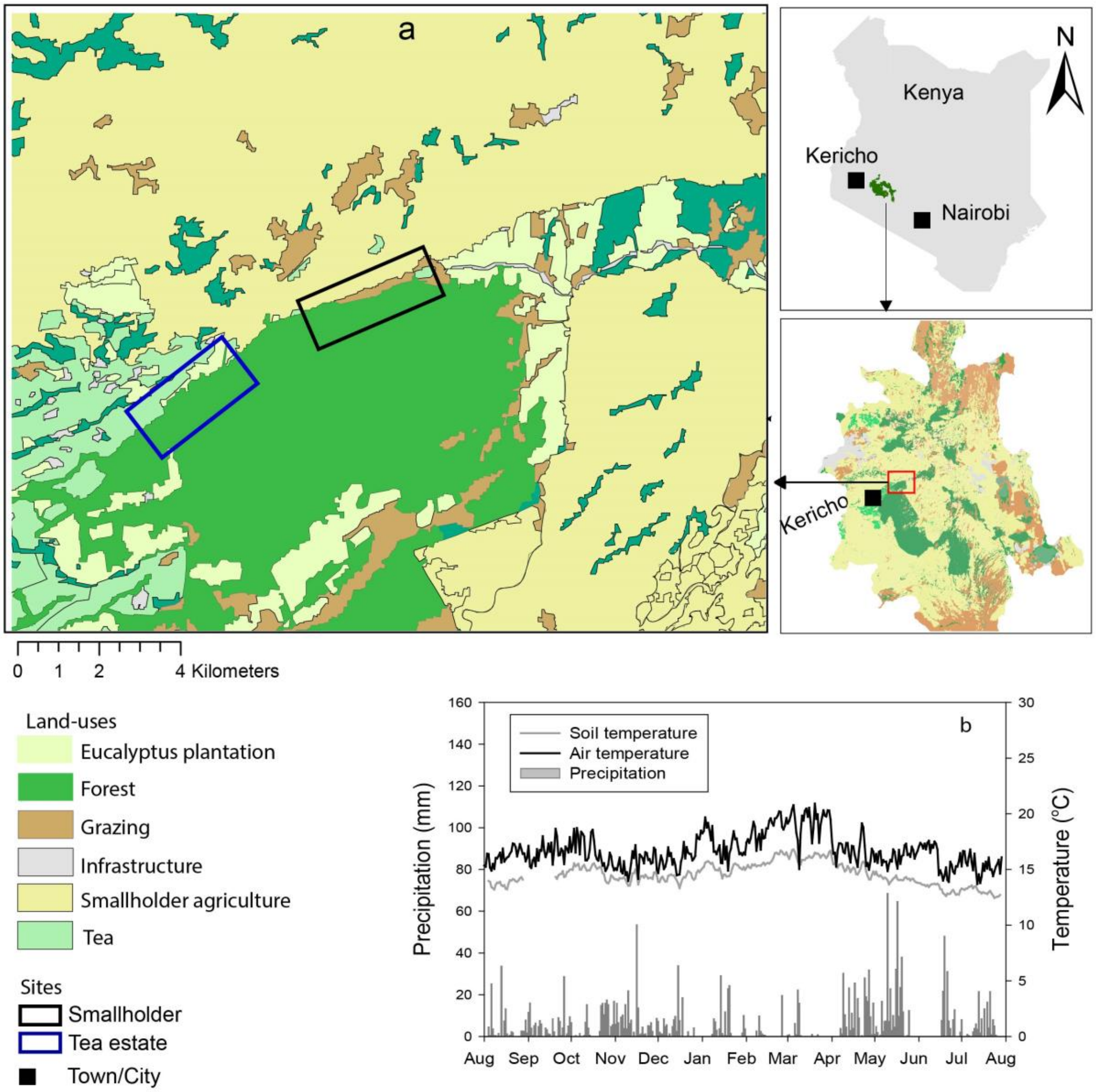

Figure 1. a) Map of the study area in the South West Mau forest. Land uses classes derived from a Swart (2016) for the smallholder and tea estate sites. b) Daily rainfall, air and soil temperature from August 2015 to August 2016 measured at the study site in the SW Mau forest of Kenya

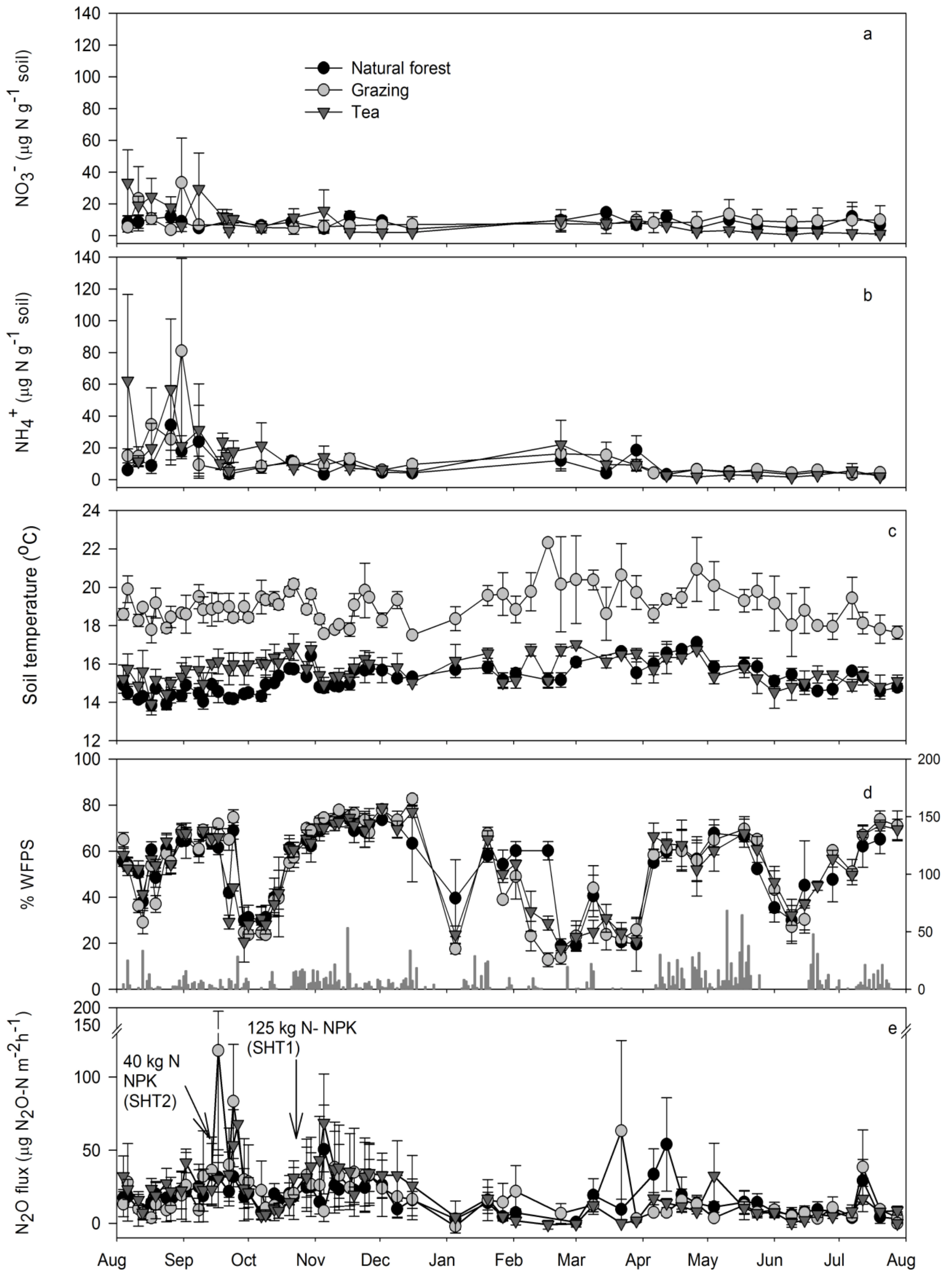

Figure 2: a) Mean (± SE) inorganic nitrogen concentrations of nitrate (NO_3^-), b) Ammonia (NH_4^+) measured bi-weekly between August 2015 to December 2015 and weekly between December 2015 to July 2016, c) Soil temperature, d) Water filled pore space (%WFPS) and precipitation (in mm) and e) Soil N_2O fluxes of different land uses (forest, grazing and tea) with three replications at the smallholder site. Fertilizer application rates and timing in the tea plots are indicated with arrows in e). Error bars are standard error of means.

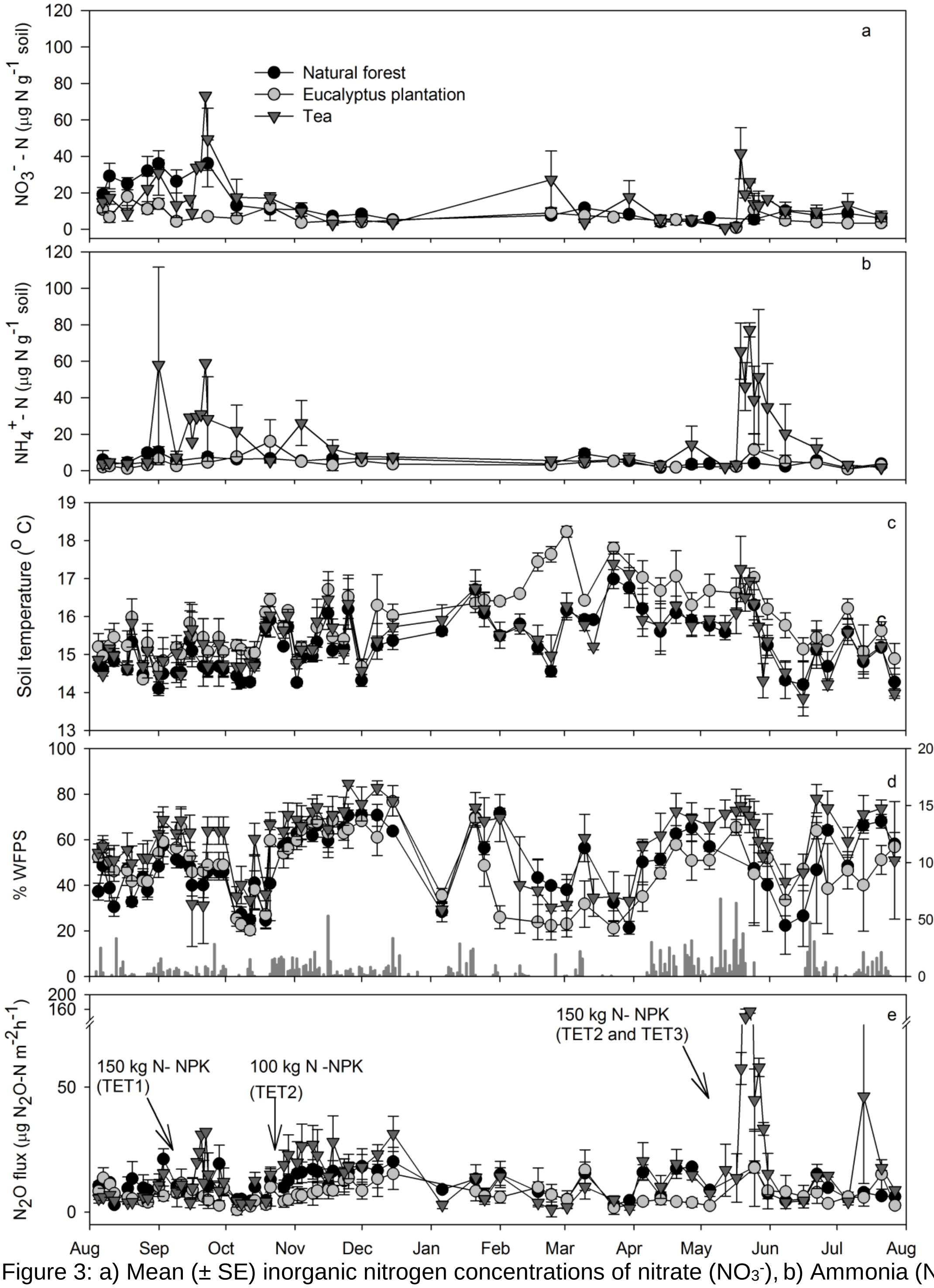

Figure 3: a) Mean (± SE) inorganic nitrogen concentrations of nitrate (NO_3^-), b) Ammonia (NH_4^+) measured bi-weekly between August 2015 to December 2015 and weekly between December 2015 to July 2016, c) Soil temperature, d) Water filled pore space (%WFPS) and precipitation (in mm) and e) Soil N_2O fluxes of different land uses (forest, grazing and tea) with three replications at the tea state site. Fertilizer application rates and timing in the tea plots are indicated with arrows in e). Error bars are standard error of means.

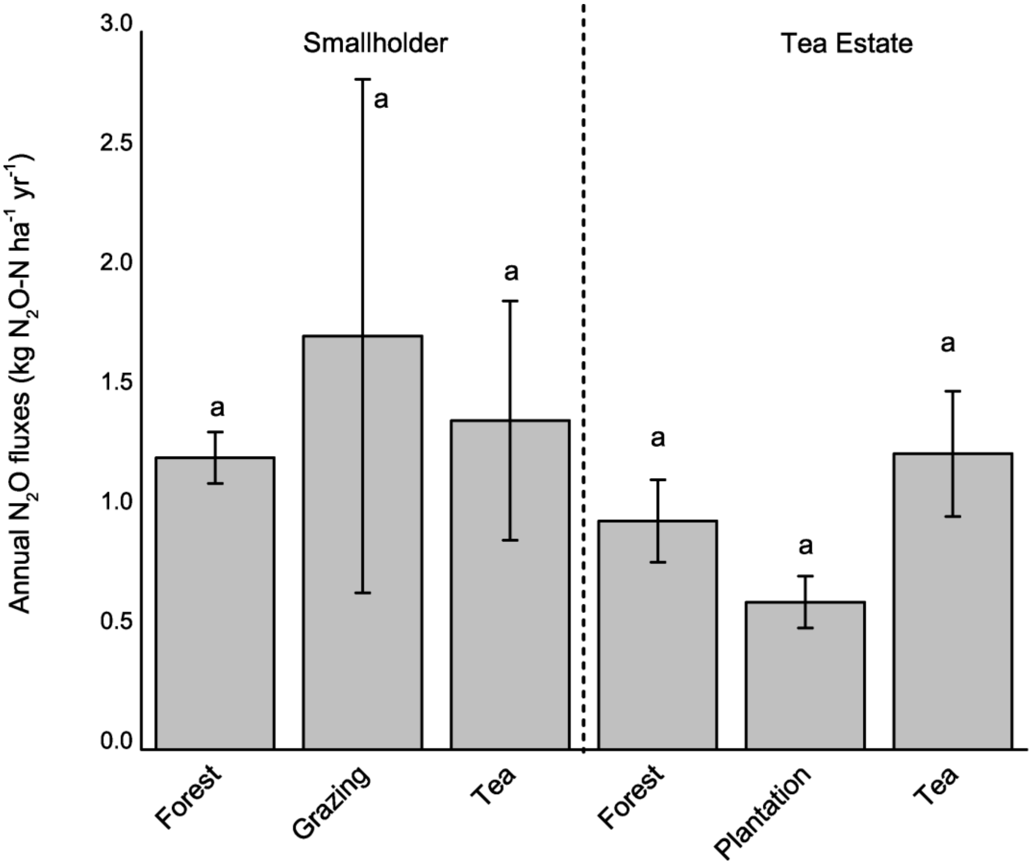

Figure 4: Annual N_2O fluxes from different land uses (Forest, Grazing, Tea and Plantation) at the smallholder and tea estate sites. Error bars are standard error of annual mean of 3 replicates for land use at each site. Analysis of variance showed no difference ($p> 0.05$) between land uses.

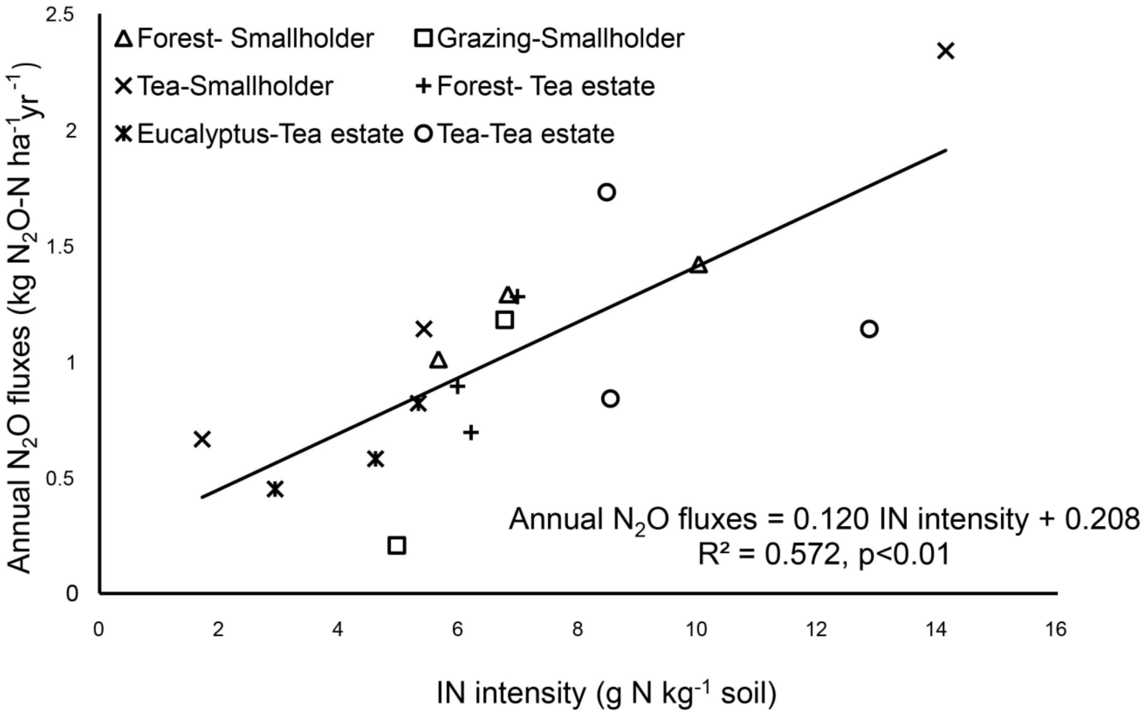

Figure 5: Relationship between annual N_2O fluxes and cumulative total IN exposure from different land uses (Grazing, Forest, Tea and Plantation) at the tea and smallholder sites.

Table 1: Characterization of the sampling plots according to dominant land use for the study site at the SW Mau forest of Kenya. Location and elevation, year in which the land use was established and the corresponding management practices for each plot are presented. The fertilizer applied in tea fields was NPK.

Site/Land use	**Code**	**Rep**	**Latitude**	**Longitude**	**Elevation (m)**	**Year established**	**Management**	**Inputs**	**Management intensity**
Smallholder agriculture									
Forest	SHF1	1	-0.2978	35.4397	2305	Native vegetation	Charcoal burning	N	1
Forest	SHF2	2	-0.2995	35.4354	2267	Native vegetation	Wood collection	N	1
Forest	SHF3	3	-0.3032	35.4235	2234	Native vegetation	Open (low tree density)	N	1
Grazing land	SHG1	1	-0.2942	35.4365	2319	1997, annual crops before	Grazing cattle, excreta deposited	Y	3
Grazing land	SHG2	2	-0.2959	35.4339	2319	1970, forest before	Grazing cattle, excreta deposited	N	3
Grazing land	SHG3	3	-0.2985	35.4203	2283	2005, annual crops before	Low density cattle, little excreta	Y	2
Tea	SHT1	1	-0.2936	35.4371	2320	1999, shrubland before	Fertilizer at 125 kg N $ha^{-1}yr^{-1}$	Y	3
Tea	SHT2	2	-0.2964	35.4327	2291	1985, forest before	Fertiliser at 40 kg N ha^{-1} yr^{-1}	Y	3
Tea	SHT3	3	-0.2987	35.4196	2294	2012, shrubland before	No fertilizer applied	N	2
Tea estates									
Forest	TEF1	1	-0.3165	35.3985	2169	Native vegetation	Little disturbance	N	1
Forest	TEF2	2	-0.3194	35.3964	2173	Native vegetation	Little disturbance	N	1
Forest	TEF3	3	-0.3225	35.3947	2170	Native vegetation	Little disturbance	N	1
Eucalyptus plantation	TEP1	1	-0.3143	35.3973	2198	2000, eucalyptus before	Timber harvested	N	2
Eucalyptus plantation	TEP2	2	-0.3172	35.3956	2163	2000, eucalyptus before	Timber harvested	N	2
Eucalyptus plantation	TEP3	3	-0.3199	35.3922	2146	2000, eucalyptus before	Timber harvested	N	2
Tea	TET1	1	-0.3133	35.3968	2208	1973, forest before	Fertiliser at 150 kg N ha^{-1} yr^{-1}	Y	3
Tea	TET2	2	-0.3159	35.3943	2176	1973, forest before	Fertiliser at 250 kg N ha^{-1} yr^{-1}	Y	3
Tea	TET3	3	-0.3187	35.3911	2168	1973, forest before	Fertiliser at 150 kg N ha^{-1} yr^{-1}	Y	3

Table 2. Soil physical and chemical characteristics for the study site at the SW Mau forest of Kenya. Values presented are means ± standard error of mean for the three replicates presented in Table 1.

Soil depth (m)	**Site**	**Land use**	**Total Nitrogen (%)**	**Total Carbon (%)**	**C:N ratio**	**pH**	**Bulk density (g cm^{-3})**	**Clay (%)**	**Sand (%)**
0-0.05	Smallholder	Forest	1.24±0.05a	13.4±0.7a	10.8±0.1b	6.6±0.1a	0.65±0.03b	22±0.1	46±2.0
	Smallholder	Grazing	0.74±0.03b	7.9±0.3b	10.9±0.1b	6.0±0.1b	0.94±0.02a	33±1.8	39±2.4
	Smallholder	Tea	0.69±0.03b	8.4±0.5b	11.9±0.2a	5.4±0.2b	0.72±0.05b	45±1.0	24±2.0
	Tea estate	Forest	0.94±0.04a	9.5±0.5a	10.1±0.1b	5.1±0.0a	0.60±0.03b	49±1.5	21±1.3
	Tea estate	Eucalyptus	0.61±0.02b	7.0±0.3b	11.3±0.7a	5.4±0.1a	0.74±0.03a	61±1.8	18±0.3
	Tea estate	Tea	0.91±0.10a	10.6±1.3a	12.0±0.1a	3.8±0.1b	0.67±0.04b	65±4.8	19±2.9
0.05-0.2	Smallholder	Forest	0.58±0.02a	5.3±0.1b	9.3±0.2 b	6.1±0.1a	0.80±0.03b	49±1.3	21±0.7
	Smallholder	Grazing	0.64±0.03a	6.7±0.3a	10.6±0.2b	6.0±0.1ab	0.93±0.02a	40±4.2	30±3.1
	Smallholder	Tea	0.46±0.01b	5.1±0.1b	11.2±0.3b	5.7±0.1b	0.84±0.03b	49±1.0	22±0.0
	Tea estate	Forest	0.44±0.02a	4.3±0.2b	9.7±0.2b	4.8±0.1b	0.68±0.04b	48±1.2	24±3.4
	Tea estate	Eucalyptus	0.42±0.02a	4.6±0.2b	10.7±0.2b	5.5±0.1a	0.79±0.03a	57±0.7	18±1.2
	Tea estate	Tea	0.46±0.01a	5.7±0.2a	12.8±0.3a	4.1±0.1c	0.74±0.02a	53±1.8	21±2.9

Mean values of soil physical and chemical characteristics ± SE followed by same letter for each soil property within a site and soil depth were not significant at p< 0.05

Table 3: Inorganic N intensities; ammonium (NH_4^+-N) intensity, nitrate (NO_3^--N) intensity and total IN (NH_4^+ -N+ NO_3^--N) intensity from 0-0.05m soil depth for the different land uses (forest, grazing land, tea and tree plantations) at the smallholder and tea estate sites from the South West Mau forest of Kenya. Values presented are means ± standard errors of the mean for three replicates. Analysis for each site was done separately.

Site	Land use	Inorganic N Intensities (g N kg^{-1})					
		NH_4^+-N	CV (%)	NO_3^--N	CV (%)	Total IN (NH_4^+-N+ NO_3^--N)	CV (%)
Smallholder	Forest	3.5±0.5a	25	4.0±0.8a	35	7.5±1.3a	30
Smallholder	Grazing	4.6±0.6a	22	1.4±0.4a	46	6.0±0.5a	15
Smallholder	Tea	4.4±2.5a	99	2.7±1.2a	74	7.1±3.7a	89
Tea estate	Forest	2.2±0.3b	21	4.2±0.5a	21	6.4±0.3b	8
Tea estate	Tea	4.5±0.2a	6	5.5±1.5a	46	10.0±1.5a	25
Tea estate	Eucalyptus	1.8±0.3b	28	2.5±0.4a	29	4.3±0.7b	28

Inorganic intensities IN (mean±SE) followed by same letter for each parameter within a site are not significant at $p<0.05$

Table 4: Spearman correlation coefficients between soil properties and annual N_2O fluxes for all plots, for all forest plots and plots with no external inputs (n=11), Forest plots (n=6), plots that received no external inputs (n=5) and plots that received external inputs (n=7)

	All plots		Forest + No external input		Forest		No external inputs		External inputs	
Soil parameter	n	N_2O	n	N_2O	n	N_2O	n	N_2O	n	N_2O
NH_4^+ Intensity	18	0.57**	11	0.36	6	0.49	5	-0.3	7	0.02
NO_3^- Intensity	18	0.47*	11	0.80***	6	0.37	5	0.4	7	-0.14
(NH_4^+ +NO_3^-) Intensity	18	0.72***	11	0.85***	6	0.71	5	0.1	7	-0.05
Total Nitrogen	18	0.35	11	0.74**	6	0.37	5	-0.1	7	0.18
Total Carbon	18	0.31	11	0.67*	6	0.37	5	-0.3	7	-0.05
C:N ratio	18	0.11	11	-0.47*	6	0.09	5	0.1	7	-0.54
Bulk density	18	0.23	11	-0.72**	6	0.14	5	-0.9*	7	0.52

•,, **, *** denote significance at p≤0.1, p≤0.05, p≤0.01 and p≤0.001, respectively*

Appendix

Table A1: Daily N_2O fluxes for three different land uses in the two study sites (smallholders and tea estate) calculated for wet and dry periods. These two periods are defined using a water filled pore space (WFPS) of 40%

			Daily N_2O fluxes (μg N_2O-N $m^{-2}h^{-1}$)		
Site	Land use	n	Wet period	Dry period	p-value
Smallholder	Forest	3	20.4±1.4	9.9±1.5	<0.001
Smallholder	Grazing	3	22.7±3.1	11.9±3.2	<0.001
Smallholder	Tea	3	28.1±2.2	7.1±1.9	<0.001
Tea estate	Forest	3	13.3±0.6	7.4±0.6	<0.001
Tea estate	Eucalyptus	3	8.1±0.6	5.2±0.8	<0.001
Tea estate	Tea	3	31.4±2.9	10.8±6.4	<0.001

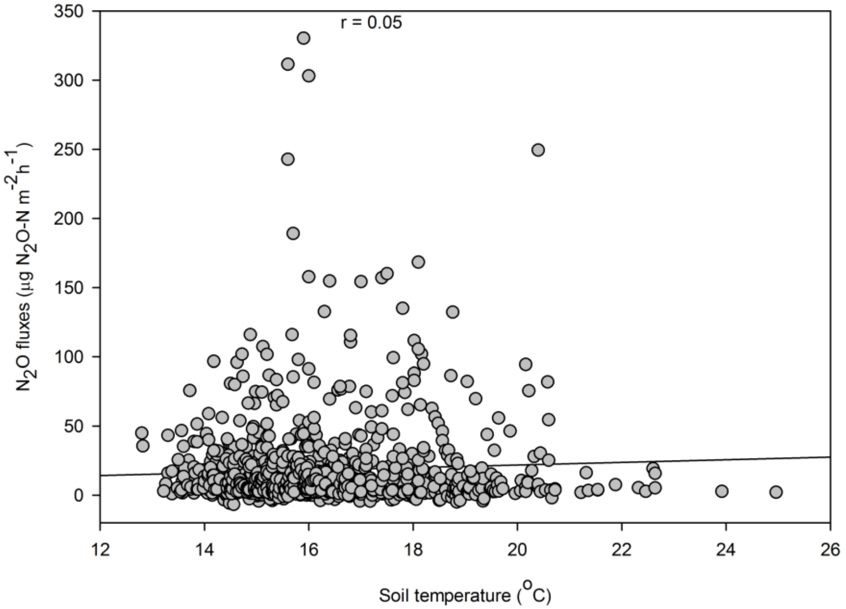

Figure A1. Correlation between N$_2$O fluxes and soil temperature

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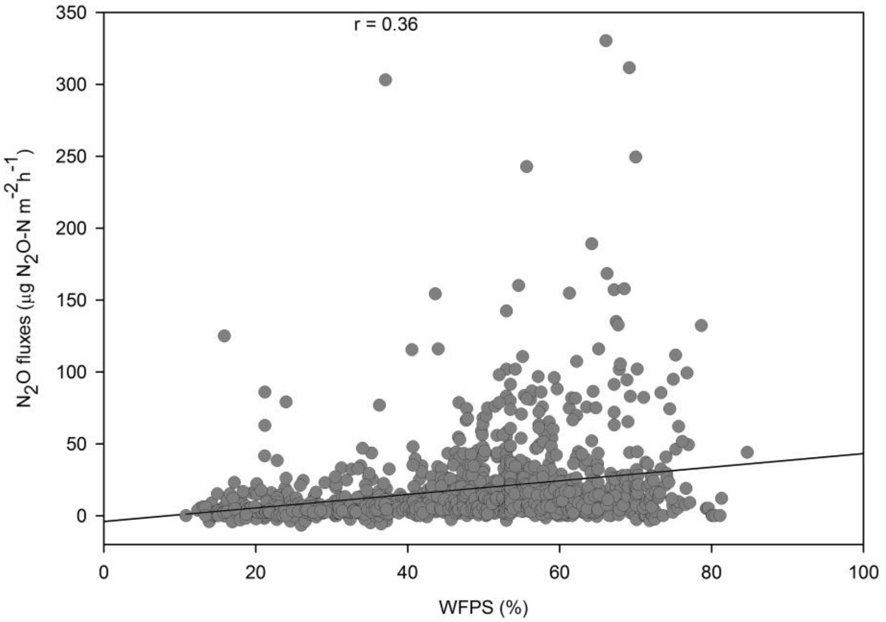

887 Figure A2. Correlation between N_2O fluxes and Water filled pore space (WFPS%)

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