Nutrient limitations regulate soil greenhouse gas fluxes from tropical forests: evidence from an ecosystem-scale nutrient manipulation experiment in Uganda

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Abstract. Soil macronutrient availability is one of the abiotic controls that alters the exchange of greenhouse gases (GHGs) between the soil and atmosphere in tropical forests. However, evidence on macronutrient regulation of soil GHG fluxes from central African tropical forests is still rare—limiting our understanding of how these biomes could respond to potential future increases in N and P deposition. ~~Tropical forests contribute significantly to the emission and uptake of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). However, studies on the soil environmental controls of greenhouse gases (GHGs) from African tropical forest ecosystems are still rare.~~ The aim of this study was to disentangle the regulation effect of soil nutrients on soil GHG fluxes ~~in a tropical forest in northwestern~~ from a Uganda n tropical forest reserve in the context of increasing N and P deposition. ~~–~~Therefore, a large-scale nutrient manipulation experiment (NME) based on 40 m x 40 m plots with different nutrient addition treatments (nitrogen (N), phosphorus (P), N + P, and control) was established in Budongo Forest Reserve. Soil CO_2, CH_4, and N_2O fluxes were measured monthly using permanently installed static chambers for 14 months. Total soil CO_2 fluxes were partitioned into autotrophic and heterotrophic components through a root trenching treatment. In addition, soil temperature, soil water content, and ~~mineral N~~nitrates were measured in parallel to GHG fluxes. N addition (N, N + P) resulted in significantly higher N_2O fluxes in the transitory phase (0-28 days after fertilization, *p < 0.01*), because N fertilization likely increased soil N beyond the microbial immobilization and plant nutritional demands leaving the excess to be nitrified or denitrified. Prolonged N fertilization however, did not elicit a significant response in background (measured more than 28 days after fertilization) N_2O fluxes. P fertilization marginally and significantly increased transitory (*p = 0.05~~2~~*) and background (*p = 0.01~~0~~*) CH_4 consumption, probably because it enhanced methanotrophic activity. Addition of N and P together (N + P) resulted in larger CO_2 fluxes in the transitory phase (*p = 0.01~~0~~*), suggesting a possible co-limitation of both N and P on soil respiration. Heterotrophic (microbial) CO_2 effluxes were significantly higher than the autotrophic (root) CO_2 effluxes (*p < 0.~~0~~01*) across all treatment plots with microbes contributing about ~~three times more~~two thirds of ~~to~~ the total soil CO_2 effluxes ~~compared to roots (p < 0.001)~~. However, neither heterotrophic nor autotrophic respiration significantly differed between treatments. The

results from this study suggest that the feedback of tropical forests to the global soil GHG budget could be disproportionately altered by ~~changes~~ increases in N and P ~~availability availabilty~~availability ~~in~~ over these biomes.

1   Introduction

45   Tropical forest soils play an important role in the earth's radiative balance by sequestering and ~~emitting~~ releasing significant amounts of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Mosier et al., 2004). It is estimated that tropical forest soils emit about 1.3 ± 0.3 Tg N_2O yr^{-1} (Butterbach-Bahl et al., 2004), capture 6.4 Tg CH_4 yr^{-1} (Dutaur and Verchot, 2007), and sequester about 10 % of the total atmospheric CO_2 via photosynthesis; and account for about 30 % of the world's soil C stocks (Jobbágy and Jackson, 2000; Malhi and Phillips, 2004).

50   The rate and magnitude of the specific plant- and soil microbial-processes that produce (CO_2: autotrophic and heterotrophic respiration, N_2O: denitrification and nitrification, CH_4: enteric fermentation and methanogenesis) and consume (CO_2: photosynthesis, CH_4: oxidation) greenhouse gases (GHGs) in and at the soil-atmospheric interface are constrained by a multiplicity of biotic and abiotic controls (Mosier et al., 2004). These controls include vegetation communities (Veber et al., 2018), soil moisture (Sjögersten et al., 2018), soil temperature (Holland et al., 2000),

55   geochem~~sists~~ry (given its control on microbial abundance (Gray et al., 2014) and soil organic carbon stabilization (Doetterl et al., 2015)), as well as macronutrient availability (especially N and P) (Oertel et al., 2016). Macronutrient replenishment in undisturbed tropical forests is inherently via litter input (for both N and P, (Tanner et al., 1998)) and rock weathering (for P, (Hedin et al., 2003)) processes. However, the past three decades have seen an increase in the levels of N and P deposition over most tropical regions (including central Africa) due to widespread

60   deforestation and biomass burning (Bauters et al., 2019; Galloway et al., 2004). Currently, the central African region receives about 18.5 kg N ha^{-1} (Bauters et al., 2019) and 1.8 – 2.5 kg P ha^{-1} (Tamatamah et al., 2005) each year due to high fire-derived N deposition (Bauters et al., 2019) and P rich biomass aerosols (Barkley et al., 2019), respectively. Increased anthropogenic N and P deposition over tropical forest biomes disrupts ecosystem stoichiometric equilibrium thereby affecting biogeochemical cycling of N and P (Bauters et al., 2019), as well as, the exchange of GHGs between

65   the soil and atmosphere (Corre et al., 2014). One way of understanding how increases in N and P availability (for instance through deposition) affect soil GHG fluxes from tropical forests is through large scale nutrient manipulation experiments (NMEs). NMEs purposely use large doses of N and P (e.g. Cleveland and Townsend, 2006 (150 kg N ha^{-1} yr^{-1} and 150 kg P ha^{-1} yr^{-1}), Hall and Matson, 2003 (100 kg N ha^{-1} yr^{-1} and 40 kg P ha^{-1} yr^{-1})) to simulate how future nutrient enrichment of tropical forests (through deposition) could affect soil GHG fluxes (among other ecosystem

70   processes) (Corre et al., 2010). To date, several NMEs have been carried out across the tropics (e.g. Corre et al., 2010; Wei et al., 2008), and ~~Understanding the role of individual controls in driving soil GHG fluxes is fundamental to our understanding of how these GHG sinks and sources respond to changes in ecosystem dynamics (Veldkamp et al., 2013). This explains to a great extent why the last two decades have seen a surge in concerted investigative efforts aimed at underpinning how~~

75   ~~macronutrients, especially N (e.g. Corre et al., 2014; Koehler et al., 2009b; Martinson et al., 2013) and P (e.g. Mori et al., 2017), influenced soil GHG fluxes from tropical forests. T~~the outcome ~~outcome of these studies~~ has been a consensus that addition of N to an already N-rich tropical forest ecosystem results in increased N_2O emissions (Corre et al., 2014; Martinson et al., 2013; Zhang et al., 2008). For N-rich forest ecosystems, an increase in available soil N

beyond the microbial immobilization and plant nutritional demands, results in the excess being nitrified or (and) denitrified by soil microbes (Corre et al., 2014). However, several studies suggest that increased availability of N not only reduces fine root biomass but also curtails microbial activity leading to reduced autotrophic (Cusack et al., 2011) and heterotrophic respiration respectively (Chen et al., 2010; DeForest et al., 2006; Koehler et al., 2009a), respectively. Notably, there are varying results on how N addition affects CH_4 uptake from tropical forest soils. For instance, Veldkamp et al. (2013) found no effect of N on CH_4 uptake while Du et al. (2019) measured reduced CH_4 consumption following addition of N to a tropical forest, with the latter study suggesting an inhibitory effect of N on CH_4 uptake (Bodelier and Steenbergh, 2014; Seghers et al., 2003; Zhang et al., 2011). Aronson and Helliker (2010) argue that the observed differences in the measured CH_4 fluxes in the two separate studies were likely due to the different amounts of N added in the respective experimental setups. They argued that low amounts of N stimulate CH_4 uptake while high amounts inhibit it.

With respect to P, it has been shown that P availability opens up the N cycle by stimulating soil organic matter mineralization, releasing excess N for soil nitrification or (both) denitrification processes (Mori et al., 2010).and results in increased N₂O emissions (Mori et al., 2017). It is also urged that P availability has a positive effect on both autotrophic and heterotrophic components of soil respiration (Mori et al., 2013). P not only stimulates fine root growth (Chen et al., 2010) but also regulates organic matter decomposition (Mori et al., 2018). However, studies elucidating P limitation of organic matter decomposition in the P deficient tropics remain rare (Cleveland and Townsend, 2006).and eEven the few available studies on the regulation effect of P on leaf litter mass loss rates are inconclusive (Cleveland and Townsend, 2006). This might explain why contrasting results were reported from two similar experiments carried out on P depleted soils in Hawaii (Hobbie and Vitousek, 2000) and the Brazilian Amazon (McGroddy et al., 2008). Hobbie and Vitousek (2000) reported an increase in litter mass loss rate while McGroddy et al. (2008) did not detect any change, suggesting that the relationship between P availability and organic matter decomposition is complex (Cleveland and Townsend, 2006). Similarly, literature on the interaction between N and P in regulating CH_4 fluxes from tropical forests remains limited.

Despite the recognition that N and P affect soil GHG fluxes and the fact that tropical forest ecosystems could subtly respond to potential future increasesshifts in N and P dynamicsdeposition (Bobbink et al., 2010; Li et al., 2006), the magnitude and direction of this response remains unclear for African tropical forests (Bobbink et al., 2010; Li et al., 2006). To date, only a handful of nutrient manipulation experiments (NMEs) focusing on tropical forests response to shifts in ecosystem N and P dynamics have been carried out. Of these studies, just a few included both N and P treatments in their experimental setups (e.g. Corre et al., 2014). Yet, P deficiency typical of tropical soils can have direct impacts on ecosystem biomass production if the limitation is lifted (John et al., 2007). Furthermore, nearly all the studies so far conducted in (sub-) tropical forest ecosystems were so far concentrated in China (Jiang et al., 2016; Yan et al., 2008; Zheng et al., 2016), Central America (Corre et al., 2014; Koehler et al., 2009a; Matson et al., 2014) and South America (Martinson et al., 2013; Müller et al., 2015; Wolf et al., 2011).

Unfortunately, no single controlled experiment has simulated the effects of elevated soil nutrient inputs on soil greenhouse gas fluxes from African tropical forests despite the projected increase in N and P deposition over theses biomes (Galloway et al., 2004) and the fact that they represent a significant proportion of global tropical forests (27 % ; Saachi et al., 2011). It was for this reason that a replicated completely randomized NME was established in a

Ugandan tropical forest reserve to investigate the role nitrogen and phosphorus have in regulating soil GHG fluxes in the context of changing N and P deposition rates over the tropics. ~~No single NME study at present has measured GHG fluxes from any of the African tropical forests, despite 27 % of the tropical forests being in Africa (Saatchi et al., 2011).~~

120 ~~However, a NME study in a tropical forest would offer valuable insights on the potential feedback of tropical forests to the global soil GHG budgets in the event that N deposition became significant over tropical Africa. Accordingly, the overarching objective of this study was to investigate how the addition of N and P regulate soil GHG fluxes in a Ugandan tropical forest.~~ It was hypothesized that:

1) addition of N or N + P to a tropical forest ecosystem would result in increased N_2O emissions coming from

125 excess availability of bio-available N beyond microbial immobilization and plant N demands, decreased CH_4 uptake due to negative effects of N addition on soil methanotrophs, and reduced CO_2 effluxes largely attributed to reduction in both root and microbial respiration upon addition of N;

2) adding P to a tropical forest ecosystem would stimulate release of N from soil organic matter and consequently lead to increased N_2O emissions, higher CO_2 effluxes linked to increased root activity and decomposition of

130 soil organic matter, and increased CH_4 uptake due to stimulation of methanotrophic activity.

2 Materials and Methods

2.1 Study site description

The study was conducted in Budongo Forest Reserve, a semi-deciduous tropical forest, located in the northwestern

135 part of Uganda (1°44'28.4" N, 31°32'11.0" E). The forest reserve spans over 825 km^2 and is extensively diverse in respect to forest communities, with *Cynometra alexandria, Chryophyllum albidum, Meosopsis eminii and Diospyros abyssinica* as the dominant tree species (Eggeling, 1947). The long-term mean annual temperature and precipitation over the study area is 25 °C and 1700 mm, respectively (Lukwago et al., 2020). ~~The study area receives about 1360 mm of rainfall annually~~ Rainfall is ~~(Climate Data.org, 2020)~~ distributed into two rainy seasons (i.e. March to May and

140 August to November) punctuated by~~,~~ a strong dry season (December to February), and a weak dry season (June to July) (Lukwago et al., 2020). It is worth noting that the amount of rainfall received during the field campaign (2385 mm, Fig. 2d) was higher than the long-term mean annual precipitation for this region. The weather data for the experiment period was obtained from a climatic station installed at Budongo Conservation Field station (2 km northwest of the study site), and was beneficial to understand how precipitation constrained soil greenhouse gas fluxes

145 given its direct control on water filled pore space. ~~Mean annual temperature over the study area is 23 °C (Climate Data.org, 2020). A Precambrian gneissic granulitic basement complex primarily dominates the geology (van Straaten, 1976).~~ The soils at the experimental site are highly weathered ~~and are~~ classified as Lixisols (IUSS Working Group WRB, 2014), and are developed on a Precambrian gneissic-granulitic basement complex (van Straaten, 1976).~~.~~

150 ### 2.2 Experimental design

The study was conducted within the framework of a running nutrient manipulation experiment (NME). The NME study used a completely randomized design to ~~which~~ investigates~~s~~ how the three macronutrients (applied individually as

nitrogen (N), phosphorus (P), and potassium (~~K)~~K) and in all possible combinations (N+ P, N+ K, P + K, N+ P + K) as treatments) constrained key ecosystem processes (~~,~~particularly nutrient cycling, and net primary productivity ~~and carbon sequestration)~~ in comparison to the unamended control. Each of the eight treatments was replicated four times (hence, n = 32 plots; 8 treatments x 4 replications). While the NME included a K treatment, ~~this~~the soil GHG flux study~~—the basis for this manuscript~~ was ~~-~~conducted ~~o~~in the N, P and N + P (combination of N and P) plots, and compared to the untreated control plots (n = 16). Only N and P (among nutrient addition plots) were exclusively considered for soil GHG flux measurements because their availability has been shown to limit soil greenhouse gas fluxes from tropical forest biomes.~~-~~ Each treatment plot measured 40 m x 40 m in size ~~with~~but measurements were conducted in the~~an~~ inner ~~core~~measurement ~~zone~~core (30 m x 30 m) to avoid boundary effects. A spacing of at least 40 m between experimental plots was ensured to prevent spillover of applied nutrients from the neighboring plots. In order to elicit an ecosystem response, N~~Nitrogen~~ was applied at a rate of 125 kg N ha^{-1} yr^{-1} in form of urea ((NH$_2$)$_2$CO), and P at 50 kg P ha^{-1} yr^{-1} as triple superphosphate (Ca(H$_2$PO$_4$)$_2$). The types of fertilizers and application rates used in this study were identical to those used in the Wright et al. (2011) NME. The fertilizer ~~were~~was applied by hand, and in four split dozes every year. Specifically, 31.3 kg N ha^{-1} ~~yr^{-1}~~and 12.5 kg P ha^{-1} ~~yr^{-1}~~were applied to the plots of the NME ~~each quarter~~every three months between May 2018 and June 2020. ~~, with these fertilizers split into four dozes annually.~~

2.3 Baseline soil physico-biochemical characterization

Prior to the first fertilizer application, soil samples were taken in all the treatment plots for baseline soil physico-biochemical analysis. These included~~;~~ texture, bulk density, soil pH, total soil organic carbon (TOC) stocks, total nitrogen stocks, C/N ratio, exchangeable bases, ECEC, and Bray extractable P.~~-~~ For the 0-10 cm depth, soil monoliths (20 cm (L) x 20 cm (W) x 10 cm (D)) were carefully taken from ten different locations within each ~~the~~plots of the NME (n = 32 plots) using a spade. However, for deeper soil layers (0-30 cm and 30- 50 cm), samples were obtained outside the established NME plots ~~layers~~in order to minimize modifications to the microenvironment inside the NME plots. Deeper soil sampling was done during a reconnaissance survey conducted at approximately 500 m from the current location of the NME site. During the reconnaissance survey, sixteen plots (n = 16) were established and samples taken from five different locations in each plot for every depth interval (i.e. 0-30 cm and 30- 50 cm) using an auger (diameter = 30 mm). ~~an Soil samples were obtained at 0 - 0.1, 0.1 - 0.3, and 0.3 - 0.5 m depth intervals at ten random locations in each plot for C and N analysis. For deeper depths, soil samples were taken from five locations in each plot.~~ The ~~Soil~~samples from each ~~the same~~ depth were ~~then pooled together in a plastic bucket~~basin, ~~thoroughly~~mixed thoroughly in a basin, and a~~bout~~ 500 g of the homogenized sample~~s were (a total of three samples i.e. one per depth per plot)~~ sent to ~~University of Göttingen in Germany~~the ~~same~~ soils laboratory of the University of Göttingen, Germany, for analysis. ~~In total, three homogeneous samples were obtained per plot for analysis (i.e. one from each of the three depths i.e. 0-10 cm, 10-30 cm, 30-50 cm).~~Soil texture was determined using a Bouyoucos hydrometer. Soil pH was determined in 1:2.5 (soil water) suspension. Soil bulk density for every depth ~~per~~in each plot was calculated from the mass of oven dried soil (at 105°C for 48 hours) and the volume of the Kopecky ring (~~v~~Volume = 251 cm^3; ~~diameter = 8 cm, height = 5 cm~~) used in collecting the soil sample. Note that soil bulk density was corrected for stone content. The ~~experimental site~~soils were tested for presence of inorganic carbon (IC) using dilute hydrochloric acid, and were

found to be devoid of any IC. Hence, TOC and N were determined using a CN elemental analyzer (Vario EL Cube, Elementar Analysis Systems GmbH, Hanau, Germany) and stocks later calculated from bulk density measurements. Exchangeable base cations (Ca, Mg, K, Na, Al, and ECEC) were determined on the 1-2 mm earth fraction of the collected soil samples.

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2.4   Soil greenhouse gas fluxes and soil environmental control measurements

Soil CO_2, CH_4 and N_2O fluxes were measured monthly over a period of fourteen months (May 2019 to June 2020). In every replicate plot's inner measurement core, four chamber bases (fabricated from a 250 mm PN10 PVC pipe and each with an area = 0.~~05~~ 044 m^2, and volume = about 12 L) were randomly installed at the soil surface, to a depth of

200   about 0.03 m. Installation of chamber bases was done at the beginning of April 2019, a month prior to the GHG flux measurements, and chamber bases remained permanently in place for the entire measurement period. Litter was not removed from the chamber. However, all the chamber bases were always maintained vegetation free throughout the gas sampling period in order to avoid measuring plant night respiration during chamber closure. On the sampling day, chamber bases were covered with vented polyvinyl hoods fitted with sampling ports. A pooled gas sample was then

205   obtained every 3, 13, 23, and 33 minutes using an airtight L~~l~~uer L~~l~~ock syringe following the pooling approach described in detail by Arias-Navarro et al. (2013). The 30 minute maximum chamber closure period used in this study was well under the threshold recommended by Pavelka et al. (2018)), but, comparable to other tropical GHG flux studies (e.g. Corre et al. (2010), Koehler et al. (2009a), Matson et al. (2017)). To check if the pooling worked correctly, both the pooled and un-pooled (an average of four individual chamber measurements) samples were taken for the month of

210   February 2020 for analysis. Both methods produced very comparable results (Supplementary Fig. 1). ~~To avoid biases in GHG fluxes introduced by diurnal temperature fluctuations, s~~Soil GHG fluxes were always measured between 9 am and 4 pm throughout the entire study period, ~~.~~ while for each measurement day, the sequence of plots to be measured were~~as~~ randomly chosen. Together with the very low diurnal variability of air (0.6 ± 0.04 °C; mean ± SE) and soil (0.2 ± 0.03 °C; mean ± SE) temperatures at this tropical forest site, time of measurement of individual gas chambers should,

215   if at all, only have a minimal effect on the measured gas fluxes. All collected gas samples were stored in Labco Exetainers (Labco Limited, Lempeter, UK) with screw-on plastic caps fitted with Labco Grey Chlorobutyl Septum because these exetainers have been demonstrated to remain airtight for periods spanning up to six months (Hassler et al., 2015). Additionally, all the plastic caps were screwed on to the exetainers by hand and "quarter turned" prior to sampling to ensure that they were airtight (Pavelka et al., 2018). ~~The~~ All the gas-filled exetainers were ~~sent~~ shipped

220   to the Department of Environmental Systems Science, ETH Zürich, Switzerland for analysis using a gas chromatograph (GC; Scion 456-GC Bruker, Germany) within a period of four months from sampling. The GC was equipped with an electron capture detector (N_2O), flame ionization detector (CH_4), thermal conductivity detector (CO_2), and auto-sampler. GC concentrations of the individual gas species of interest (CO_2, CH_4 and N_2O) were then calculated by comparing the peak areas of the measured samples to the respective peak areas of a suite of standard gas samples. Next,

225   flux rates of individual gases at the soil-atmospheric interface were calculated based on either linear increase or decrease in gas concentrations during chamber closure following Eq. 1 in Butterbach-Bahl et al. (2011).

$$GHG_{flux} = \frac{V_{ch} * GHG_m * S * 10^6 * 60}{A_{ch} * GHG_v * 10^9}$$ (1)

where GHG_{flux} is given as a positive flux to the atmosphere and a negative flux into the soil [µg m^{-2} h^{-1}], V_{ch} is the chamber volume [m^3], GHG_m is the molar mass of the different gases [g mol^{-1}], S is the slope of a linear regression calculated based on the increase or decrease in gas concentrations during chamber closure [ppm min^{-1}], A_{ch} is the chamber ground area [m^2], GHG_v is the molar volume of the different gases [m^3 mol^{-1}]. Note that the constants 10^6, 10^9, and 60 were used to convert grams into micrograms, parts per million into cubic meters, and minutes into hours. GHG_v was adjusted to air temperature and pressure in the field using ideal gas law following Eq. 2:

$$GHG_v = 0.02241 * \frac{273.15 + T_f}{273.15} * \frac{P_f}{P_s}$$

(2)

where T_f is the air temperature [°C] and P_f is the pressure [Pa] at the field site, while P_s is the pressure at sea level [Pa]. As a quality check, the linearity of CO_2 increase during chamber closure was inspected by comparing the CO_2 concentrations (of each chamber measurement) with time since chamber closure, and thereafter, determined the goodness of fit for the linear regression model (the R^2). The R^2 for all measurement was 0.992 ± 0.001 (mean ± SE). For purposes of quality assurance, Additionally, the measured gas concentrations from the GC were checked against the standards and the GC's minimum detection limit to ensure that the changes in gas concentrations during chamber closure were well above its minimum detection limit.

In parallel to gas flux measurements, soil environmental controls particularly soil temperature, volumetric water content, and mineral nsoil mineral nitrogenitrogen (ammonia ammonium (NH_4^+) and nitrate (NO_3^-)) were measured. Soil temperature and volumetric water content were determined at 0.05 m soil depth adjacent to each of the four-installed chamber bases per replicate plot. A digital thermometer (Greisinger GMH 3230, Germany) fitted with an insertion probe and a calibrated ML3 ThetaProbe soil moisture sensor (AT Delta-T Devices Limited, United Kingdom) were used to determine soil temperature and soil volumetric water content, respectively. Soil mineral nmineral nitrogen itrogen was determined by obtaining a soil sample in a Kopercky ring at 0.05 m depth (from the soil surface) and 1 m distance from each of the installed chamber per replicate. The obtained soil samples (from each replicate plot) were pooled together and thoroughly mixed. Next, 100 and 150 g of the pooled soil samples were extracted with 100 and 600 mL $CaCl_2$ solution for determination of NO_3^- and NH_4^+ concentrations respectively using the RQflex® 10 reflectometer. RQflex® 10 reflectometer is part of the reflectoquant system comprising of a reflectometer, batch-specific barcode and test strips. The test strips used in this study had a 3 - 90 and 0.2 - 7 mg L^{-1} detection range for nitrates (NO_3-N) and ammonium (NH_4-N), respectively.

To understand the contribution of autotrophic (root) and heterotrophic (microbial) sources to total soil respiration, a trenching treatment was done in all the plots following the protocol of Wang and Yang (2007). Prior to trenching, root biomass distribution with depth was determined in order to establish where most roots were located. Root biomass estimation involved digging three profile pits measuring 1 m (L) x 1 m (W) x 1.1 m (D) at the forest site. In every pit, ten soil monoliths (each measuring: 20 cm (L) x 20 cm (W)) were carefully cut out (using a spade and hoe) following a 10 cm depth interval from the surface down to 1 m. The soil monoliths were thoroughly washed to isolate the roots from the bulk soil. The root samples were oven dried at 60 °C for 48 hours and weighed to determine the root biomass per depth increment. The root biomass for each depth interval was calculated as the mean of the root biomass from the three pits for that interval. It was established that over 90 % of the roots were within the top 0.6 m of the soil profile. Therefore, Aa circular trench (about 0.60 m in diameter) was dug to a depth of about 0.6 m at the center of all the

265 plots, thereby creating a soil mass free of roots. ~~The depth of the trench was based on an earlier root biomass inventory, which indicated that over 90 % of the roots were within the top 0.6 m of the soil profile.~~ All the trenches were lined with a heavy-duty plastic sheet to prevent roots from growing back into the trenched soil mass. The trenched soil mass and the proximally neighboring un-trenched (reference) zone (about 1 m apart) were respectively installed with a chamber base. Both the trenched and reference chamber bases had a design (area = 0.044 m^2, and volume = about 12

270 L) identical to the one used in the NME soil GHG flux study. The installed chamber bases were left standing for six months, before the first measurements began in November 2019. This ensured that a large proportion of the cut roots in the trenched soil mass decomposed before the start of the CO_2 measurements. CO_2 measurements were conducted monthly for a period of 4 months (starting in November 2019 ~~to~~ and ending in February 2020). The selected measurement time window represented the transition between the wet season and the long dry season, allowing us to

275 capture how soil moisture constrained the different soil CO_2 efflux sources. After the completion of flux measurements, root coring was done to a depth of 0.30 m at two locations directly adjacent to both the trenched and un-trenched chambers, in order to determine if the trenching approach was effective in reducing the amount of living root biomass in the trenched zone. It was established that there was a 73 % and 63 % reduction in fine root biomass, and coarse root biomass, respectively, in the trenched zone in comparison to the reference zone. Heterotrophic (microbial) respiration

280 was equal to the CO_2 effluxes from the trenched chamber while autotrophic (root) respiration was the difference between CO_2 effluxes from the reference and trenched chambers.

2.5   Statistical Analysis

Prior to statistical analysis, transitory N_2O fluxes from N addition plots (N, and N + P) were detrended to compensate

285 for absence of frequent measurements immediately after fertilization coming from sampling GHGs monthly. Detrending involved using a log-normal fit between the measured N_2O fluxes and time since fertilization (until day 42), and this explained 43 % of the observed variability in the N_2O data during the transitory phase ($p < 0.05$). Additionally, GHG flux and soil environmental control data were aggregated based on seasons (wet and dry) and phases (transitory; 0-28 days from the date of fertilization, and background; more than 28 days after fertilization).

290 Furthermore, d ~~D~~espite monitoring soil NO_3^- and NH_4^+ on a monthly basis throughout the measurement period, ~~these data were aggregated (later in the text referred to as soil mineral N)~~ only the soil NO_3^- data set was used in the a~~to overcome skewness introduced by~~nalysis b~~b~~ecause soil NH_4^+ ~~, which~~ was mostly low and often below the detection limit of the reflectometer at majority of the sampling time points.

Data was checked for normality and homogeneity of variance (homoscedasticity) across treatment groups, seasons,

295 and phases before implementing parametric tests (i.e. linear mixed effects model (LMEMs), and one-way analysis of variance (ANOVA)). Normality of the respective data was inspected by use of diagnostic plots (histograms and quantile-quantile plots), and the Shapiro-Wilk normality test, while heteroscedasticity was determined with the Levene test and by inspecting residual plots of fitted values. In case of heteroscedasticity and non-normal distribution of the data, either a logarithmic or a Tukey transformation was applied on the dataset. However, if normality of the data and

300 homogeneity of variance were not restored by the transformations, an equivalent non-parametric statistical test was selected. The Spearman's correlation coefficient test was used to check the relationship between the measured background soil GHG fluxes and soil environmental controls.

To determine differences in mean soil GHG fluxes between treatments, one way ANOVA test was used with GHG species and treatments included in the model as response and predictor variables, ~~-~~respectively. In order to determine

305 the effect of the added nutrients on soil GHG fluxes (CO_2, CH_4, and N_2O), soil CO_2 sources (heterotrophic and autotrophic), and soil environmental controls (water filled pore space, soil temperature, and ~~mineral N~~nitrates), LMEMs were employed. LMEMs effectively deal with temporal pseudo-replication (coming from repeated measurements) hence safeguard against inflation of the degrees of freedom, which would ~~dramatically~~ significantly compromise the power of the statistical test. Added nutrients (treatments), seasons (wet and dry), CO_2 sources

310 (autotrophic and heterotrophic) and phases (transitory and background) were included in the LMEMs as fixed effects while sampling days and replicate plots were included as random effects. Some of the LMEMs were extended to either include a variance function (to account for variation in the response variable per level of the fixed effect), or a first order temporal auto regressive process (to control for correlation between closely spaced measurements in time) or both. The extensions were included in the LMEMs on the premise that they improved the relative goodness of model

315 fit based on Akaike Information Criteria (AIC).

All the statistical data analyses were performed using R 3.6.3 (R Development Core Team, 2019) using *nlme' and 'car'* packages for LMEMs and one-way ANOVA tests, respectively. Throughout the paper, statistical significance in all the tests was inferred if *$p \leq 0.05$*. Annual soil GHG fluxes were estimated through a trapezoidal interpolation on the measured monthly soil GHG fluxes.

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3   Results

3.1   Soil physico-chemical characteristics, water filled pore space, soil temperature and ~~mineral N~~nitrates

Soil characteristics did not significantly differ ~~between the nutrient treatment plots and the control~~across plots; hence,

325 the parameters presented in Table 1 represent the soil physico-chemical characteristic for the NME site.

Table 1. ~~Mean (± SE, n = 32)~~ Ssoil physico-chemical properties in three depths and vegetation characteristics of the study site located in Budongo forest, northwestern Uganda.

Soil physico-chemical properties	Soil depth (m)		
	0 - 0.10	0.10 - 0.30~~‡~~	0.30 - 0.50~~‡~~
Soil bulk density (g cm^{-3})	1.2 ± 0.2	1.5 ± 0.2	1.3 ± 0.2
Soil pH (1:2.5)	6.4 ± 0.2	6.2 ± 0.2	6.0 ± 0.2
Soil total carbon (C) (kg C m^{-2})	4.1 ± 0.0	3.1 ± 0.0	1.8 ± 0.0
Soil total nitrogen (N) (g N m^{-2})	423 ± 1.0	387 ± 0.2	249 ± 0.6
Soil C/N ratio	9.5 ± 0.3	8.0 ± 0.3	7.2 ± 0.3
~~ᵃ15N (‰)~~	~~8.4 ± 0.3~~	~~9.2 ± 0.2~~	~~9.5 ± 0.2~~
~~ᵃ13C (‰)~~	~~-26.5 ± 0.18~~	~~-24.4 ± 0.17~~	~~-23.8 ± 0.07~~
Sand (%)	55 ± 2	55 ± 2	49 ± 1
Silt (%)	27 ± 2	21 ± 1	14 ± 1
Clay (%)	18 ± 1	23 ± 1	38 ± 1
ECEC ($mmol_c$ kg^{-1})	149 ± 8	76 ± 4	62 ± 4
Exchangeable aluminum (g Al m^{-2})	0.10 ± 0.06	0.11 ± 0.15	0.14 ± 0.20

Exchangeable calcium (g Ca m^{-2})	75.6 ± 4.10	39.0 ± 8.51	34.7 ± 8.59
Exchangeable magnesium (g Mg m^{-2})	17.0 ± 0.90	12.3 ± 2.7	11.7 ± 1.0
Bray II extractable phosphorus (g P m^{-2})	1.80 ± 0.20	1.01 ± 0.14	0.838 ± 0.159
Base saturation (%)	99 ± 1	97 ± 1	98 ± 1
Plant-available phosphorus (g P m^{-2}) ‡	1.7 ± 0.0	-	-
Plant-available molybdenum (mg Mo m^{-2}) ‡	14 ± 5.0	-	-
Vegetation characteristics (≥ 10 cm DBH)			
Forest type	Moist semi-deciduous tropical forest		
Most abundant tree species	Funtumia elastica, Celtis mildbraedii, Cynometra alexandri, Celtis zenkeri		
Stand height (m)	18.7 ± 0.1		
Mean basal area (m^2 ha^{-1})	34.0 ± 1.0		
Tree density (trees ha^{-1})	621 ± 13		
N fixing trees at the site (trees ha^{-1})	~ 42		

Notes: DBH is diameter at breast height. ECEC is effective cation exchange capacity. ‡ data in respective columns and rows were generated from a reconnaissance evaluation study carried out in close proximity (~ 500 m) of the current location of the nutrient manipulation experiment.

The soils have a high bulk density (specifically 10 - 30 cm), slightly acidic pH, sandy texture, relatively high effective cation exchange capacity (ECEC), high base saturation (dominated by Ca and Mg), low in plant available phosphorus, and a low C/N (Table 1). There is also a high natural abundance ^{15}N signature and the soils are low in plant available phosphorus (Table 1). Water filled pore space (WFPS) was significantly higher in the wet season (March to December; 55 ± 1.0 %) compared to the dry season (January to February; 43 ± 1.7 %) (Fig. 1a, Fig. 2a, $p < 0.001$). WFPS was higher in N and N + P addition plots compared the control plots both in the dry (N; $p = 0.01602$, N + P; $p = 0.044$) and wet (N; $p = 0.0215$, N + P; $p = 0.050$) seasons (Fig. 1a). Soil temperature varied minimally (0.6 °C) across treatments and seasons ranged ranging between 20.1 and 212.24 °C in the dry season, and between 19.7 and 22.9 °C in the wet season, with minimal variation (0.6 °C) across treatments and seasons (mean annual temperature of 20.9 °C) (Fig. 1b, Fig. 2b). Soil mineral N contents nitrate contents measured across all treatment plots were significantly larger in the dry season compared to the wet season (Fig. 1c, $p < 0.01$). varied between 43 and 66 mg N kg^{-1} in the dry season and between 33 and 55 mg N kg^{-1} in the wet season (Fig. 1c), with the highest and lowest soil mineral N contents measured in May and January respectively (Fig. 2c). Mineral N Soil nitrate content from the N ($p = 0.0106$) and N + P ($p = 0.023$) addition plots was significantly higher than the control plots in the wet season (Fig.1c), but no significant difference was detected between the nutrient addition treatments and the control in the dry season (Fig. 1c). Mineral N contents were significantly lower across all treatments plots in the wet season than the dry season (Fig. 1c, $p < 0.001$). Strong mineral N nitrate peaks were observed in N and N + P addition plots in September 2019 and June 2020 shortly after fertilization (Fig. 2c).

Table 2. Mean (± SE, n = 4) soil GHG fluxes (CO_2, CH_4, N_2O) as well as annual soil GHG fluxes measured between May 2019 and June 2020 from control (Ctrl), nitrogen (N), phosphorus (P), and N + P plots of a the nutrient manipulation experiment.

Treatment [a]	CO_2 fluxes (mg C m^{-2} h^{-1})	Annual CO_2 fluxes†* (Mg C ha^{-1} yr^{-1})	CH_4 fluxes (µg C m^{-2} h^{-1})	Annual CH_4 fluxes†* (kg C ha^{-1} yr^{-1})	N_2O fluxes (µg N m^{-2} h^{-1})	Annual N_2O fluxes†* (kg N ha^{-1} yr^{-1})
Ctrl	164 ± 5.3[a]	14.5 ± 0.6[a]	-30.5 ± 4.9[a]	-2.7 ± 0.4[a]	20.5 ± 3.2[a]	1.8 ± 0.3[a]

N	186 ± 6.5^a	$16.4 \pm 0.9^{\underline{a}}$	-39.7 ± 4.4^a	$-3.4 \pm 0.4^{\underline{a}}$	50.2 ± 11^{be}	$4.\underline{8} \pm 1.5^{\underline{b}}$
P	186 ± 5.3^a	$16.4 \pm 1.0^{\underline{a}}$	-56.2 ± 3.8^b	$-4.7 \pm 0.7^{\underline{b}}$	21.8 ± 2.4^a	$1.9 \pm 0.3^{\underline{a}}$
N + P	197 ± 5.4^b	$17.3 \pm 0.8^{\underline{b}}$	-39.3 ± 6.3^a	$-3.3 \pm 0.7^{\underline{a}}$	53.8 ± 10^{be}	$4.\underline{6} \pm 0.4^{\underline{b}}$

Notes: [a]Means followed by different lower-case letters indicate significant differences among treatments (One way analysis of variance, $p \leq 0.05$); †*Annual soil CO_2 fluxes, CH_4 fluxes, and N_2O fluxes were approximated by applying the trapezoid rule on time intervals between measured flux rates, assuming constant flux rates per day. Annual soil GHG fluxes were not tested for statistical differences because they are interpolations. The mean and annual soil GHG fluxes included both transitory and background flux measurements. Note: Transitory N_2O fluxes (measured within 28 days from fertilization) from N addition plots (N, N + P) were detrended to compensate for absence of frequent measurements immediately after fertilization coming from sampling GHGs monthly.

3.2   Soil CO₂ fluxes

Soil CO_2 fluxes (both transitory and background) varied between 60 and 330 mg C m^{-2} h^{-1} during the measurement period across all treatments. However, (Fig. 3a, Fig. 4a, d) with the highest CO_2 fluxes were measured in December (at the interface between wet and dry season) (Fig. 3a). Fertilization resulted in an immediate increase in CO_2 fluxes across all nutrient addition plots (N; 15 %, P; 14 %, N + P; 24 %) in the transitory phase. However, this increase was only significant in the N + P plots (p = 0.010) (Fig. 4a, $p = 0.01$). There was no significant effect of fertilization on background CO_2 fluxes between nutrient addition treatments and the control plots (Fig. 4d).
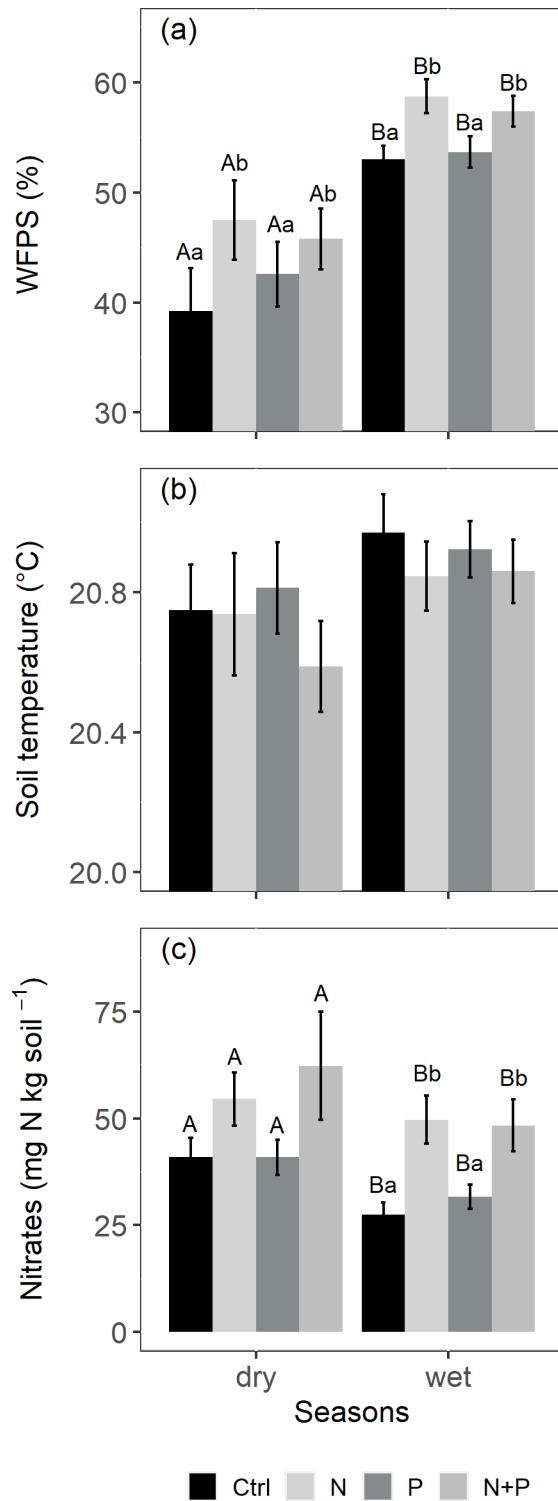
Figure 1. Mean (± SE, n = 4) water filled pore space (WFPS) (a), soil temperature (b), and ~~mineral N~~ nitrates (c) in the top 0.05 m of the control (Ctrl), nitrogen (N), phosphorus (P), and N + P plots of ~~a~~ the nutrient manipulation experiment measured during the dry (January and February; monthly precipitation < 100 mm) and wet (March to December; monthly precipitation > 100 mm) seasons. Different lower-case letters indicate significant differences between treatments and the

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control while different upper-case letters indicate significant differences between seasons (linear mixed effects models; $p \leq 0.05$).

Similarly, no significant differences in the background CO_2 fluxes were detected between seasons despite measuring
380   marginally lower background CO_2 fluxes in the wet season compared to the dry season (Fig. 4d). Additionally, no significant differences were detected between transitory and background CO_2 fluxes (Fig. 4a, ~~-~~d). Heterotrophic (microbial) CO_2 effluxes were significantly higher than the autotrophic (root) CO_2 effluxes (Fig. 5, $p < 0.0\theta1$) across all treatment plots with microbes contributing about three times more to the total soil CO_2 effluxes compared to roots (Fig. 5, $p < 0.0\theta1$). Neither heterotrophic nor autotrophic respiration significantly differed between treatments (Fig.
385   5). Overall, there was relatively low variability in annual CO_2 fluxes across treatments (CV = 14.8 ± 2.2 %). The Spearman's correlation coefficient indicated that background soil CO_2 fluxes did not correlate to any of the measured soil environmental ~~(WFPS, soil temperature, and mineral N)~~ controls (WFPS, soil temperature, and nitrates) ~~-~~across all treatment plots (Fig. 6a, b, c).

390   **3.3   Soil CH₄ fluxes**

Across all treatments, phases (transitory and background) and seasons, soil CH_4 fluxes varied between an up-take of -278 mg C m^{-2} h^{-1} ~~(Fig. 3b, Fig. 4b, e)~~. In the transitory phase, CH_4 consumption increased slightly but not significantly in the N (2 %) and N + P (6 %) plots. A larger but still not significant increase was found in the case of P plots (54 %; $p = 0.05\cancel{2}$) (Fig. 4b). Beyond 28 days from fertilization, no significant difference
395   in background soil CH_4 fluxes between treatments was detected in the dry season (Fig. 4e). However, a significantly higher background soil CH_4 consumption was measured in P plots in the wet season (Fig. 4e, $p = 0.01\theta$). Soil CH_4 consumption in the dry season was on average 1.5 times larger than the wet season across all treatments (Fig. 4e, $p = 0.01\cancel{07}$). Soil CH_4 uptake across all treatment plots measured during the transitory phase (-39.0 ± 3.7 mg C m^{-2} h^{-1}) did not significantly differ from the CH_4 uptake in the background phase (-42.8 ± 3.4 mg C m^{-2} h^{-1}) (Fig. 4b, e). Annual
400   CH_4 uptake ranged between -2.7 ~~± 0.40~~ and -4.7 ~~± 0.74~~ kg C ha^{-1} yr^{-1}, with soils in all the treatment plots acting as net sinks for CH_4 (Table 2). The Spearman's correlation coefficient test indicated that background CH_4 fluxes were strongly and positively correlated to WFPS (Fig. 6d) while soil temperature (Fig. 6e) and ~~mineral N~~nitrates (Fig. 6f) were also significant but negatively correlated.

405   **3.4   Soil N₂O fluxes**

Soil N_2O fluxes across treatments, phases (transitory and background), and seasons varied between an uptake of -18 and a release of ~~507~~ 499 µg N m^{-2} h^{-1} ~~(Fig. 3c)~~. A strong increase of N_2O was measured immediately after fertilization (September and December 2019, April and June 2020) in all N addition plots with increases of ~~400~~ 445 % in N plots ($p < 0.0\theta1$) and ~~419~~ 455 % in the N + P plots ($p < 0.0\theta1$) compared to the control plots in the transitory phase (Fig.
410   4c). The soil N_2O peaks in September 2019 and June 2020 (Fig. 3c) coincided with the peaking in soil ~~mineral N~~nitrate concentrations (Fig. 2c). Background soil N_2O fluxes did not differ significantly between nutrient addition plots and the control plots both in the dry and wet seasons (Fig. 4f). Annual N_2O fluxes ranged between 1.8 ~~± 0.3~~ and 4.~~2~~8 ±

~~1.5~~ kg N ha⁻¹ yr⁻¹, with soils in all the treatment plots acting as net sources for N_2O (Table 2). The Spearman's correlation coefficient indicated that background soil N_2O fluxes were strongly and positively correlated to WFPS (Fig. 6g) in all treatment plots. Majority of the background soil N_2O fluxes higher than 15 µg N m⁻² h⁻¹ (constituting 74 % of the averages background soil N_2O fluxes) corresponded to WFPS greater than 49 % (wetter conditions) (Fig. 6g). Background soil N_2O fluxes negatively correlated to soil temperature (Fig. 6h) and ~~mineral N~~nitrates (Fig. 6i) in all treatment plots.
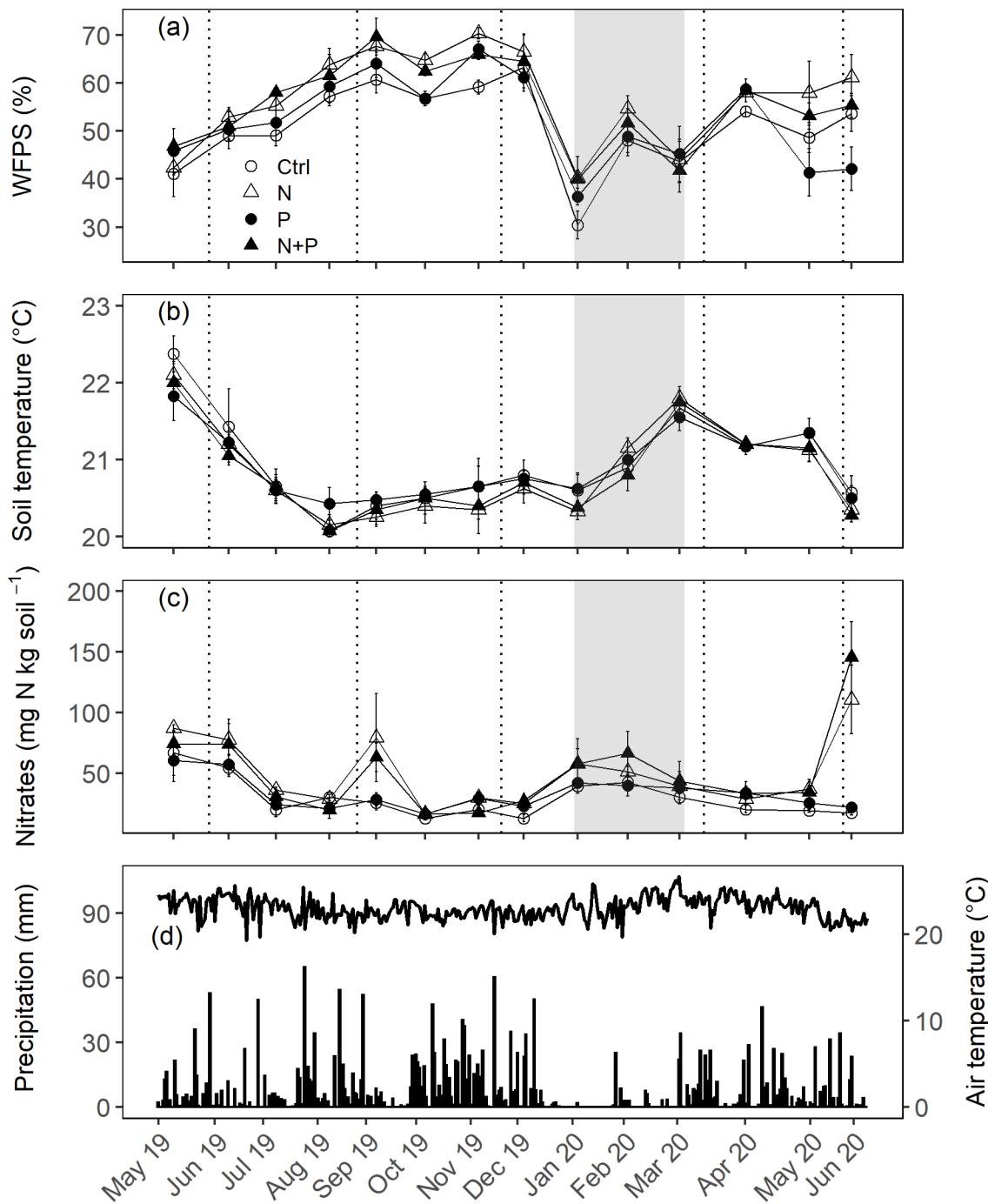
Figure 2. Mean (± SE, n = 4) water filled pore space (a), soil temperature (b), and ~~mineral N~~nitrates (c) in the top 0.05 m measured monthly (May 2019 to June 2020) from control (Ctrl), nitrogen (N), phosphorus (P), and N + P plots of the nutrient manipulation experiment. Vertical lines indicate the timing of each split dose of N (31.3 kg N ha^{-1}~~yr~~$^{-1}$), P (12.5 kg P ha^{-1}~~yr~~$^{-1}$) and N (31.3 kg N ha^{-1}~~yr~~$^{-1}$) + P (12.5 kg P ha^{-1}~~yr~~$^{-1}$) fertilization every three months. The gray shaded rectangle (in a, b, and c) marks the beginning and end of the dry season (January and February; monthly precipitation < 100 mm), while (d) gives the daily precipitation (bars) and air temperature (line) between May 2019 and June 2020. Climatic data was obtained from a weather station installed at Budongo Conservation Field Station, 2 km from the location of the nutrient manipulation experiment in Budongo forest, northwestern Uganda.
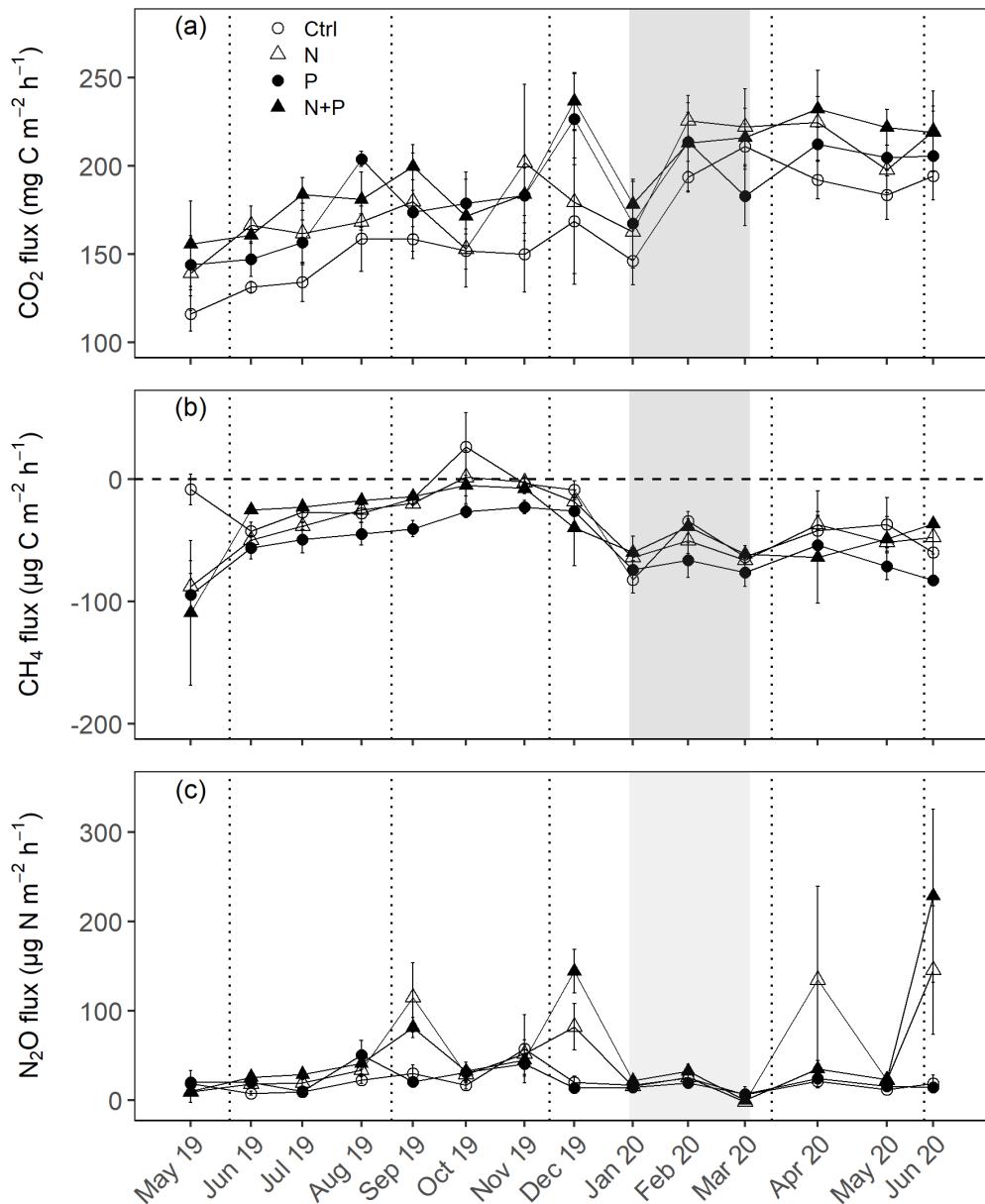
Figure 3. Mean (± SE, n = 4) soil CO_2 fluxes (a), CH_4 fluxes (b), and N_2O fluxes (c) measured monthly (between May 2019 and June 2020) from control (Ctrl), nitrogen (N), phosphorus (P), and N + P plots of the nutrient manipulation experiment. Vertical lines indicate the timing of each split dose of N (31.3 kg N ha^{-1}~~yr~~$^{-1}$), P (12.5 kg P ha^{-1}~~yr~~$^{-1}$) and N (31.3 kg N ha^{-1}~~yr~~

-¹) + P (12.5 kg P ha⁻¹ yr⁻¹) fertilization every three months. The gray shaded rectangle marks the beginning and end of the dry season (January and February; monthly precipitation < 100 mm). Note: Transitory N_2O fluxes (measured within 28 days from fertilization) from N addition plots (N, N + P) were detrended to compensate for absence of frequent measurements immediately after fertilization coming from sampling GHGs monthly.

4 Discussion

4.1 Effect of -N and P addition and soil environmental controls on soil CO_2 fluxes

The annual soil CO_2 effluxes from control plots (Table 2) were lower than those measured from tropical forests in Thailand (Hashimoto et al., 2004) and Hawaii (Townsend et al., 1995); within range to those from the Democratic Republic of Congo (Baumgartner et al., 2020), Panama (Koehler et al., 2009a; Pendall et al., 2010), Brazil (Sousa Neto et al., 2011), and Cameroon (Verchot et al., 2020); and higher than those reported from Kenya (Wanyama et al., 2019), and Indonesia (van Straaten et al., 2011). The differences in soil CO_2 fluxes between the control plots in this study and studies done in other tropical forest sites may be due to differences in soil environmental characteristics e.g. soil C quality and quantity, soil temperature, and moisture availability at the respective sites (Nottingham et al., 2015).

The alleviation of nutrient limitations on soil biological activity (in microbial communities and in root respiration) through fertilizer addition was particularly reflected by the significant increase in transitory CO_2 effluxes following addition of both N and P together (Fig. 4a). The transitory phase (< 28 days from fertilization) is the period where addition of nutrients (N, P, N + P) is expected to result in a large pulse of microbial activities. However, the fact that the increase in soil CO_2 effluxes was significant only in plots where N and P were added simultaneously (N + P), suggests a possible co-limitation between N and P on soil biological activity (Bréchet et al., 2019). These results seemingly align with the proposed multiple element limitation concept, which suggests a strong response in microbial mediated processes upon supply of limiting nutrients (Fanin et al., 2015). Furthermore, the results likely indicate that some soil respiration sources may respond positively to N addition (Yan et al., 2017), while others may respond positively to P addition (Ma et al., 2020), yielding an overall additive response when added together. An increase in soil CO_2 effluxes following addition of N and P simultaneously together has also been reported in studies like Bréchet et al. (2019), and Soong et al. (2018) from Panamanian tropical forests.

In contrast, the lack of significant treatment effects on background soil CO_2 efflux (Fig. 4a, d) and its different components (heterotrophic and autotrophic; Fig. 5) may suggest that ~~the test site is complex with~~ numerous counteracting processes could be happening at the same time, hence masking treatment effects. Some studies have for instance demonstrated that addition of N subdues exoenzymes (Li et al., 2018), decreases microbial biomass (Burton et al., 2004; Hicks et al., 2019), increases net primary productivity (Adamek et al., 2009), reduces fine root biomass (Cusack et al., 2011), while other studies have reported that P addition increases soil organic matter decompsition in tropical forest ecosytems (Cleveland and Townsend, 2006). The possibility of counteracting processes at ~~T~~the experimental site ~~complexity~~ is further exemplified by the lack of a relationship between all the measured soil environmental controls (soil temperature, ~~mineral N~~ nitrates and soil moisture) and background CO_2 effluxes (Fig 6a, b, c). Although these results are consistent with the findings by Baumgartner et al. (2020) in the Congo basin, they

contrast several GHG studies located in tropical forests that have reported a strong correlation between CO_2 effluxes

470 and soil moisture (Matson et al., 2017; van Straaten et al., 2011). For this experiment site, it could be that the minimal temporal fluctuation in soil temperature (Fig. 1b), together with the fact that water filled pore space was mostly > 40 % (Fig. 1a) during the sampling campaign dampened the effect of soil temperature and moisture on soil CO_2 fluxes.

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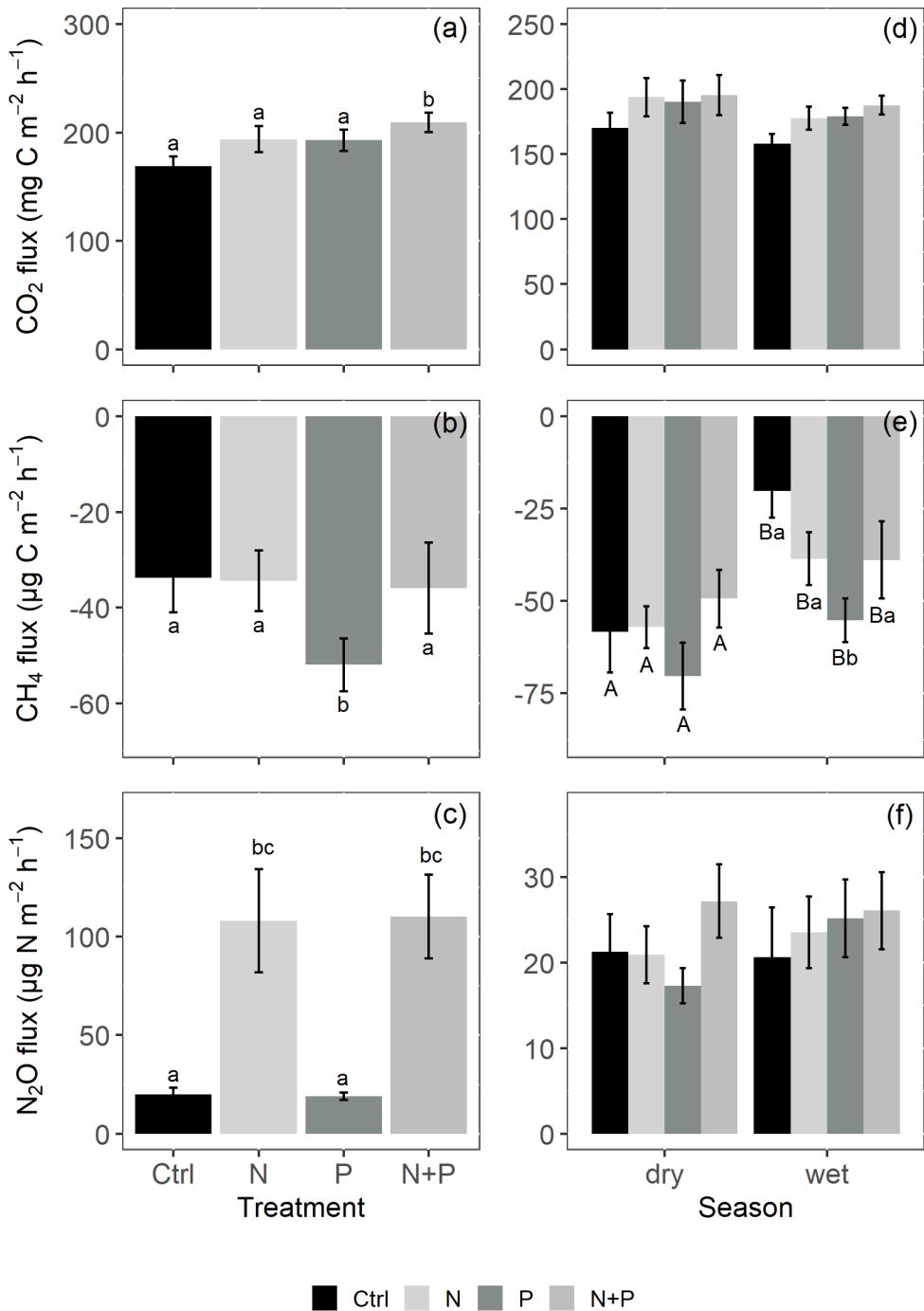
Figure 4. Mean (± SE, n = 4) soil CO_2 fluxes (a, d), CH_4 fluxes (b, e), and N_2O fluxes (c, f) from the control (Ctrl), nitrogen (N), phosphorus (P), and N + P plots of ~~a~~ the nutrient manipulation experiment. Column 1 (a, b, and c) includes only fluxes measured during the transitory phase (0 to 28 days after fertilization; and all the transitory fluxes were in the wet season (monthly precipitation >100 mm)). Column 2 (d, e, and f) includes only background level fluxes (fluxes measured more than 28 days after fertilization). Different lower-case letters indicate significant differences between nutrient addition treatments and the control while different upper-case letters indicate significant differences between seasons (linear mixed effects models; $p \leq 0.05$). Note: Transitory N_2O fluxes (measured within 28 days from fertilization) from N addition plots (N, N +

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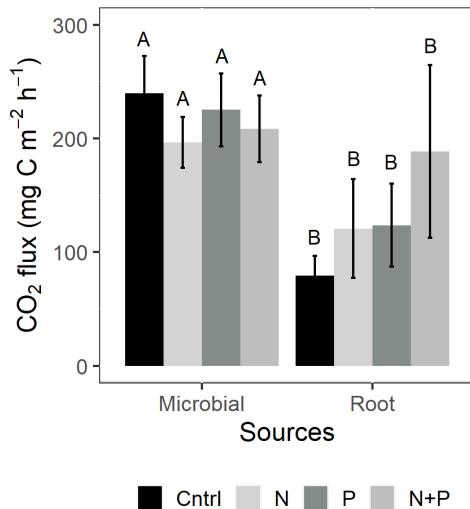
 P) were detrended to compensate for absence of frequent measurements immediately after fertilization coming from sampling GHGs monthly.

490 **Figure 5. Mean (± SE, n = 4) soil CO_2 flux~~flux~~ from the control (Ctrl), nitrogen (N), phosphorus (P), and N + P plots of a trenching treatment separated into microbial and root sources. Different upper-case letters indicate significant differences between microbial and root contribution to total CO_2 flux (linear mixed effects models; $p \leq 0.05$).**

4.2 Effect of N and P addition and soil environmental controls on soil CH_4 fluxes

495 The annual soil CH_4 fluxes from the control plots (Table 2) were at the upper end of CH_4 fluxes measured in lowland tropical forests (Aronson et al., 2019; Veldkamp et al., 2013; Zheng et al., 2016), and at the lower end of those measured in (sub-) montane tropical forest ecosystems (Sousa Neto et al., 2011; Yan et al., 2008). The difference in soil texture and soil moisture regimes between this experimental site and the other study sites might explain why CH_4 uptake at the respective sites was different. It is recognized that soil physical properties, particularly texture (Sousa Neto et al., 500 2011), along with soil moisture content directly control the entry and diffusivity of CH_4 from the atmosphere to the oxidative sites in the soil (Veldkamp et al., 2013).

In this experiment, the significantly higher CH_4 consumption from the P addition plots compared to the control during both the transitory and background periods (Fig. 4b, e) is attributed to the alleviation of P limitations affecting methanotrophic activity. Similar findings were reported by Zhang et al. (2011), and Yu et al. (2017), but contrasted 505 those of Bréchet et al. (2019) and Zheng et al. (2016). It is worth noting that although all these studies were located in tropical forests, they differed fundamentally in their experimental designs, type and amount of fertilizers applied, and the frequency of fertilizer application, which could have influenced the reported CH_4 uptake rates at the respective sites.

The lack of a response in background CH_4 consumption following N fertilization (Fig. 4e) is likely because there were 510 contrasting ecosystem responses to N addition. On the one hand, the addition of nitrogen significantly increased soil water filled pore space in comparison to the control (Fig. 1a; possibly as a result of reduced fine root biomass (Cusack

et al., 2011)), which could have resulted in a decrease in methane uptake. On the other hand, the negative correlation between ~~mineral N~~nitrates and background CH_4 fluxes (Fig. 6f) indicates that increases in soil ~~mineral nitrogen~~ nitrate content should increase CH_4 uptake. Additionally, the lack of a clearer signal in background CH_4 uptake may have to

515   do with the high variability in the measured CH_4 fluxes (CV = 97 ± 58 %) potentially caused by localized termite activity (Brune, 2014; Nauer et al., 2018).

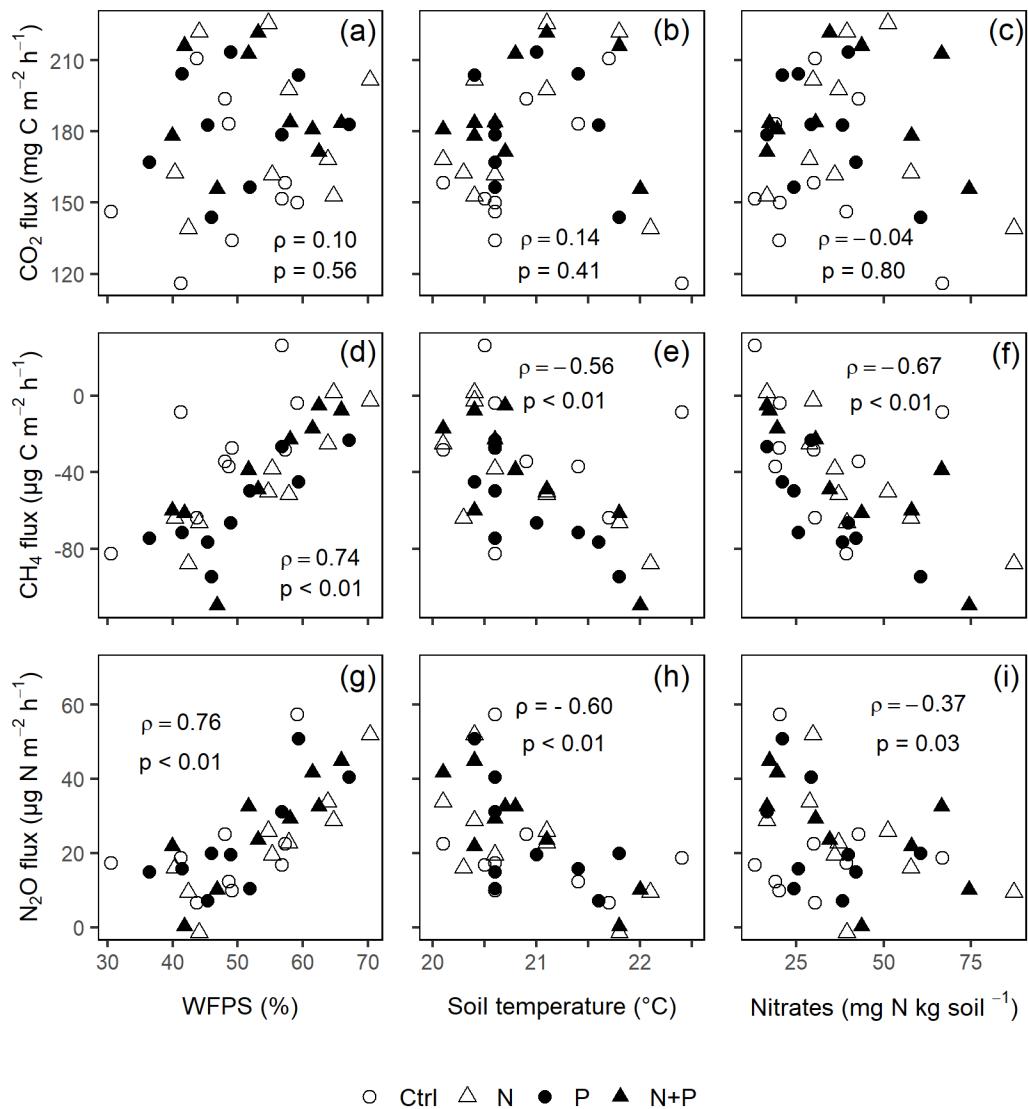



Figure 6. Spearman's correlation coefficient between mean background CO_2 (a-c), CH_4 (d-f), and N_2O (g-i) fluxes and WFPS

520   **(column 1), soil temperature (column 2) and ~~mineral nitrogen~~nitrates (column 3) using monthly measurement means of four replicate treatment plots taken between May 2019 and June 2020 ($p \leq 0.05$, n = 16 (four replicate plots in each of the four treatments)) . ρ is the spearman's correlation coefficient.**

4.3   Effect of -N and P addition and soil environmental controls on soil N$_2$O fluxes

525   The annual soil N$_2$O fluxes from the control plots (Table 2) were at the higher end of those measured in (sub-) montane tropical forests (Iddris et al., 2020, Arias-Navarro et al., 2017, Gütlein et al., 2018), and at the lower end of those measured in lowland tropical forest sites (e.g. Koehler et al., 2009b). This may either be due to the differences in soil N cycling rates (Koehler et al., 2009b) or the differences in spatial abundance of leguminous trees (Xu et al., 2020) at the respective sites.

530   The immediate flush of N$_2$O following fertilization (in the transitory phase) both in the N and N + P addition plots (Fig. 3c, Fig. 4c), is due to the increase in soil N concentrations beyond microbial immobilization and plant N needs (Davidson et al., 2000), which is typical of an open or leaky N cycle (Koehler et al., 2009b). Contrary to Kaspari et al. (2008) and Koehler et al. (2009b), sustained N fertilization did not trigger a significant response in background soil N$_2$O fluxes from N addition plots (Fig. 4f). This was unexpected, but given the rapid drainage at the site (sandy texture,

535   Table 1), there could have been substantial loss of added N via leaching, which possibly rid the ecosystem of excess ~~mineral N~~ nitrates (Lohse and Matson, 2005; Martinson et al., 2013). Notably, sustained P addition did not result in increased background N$_2$O fluxes (Fig. 4f), which contrasts the findings by Mori et al. (2017) who reported that P availability opens up the N cycle by stimulating mineralization of soil organic matter releasing excess N that is lost as ~~and leads to increased~~ N$_2$O emissions. At this study site, it could be that either the amount of P added in the experiment

540   was not sufficient to trigger a response in background soil N$_2$O fluxes or P is not a limiting nutrient for N$_2$O fluxes given the relatively high pH of the site (Table 1).

Unexpectedly, ~~mineral N~~nitrates correlated negatively to background N$_2$O fluxes (Fig. 6i), yet many studies (e.g. Corre et al., 2014; Zhang et al., 2020) have found that ~~mineral N~~nitrates and N$_2$O fluxes were positively correlated. The likely explanation for such a relationship is the transformation of N$_2$O to N$_2$ under wet conditions, which further reduced the

545   amount of ~~mineral N~~ nitrates ~~(particularly NO$_3^-$)~~ in soil (Matson et al., 2017). Despite the minimal influence of seasonality on background N$_2$O fluxes (Fig. 4f), a strong positive correlation between background N$_2$O fluxes and WFPS was observed (Fig 6g), which conforms to the explanation given by the conceptual hole in the pipe (HIP) model. The HIP model places soil aeration status (approximated by WFPS) second to N availability in controlling soil N$_2$O fluxes. Soil aeration not only directly controls oxygen entry into the soil but also determines how N$_2$O is produced

550   (denitrification or nitrification), and transported out of the soil (Davidson et al., 2000). Whereas there seems to be a balance between denitrification and nitrification process at this forest site (given that majority of the measurements corresponded to WFPS of $\leq$ 60 %, Fig. 6g), the considerable N$_2$O fluxes at higher WFPS values ($\geq$ 60 %, Fig. 6g) seem to suggest that denitrification is more dominant than nitrification in producing N$_2$O in these biomes.

555   **4.4   Implications of increasing N and P deposition rates on soil greenhouse gases from tropical forests**

While this experiment was established to investigate how nutrient limitations constrain soil GHG fluxes, it also sheds valuable insights on how anthropogenic nutrient inputs (through deposition) may affect future soil GHG fluxes from African tropical forests and other tropical sites with a similarly strong seasonality, soil, and vegetation characteristics (Table 1). Nutrient depositions are often highest immediately after the onset of the rainy season (Wang et al., 2020),

560   especially due to aerosol deposition following burning activities associated with deforestation during the dry season

(Giglio et al., 2006; Roberts et al., 2009). Accordingly, we suspect that the increased N inputs during this short time may yield similar responses to those observed in the transitory period measured at this study site; namely—N_2O flushes when reactive nitrogen enters the soil. Although N additions did not elicit a positive N_2O response during the background period, it is quite likely that our fertilization activities (from year 1 to year 2 of the study) had not gone on for long enough to simulate chronic long-term N additions. A study conducted by Koehler et al. (2009b) in Panama showed that 11 years of chronic N addition significantly increased both transitory and background soil N_2O emissions. In addition, this study shows that future increases in P deposition over tropical forests may significantly ~~boost~~increase the CH_4 sink capacity of tropical forest soils. Also, it was interesting to observe that the addition of N and P simultaneously resulted in increased CO_2 effluxes immediately after fertilization likely suggesting a co-limitation of N and P on soil respiration. This means that future increases in deposition of N and P rich ashes (from biomass burning), might result in significant soil CO_2 emissions from these biomes, while it is unclear if this is compensated via an increase in photosynthetic CO_2 uptake as indicated by Cernusak et al. (2013). In this context, it is important to note that ~~i~~It has been demonstrated by Barkley et al. (2019) that P derived from biomass burning aerosols is more soluble than the P from dust aerosols, hence, the former would have an immediate impact on ecosystem processes.

5. Conclusion

Nutrient manipulation studies premised in tropical forests are crucial to understand how these under-studied yet very important sinks and sources of soil GHGs subtly respond to changes in soil macro nutrient availability. ~~A nutrient manipulation experiment established in a pristine tropical forest in northwestern Uganda was used as basis to determine the soil greenhouse gas (GHG; CO_2, CH_4 and N_2O) flux response to different N, P, and N + P fertilizer additions.~~ N fertilization (N and~~,~~ N + P) significantly increased N_2O fluxes immediately after fertilization (transitory phase), ~~while~~ but had no significant effect ~~was found for~~on~~-~~ background N_2O fluxes, which might occur if the system would gain N over longer time spans. Against our expectations, neither background CO_2 effluxes nor CH_4 uptake decreased following addition of N, indicating neither a negative effect of a potential surplus of soil N on root and microbial respiration nor a negative effect on methanotrophs. CO_2 effluxes even showed a significant increase during the transitory phase following N and N + P fertilization. However, this effect was only significant for N + P addition, indicating some N and P co-limitation. ~~There was also an immediate significant increase in CO_2 effluxes shortly after adding N and P simultaneously together, indicating the co-limitation of N and P for soil respiration. The unexpected lack of a response in background CH_4 uptake and background CO_2 effluxes (including the autotrophic and heterotrophic components of CO_2 production) to N fertilization is in part attributed to the complex nature of the ecosystem.~~ An increase in CH_4 uptake was found both shortly and after sustained P fertilization; supporting our second hypothesis, which suggested that lifting the P limitation on soil methanotrophs, would significantly increase CH_4 consumption. ~~. This is consistent with the assumption that the alleviation of a P limitation will increase methanotrophic activity.~~ Surprisingly, both transitory and background N_2O and CO_2 fluxes (including its different components) were not significantly affected by P fertilization. Overall, the results from this first nutrient manipulation GHG study from a wet African tropical forest site, in general, indicate our limited knowledge about the counteracting interactions between N and P inputs and GHG

fluxes from different tropical forest ecosystems. This confines any general conclusions and equally limits our ability to parametrize tropical forest ecosystems in Earth System Models. Nevertheless, the contribution of tropical forest biomes to the global soil GHG budgets maybe disproportionately altered via potential future increases in N and P availability.~~Overall, this first nutrient manipulation GHG study from a tropical forest site in the wet tropics of Africa indicates that more studies are needed to understand the complex interaction between N and P inputs and GHG fluxes from these ecosystems. In general, our results suggest that the contribution of tropical forest biomes to the global soil GHG budgets may be disproportionately altered by external nutrient inputs.~~

Declaration on Conflict of Interest. We declare that there is no conflict of interest.

Author contribution. JT and OvS conceptualized the study. OvS established the nutrient manipulation experiment. JT conducted the fieldwork, did data analysis and prepared the manuscript. OvS, PF, and SD provided significant input on the experimental set-up and data analysis. RH and BM did laboratory measurements and gave critical feedback on the manuscript. OvS, PF, SD, MG, and LFT critically reviewed and gave feedback on the manuscript.

Data availability. Data is available on request.

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References

Adamek, M., Corre, M. D. and Hölscher, D.: Early effect of elevated nitrogen input on above-ground net primary production of a lower montane rain forest, Panama, J. Trop. Ecol., 256, 637–647, doi:10.1017/S0266467409990253, 2009.

635 Arias-Navarro, C., Díaz-Pinés, E., Kiese, R., Rosenstock, T. S., Rufino, M. C., Stern, D., Neufeldt, H., Verchot, L. V. and Butterbach-Bahl, K.: Gas pooling: A sampling technique to overcome spatial heterogeneity of soil carbon dioxide and nitrous oxide fluxes, Soil Biol. Biochem., 67, 20-23, doi:10.1016/j.soilbio.2013.08.011, 2013.

Arias-Navarro, C., Díaz-Pinés, E., Zuazo, P., Rufino, M. C., Verchot, L. V. and Butterbach-Bahl, K.: Quantifying the contribution of land use to N_2O, NO and CO_2 fluxes in a montane forest ecosystem of Kenya, Biogeochemistry., 134,

640 95–114, doi:10.1007/s10533-017-0348-3, 2017.

Aronson, E. L. and Helliker, B. R.: Methane flux in non-wetland soils in response to nitrogen addition: A meta-analysis, Ecology., 91, 3242–3251, doi:10.1890/09-2185.1, 2010.

Aronson, E. L., Dierick, D., Botthoff, J. K., Oberbauer, S., Zelikova, T. J., Harmon, T. C., Rundel, P., Johnson, R. F., Swanson, A. C., Pinto-Tomás, A. A., Artavia-León, A., Matarrita-Carranza, B. and Allen, M. F.: ENSO-influenced

645 drought drives methane flux dynamics in a tropical wet forest soil, J. Geophys. Res., 124, 2267–2276, doi:10.1029/2018JG004832, 2019.

Barkley, A. E., Prospero, J. M., Mahowald, N., Hamilton, D. S., Popendorf, K. J., Oehlert, A. M., Pourmand, A., Gatineau, A., Panechou-Pulcherie, K., Blackwelder, P. and Gaston, C. J.: African biomass burning is a substantial source of phosphorus deposition to the Amazon, Tropical Atlantic Ocean, and Southern Ocean, Proc. Natl. Acad. Sci.

650 U. S. A., 116(33), 16216–16221, doi:10.1073/pnas.1906091116, 2019.

Baumgartner, S., Barthel, M., Drake, T., Bauters, M., Makelele, I. A., Mugula, J. K., Summerauer, L., Gallarotti, N., Ntaboba, L. C., Van Oost, K., Boeckx, P., Doetterl, S., Werner, R. and Six, J.: Seasonality, drivers, and isotopic composition of soil CO_2 fluxes from tropical forests of the Congo Basin, Biogeosciences., 17, 6207–6218, doi:10.5194/bg-17-6207-2020, 2020.

655 Bauters, M., Verbeeck, H., Rütting, T., Barthel, M., Bazirake Mujinya, B., Bamba, F., Bodé, S., Boyemba, F., Bulonza, E., Carlsson, E., Eriksson, L., Makelele, I., Six, J., Cizungu Ntaboba, L. and Boeckx, P.: Contrasting nitrogen fluxes in African tropical forests of the Congo Basin, Ecol. Monogr., 89(1), 1–17, doi:10.1002/ecm.1342, 2019.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J. W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L. and De Vries, W.:

660 Global assessment of nitrogen deposition effects on terrestrial plant diversity: A synthesis, Ecol. Appl., 20, 30-59, doi:10.1890/08-1140.1, 2010.

Bodelier, P. L. E. and Steenbergh, A. K.: Interactions between methane and the nitrogen cycle in light of climate change, Curr. Opin. Environ. Sustain., 9-10, 26-36, doi:10.1016/j.cosust.2014.07.004, 2014.

Bréchet, L., Courtois, E. A., Saint-Germain, T., Janssens, I. A., Asensio, D., Ramirez-Rojas, I., Soong, J. L., Van

665 Langenhove, L., Verbruggen, E. and Stahl, C.: Disentangling drought and nutrient effects on soil carbon dioxide and methane fluxes in a tropical forest, Front. Environ. Sci., 7, 180, doi:10.3389/fenvs.2019.00180, 2019.

Brune, A.: Symbiotic digestion of lignocellulose in termite guts, Nat. Rev. Microbiol., 12, 168–180, doi:10.1038/nrmicro3182, 2014.

Burton, A. J., Pregitzer, K. S., Crawford, J. N., Zogg, G. P. and Zak, D. R.: Simulated chronic NO_3^- deposition reduces

670 soil respiration in northern hardwood forests, Glob. Chang. Biol., 10, 1080–1091, doi:10.1111/j.1365-2486.2004.00737.x, 2004.

Butterbach-Bahl, K., Kiese, R. and Liu, C.: Measurements of biosphere atmosphere exchange of CH_4 in terrestrial ecosystems, 1st ed, Methods Enzymol, Elsevier Inc., 495:271-87, doi: 10.1016/B978-0-12-386905-0.00018-8, 2011.

Butterbach-Bahl, K., Kock, M., Willibald, G., Hewett, B., Buhagiar, S., Papen, H. and Kiese, R.: Temporal variations of fluxes of NO, NO_2, N_2O, CO_2, and CH_4 in a tropical rain forest ecosystem, Glob. Biogeochem. Cycles., 18, doi:10.1029/2004GB002243, 2004.

Cernusak, L.A., Winter, K., Dalling, J.W., Holtum, J.A., Jaramillo, C., Körner, C., Leakey, A.D., Norby, R.J., Poulter, B., Turner, B.L. and Wright, S.J.: Tropical forest responses to increasing atmospheric CO_2: current knowledge and opportunities for future research. Funct. Plant Biol., 40, 531-551, doi.org/10.1071/FP12309, 2013.

Chen, D., Zhou, L., Rao, X., Lin, Y. and Fu, S.: Effects of root diameter and root nitrogen concentration on in situ root respiration among different seasons and tree species, Ecol. Res., 25, 983–993, doi:10.1007/s11284-010-0722-2, 2010.

Cleveland, C. C. and Townsend, A. R.: Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere, Proc. Natl. Acad. Sci. U.S.A., 103, 10316-10321, doi:10.1073/pnas.0600989103, 2006.

Climate Data.org: Masindi climate, available at: https://en.climate-data.org/africa/uganda/western-region/masindi-55265/, last access: 9 December 2020.

Corre, M. D., Sueta, J. P. and Veldkamp, E.: Nitrogen-oxide emissions from tropical forest soils exposed to elevated nitrogen input strongly interact with rainfall quantity and seasonality, Biogeochemistry., 118, 103-120, doi:10.1007/s10533-013-9908-3, 2014.

Corre, M. D., Veldkamp, E., Arnold, J., and Wright, S. J.: Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama. Ecology., 91, 1715–1729, doi: 10.1890/09-0274.1, 2010.

Cusack, D. F., Silver, W. L., Torn, M. S., Burton, S. D. and Firestone, M. K.: Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests, Ecology., 92, 621–632, doi:10.1890/10-0459.1, 2011.

Davidson, E. A., Keller, M., Erickson, H. E., Verchot, L. V. and Veldkamp, E.: Testing a conceptual model of soil emissions of nitrous and nitric oxides, Bioscience., 50, 667–680, doi:10.1641/0006-3568(2000)050[0667:TACMOS]2.0.CO;2, 2000.

DeForest, J. L., Noormets, A., McNulty, S. G., Sun, G., Tenney, G. and Chen, J.: Phenophases alter the soil respiration-temperature relationship in an oak-dominated forest, Int. J. Biometeorol., 51, 135-144, doi:10.1007/s00484-006-0046-7, 2006.

Doetterl, S., Stevens, A., Six, J., Merckx, R., Oost, K. Van, Pinto, M. C., Casanova-Katny, A., Muñoz, C., Boudin, M., Venegas, E. Z. and Boeckx, P.: Soil carbon storage controlled by interactions between geochemistry and climate, Nature Geoscience., 8, 780-783, doi:10.1038/NGEO2516, 2015.

Du, E., Xia, N. and de Vries, W.: Effects of nitrogen deposition on growing-season soil methane sink across global forest biomes, Biogeosciences Discuss., 1–16, doi:10.5194/bg-2019-29, February, 2019.

Dutaur, L. and Verchot, L. V.: A global inventory of the soil CH_4 sink, Glob. Biogeochem. Cycles., 21, 1–9, doi:10.1029/2006GB002734, 2007.

Eggeling, W. J.: Observations on the ecology of the Budongo rainforest, Uganda, J. Ecol., 34, 20-87, doi:10.2307/2256760, 1947.

Fanin, N., Hättenschwiler, S., Schimann, H. and Fromin, N.: Interactive effects of C, N and P fertilization on soil microbial community structure and function in an Amazonian rain forest, Funct. Ecol., 29, 140–150, doi:10.1111/1365-2435.12329, 2015.

Galloway, J., Dentener, F., Capone, D., Boyer, E. and Howarth, R.: Nitrogen cycles: past, present, and future, Biogeochemistry., 70, 2004.

Giglio, L., Csiszar, I., and Justice, C. O.: Global distribution and seasonality of active fires as observed with Terra and Aqua Moderate Resolution Imaging Spectroradiometers (MODIS) sensors, J. Geophys. Res., 111, G02016, doi:10.1029/2005JG000142, 2006.

Gray, N. D., McCann, C. M., Christgen, B., Ahammad, S. Z., Roberts, J. A. and Graham, D. W.: Soil geochemistry confines microbial abundances across an arctic landscape; implications for net carbon exchange with the atmosphere, Biogeochemistry., 120, 307–317, doi:10.1007/s10533-014-9997-7, 2014.

Gütlein, A., Gerschlauer, F., Kikoti, I. and Kiese, R.: Impacts of climate and land use on N_2O and CH_4 fluxes from tropical ecosystems in the Mt. Kilimanjaro region, Tanzania, Glob. Chang. Biol., 24, 1239–1255, doi:10.1111/gcb.13944, 2018.

Hall, S. J., and Matson P. A.: Nutrient status of tropical rain forests influences soil N dynamics after N additions. Ecological Monographs., 73, 107–129, jstor.org/stable/3100077, 2003.

Hashimoto, S., Tanaka, N., Suzuki, M., Inoue, A., Takizawa, H., Kosaka, I., Tanaka, K., Tantasirin, C. and Tangtham, N.: Soil respiration and soil CO_2 concentration in a tropical forest, Thailand, J. For. Res., 9, 75–79, doi:10.1007/s10310-003-0046-y, 2004.

Hassler, E., Corre, M. D., Tjoa, A., Damris, M., Utami, S. R., and Veldkamp, E.: Soil fertility controls soil–atmosphere carbon dioxide and methane fluxes in a tropical landscape converted from lowland forest to rubber and oil palm plantations. Biogeosciences., 12(19), 5831-5852, doi.org/10.5194/bg-12-5831, 2015.

Hedin, L.O., Vitousek, P.M. and Matson, P.A.: Nutrient losses over four million years of tropical forest development. Ecology., 84(9), 2231-2255, doi.org/10.1890/02-4066, 2003.

Hicks, L. C., Meir, P., Nottingham, A. T., Reay, D. S., Stott, A. W., Salinas, N. and Whitaker, J.: Carbon and nitrogen inputs differentially affect priming of soil organic matter in tropical lowland and montane soils, Soil Biol. Biochem., 129, 212–222, doi:10.1016/j.soilbio.2018.10.015, 2019.

Hobbie, S. E. and Vitousek, P. M.: Nutrient limitation of decomposition in Hawaiian forests, Ecology., 81, 1867-1877, doi:10.1890/0012-9658(2000)081[1867:NLODIH]2.0.CO;2, 2000.

Holland, E. A., Neff, J. C., Townsend, A. R. and McKeown, B.: Uncertainties in the temperature sensitivity of decomposition in tropical and subtropical ecosystems: Implications for models, Glob. Biogeochem. Cycles., 14, 1137-1151, doi:10.1029/2000GB001264, 2000.

Iddris, N. A.-A., Corre, M. D., Yemefack, M., van Straaten, O. and Veldkamp, E.: Stem and soil nitrous oxide fluxes from rainforest and cacao agroforest on highly weathered soils in the Congo Basin, Biogeosciences., 17, 5377–5397, doi:10.5194/bg-17-5377-2020, 2020.

750   ~~Iddris, N. A. A., Corre, M. D., Yemefack, M., van Straaten, O. and Veldkamp, E.: Stem and soil nitrous oxide fluxes from rainforest and cacao agroforest on highly weathered soils in the Congo Basin, Biogeosciences, 17, 5377–5397, doi:10.5194/bg 17-5377-2020, 2020.~~

IUSS Working Group WRB: World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps., 106, 2014.

755   Jiang, X., Chen, H., Peng, C., Li, Y., He, Y., Chen, D., Lin, M., Hu, J., Ma, T., Liu, L., Liu, X., Xia, M. and Liu, Y.: Soil carbon dioxide fluxes from three forest types of the tropical montane rainforest on Hainan Island, China, Water. Air. Soil Pollut., 227, doi:10.1007/s11270-016-2904-1, 2016.

Jobbágy, E. G. and Jackson, R. B.: The vertical distribution of soil organic carbon and its relation to climate and vegetation, Ecol. Appl., 10, 423-436, doi:10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2, 2000.

760   John, R., Dalling, J. W., Harms, K. E., Yavitt, J. B., Stallard, R. F., Mirabello, M., Hubbell, S. P., Valencia, R., Navarrete, H., Vallejo, M. and Foster, R. B.: Soil nutrients influence spatial distributions of tropical trees species, Proc. Natl. Acad. Sci. U.S.A., 104, 864-869, doi:10.1073/pnas.0604666104, 2007.

Kaspari, M., Garcia, M. N., Harms, K. E., Santana, M., Wright, S. J. and Yavitt, J. B.: Multiple nutrients limit litterfall and decomposition in a tropical forest, Ecol. Lett., 11, 35–43, doi:10.1111/j.1461-0248.2007.01124.x, 2008.

765   Koehler, B., Corre, M. D., Veldkamp, E. and Sueta, J. P.: Chronic nitrogen addition causes a reduction in soil carbon dioxide efflux during the high stem-growth period in a tropical montane forest but no response from a tropical lowland forest on a decadal time scale, Biogeosciences., 6, 2973–2983, doi:10.5194/bg-6-2973-2009, 2009a.

Koehler, B., Corre, M. D., Veldkamp, E., Wullaert, H. and Wright, S. J.: Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input, Glob. Chang. Biol., 15, 2049–2066,
770   doi:10.1111/j.1365-2486.2008.01826.x, 2009b.

Li, Y., Sun, J., Tian, D., Wang, J., Ha, D., Qu, Y., Jing, G. and Niu, S.: Soil acid cations induced reduction in soil respiration under nitrogen enrichment and soil acidification, Sci. Total Environ., 615, 1535–1546, doi:10.1016/j.scitotenv.2017.09.131, 2018.

Li, Y., Xu, M. and Zou, X.: Effects of nutrient additions on ecosystem carbon cycle in a Puerto Rican tropical wet
775   forest, Glob. Chang. Biol., 12, 284–293, doi:10.1111/j.1365-2486.2005.01096.x, 2006.

Lohse, K. A. and Matson, P.: Consequences of nitrogen additions for soil losses from wet tropical forests, Ecol. Appl., 15, 1629–1648, doi:10.1890/03-5421, 2005.

Lukwago, W., Behangana, M., Mwavu, E. N. and Hughes, D. F.: Effects of selective timber harvest on amphibian
780   species diversity in Budongo forest Reserve, Uganda, Forest. Ecol. Manage., 458, doi:10.1016/j.foreco.2019.117809, 2020.

Ma, S., Chen, G., Tian, D., Du, E., Xiao, W., Jiang, L., Zhou, Z., Zhu, J., He, H., Zhu, B. and Fang, J.: Effects of seven-year nitrogen and phosphorus additions on soil microbial community structures and residues in a tropical forest in Hainan Island, China, Geoderma., 361, 114034, doi:10.1016/j.geoderma.2019.114034, 2020.

785 Malhi, Y. and Phillips, O. L.: Tropical forests and global atmospheric change: A synthesis, Philos. Trans. R. Soc. B Biol. Sci., 359, 549–555, doi:10.1098/rstb.2003.1449, 2004.

Martinson, G. O., Corre, M. D. and Veldkamp, E.: Responses of nitrous oxide fluxes and soil nitrogen cycling to nutrient additions in montane forests along an elevation gradient in southern Ecuador, Biogeochemistry., 112, 625-636, doi:10.1007/s10533-012-9753-9, 2013.

790 Matson, A. L., Corre, M. D. and Veldkamp, E.: Nitrogen cycling in canopy soils of tropical montane forests responds rapidly to indirect N and P fertilization, Glob. Chang. Biol., 20, 3802–3813, doi:10.1111/gcb.12668, 2014.

Matson, A. L., Corre, M. D., Langs, K. and Veldkamp, E.: Soil trace gas fluxes along orthogonal precipitation and soil fertility gradients in tropical lowland forests of Panama, Biogeosciences., 14, 3509–3524, doi:10.5194/bg-14-3509-2017, 2017.

795 McGroddy, M. E., Baisden, W. T. and Hedin, L. O.: Stoichiometry of hydrological C, N, and P losses across climate and geology: An enviornmental matrix approach across New Zealand primary forests, Glob. Biogeochem. Cycles., 22, doi:10.1029/2007GB003005, 2008.

Mori, T., Lu, X., Aoyagi, R. and Mo, J.: Reconsidering the phosphorus limitation of soil microbial activity in tropical forests, Funct. Ecol., 32, 1145–1154, doi:10.1111/1365-2435.13043, 2018.

800 Mori, T., Ohta, S., Ishizuka, S., Konda, R., Wicaksono, A., Heriyanto, J. and Hardjono, A.: Effects of phosphorus application on root respiration and heterotrophic microbial respiration in Acacia mangium plantation soil, Tropics., 22, 113–118, doi:10.3759/tropics.22.113, 2013.

Mori, T., Ohta, S., Ishizuka, S., Konda, R., Wicaksono, A., Heriyanto, J. and Hardjono, A.: Effects of phosphorus addition on N_2O and NO emissions from soils of an Acacia mangium plantation, Soil Sci. Plant Nutr., 56(5), 782–788,
805 doi:10.1111/j.1747-0765.2010.00501.x, 2010.

Mori, T., Wachrinrat, C., Staporn, D., Meunpong, P., Suebsai, W., Matsubara, K., Boonsri, K., Lumban, W., Kuawong, M., Phukdee, T., Srifai, J. and Boonman, K.: Effects of phosphorus addition on nitrogen cycle and fluxes of N_2O and CH_4 in tropical tree plantation soils in Thailand, Agric. Nat. Resour., 51, 91–95, doi:10.1016/j.anres.2016.03.002, 2017.

810 Mosier, A., Wassmann, R., Verchot, L., King, J. and Palm, C.: Methane and nitrogen oxide fluxes in tropical agricultural soils: Sources, sinks and mechanisms, Environ. Dev. Sustain., 6, 11-49, doi:10.1023/B:ENVI.0000003627.43162.ae, 2004.

Müller, A. K., Matson, A. L., Corre, M. D. and Veldkamp, E.: Soil N_2O fluxes along an elevation gradient of tropical montane forests under experimental nitrogen and phosphorus addition, Front. Earth Sci., 3, 66,
815 doi:10.3389/feart.2015.00066, 2015.

Nauer, P. A., Hutley, L. B. and Arndt, S. K.: Termite mounds mitigate half of termite methane emissions, Proc. Natl. Acad. Sci. U. S. A., 115, 13306–13311, doi:10.1073/pnas.1809790115, 2018.

Nottingham, A. T., Whitaker, J., Turner, B. L., Salinas, N., Zimmermann, M., Malhi, Y. and Meir, P.: Climate Warming and Soil Carbon in Tropical Forests: Insights from an Elevation Gradient in the Peruvian Andes, Bioscience., 65, 906–
820 921, doi:10.1093/biosci/biv109, 2015.

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F. and Erasmi, S.: Greenhouse gas emissions from soils—A review, Chemie der Erde., 76, 327-352, doi:10.1016/j.chemer.2016.04.002, 2016.

Pavelka, M., Acosta, M., Kiese, R., Altimir, N., Brümmer, C., Crill, P., Darenova, E., Fuß, R., Gielen, B., Graf, A. and Klemedtsson, L.: Standardization of chamber technique for CO_2, N_2O and CH_4 fluxes measurements from terrestrial

825   ecosystems, Int. Agrophys., 32, 569-587, doi: 10.1515/intag-2017-0045, 2018.

Pendall, E., Schwendenmann, L., Rahn, T., Miller, J. B., Tans, P. P. and White, J. W. C.: Land use and season affect fluxes of CO_2, CH_4, CO, N_2O and H_2 and isotopic source signatures in Panama: Evidence from nocturnal boundary layer profiles, Glob. Chang. Biol., 16, 2721–2736, doi:10.1111/j.1365-2486.2010.02199.x, 2010.

R Development Core Team: a language and environment for statistical computing. R Foundation for Statistical

830   Computing, Vienna, Austria, 2019.

Roberts, G., Wooster, M.J. and Lagoudakis, E.: Annual and diurnal African biomass burning temporal dynamics. Biogeosciences., 6(5), 849-866, doi.org/10.5194/bg-6-849-2009, 2009.

Saatchi, S. S., Harris, N. L., Brown, S., Lefsky, M., Mitchard, E. T. A., Salas, W., Zutta, B. R., Buermann, W., Lewis, S. L., Hagen, S., Petrova, S., White, L., Silman, M. and Morel, A.: Benchmark map of forest carbon stocks in tropical

835   regions across three continents, Proc. Natl. Acad. Sci. U.S.A., 108, 9899-904, doi:10.1073/pnas.1019576108, 2011.

Seghers, D., Top, E. M., Reheul, D., Bulcke, R., Boeckx, P., Verstraete, W. and Siciliano, S. D.: Long-term effects of mineral versus organic fertilizers on activity and structure of the methanotrophic community in agricultural soils, Environ. Microbiol., 5, 867-77, doi:10.1046/j.1462-2920.2003.00477.x, 2003.

Sjögersten, S., Aplin, P., Gauci, V., Peacock, M., Siegenthaler, A. and Turner, B. L.: Temperature response of ex-situ

840   greenhouse gas emissions from tropical peatlands: Interactions between forest type and peat moisture conditions, Geoderma., 324, 47-55, doi:10.1016/j.geoderma.2018.02.029, 2018.

Soong, J. L., Marañon-Jimenez, S., Cotrufo, M. F., Boeckx, P., Bodé, S., Guenet, B., Peñuelas, J., Richter, A., Stahl, C., Verbruggen, E. and Janssens, I. A.: Soil microbial CNP and respiration responses to organic matter and nutrient additions: Evidence from a tropical soil incubation, Soil Biol. Biochem., 122, 141–149,

845   doi:10.1016/j.soilbio.2018.04.011, 2018.

Sousa Neto, E., Carmo, J. B., Keller, M., Martins, S. C., Alves, L. F., Vieira, S. A., Piccolo, M. C., Camargo, P., Couto, H. T. Z., Joly, C. A. and Martinelli, L. A.: Soil atmosphere exchange of nitrous oxide, methane and carbon dioxide in a gradient of elevation in the coastal Brazilian Atlantic forest, Biogeosciences., 8, 733–742, doi:10.5194/bg-8-733-2011, 2011.

850   Tamatamah, R. A., Hecky, R. E. and Duthie, H. C.: The atmospheric deposition of phosphorus in Lake Victoria (East Africa), Biogeochemistry., 73(2), 325–344, doi:10.1007/s10533-004-0196-9, 2005.

Tanner, E. V. J., Vltousek, P. M. and Cuevas, E.: Experimental investigation of nutrient limitation of forest growth on wet tropical mountains, Ecology., 79(1), 10–22, doi:10.1890/0012-9658(1998)079[0010:EIONLO]2.0.CO;2, 1998.

Townsend, A. R., Vitousek, P. M. and Trumbore, S. E.: Soil organic matter dynamics along gradients in temperature

855   and land use on the island of Hawaii, Ecology., 76, 721–733, doi:10.2307/1939339, 1995.

van Straaten, H.P.: Präkambrium und junges Western Rift im Bunyoro Distrikt, NW- Uganda (Ostafrika), Geologishes Jahbuch. Reihe B, Heft 18. Hanover, 1976.

van Straaten, O., Veldkamp, E. and Corre, M. D.: Simulated drought reduces soil CO_2 efflux and production in a tropical forest in Sulawesi, Indonesia , Ecosphere., 2, 1-22, doi:10.1890/es11-00079.1, 2011.

860   Veber, G., Kull, A., Villa, J. A., Maddison, M., Paal, J., Oja, T., Iturraspe, R., Pärn, J., Teemusk, A. and Mander, Ü.: Greenhouse gas emissions in natural and managed peatlands of America: Case studies along a latitudinal gradient, Ecol. Eng.,114, 34-45, doi:10.1016/j.ecoleng.2017.06.068, 2018.

Veldkamp, E., Koehler, B. and Corre, M. D.: Indications of nitrogen-limited methane uptake in tropical forest soils, Biogeosciences., 10, 5367–5379, doi:10.5194/bg-10-5367-2013, 2013.

865   Verchot, L. V., Dannenmann, M., Kengdo, S. K., Njine-Bememba, C. B., Rufino, M. C., Sonwa, D. J. and Tejedor, J.: Land-use change and Biogeochemical controls of soil CO_2, N_2O and CH_4 fluxes in Cameroonian forest landscapes, J. Integr. Environ. Sci., 00, 1–23, doi:10.1080/1943815X.2020.1779092, 2020.

Wang, C. K., and Yang J. Y.: Rhizospheric and heterotrophic components of soil respiration in six Chinese temperate forests. Glob. Chang. Biol., 13:123–131, doi.org/10.1111/j.1365-2486.2006.01291.x, 2007.

870   Wang, Z., Zhang, X., Liu, L., Cheng, M. and Xu, J.: Spatial and seasonal patterns of atmospheric nitrogen deposition in North China, Atmospheric Sci. Lett., 13(3), 188-194, doi.org/10.1080/16742834.2019.1701385, 2020.

Wanyama, I., Pelster, D. E., Butterbach-Bahl, K., Verchot, L. V., Martius, C. and Rufino, M. C.: Soil carbon dioxide and methane fluxes from forests and other land use types in an African tropical montane region, Biogeochemistry., 875   143, 171–190, doi:10.1007/s10533-019-00555-8, 2019.

Wei, Z., Jiangming, M., Yunting, F., Xiankai, L. and Hui, W.: Effects of nitrogen deposition on the greenhouse gas fluxes from forest soils, Acta Ecol. Sin., 28(5), 2309–2319, doi:10.1016/S1872-2032(08)60047-5, 2008.

Wolf, K., Veldkamp, E., Homeier, J. and Martinson, G. O.: Nitrogen availability links forest productivity, soil nitrous oxide and nitric oxide fluxes of a tropical montane forest in southern Ecuador, Global Biogeochem. Cycles., 25, 880   doi:10.1029/2010GB003876, 2011.

Wright, S. J., Yavitt, J. B., Wurzburger, N., Turner, B. L., Tanner, E. V., Sayer, E. J., Santiago, L. S., Kaspari, M., Hedin, O, L., Harms, E, K., Garcia, N. M., and Corre, M. D.: Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. Ecology., 92(8), 1616-1625, doi: 10.1890/10-1558.1, 2011.

Xu, H., Detto, M., Fang, S., Chazdon, R. L., Li, Y., Hau, B. C. H., Fischer, G. A., Weiblen, G. D., Hogan, J. A., 885   Zimmerman, J. K., Uriarte, M., Thompson, J., Lian, J., Cao, K., Kenfack, D., Alonso, A., Bissiengou, P., Memiaghe, H. R., Valencia, R., Yap, S. L., Davies, S. J., Mi, X. and Yao, T. L.: Soil nitrogen concentration mediates the relationship between leguminous trees and neighbor diversity in tropical forests, Commun. Biol., 3, 1–8, doi:10.1038/s42003-020-1041-y, 2020.

~~Yan, G., Xing, Y., Xu, L., Wang, J., Dong, X., Shan, W., Guo, L. and Wang, Q.: Effects of different nitrogen additions~~ 890   ~~on soil microbial communities in different seasons in a boreal forest, Ecosphere, 8, e01879, doi:10.1002/ecs2.1879,~~ ~~2017.~~

Yan, Y., Sha, L., Cao, M., Zheng, Z., Tang, J., Wang, Y., Zhang, Y., Wang, R., Liu, G., Wang, Y. and Sun, Y.: Fluxes of CH_4 and N_2O from soil under a tropical seasonal rain forest in Xishuangbanna, Southwest China, J. Environ. Sci., 20, 207–215, doi:10.1016/S1001-0742(08)60033-9, 2008.

895   Yu, L., Wang, Y., Zhang, X., Dörsch, P. and Mulder, J.: Phosphorus addition mitigates N_2O and CH_4 emissions in N-saturated subtropical forest, SW China, Biogeosciences., 14, 3097–3109, doi:10.5194/bg-14-3097-2017, 2017.

Zhang, T., Zhu, W., Mo, J., Liu, L. and Dong, S.: Increased phosphorus availability mitigates the inhibition of nitrogen deposition on CH$_4$ uptake in an old-growth tropical forest, southern China, Biogeosciences., 8, 2805–2813, doi:10.5194/bg-8-2805-2011, 2011.

900 Zhang, W., Mo, J., Yu, G., Fang, Y., Li, D., Lu, X. and Wang, H.: Emissions of nitrous oxide from three tropical forests in Southern China in response to simulated nitrogen deposition, Plant Soil., 306, 221–236, doi:10.1007/s11104-008-9575-7, 2008.

Zhang, Y., Ma, M., Fang, H., Qin, D., Cheng, S. and Yuan, W.: Impacts of nitrogen addition on nitrous oxide emission : Comparison of five nitrous oxide modules or algorithms, Ecol. Modell., 421, 108963,

905 doi:10.1016/j.ecolmodel.2020.108963, 2020.

Zheng, M., Zhang, T., Liu, L., Zhang, W., Lu, X. and Mo, J.: Effects of nitrogen and phosphorus additions on soil methane uptake in disturbed forests, J. Geophys. Res., 121, 3089–3100, doi:10.1002/2016JG003476, 2016.

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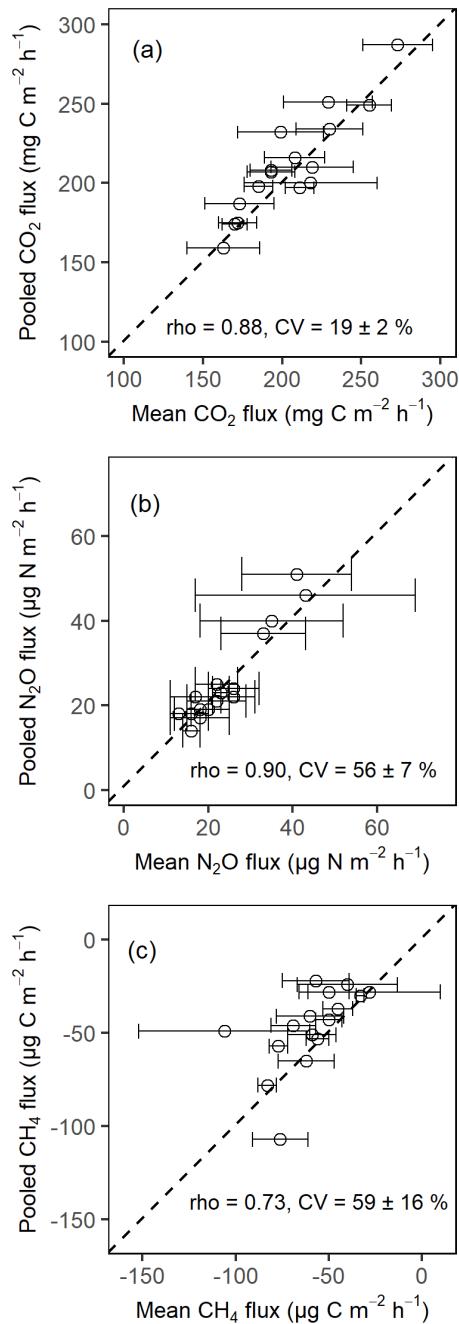
     Supplementary figures

Supplementary figure 1. Comparison of the soil CO_2 fluxes (a) soil N_2O fluxes (b), and soil CH_4 fluxes (c) from pooled sampling
940   and the mean of four chamber measurements for the month of February 2020 in Budongo Forest reserve. Rho is the spearman
correlation coefficient, and CV is the coefficient of variation. Error bars are derived from standard error of the mean.