https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

SOIL Discussions Open Access EGU

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. 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 Uganda. 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. 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 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 ($p = 0.052$) and background ($p = 0.010$) 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 ($p = 0.010$), suggesting a possible co-limitation of N and P on soil respiration. Heterotrophic (microbial) CO_2 effluxes were significantly higher than the autotrophic (root) CO_2 effluxes ($p < 0.001$) across all treatment plots with microbes contributing about three times more 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 in N and P availability in these biomes.

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

1 Introduction

Tropical forest soils play an important role in the earth's radiative balance by sequestering and emitting significant amounts of carbon dioxide (CO_2), methane (CH_4), 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).

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) 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), geochemsitsry 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).

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 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. The 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 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). 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 and results in increased N_2O 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). Even 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

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

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 shifts in N and P dynamics, the magnitude and direction of this response remains unclear (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 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). 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). 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 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 organic matter and consequently lead to increased N_2O emissions, higher CO_2 effluxes linked to increased root activity and decomposition of 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 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 study area receives about 1360 mm of rainfall annually (Climate-Data.org, 2020) distributed into two rainy seasons (i.e. March to May and August to November), 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. Mean annual temperature over the study area is 23 °C (Climate-Data.org, 2020).

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

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).

2.2 Experimental design

The study was conducted within the framework of a running nutrient manipulation experiment (NME) which investigates how the three macronutrients (N, P, and potassium (K)) constrained key ecosystem processes, particularly nutrient cycling, net primary productivity and carbon sequestration. While the NME included a K treatment, this study was conducted in the N, P and N + P (combination of N and P) plots, and compared to the untreated control plots (n = 16). Each treatment plot measured 40 m x 40 m in size with an inner core measurement zone (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. Nitrogen was applied at a rate of 125 kg N ha^{-1} yr^{-1} in form of urea ($(NH_2)_2CO$), and P at 50 kg P ha^{-1} yr^{-1} as triple superphosphate ($Ca(H_2PO_4)_2$), 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. 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. Soil samples from the same depth were then pooled together in a plastic bucket, thoroughly mixed, and a 500 g homogenized sample (a total of three samples i.e. one per depth per plot) sent to University of Göttingen in Germany for analysis. 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 plot was calculated from the mass of oven dried soil (at 105°C for 48 hours) and the volume of the Kopecky ring (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.

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 m^2, and volume = about 12 L) were randomly installed at the soil surface, to a depth of 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. On the sampling day, chamber bases were covered with vented polyvinyl hoods fitted with sampling ports. A pooled gas sample was

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

then obtained every 3, 13, 23, and 33 minutes using an airtight luer lock syringe following the pooling approach described in detail by Arias-Navarro et al. (2013). To check if the pooling worked correctly, both the pooled and un-
150 pooled (an average of four individual chamber measurements) samples were taken for the month of February 2020 for analysis. Both methods produced very comparable results. To avoid biases in GHG fluxes introduced by diurnal temperature fluctuations, soil GHG fluxes were always measured between 9 am and 4 pm throughout the entire study period. The gas-filled exetainers were sent to the Department of Environmental Systems Science, ETH Zürich, Switzerland for analysis using a gas chromatograph (GC; Scion 456-GC Bruker, Germany) equipped with an electron
155 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, 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).

160 $$GHG_{flux} = \frac{V_{ch} * GHG_m * S * 10^6 * 60}{A_{ch} * GHG_v * 10^9} \quad (1)$$

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,
165 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:

$$GHG_v = 0.02241 * \frac{273.15 + T_f}{273.15} * \frac{P_f}{P_s} \quad (2)$$

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]. For purposes of quality assurance, the measured gas concentrations from the GC were checked against the standards
170 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 nitrogen (ammonia (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
175 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 nitrogen 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,
180 100 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.

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

To understand the contribution of autotrophic (root) and heterotrophic (microbial) sources to total soil respiration, a trenching treatment was done in all the plots. A circular trench (about 0.60 m in diameter) was dug to a depth of about 0.6 m at the center of all the 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. 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 for 4 months (November 2019 to February 2020). 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 the amount of living fine root biomass that was still present in the trenched chamber. 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.

2.5 Statistical Analysis

Prior to statistical analysis, 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). Despite 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) to overcome skewness introduced by soil NH_4^+, which was mostly 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, 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 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 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), 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 compromise the power of the statistical test. Added nutrients (treatments), seasons (wet and dry), CO_2 sources (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

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

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 fit based on Akaike Information Criteria (AIC). All the statistical data analyses were performed using R 3.6.3 (R Development Core Team, 2019). Throughout the paper, statistical significance in all the tests was inferred if $p \leq 0.05$.

3 Results

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

Soil characteristics did not significantly differ between the nutrient treatment plots and the control; hence, the parameters presented in Table 1 represent the soil physico-chemical characteristic for the NME site.

Table 1. Mean (± SE, n = 32) soil 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
$\delta^{15}N$ (‰)	8.4 ± 0.3	9.2 ± 0.2	9.5 ± 0.2
$\delta^{13}C$ (‰)	-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.

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

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), 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; $p = 0.016$, N + P; $p = 0.044$) and wet (N; $p = 0.015$, N + P; $p = 0.050$) seasons (Fig. 1a). Soil temperature ranged between 20.1 and 22.2 °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 varied between 41 and 63 mg N kg^{-1} in the dry season and between 32 and 54 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 from the N ($p = 0.007$) and N + P ($p = 0.024$) 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 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 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	-30.5 ± 4.9^{a}	-2.7 ± 0.4	20.5 ± 3.2^{a}	1.8 ± 0.3
N	186 ± 6.5^{a}	16.4 ± 0.9	-39.7 ± 4.4^{a}	-3.4 ± 0.4	50.2 ± 11^{bc}	4.2 ± 1.5
P	186 ± 5.3^{a}	16.4 ± 1.0	-56.2 ± 3.8^{b}	-4.7 ± 0.7	21.8 ± 2.4^{a}	1.9 ± 0.3
N + P	197 ± 5.4^{b}	17.3 ± 0.8	-39.3 ± 6.3^{a}	-3.3 ± 0.7	53.8 ± 10^{bc}	4.1 ± 0.4

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.

3.2 Soil CO_2 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 (Fig. 3a, Fig. 4a, d) with the highest CO_2 fluxes 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). There was no significant effect of fertilization on background CO_2 fluxes between nutrient addition treatments and the control plots (Fig. 4d).

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

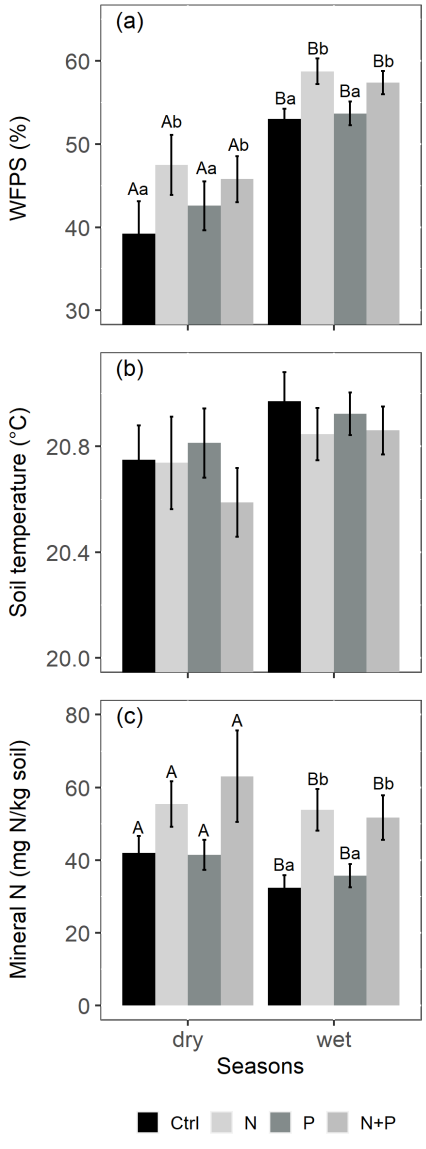

Figure 1. Mean (± SE, n = 4) water filled pore space (WFPS) (a), soil temperature (b), and mineral N (c) in the top 0.05 m of the control (Ctrl), nitrogen (N), phosphorus (P), and N + P plots of a nutrient manipulation experiment measured during the dry (January and February; monthly precipitation < 100 mm) and wet (March to December; monthly precipitation > 100 mm) seasons. Different lower-case letters indicate significant differences between treatments and the 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 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

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

(microbial) CO_2 effluxes were significantly higher than the autotrophic (root) CO_2 effluxes (Fig. 5, $p < 0.001$) across all treatment plots with microbes contributing about three times more to the total soil CO_2 effluxes compared to roots (Fig. 5, $p < 0.001$). Neither heterotrophic nor autotrophic respiration significantly differed between treatments (Fig. 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 across all treatment plots (Fig. 6a, b, c).

3.3 Soil CH_4 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} and a release of 77 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 %; $p = 0.052$) (Fig. 4b). Beyond 28 days from fertilization, no significant difference 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, $p = 0.010$). Soil CH_4 consumption in the dry season was on average 1.5 times larger than the wet season across all treatments (Fig. 4e, $p = 0.007$). 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 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 (Fig. 6f) were also significant but negatively correlated.

3.4 Soil N_2O fluxes

Soil N_2O fluxes across treatments, phases (transitory and background), and seasons varied between an uptake of -18 and a release of 507 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 % in N plots ($p < 0.001$) and 419 % in the N + P plots ($p < 0.001$) compared to the control plots in the transitory phase (Fig. 4c). The soil N_2O peaks in September 2019 and June 2020 (Fig. 3c) coincided with the peaking in soil mineral N 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 ± 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 (Fig. 6i) in all treatment plots.

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Preprint. Discussion started: 4 January 2021

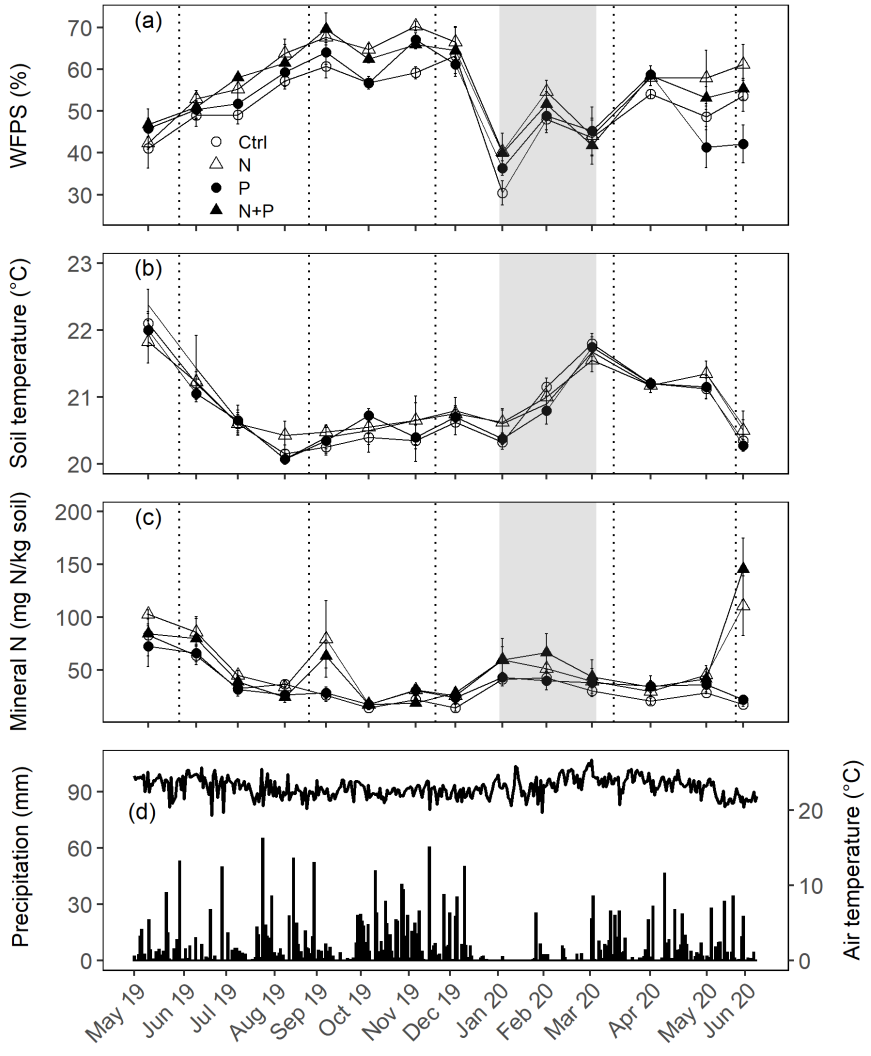

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Figure 2. Mean (± SE, n = 4) water filled pore space (a), soil temperature (b), and mineral N (c) in the top 0.05 m measured monthly (May 2019 to June 2020) from control (Ctrl), nitrogen (N), phosphorus (P), and N + P plots of the nutrient manipulation 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. 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.

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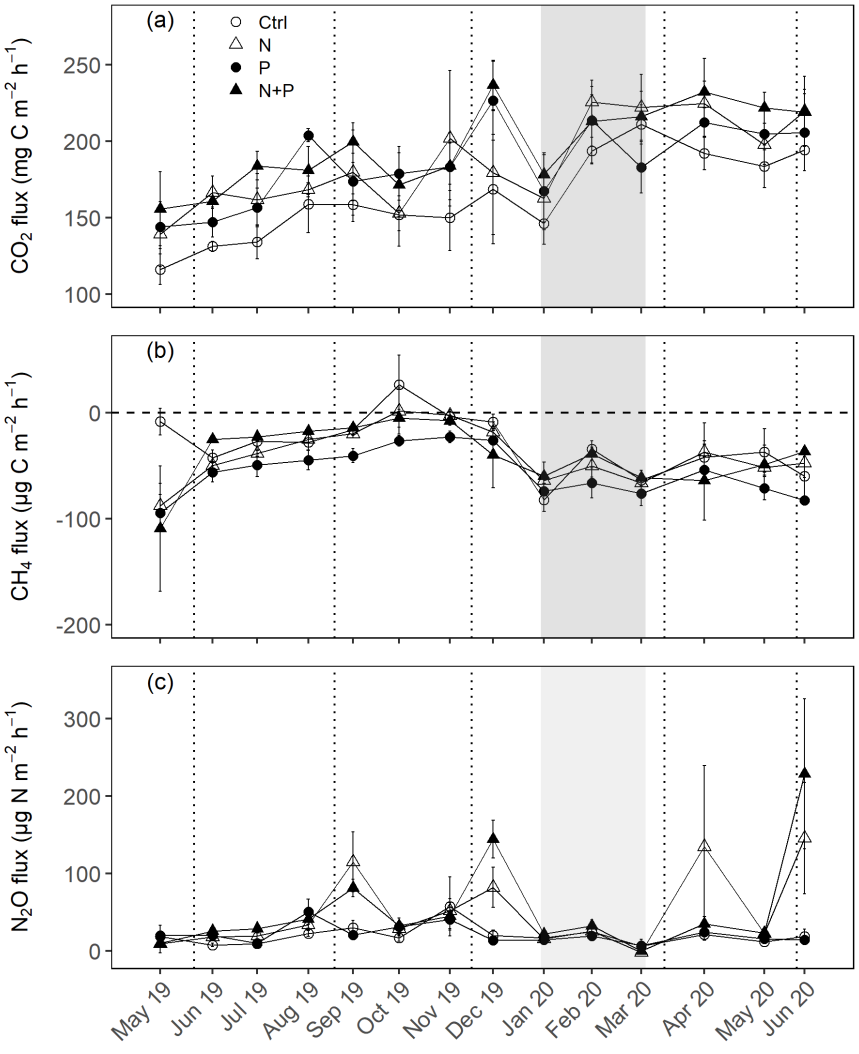
Preprint. Discussion started: 4 January 2021



325 **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. The gray shaded rectangle marks the beginning and end of the dry season (January and February; monthly precipitation < 100 mm).**

330

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

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.

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 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 mirobial 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 experimental site complexity is further exemplified by the lack of a relationship between all the measured soil environmental controls (soil temperature, mineral N 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 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.

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

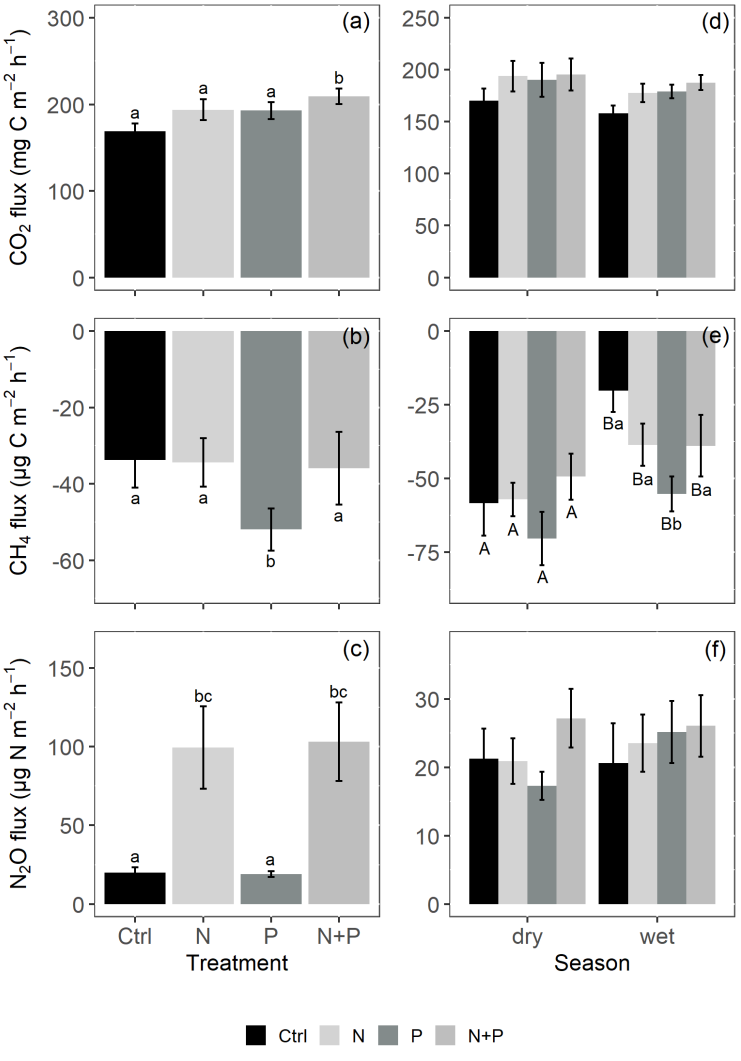


Figure 4. Mean (± SE, n = 4) soil CO_2 fluxes (a, d), CH_4 fluxes (b, e), and N_2O fluxes (c, f) from the control (Ctrl), nitrogen (N), phosphorus (P), and N + P plots of a nutrient manipulation experiment. Column 1 (a, b, and c) includes only fluxes measured during the transitory phase (0 to 28 days 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; $p \leq 0.05$*).

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Preprint. Discussion started: 4 January 2021

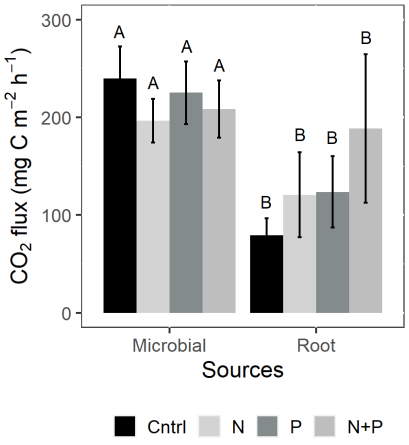

Figure 5. Mean (± SE, n = 4) soil CO_2 flux from the control (Ctrl), nitrogen (N), phosphorus (P), and N + P plots of a trenching treatment separated into microbial and root sources. Different upper-case letters indicate significant differences between microbial and root contribution to total CO_2 flux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

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., 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 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 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 and background CH_4 fluxes (Fig. 6f) indicates that increases in mineral nitrogen should increase CH_4 uptake. Additionally, the lack of a clearer signal in background CH_4 uptake may have to do with the high

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

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

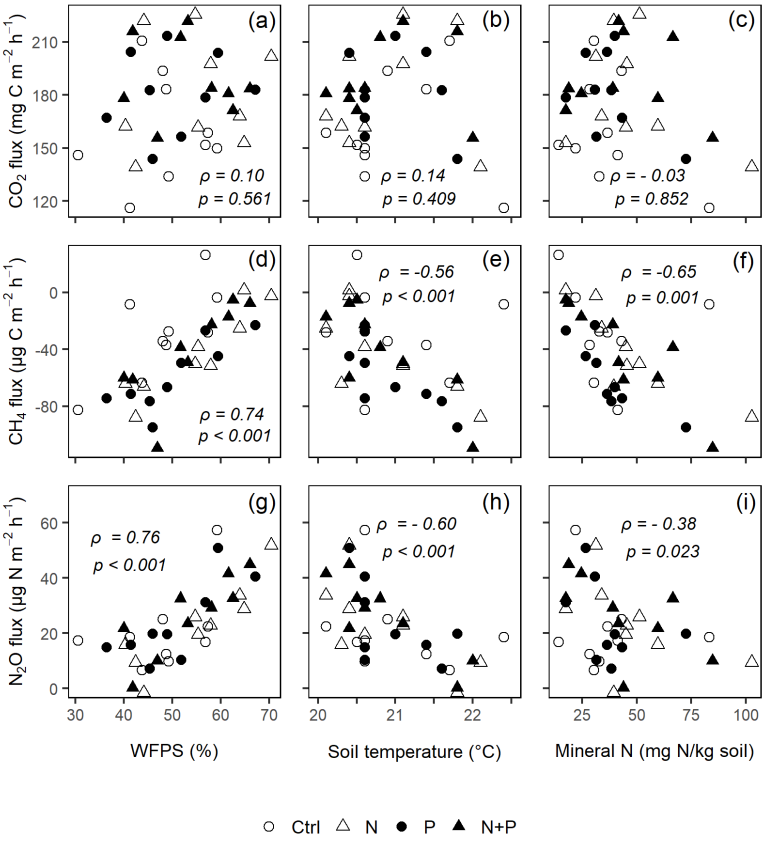

Figure 6. Spearman's correlation coefficient between mean background CO_2 (a-c), CH_4 (d-f), and N_2O (g-i) fluxes and WFPS (column 1), soil temperature (column 2) and mineral nitrogen (column 3) using monthly measurement means of four replicate treatment plots taken between May 2019 and June 2020 ($p \leq 0.05$, 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_2O fluxes

The annual soil N_2O 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.

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

The immediate flush of N_2O 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_2O fluxes from N addition plots (Fig. 4f). This was unexpected, but given the rapid drainage at the site (sandy texture, Table 1), there could have been substantial loss of added N via leaching, which possibly rid the ecosystem of excess mineral N (Lohse and Matson, 2005; Martinson et al., 2013). Notably, sustained P addition did not result in increased background N_2O fluxes (Fig. 4f), which contrasts the findings by Mori et al. (2017) who reported that P availability opens up the N cycle and leads to increased N_2O emissions. At this study site, it could be that either the amount of P added in the experiment was not sufficient to trigger a response in background soil N_2O fluxes or P is not a limiting nutrient for N_2O fluxes given the relatively high pH of the site (Table 1).

Unexpectedly, mineral N correlated negatively to background N_2O fluxes (Fig. 6i), yet many studies (e.g. Corre et al., 2014; Zhang et al., 2020) have found that mineral N and N_2O fluxes were positively correlated. The likely explanation for such a relationship is the transformation of N_2O to N_2 under wet conditions, which further reduced the amount of mineral N (particularly NO_3^-) in soil (Matson et al., 2017). Despite the minimal influence of seasonality on background N_2O fluxes (Fig. 4f), a strong positive correlation between background N_2O 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_2O fluxes. Soil aeration not only directly controls oxygen entry into the soil but also determines how N_2O is produced (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 ≤ 60 %, Fig. 6g), the considerable N_2O fluxes at higher WFPS values (≥ 60 %, Fig. 6g) seem to suggest that denitrification is more dominant than nitrification in producing N_2O in these biomes.

5 Conclusion

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, N + P) significantly increased N_2O fluxes immediately after fertilization, while no effect was found for background N_2O fluxes. 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. 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 affected by P fertilization. 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

https://doi.org/10.5194/soil-2020-94
Preprint. Discussion started: 4 January 2021

455 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.

460 **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.

465 **Data availability.** Data is available on request

Acknowledgement. We thank F. Babweteera and Budongo Conservation Field Station management for hosting the nutrient manipulation experiment, providing us with working space and the climatic data. Special thanks goes to Johan Six's laboratory ETH-Zürich for analyzing the gas samples. We are grateful to the German Academic Exchange
470 (DAAD) (grant number: 57381412) for JT's stipendium and meeting his travel costs between Uganda and Germany. We also thank the International Foundation of Science (IFS) for the financial support (grant number: D/6293-1) towards JT's fieldwork in Uganda, and the National Agricultural Research Organization (NARO) for the institutional support and administration of the IFS grant. We thank the DFG funded Emmy Noether Junior Research Group "TropSOC" (Gepris - project number 387472333) for the additional support towards this study and the DFG funded
475 Individual Research project (RELIANCE; grant number STR 1375/1-1) for setting up the nutrient manipulation experiment. Lastly, we thank G. B. Ayo and M. Adriko for supporting our field measurements.

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