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Nitrous oxide emission from pigeon pea – maize rotation in response to conservation agriculture and biochar amendments in a Ferralsol, northern Uganda

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Abstract

Smallholder agriculture in sub-Saharan Africa (SSA) commonly involves limited use of mineral or organic fertilizer, often resulting in severe nutrient limitation. Conservation Agriculture (CA), including crop rotation with legumes and biochar amendments, has been advocated to enhance soil fertility and plant available nitrogen (N). However, CA may affect nitrous oxide (N_2O) emissions even in unfertilized agroecosystems. N_2O is an important greenhouse gas, and understanding the trade-offs between N_2O emissions and crop yields in N-poor agroecosystems in SSA is essential. Here we studied crop yield, soil N and N_2O emissions in a double cropping system (pigeon pea – maize rotation) throughout two consecutive cropping seasons (April-October 2023 and October 2023-January 2024) in a Ferralsol in Northern Uganda. The study, conducted at a site which had been left fallow for 3 years, involved pairwise comparison of conventionally tilled systems under crop rotation

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(Conventional) and continuous maize monocropping (ConventMM). In addition, the effect of tillage systems (Conventional, CA and CA+biochar) under pigeon pea – maize rotation was investigated. We defined CA as reduced tillage with planting basins and crop residue retention, whereas conventional tillage involved overall ploughing. Grain yield was not significantly affected by rotation or tillage system. N_2O fluxes were small, ranging from 1.02 – 51.19 µg N m^2 h^{-1} over the entire period. Short-lived emission peaks were observed following pigeon pea harvest in the crop rotation, which were absent in maize monocropping. Overall, across all seasons, cumulative growing-season (279 days) N_2O emissions ranged from 0.44 – 1.11 kg N ha^{-1}. Biochar amendments in CA systems did not affect daily N_2O emissions in planting basins. In the first season, yield-scaled N_2O emissions and N yield scaled N_2O emissions were significantly smaller in CA systems with biochar compared to conventional tillage, suggesting that CA and biochar was effective in minimising emissions without penalising pigeon pea productivity.

Key words: N_2O, reduced tillage, legume-cereal rotation, biochar, low input systems.

1. Introduction

Nitrous oxide (N_2O) is a long-lived tropospheric greenhouse gas (GHG) with a lifetime of 116 years, and a global warming potential approximately 300 times greater than that of carbon dioxide (CO_2) (Tian et al., 2020). Atmospheric N_2O is also implicated in the breakdown of ozone in the stratosphere (Portmann et al., 2012). On a global scale, agriculture is a major source of atmospheric N_2O, contributing approximately 60% to global N_2O emissions (Adegbeye et al., 2020; Kim et al., 2016). In sub-Saharan Africa (SSA), N_2O emissions are mainly associated with forest clearing, livestock manure, and crop production (Boateng et al., 2019; Hickman et al., 2011). Although acidic soils tend to have high N_2O emissions (Wang et

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al., 2018), the limited N inputs in smallholder farming systems in SSA reduces soil N availability, consequently leading to relatively low N_2O emissions. Only ~3% of the globally applied inorganic fertilizer, a key driver for soil N_2O emissions, is used in Africa (Hickman et al., 2011). The recent 2024 Nairobi Declaration, targeting increased fertilizer use in Africa (Africa Union, 2024), might change future trajectories of fertilizer consumption in SSA, potentially increasing soil N availability and N_2O emissions over time. Besides increasing mineral N fertilization, additional plant-available N can be derived from introducing legumes in crop rotation (Jensen et al., 2012), or using organic fertilizers such as animal manure. These strategies are central to conservation agriculture (CA) in subsistence farming systems, but little is known about how they affect baseline N_2O emissions. Also, climate smart practices such as biochar amendments, have been proposed to enhance crop yield and soil fertility (Namatsheve et al., 2024; Schmidt et al., 2021) and to reduce N_2O emissions (Zhang et al., 2021).

Nitrous oxide is an intermediate or by-product in soil N transformations, that include nitrification, denitrification and nitrifier denitrification (Meier et al., 2020). The biogeochemistry of N_2O in soil is to a large extent regulated by complex interactions between environmental and biological factors such as temperature, water, oxygen levels, acidity and substrate availability (Case et al., 2015; Tian et al., 2020). Nitrification occurs under predominately aerobic soil conditions, whereby autotrophic bacteria or archaea oxidize NH_4^+ to NO_2^-, which is further oxidized to nitrite (NO_3^-) by nitrate-oxidizing bacteria (Dick et al., 2008; Fungo et al., 2019). Denitrification occurs in predominately anaerobic soils, or soil aggregates, and is an anoxic respiratory process mediated by bacteria and some fungi, reducing NO_3^- stepwise to N_2 via the intermediates NO_2^-, NO and N_2O (Saggar et al., 2013; Scheer et al., 2020). Nitrifier denitrification occurs when nitrifying bacteria reduce NO_2^- under hypoxic conditions, analogously to the denitrification pathway (Wrage-Mönnig et al., 2018).

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In a quest to improve crop production and soil fertility, sustainable agricultural practices such as conservation agriculture (CA) and biochar amendment have been promoted in SSA (Namatsheve et al., 2024). Conservation agriculture may improve crop production (Giller et al., 2015; Hobbs et al., 2008) and is based on three core principles: the first principle is minimum soil disturbance, which may enhance water retention and soil organic matter content (Pittelkow et al., 2015; Powlson et al., 2011). In addition to increased crop production, this may lead to increased N_2O emission (Guenet et al., 2021; Shakoor et al., 2021). Availability of N in non-fertilized systems can be improved through the second principle of CA, which involves incorporating legumes in cereal dominated farming systems (Namatsheve et al., 2021) and the third principle, crop residue retention (Fang et al., 2007; Turmel et al., 2015), both of which may stimulate N_2O emissions (Abalos et al., 2022). Mitigation of N_2O emission in low-input crop production systems ultimately depend on synchronizing the release of mineral N from legumes and crop residues with N uptake by crops. Besides CA, also, biochar, a carbon rich material produced by pyrolysis of organic waste has been claimed to enhance yields (Cornelissen et al., 2016; Lehmann, 2007). Interest in biochar emerges from pioneering research on Brazil's *Terra Preta* soils, which were enriched over time by repeated application of charcoal and organic matter, resulting in soils with a stable organic matter pool (Glaser et al., 2001). Biochar tends to increase soil pH which favours N_2 over N_2O as a main product of denitrification (Wang et al., 2018). Although biochar contributes to the retention of exchangeable plant-available NH_4^+, it also may immobilize soil N (Nguyen et al., 2017), thereby reducing N availability and N_2O emission (Jeffery et al., 2015; Namatsheve et al., 2024). However, Weldon et al., (2022) reported that the magnitude for sorption capacity of biochar for NH_4^+ is low, with a huge uncertainty range. In addition, the role of biochar on N cycling in unfertilised tropical soils remains unclear (Namatsheve, 2025).

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Indeed, earlier studies reported increased N_2O emissions in SSA under CA (Baggs et al., 2006; Raji and Dörsch, 2020; Shumba et al., 2023), while biochar amendments have been shown to reduce N_2O emissions (Fungo et al., 2017, 2019; Namoi et al., 2019). However, these studies were carried out in systems that received inorganic N fertilizers, which do not represent the realities of unfertilized smallholder tropical agroecosystems typical of Uganda and other countries in SSA. Our meta-analysis study indicated that residue retention increases NO_3 availability and subsequently N_2O emissions (Namatsheve et al., 2024). Building on this, we recently demonstrated that integrating biochar into CA systems enhanced biological N_2-fixation of pigeon pea in unfertilized systems of Uganda (Namatsheve et al., 2025). This raises questions on the implication of the additional N from biological N_2-fixation on NO_3 and N_2O emissions in unfertilized systems, with tight N cycling. As far as we know, there are no published studies that examine the synergy of CA and biochar on N_2O emission in unfertilized tropical agroecosystems.

In this study we investigated the effect of conservation agriculture on grain yield, N_2O emissions, mineral N dynamics, and yield-scaled N_2O emissions on an unfertilized Ferralsol in northern Uganda over two consecutive cropping seasons. Specifically, we compared crop rotation (pigeon pea – maize) with maize monocropping under conventional tillage (overall digging). In addition, we compared pigeon pea – maize rotation under three practices, i.e. conventional tillage, CA (reduced tillage), and CA in combination with biochar (CA+biochar). We hypothesised that rotation with pigeon pea increases N_2O emission compared to maize monocropping, while CA+Biochar reduces N_2O emissions, both compared to CA and to conventionally tilled soil.

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2. Methodology

2.1. Site description

A field experiment was carried out in Gulu, Northern Uganda (*2° 47′ 46″ N, 32° 20′ 45″ E*). Uganda has a bimodal rainfall pattern and eight distinct agro-ecological regions, where Gulu lies in the Northern savannah grasslands (Mubiru et al., 2012). Soils in Gulu are acric Ferralsols, and the texture is a loamy sand (Wortmann and Eledu, 1999). Average soil organic C and total N are 1.52% w/w and 0.11% w/w, respectively, while average soil pH is 6.71 (Table S19). The research site has a double cropping system i.e., one during the first rain season from April to August and a second one during the second rain season August to December, a dry period is from December to February. The average annual temperature is 24 °C, and the annual rainfall in 2023 was 1238 mm, of which 818 mm was received in the first season and 419 mm in the second. The weather data were obtained from the Gulu weather station which is about 6 km from the experimental site.

2.2. Experimental design, crop establishment and management

The experiment was established on a field that has been a fallow for the 3 previous years; before that it was used for maize and cassava production without mineral fertilizer. Prior to the establishment of the experiment, a dense vegetation of grasses was removed by slashing and chemical weeding using glyphosate [N-(phosphonomethyl) glycine]. Plots (6 m × 5 m) under conventional management were prepared by overall digging using hand hoeing (100% tillage) and plots of the same size under CA by manually digging 10-L planting basins (35cm long × 15cm wide × 20cm deep) spaced 70 cm × 35 cm (interrow × within row spacing). Given these basin dimensions about 10% - 12% of the land under CA was tilled. The experiment had four treatments, replicated four times, and randomised in a complete block design (RCBD). The treatments were ConventMM, Conventional, CA and CA+BC (Table 1). Biochar was

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homogeneously mixed into the basins of the CA+BC plots when preparing the planting basins before sowing. In the treatments with crop rotation, pigeon pea was sown in the 1st season, and maize in the 2nd season. Maize monocropping had maize in both seasons. Dates of sowing and harvesting are indicated in Fig. 1a.

A pigeon pea variety SEPI 1 (bred at ICRISAT, Malawi and released by the National Agricultural Research Organisation, Uganda) was sown uniformly in Conventional, CA and CA+BC treatments. SEPI 1 is a medium maturity variety, with 77 – 87 days to flowering and 105 – 139 days to 75% maturity. It is an indeterminate variety with semi-branching growth, the main stem continuing to elongate indefinitely; potential grain yields range from 1.8 – 3.4 Mg ha^{-1}. A maize variety, Longe 10H, which is a hybrid variety with 100 – 120 days to maturity and a yield potential of 7 – 9 Mg ha^{-1} was sown in the ConventMM treatment (1st season) and in all treatments in the 2nd season. During sowing in CA treatments, three seeds were planted in each basin spaced 10 cm from each other giving a total planting population of 56 000 plants per ha, equalling planting population in conventional tillage treatments.

To mimic subsistence farming systems in Uganda, no inorganic fertilizer was applied. For CA, weeds were controlled by spraying glyphosate at a rate of 1.03 L ha^{-1}, immediately after sowing and hand pulling throughout the season. Weed control in the conventional treatment was done by hand hoeing at planting and throughout the season.

2.3. Biochar production and application

Biochar was prepared from pigeon pea stems and twigs using the flame curtain “Kon Tiki” kiln (Cornelissen et al., 2016; Munera-Echeverri et al., 2020). The kiln consists of a conically shaped pit with a depth of 1m and a diameter of 3 m. The pyrolysis temperature was 600 °C. After weighing, the pigeon pea feedstock was pyrolyzed, quenched with water, covered with

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banana leaves and soil, and recovered after 3 days. The biochar was weighed (dry matter), ground and packed. The feedstock to biochar conversion ratio was 4:1, and the biochar had a pH of 9.74, carbon (C) concentration of 51%, nitrogen (N) concentration of 0.76%, cation exchange capacity (CEC) of 80.94 $cmol_c\ kg^{-1}$ and plant available P of 703 mg kg^{-1} (Table S8).

During biochar application, manually dug 10-L planting basins (35cm long × 15cm wide × 20cm deep) spaced 70 cm × 35 cm (interrow × within row spacing) were opened and the soil was mixed evenly with 1 litre of biochar (240g w/w) per basin for the CA+BC treatment, and 500 ml of biochar (120g w/w) for the CA+BC+BC treatment in the first year, and the other 120g w/w in the second year. Afterwards, the planting basins were covered with a thin layer of soil.

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Table 1: Treatment description and management of the experiment site at Gulu, Northern Uganda

Treatment name and abbreviation	Treatment description and management
1. Conventional tillage, maize monocropping, no rotation (ConventMM)	Shallow conventional tillage by hand hoeing (overall digging) to a depth of 10 cm (100% tillage). Maize was grown in both seasons. Crop residues were left on the soil surface and spread evenly after harvesting. Plant spacing 70 cm × 35 cm (interrow × within row spacing). Weed control by hand weeding during sowing and throughout the season.
2. Conventional tillage, pigeon pea – maize rotation (Conventional)	Shallow conventional tillage by hand hoeing (overall digging) to a depth of 10cm (100% tillage). Pigeon pea – maize rotation; crop residues were left on the soil surface and spread evenly after harvesting, while stems were removed from the field. Plant spacing 70 cm × 35 cm (interrow × within row spacing). Weed control by hand weeding during sowing and throughout the season.
3. Basins, pigeon pea – maize rotation, incorporating residues (CA)	Manually dug 10-L sized planting basins (approximately 35 cm long, 15 cm in diameter and cm deep) created by hand hoeing at the beginning of the experiment with a spacing of 70 cm × 35 cm (interrow × within row spacing). Pigeon pea – maize rotation; crop residues were left on soil surface and spread evenly after harvesting. Weed control by herbicides (glyphosate) during sowing and hand pulling throughout the season.
4. Basins, pigeon pea – maize rotation, incorporating residues, 4 Mg ha^{-1} of biochar applied once (CA+BC)	As CA, but with biochar mixed into the basins at a rate of 4 Mg ha^{-1} during the first season, before sowing.

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2.4. Soil sampling and analysis for chemical characterization

Soils were sampled before establishing the trials in March 2023 (background sampling) for general characterisation of the research site. Using an auger, 3 samples from 0-20 cm depth were randomly taken from the experimental site and mixed into a single composite sample (Table S19). Plot wise sampling was carried out at the onset (April 2023) and end (October 2023) of the first growing season from planting basins in CA and CA+Biochar treatments and in the planting rows in conventional treatments to assess the effect of different treatments on soil properties. The soils were sampled at 0 – 20 cm depth in each plot. Prior to analysis, samples from each treatment in each of the four blocks were bulked (viz., n=4 for the onset and n=4 for the end for each treatment), air dried and passed through a 2 mm sieve. Soil pH was determined in water (2.5:1) (Gee and Bauder, 1986). Soil organic carbon (SOC) and N was determined using a Thermo Finnigan EA attached to the Elemental Analyzer Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS).

2.5. Nitrous oxide flux sampling and analysis

The static chamber method was used to estimate N_2O emissions. We used cylindrical 20 cm high, custom-made PVC chambers manufactured from 16 cm diameter, grey opaque sewage pipes with a self-sealing rubber septum on the top for gas sampling. Permanent gas sampling plots were established by inserting 17 cm diameter PVC rings (the base) to a depth of 7 cm into the soil in April 2023, during sowing in the 1st season. We used two chamber positions in each plot. One was placed in the interrow, between two rows and another in-row between two plants within a row or in the planting basin (CA) (Fig S1, S2).

The chambers were deployed by carefully inserting them 3 cm into the pre-installed collars to obtain an airtight fit. To facilitate chamber deployment, the contact area between the collar and

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chamber was sealed with a thin layer of petroleum jelly. Each chamber covered an area of 0.020 m^2 and had a total headspace volume of 0.004 m^3. For each flux measurement, four gas samples were drawn from the chamber headspace 1, 15, 30 and 60-minutes after deployment using a 20 mL polypropylene syringe equipped with a three-way valve. Collected gas samples were transferred to pre-evacuated 12 mL glass vials, which were crimp-sealed with butyl septa. When sampling the chamber headspace, the plunger of the syringe was moved slowly in and out for three times to mix the gas and obtain a representative sample. Air and chamber temperature was recorded before removing the chambers using a handhold thermometer which was placed inside a chamber, before and after sampling.

Gas sampling was done approximately biweekly, resulting in 17 sampling campaigns between May 2023 and January 2024. The vials were shipped to the Norwegian University of Life Sciences for CO_2 and N_2O analysis by gas chromatography. CO_2 measurements were done for quality check. He-filled vials were included as blanks to check for contamination during storage and shipment of the vials. The vials were analysed on a gas chromatograph (GC; model 7890A, Agilent, Santa Clara, CA, USA) connected to an auto-sampler (Gilson). N_2O was quantified by an electron capture detector and CO_2 by a thermal conductivity detector as described by (Žurovec et al., 2017).

Flux rates were estimated by fitting a linear or second order (polynomial) function to the concentration change over time in the chamber headspace. Changes in concentration were converted to area flux as follows:

$$F = \frac{dc}{dt} . \frac{Vc}{A} . \frac{Mn}{Vn} . 60, \quad (1)$$

where F is the N_2O flux (μg N_2O-N m^{-2} h^{-1}), $\frac{dc}{dt}$ the rate of change in concentration over time in the chamber headspace (ppm min^{-1}), Vc the volume of the chamber, 0.004 m^3, A the area

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covered by the chamber, 0.020 m^2, Mn the molar mass of N in N_2O (g mol^{-1}) and Vn the molecular volume of N_2O or CO_2 at chamber temperature (m^3 mol^{-1}). A quadratic fit was used if N_2O concentration in the chamber showed a convex downwards trend with time. Fluxes were cumulated plot-wise by linear interpolation. The cumulative N_2O emissions (kg N_2O-N ha^{-1}) were calculated as follows:

$$cumulative\ N_2O = \sum(f_i + (f_{i+1})/2 \times (t_{i+1} - t_i) \times 24 \times 10^{-5} \qquad (2)$$

where f represents the N_2O flux (μg N_2O-N m^{-2} h^{-1}), i the ith measurement, $(t_{i+1} - t_i)$ the number of days between two adjacent measurements, and 24×10^{-5} was used for unit conversion. We also scale up the N_2O-N cumulative emissions to hectare using a scaling factor of 0.12 for basin and 0.88 for interrows in CA treatments, while 0.50 was used for both inrows and interrows in conventional treatments.

2.6. Soil moisture and mineral N content

Directly after each flux sampling, soils were sampled from both planting stations (Conventional and ConventMM) and basins (CA and CA+Biochar) and from interrow positions (all treatments), for analyzing mineral N (NO_3-N and NH_4^+-N) and soil moisture. Soils were sampled from 0-20 cm depth, using a 10 mm diameter corer with a height of 20 cm. Only one core was taken to prevent excessive perturbation, particularly in the planting basins. The cores were stored in a cooling box on ice and shipped to Gulu University which is located 5 km from the experimental site. Mineral N was extracted from 11g of field moist soil in 40 ml of 2M potassium chloride (KCl), after 1 hour of horizontal shaking at 200 strokes per minute and passing the supernatant through Whatman filters grade 589/3. The supernatants were frozen for subsequent analysis of NO_3-N and NH_4^+-N at the Norwegian University of Life Sciences by flow injection analysis (FIA star 5020, Tecator, Sweden).

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The remaining soil was dried at 105°C for 72h to determine gravimetric moisture content and bulk density (BD). BD was calculated by dividing weight of oven dried soil with the volume of the soil core (15.714 cm^3) and its gravimetric soil moisture content calculated by dividing weight of water (difference between fresh soil weight and oven dried soil) by the weight of oven dried soil.

The bulk density (BD) was then used to calculate water filled pore space (WFPS) as follows:

$$WFPS = \frac{\theta g \times BD}{\left(1 - \frac{BD}{PD}\right)} \times 100 \qquad (3)$$

where θg is the gravimetric water content, *BD* the soil bulk density (1.29 ± 0.01 g cm^{-3}) and *PD* the soil particle density (2.65g cm^{-3}). Daily rainfall and temperature data were obtained from the Gulu meteorological station which is located 6 km from the experimental site.

2.7. Yield and yield-scaled emissions

Crops were harvested at physiological maturity, 6 months after sowing for pigeon pea and 4 months for maize. To compare crop yields under conventional and CA management and in CA+BC treatments, all values for dry biomass and grain (moisture content of 12.5% for maize and 15% for pigeon pea) were extrapolated from the plot to the hectare. Yield-scaled N_2O emissions (kg N_2O-N kg^{-1} grain yield) and N yield scaled emissions (kg N_2O-N kg^{-1} grain N) were estimated for each season by dividing the scaled cumulative N_2O emissions with grain yield or N content of the grain (N concentration x grain yield). A scaling factor of 0.12 for N_2O emissions in planting basins and 0.88 for N_2O emissions in interrows was used to calculate yield-scaled N_2O emissions in CA and CA+BC treatments. In conventional treatments, scaling factor of 0.50 was used for both inrows and interrows.

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2.8. Data analysis

All data were analysed using R software, version 4.3.2. A random intercept, fixed slope linear mixed-effect model using the *lmer* function from *lme4* packages (Bates, 2010) with treatment, chamber position (interrow and in row) and season as fixed factors was used to evaluate treatment effects on N_2O emissions and soil mineral N. On soil parameters, yield and yield-scaled emissions, fixed factors were treatments and seasons while block was a random factor (Table S1 – S20). Variation associated with sampling day (17 levels) and blocks was modelled by introducing random effects to account for repeated measurement on the same plot, on N_2O fluxes and soil mineral N. The most parsimonious model was selected after model comparisons based on goodness of-fit assessed by the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), and stepwise model reduction (Aho et al., 2014). We assumed normal error structure and homoscedasticity and validated the model assumptions by checking quantile plots of residuals against fitted values (Zuur et al., 2009). Model parameters (estimated marginal means) were extracted using the "*emmeans*" package (Lenth, 2016), and multiple comparisons were performed using multcomp (Hothorn et al., 2008) with adjusted *p* values (Tukey post-hoc test at 0.05 probability level) (Lenth, 2016). The 95% confidence intervals (CI) were retrieved using lsmeans function. Differences between levels of the fixed effects were assessed using multicomp package. Linear regression analyses were performed to analyse the relationship between N_2O fluxes with WFPS and mineral N. Visualization of the fitted models was achieved using the package ggplot2 (Wickham, 2016).

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3.0 Results

3.1. Soil parameters

Soils were near neutral with a background pH of 6.71 (Table S18). During the onset of the first season, soil pH ranged from 6.71 – 6.97. CA+BC significantly ($p < 0.05$) increased pH compared to CA systems at the onset of the first season (Table 2). Generally, pH decreased from the beginning to the end of the first season, after which no significant pH differences among treatments were found. SOC ranged from 1.25 – 2.23% and biochar significantly increased C, from 1.30% in CA to 2.23% in the CA+BC treatment (Table 2). At the end of the first season, CA+BC had significantly higher C than other non-BC treatments. Different treatments did not affect soil mineral N at the beginning and end of the first season (Table 2).

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Table 2: Treatment effects on inrow (conventional treatments) and within planting basin (CA treatments) soil properties (pH, C, N, and BD). Soils were sampled at sowing (onset) and at the end of the first season, in Gulu, Uganda. Means are shown with standard errors of means (N=4). Lowercase letters compare treatments at onset or end of sampling, while uppercase letters compare change between samples taken at onset and end of the season. Different letters represent significant differences ($p < 0.05$), determined at 5% level using Tukey test.

	pH		C (%)		N (%)	
Treatment	onset	end	onset	end	onset	end
Conventional	6.64±0.14 b A	6.36±0.10 a A	1.41±0.06 b A	1.31±0.07 b A	0.11±0.01 a A	0.10±0.00 a A
CA	6.61±0.08 b A	6.35±0.07 a A	1.30±0.04 b A	1.25±0.05 b A	0.10±0.01 a A	0.09±0.00 a A
CA+BC	6.97±0.13 a A	6.28±0.20 a B	2.23±0.27 a A	2.15±0.11 a A	0.11±0.01 a A	0.12±0.00 a A
ConventMM	6.71±0.04 ab A	6.59±0.12 a A	1.52±0.18 b A	1.35±0.06 b A	0.12±0.01 a A	0.10±0.00 a A
P-value	<0.001	0.364	<0.001	*<0.001*	0.569	*0.569*

3.2. N_2O emission dynamics

Treatment and chamber position significantly ($p<0.05$) affected N_2O hourly fluxes (Table S2, S4). N_2O fluxes were small in all treatments throughout the entire observation period (Fig. 1a) with treatment averages ranging from 1.02 – 51.19 µg m^{-2} h^{-1}. The fluxes peaked in mid-October following pigeon pea harvest and sowing of maize, were conventionally tilled soil with pigeon pea – maize rotation (Conventional) had the largest emissions, while conventional tillage with maize monocropping (ConventMM) showed a far less pronounced N_2O emission peak in October. N_2O emissions levelled off towards the end of the second season, in January, coinciding with a longer dry spell (Fig 1a).

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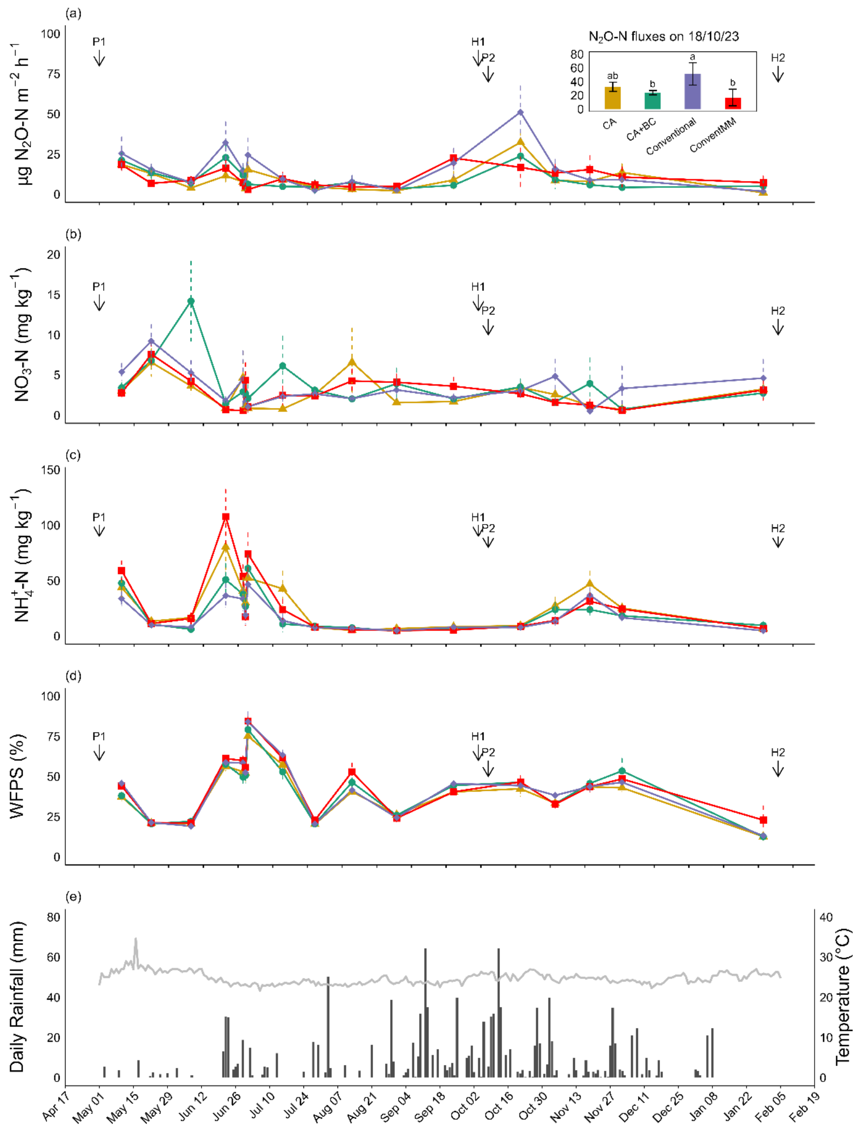

Fig 1: Mean (± se) of (a) N_2O emission fluxes, (b) KCl-extractable NO_3^- and (c) NH_4^+, (d) WFPS, as well as (e) daily rainfall and air temperature (°C) during the two cropping seasons between May 2023 – January 2024 in Gulu, Uganda. P1 and P2 indicate planting date for the 1st and 2nd second season (18 April 2023 and 05 October 2023, respectively). The data is based on 8 observations, average of 2 chamber positions and 4 blocks. H1 and H2 indicate harvesting dates (01 October 2023 and 30 January 2024, respectively. The insert in (a) shows mean ± se N_2O fluxes during peak emission on 18 October 2023.

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3.3. Mineral N dynamics

NO_3^-N concentrations ranged from 0 – 15 and 0 – 5 mg kg^{-1} in the first and second season, respectively (Fig 1b), while NH_4^+ ranged from 10 – 110 and 10 – 45 mg kg^{-1} in the first and second respectively (Fig 1c). Generally, both NH_4^+ and NO_3^- were more variable in season one than in season two (Fig 2). The rotation effect on NH_4^+ was significant in the first season ($p < 0.05$), with more extractable NH_4^+ in the ConventMM (31.22 mg kg^{-1}, 20 – 42.40 CI) than the Conventional treatment (19.20 mg kg^{-1}, 8 – 30.50 CI). (Fig 2a).

Tillage system significantly ($p < 0.05$) affected NH_4^+, CA (27.19 mg kg^{-1}, 22.5 – 31.9 CI) increased NH_4^+ compared to other treatments. NO_3^- ranged from 0 – 14 mg kg^{-1} and there was no significant ($p > 0.05$) season and treatment effect (Fig 3b). N_2O fluxes were affected by Mineral N (NH_4^+ and NO_3^-), but with a weak coefficient of determination ($R^2 = 0.04$, $p < 0.001$) (Fig 4).

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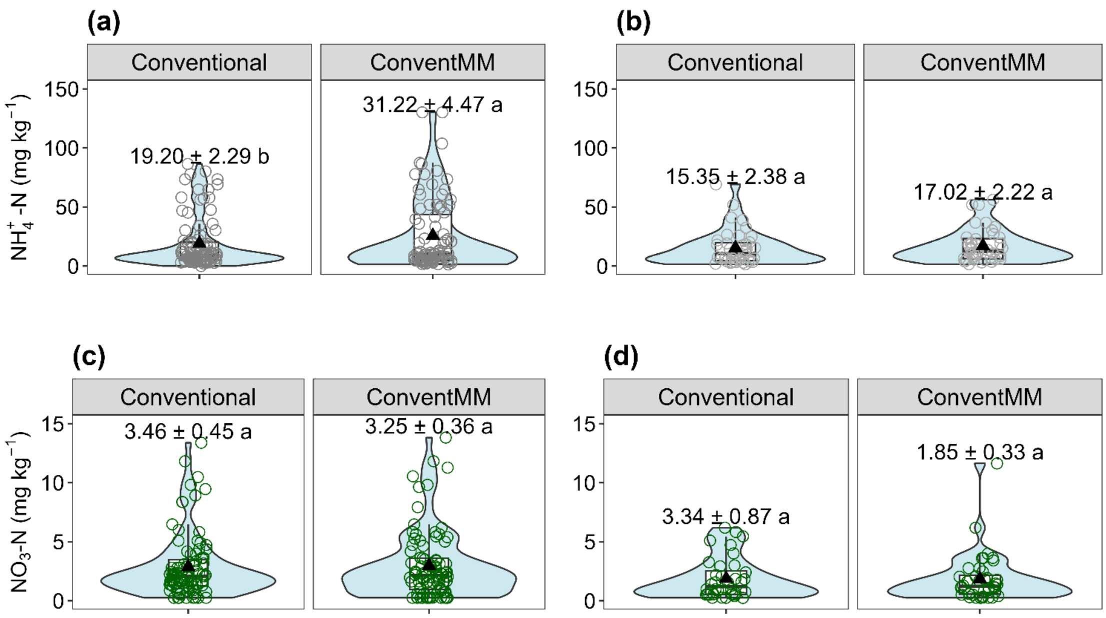

Fig 2: Violin and box-whisker plot showing the effect of rotation on NH_4^+ in season 1 (a) and season 2 (b), and NO_3^- in season 1 (c) and season 2 (d) in Gulu, Uganda. Upper and lower edges of boxes indicate 75th and 25th percentiles, horizontal lines within boxes indicate median, whiskers below and above the boxes indicate the 10th and 90th percentiles, and triangles indicate arithmetic mean. Differences between treatments were tested using the Tukey post-hoc test. Different letters represent significant differences ($p < 0.05$).

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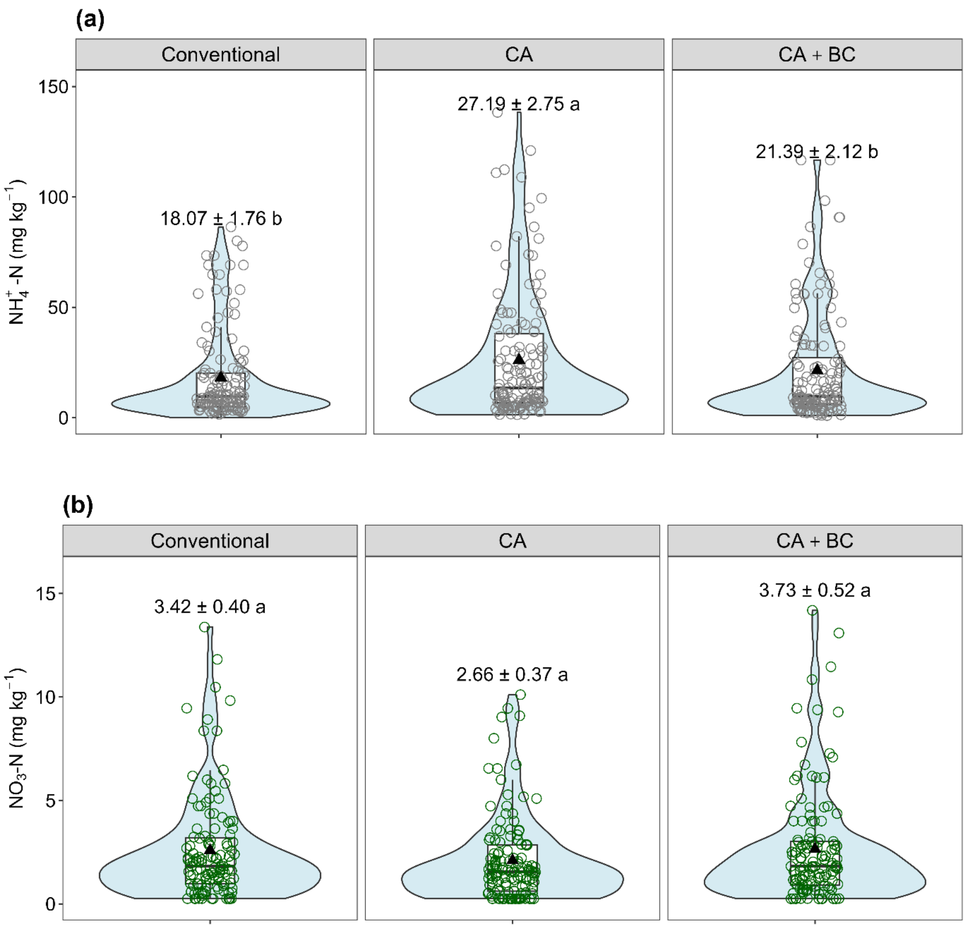

Fig 3: Violin and box-whisker plot showing the effect of tillage systems on (a) NH_4^+ and (b) NO_3^- for both seasons in Gulu, Uganda. Upper and lower edges of boxes indicate 75th and 25th percentiles, horizontal lines within boxes indicate median, whiskers below and above the boxes indicate the 10th and 90th percentiles, and triangles indicate arithmetic mean. Data plotted is the average of season 1 and 2. Significant differences (p <0.05) between treatments were tested using the Tukey post-hoc test. Different lowercase letters indicate differences between treatments within a season.

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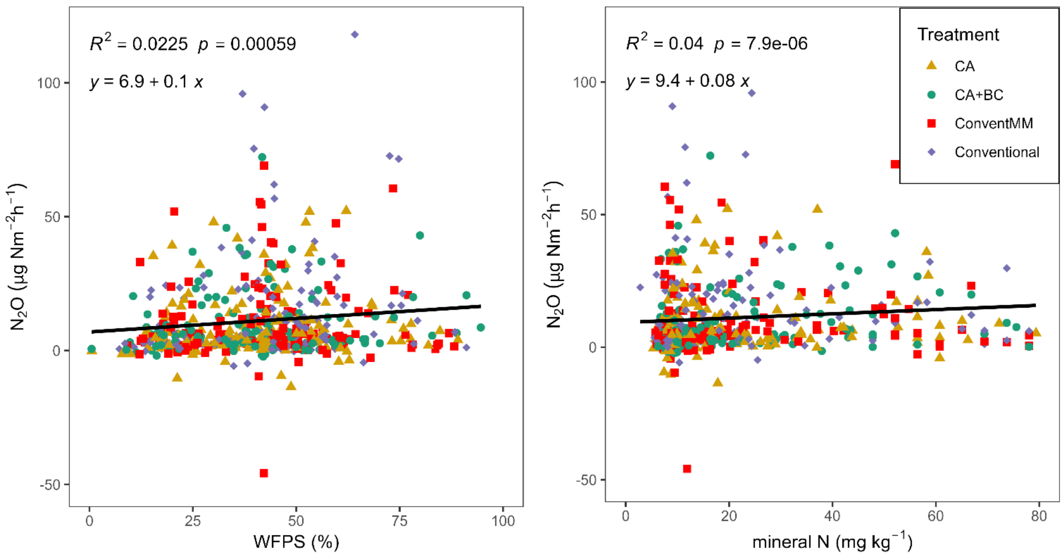

Fig 4: Relationship between hourly N_2O fluxes and water filled pore space (WFPS) and mineral N (mg kg^{-1}) in Gulu. Data points are for 17 sampling dates.

3.4. Effect of rotations and tillage on cumulative N_2O emissions

Cumulative N_2O emissions ranged from 0.24 – 0.50 kg N ha^{-1} in the 1st season (134 days), and from 0.19 – 0.61 kg N ha^{-1} in the 2nd season (125 days). Cumulative N_2O emissions for the entire measurement period May 2023 to January 2024 ranged from 0.44 – 1.11 kg N ha^{-1} (279 days) (Fig. 4, Fig. 5, Fig. S3). Chamber position did not significantly explain variations in cumulative N_2O emissions and there were no significant differences ($p > 0.05$) between conventionally tilled pigeon pea – maize rotation and conventionally tilled continuous maize monocropping (Table S17, S18, Fig. 5, Fig S3). Treatments and the interaction of treatment and position showed a significant effect ($p < 0.05$) of tillage system (Conventional, CA, and CA+BC) on cumulative N_2O emissions in season 1 and for the entire sampling period (season 1 and 2 combined) (Table S20). Cumulative N_2O emissions in season 2 were only affected (p

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< 0.05) by treatment (Fig. 6). There was no significant difference in cumulative N_2O between season one and two (Fig. 6a, b). Average cumulative N_2O were highest in conventional interrow with no significant differences between the other treatments, irrespectively of position. The cumulative N_2O for the whole sampling period (season 1 and 2) was significantly smaller under CA+BC compared to conventional in the interrows (Fig 6c). Significantly ($p < 0.05$) larger emissions were recorded in inter-rows compared to in-rows in conventional treatment during the first season (Fig 6).

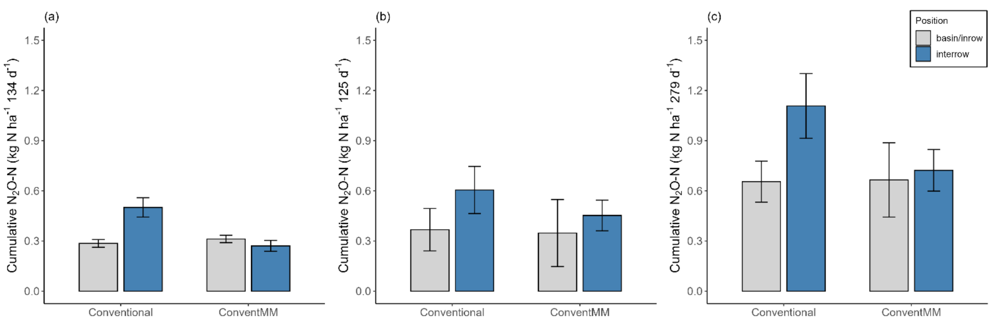

Fig 5: Cumulative N_2O emissions (kg N ha^{-1}) under conventional tillage with pigeon pea rotation (Conventional) and maize monocropping (ConventMM) in Gulu, Uganda. Shown are treatment arithmetic means and SE for conventionally tilled continuous maize monocropping (MM) and conventionally tilled PP-maize rotation in (a) first season (May 2023 to September 2023), (b) second season (October 2023 to January 2024) and (c) sum of both seasons (May 2023 – January 2024).

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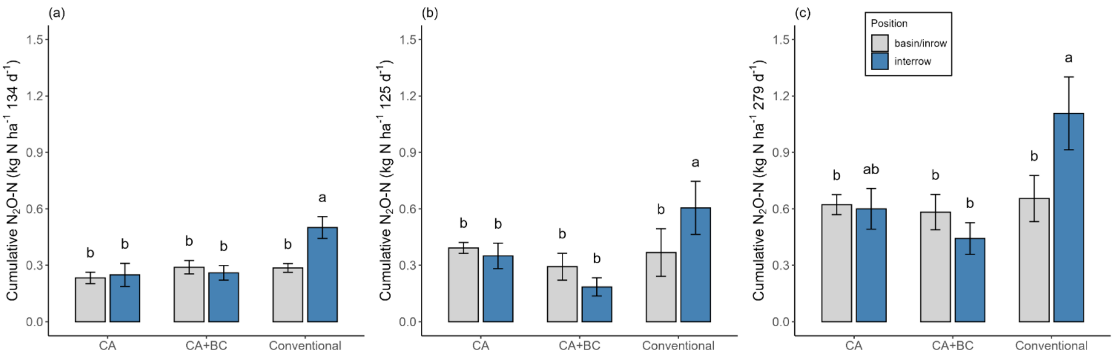

Fig 6: Cumulative N_2O emissions (kg N ha^{-1}) for systems under pigeon pea – maize rotation (a) first season with pigeon pea (measured from May 2023 to September 2023), (b) second season with maize (measured from October 2023 to January 2024) and (c) sum of both seasons (May 2023 – January 2024), in Gulu, Uganda. Error bars represent standard errors (N = 4). Different letters represent significant differences between treatments for each of the seasons or the cumulative of all the seasons ($p < 0.05$) using the Tukey post-hoc test.

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Table 3: Grain yield, grain N yield, yield scaled N_2O emissions and N yield scaled N_2O emissions during the first and second rain season, in northern Uganda. Means are shown with standard errors of means (N=4). Uppercase letters compare seasons specifically for ConventMM treatment. Lowercase letters compare treatments with rotation (Conventional, CA, CA+BC) within a season. Different letters represent significant differences ($p < 0.05$), determined at 5% level using Tukey test, ns represents not significant.

Season	Treatment	Crop	Grain yield	Grain N yield	Yield scaled emissions	N yield scaled emissions
			(kg ha^{-1})		(g N_2O-N kg^{-1} grain)	(g N_2O-N kg^{-1} N grain)
First season	ConventMM	Maize	856.67±157.12 B	10.46±2.17 B	0.36±0.04 A	29.86±3.44 A
	Conventional	pigeon pea	1266.67±83.78 a	40.30±2.75 a	0.32±0.04 a	9.93±1.05 a
	CA	pigeon pea	1539.17±175.70 a	50.79±6.18 a	0.16±0.03 b	4.95±0.85 b
	CA+BC	pigeon pea	1580.50±89.67 a	50.73±2.76 a	0.18±0.03 b	5.31±0.97 b
	P-value		ns	ns	< 0.05	< 0.05
Second season	ConventMM	maize	2590.91±156.72 a A	29.86±2.17 a A	0.15±0.04 a B	13.46±3.87 a B
	Conventional	maize	2658.20±122.73 a	34.82±4.08 a	0.19±0.05 a	14.83±4.13 a
	CA	maize	2743.51±310.59 a	34.42±3.23 a	0.14±0.01 a	10.39±1.65 a
	CA+BC	maize	2758.31±175.24 a	39.27±2.76 a	0.08±0.02 a	5.11±1.34 a
	P-value		ns	ns	ns	ns

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3.5. Treatment effects on grain yield and yield scaled N_2O-N emissions

Different treatments did not affect ($p > 0.05$) grain yield in neither season. Pigeon pea grain yield ranged from 1.3 – 1.6 t ha^{-1} in the first season, and maize grain yield ranged from 2.6 – 2.8 t ha^{-1} in the second season. Grain N yield was also not affected by treatments and it ranged from 10 – 51 kg N ha^{-1} in the first season, and 30 – 39 kg N ha^{-1} in the second season (Table 3). Yield scaled emissions and N yield scaled emissions ranged from 0.16 – 0.32 g N_2O-N kg^{-1} grain and 5.11 – 29.86 g N_2O-N kg^{-1} grain N, respectively; with significantly lower values for CA and CA+BC than for Conventional in the first season. In the second season, treatments had no effect ($p > 0.05$) on yield and yield scaled emissions. Maize yield and yield scaled emissions in the conventional treatment (ConventMM) were significantly higher ($p < 0.05$) in the second season compared to the first season.

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4.0. Discussion

4.1. Dynamics of N_2O-N fluxes

Averaged daily N_2O fluxes ranged from 1.0 – 51.2 µg N m^2 h^{-1} (Fig 1a), and the cumulative N_2O-N emissions from May 2023 – January 2024 were less than 1.2 kg N_2O-N ha^{-1} (Fig 4, 5). These ranges are consistent with other research carried out acidic tropical soils under conservation agriculture with (Fungo et al., 2017; Munera-Echeverri et al., 2022) or without biochar addition (Shumba et al., 2023). Low N_2O emissions can be attributed to low soil mineral N contents (Fig 1b, c) (Chapuis-Lardy et al., 2009). Higher N_2O-N fluxes were recorded in October 2023 after harvesting pigeon pea and immediate sowing for the second season (Fig 1a). These emissions might have been associated with decomposition of pigeon pea residues, leaf litter and root turnover. This would indicate that crop residues were a source of C and N substrates that induced significant N_2O production. Additionally, consumption of labile C by heterotrophs might have created anaerobic microsites that promoted denitrification, especially as this period coincided with heavy rainfalls and high WFPS values (Fig. 2d). A similar, but smaller emission peak was seen in June when abundant rainfalls terminated a dry spell. Rewetting of dry soil triggers increased N_2O emissions likely due to increased nitrification and denitrification fueled by release of readily available N and C from dead microbial biomass (Namoi et al., 2019). Likewise, after harvest and sowing, plant N uptake is small, which might have supported elevated microbial C and N turnover. N_2O emission peaks were short-lived lasting for only 2 weeks (Fig 1a).

4.2. Mineral N and WFPS

Daily N_2O emissions were weakly corelated to mineral N. The Gulu site has a relatively low soil $\delta^{15}N$ value of 4.64 and a soil N content of 0.11% (Namatsheve et al., 2025), indicating a

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highly efficient and tight N cycling (Craine et al., 2015).This could mean that microbes compete well for mineral N in these soils, probably immobilizing available N rather than releasing it for microbial N transformations like nitrification and denitrification. We anticipated that biological N_2-fixation by pigeon pea in the first season would result in higher N_2O emissions in the second season, especially in CA treatments where N_2-fixation was high (Namatsheve et al., 2025). However, N_2O fluxes and mineral N did not appear to be driven by this N_2-fixation. Our results imply that the process of symbiotic N fixation *per se*, and residue retention do not affect soil mineral N and N_2O emissions in unfertilized soils with inherently low N. Rochette et al. (2004) also reported that there is considerable uncertainty related to the emissions of N_2O from soils under legumes, and the soil mineral N alone was a poor indicator of N_2O emissions for two seasons in acidic soils in Canada.

Generally, NH_4^+ and NO_3^- contents were more variable in the first season (May – October) than the second season (October – January) (Fig 1b, 1c, 3). At the onset of the experiment, mineral N was most likely from mineralisation of chemically mulched grasses having grown on the fallow for 3 years prior to the experiment. Across all seasons, significantly higher NH_4^+ was recorded in CA systems than conventional treatments, mainly due to mineralisation of pigeon pea crop residues. Biochar amendments in CA systems did not affect NH_4^+ and NO_3^-. Our result for mineral N aligns with the findings of Munera-Echeverri et al. (2022), who reported similarly low concentrations of NO_3^- (0.8 – 4 mg kg^{-1}) and NH_4^+ (4.3 – 10 mg kg^{-1}) in a fertilised Arenosol in Zambia.

High N_2O emission rates go often along with high WFPS values, increasing the anaerobic volume and hence denitrification in soils (Hao et al., 2025; Wang et al., 2023). Our results indicated a weak positive relationship between WFPS and N_2O emissions ($R^2 = 0.02$, $p < 0.001$; Fig. 7). High evaporation under tropical conditions due to high temperatures (mean 25 °C, range 23 – 34 °C) results in rapid water loss, which drastically reduced the time the soil would

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be above 60% WFPS, despite high rainfalls throughout the sampling period (Fig 1d, e). Apart from June – July 2023, WFPS was <60%, which is often considered a threshold for denitrification-driven N_2O emissions. This may, in part, explain the weak correlation between WFPS and N_2O fluxes in our study.

4.3. Effects of crop rotation, tillage and biochar on N_2O emissions

Cumulative N_2O emission in the conventionally tilled pigeon pea–maize rotation (Conventional) did not differ from the conventionally tilled maize monocrop (ConventMM) (Fig 5, Fig S3). The trials were established in a soil with low organic N content without applying N fertilizer. This is in line with Phiri et al. (2025) who did not find any effect of conservation agriculture on soil N over a short period of time. However, after some seasons, nutrient cycling, inputs from crop residues and biological N_2-fixation might enhance soil fertility. Jeuffroy et al. (2013) reported a 75 – 80% reduction in N_2O emissions in a 4-year study under pea rotation without N input compared to a fertilized monocrop, illustrating the significance of N input for N_2O emissions, rather than the effect of rotation itself. We did not find the effect of CA (reduced tillage) on N_2O emissions. However, Jantalia et al. (2008) and Ruan and Philip Robertson (2013) reported higher N_2O emissions under conventional tillage compared to reduced tillage, which they attributed to soil disturbance, increased soil aeration and accelerated organic matter breakdown.

Biochar applied at a rate of 4 Mg ha^{-1} in planting basins in the CA system did not affect N_2O emissions. In theory, biochar with a high C:N ratio of >60, as applied in this study, could reduce the bioavailability of inorganic N through microbial immobilisation (Namoi et al., 2019) or sorption of NO_3 due to unconventional H-bonding between NO_3^- ions and biochar surface functional groups (Kammann et al., 2017; Nguyen et al., 2017). However, these mechanisms

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appear to be relevant for N_2O emissions only when inorganic N fertilizers are applied. This is a newly established experiment, and the treatment effects may not have manifested yet. In a similar short-term study, Munera-Echeverri et al. (2022) reported that biochar amendments did not affect N_2O emissions despite increased gross nitrification rates in the biochar treatments. Contrary, biochar was shown to effectively reduce N_2O emissions in long-term studies where inorganic N fertilizers were applied. Fungo et al. (2019) reported a 22% reduction in emissions, over three years in a fertilized Ultisol in western Kenya. Similarly, Case et al. (2015) reported that biochar suppressed N_2O emissions in a sandy loam soil fertilized with 140 kg N ha^{-1} yr^{-1}, over a period of 3 years. Lentz et al. (2014) also found that biochar reduced N_2O emissions by 50%, indicating that biochar inhibited nitrification and N immobilisation.

4.4. Yield and yield scaled N_2O emissions

Treatments had no significant effect on grain and grain N yields in either season. Again, as these were newly established trials, we did not expect to see immediate effects of CA and biochar amendment. Rusinamhodzi et al. (2011) highlighted that high inputs of especially N fertilizer are required to realize yield benefits of CA. When inorganic fertilizer is applied, positive effects of CA become more prominent in the long term. In Zambia, benefits of biochar and/or CA on grain yield were reported after several seasons (Martinsen et al., 2014, 2017; Munera-Echeverri et al., 2020). Yields in the first season under conventional tillage with maize monocropping (ConventMM) were low, <1.6 t ha^{-1} the average maize yield in Uganda without N fertilization (Kaizzi et al., 2012). Low rainfall received from early May to mid-June, during the critical growth stage for maize (tasselling and grain filling) drastically reduced maize grain yield in the first season.

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Reducing yield-scaled N_2O emission has been pointed out as one of the most promising strategies to increase crop yield while reducing N_2O emissions. In this study, yield-scaled N_2O emission were 0.16 – 0.32 g N kg^{-1} grain in the first season, and < 0.20 g N kg^{-1} grain in the second season. During the first season, yield-scaled N_2O emission in CA and CA+BC were significantly reduced by 50 % compared to conventional practices, indicating that N use efficiency was high. These practices were effective in minimising emissions without penalising pigeon pea productivity, supporting CA as a sustainable agricultural practice. In the second season, yield scaled emissions were low, but no treatment effect was recorded. Our results are in line with other studies in SSA, although they applied mineral N fertilizer; for example, Shumba et al. (2023) reported yield scaled emissions of 0.09 – 0.19 in maize after applying 58 kg N ha^{-1} in a Ferralsol and Lixisol in Zimbabwe.

5. Conclusions

Nitrous oxide emissions were not affected by conservation agriculture and biochar, likely because in low-input systems without fertilization microbial immobilization prevails over the influence of biochar on mineral N availability. We established that nitrous oxide emissions peaks following rainfall events after dry spells and the incorporation of high-quality pigeon pea residues were short lived, indicating that residue management may have temporary effects on nitrous oxide emissions in unfertilized systems. Yield scaled nitrous oxide emissions were substantially lower under conservation agriculture alone, and conservation agriculture with biochar, implying that nitrous oxide emissions can be reduced without penalising pigeon pea grain yield.

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