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Article

Effect of Manure and Urea Fertilization on Yield, Carbon Speciation and Greenhouse Gas Emissions from Vegetable Production Systems of Nigeria and Republic of Benin: A Phytotron Study

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Abstract: Fertility management techniques being promoted in sub-Saharan Africa (SSA) seek to grow indigenous vegetables economically and sustainably. This study was conducted in a phytotron chamber and compared yield, soil carbon (C) speciation and greenhouse gas (nitrous oxide (N_2O) and carbon dioxide (CO_2)) emissions from SSA soils of two ecoregions; the dry savanna (Ina, Republic of Benin) and rainforest (Ife, Nigeria) cultivated with local amaranth (*Amaranthus cruentus*) under manure (5 t/ha) and/or urea (80 kg N/ha) fertilization. Vegetable yield ranged from 1753 kg/ac to 3198kg/ac in the rainforest, *RF*, soils and 1281 kg/ac to 1951 kg/ac in the dry savanna, *DS*, soils. Yield in the urea treatment was slightly higher compared to the manure+urea treatment, but the difference was not statistically significant. Cumulative CO_2 emissions over 21 days ranged from 497.06 to 579.47 g CO_2 in the *RF*, and 322.96 to 624.97 g CO_2 in the *DS*, while cumulative N_2O emissions ranged from 60.53 to 220.86 mg N_2O in the *RF*, and 24.78 to 99.08 mg N_2O in the *DS*. In the *RF* samples, the combined use of manure and urea reduced CO_2 and N_2O emissions but led to an increase in the *DS* samples. ATR-FTIR analysis showed that the combined use of manure and urea increased the rate of microbial degradation in the soils of the *DS*, but no such effect was observed in soils of the *RF*. We conclude that combining manure and urea fertilization has different effects on soils of the two ecoregions, and that *RF* farmers can reduce agricultural emissions without compromising soil productivity and yield potential.

Keywords: Sub-Saharan Africa; FTIR spectroscopy; fertilizer microdosing; African leafy vegetables; greenhouse gas mitigation; sustainability; tropical agriculture; soil fertility

1. Introduction

Agriculture is a major contributor to global anthropogenic greenhouse gas (GHG) emissions, accounting for 56% of emissions in 2005, primarily from the use of mineral fertilizers and manures to crop and soil systems, as well as cultivation of peatlands [1,2]. Africa currently contributes an estimated 15% of the global N_2O emissions from agricultural soils [3] despite its current low average fertilizer application

rates of 9 kg ha^{-1}; which is very low compared to 135kg/ha in Asia and 73 kg/ha in Latin America [4]. N fertilization is expected to increase in SSA by up to six-fold from the current levels in this century [5,6], since agricultural productivity in sub-Saharan Africa (SSA) is limited by low soil fertility [7]. Farmers in SSA are adopting the combined use of inorganic fertilizers and manures/crop residues to increase agricultural yields [8,9] as part of a widely accepted package of practices called Integrated Soil Fertility Management (ISFM); however little research has been performed on the environmental sustainability (including GHG emissions) of ISFM practices in African agricultural systems.

Increasing N fertilizer rates is known to increase soil N_2O emission and contribute towards global warming [5]. The magnitude of soil N_2O emissions is dependent on factors such as N fertilization rates, N fertilizer form [10], soil properties such as aeration, C bioavailability, and N utilization efficiency [11,12]. However, many studies [9,12-14] have reported that combining organic and inorganic fertilizers led to a reduction in soil N_2O emissions and an increase in crop yields in Mali and Zimbabwe. These studies suggested that a combination of organic fertilization with low rates of inorganic N can be used as a mitigation option for reducing N_2O emissions while retaining similar crop yields. Meanwhile, organic materials have also been shown to enhance the emissions of N_2O and CO_2 when in combination with urea fertilizers [15,16], primarily due to the production of CO_2 by the hydrolysis of urea to CO_2 and ammonia, as well as stimulation of heterotrophic microbial activity [17].

Changes in land management practices and cropping systems can significantly influence nutrient cycling and GHG emissions by altering soil chemical, physical and biological properties [11,18-20]. Dick et al (2008) [13] reported that 4.1% of urea treatment added to a pearl millet field in Mali was lost as N_2O within the first year, while Singh and Verma (2007) [21] estimated that 70% of current N demand by plants is supplied by inorganic fertilizers, and as much as 50-70% of it is lost to nitrification processes such as nitrate leaching and nitrous oxide emission.

Because organic matter is so tightly coupled to GHG emissions, advanced spectroscopic techniques, such as attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy that can examine changes in the chemical forms of organic compounds [22] are vital to assessing SOC quality in cropping systems. ATR-FTIR analysis of soils uses the vibrational characteristics of organic compounds to characterize the functional group chemistry of SOC [23,24] and investigate the effect of agronomic management practices on the composition and dynamics of SOC at molecular scale in the soils [24-26]. Currently, we have little information on how N fertilization affects C speciation/transformation, and N_2O and CO_2 emissions in vegetable production systems of SSA. Therefore, understanding how C and N cycles under cultivation of indigenous vegetables on West African soils respond to the application of organic manure and fertilizer microdosing is crucial in estimating the sustainability and productivity of the soil.

The objective of this study is to investigate the effect of manure and fertilizer treatments on both GHG emissions and SOM speciation by determining and quantifying the impact of reduced mineral nitrogen fertilization and organic manure on vegetable yield, emission of CO_2 and N_2O from SSA soils cultivated with indigenous vegetables in a controlled environment.

2. Materials and Methods

2.1. Study sites

Soil sampling for this study was performed at two field sites - (a) Ina, Benin Republic (9° 95 N, 2°72 E) with an elevation of 380m, annual precipitation of 1073mm and a mean temperature of 26.5°C and (b) Ife, Nigeria (07° 29' 21.9" N 004° 34' 17.3" E) with an altitude of 293 m, annual precipitation of 1317mm and a mean temperature of 25.6°C, was collected and used in this phytotron study. The field site at Ina is in the dry savanna (*DS*) ecoregion, while the site at Ife falls within the rainforest (*RF*) ecoregion of Nigeria. The soils were classified as Haplic Lixisols (LXha) and Plethnic Plinthosols (PTpx), for the two eco-regions respectively [27]. Lixisols are slightly acidic soils dominated by kaolinite clays, while Plinithosols are rich

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in iron and manganese and have a mix of kaolinite, quartz and other minerals [27]. Representative soil samples were collected systematically from field sites in both ecoregions up to a depth of 20 cm and homogenized into one composite sample. Soils were air dried, collected in plastic bags and shipped to the University of Saskatchewan for the phytotron experiment.

The soils were randomly distributed into different pre-designed treatments soil pots (in equal volumes), mixed with manure (for the treatments requiring manure), cultivated with the local amaranth (*Amaranthus cruentus*) and grown in the phytotron. The growth chambers were set for 16 hours at 25°C (day) and 8 hours at 18°C (night), relative humidity was approximately 55(±5) %. The experimental pots consisted of a control (C) treatment, manure only (M) treatment, Manure + 80 kg N/ha (M80) treatment, No manure + 80 kg N/ha (O80) treatment and an empty soil pot microdosed (with 80 kg N/ha) without any vegetables (S80). N was applied as urea. The experimental pots were arranged in a randomized complete block design and replicated 3 times for the two ecozones (i.e. *DS* and *RF*).

Soil samples were collected before planting and after harvest, air-dried, sieved (>2mm), and analyzed for particle size, pH, EC, total N, total P, total organic C, and C FTIR using standard methods. The vegetables were grown for 45 days after emergence. Particle size analysis was done using the hydrometer method [28], pH was measured in triplicate using a glass electrode in a 2:1 water:soil suspension, with 10 mL of water and 5 g of soil [28]. The TOC was analyzed using triplicate 0.25 g of very fine (< 250 μm particle size) samples on an automated C632 LECO analyzer (LECO © Corporation, 1987) with a preset combustion temperature of 1100 °C. Low C standard reference materials was used for calibration and a quality control sample was measured after every 20 samples. Total N and P were measured in triplicate using the H_2SO_4 – H_2O_2 acid block digestion method of Thomas et al. (1967). Digests were then allowed to cool to room temperature, diluted and analyzed on a Folio AA3 auto-analyzer. A standard soil of known concentration of N was used for quality control.

2.2. Measurement of GHG emissions

The GHG experiment was conducted under controlled conditions in the phytotron. Gas samples were collected at 0, 1, 2, 3, 4, 5, 8, 14 and 21 days from the experimental pots to determine N_2O and CO_2 emissions. Air samples were collected from the headspace of the soil pot using a 30 mL gas-tight syringe at time 0 and then at 60 minute intervals (the chambers did not have fans to circulate the air, so we assumed that the air would equilibrate after 60 minutes). The collected air samples were transferred to a pre-evacuated 12-mL Exetainer vial (Labco Inc.; Ceredigion, UK) that was then analyzed via gas chromatography to determine the total concentrations of N_2O and CO_2 in the headspace samples. Ambient air samples were collected, and the ambient air temperature inside the chambers was also recorded. Total concentrations of N_2O and CO_2 in the headspace gas were determined using a Scion 456-GC gas chromatograph equipped with an electron capture detector (ECD) for the determination of N_2O and a thermal conductivity detector (TCD) for the determination of CO_2. To avoid 'pulsing' or the 'Birch effect' [29], soil pots were watered at least 12 hours before gas samples were taken. The N_2O and CO_2 emissions was calculated as the product of the increase in N_2O and CO_2 concentration above ambient air and the volume of the headspace divided by the time the headspace was sealed and the soil surface area.

2.3. FTIR spectroscopy

Speciation of SOC for the finely ground soil samples was investigated using ATR-FTIR spectroscopy using a Bruker Optics Equinox 55 FTIR spectrometer equipped with a Mercury Cadmium Teluride (MCT) detector and single bounce PlatinumIR ATR accessory with diamond coated ZnSe optics. Spectra were collected by averaging 128 scans at 4 cm^{-1} resolution over a spectral range of 4000–400 cm^{-1} and were background corrected by using the spectrum of the empty ATR crystal with ambient air as reference.– The 1800–900 cm^{-1} region was considered for the analysis of C functional groups in this study; the spectral region between 900 to 400 cm^{-1} was excluded due to being dominated by vibrations of soil minerals [30],

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while the bands between 2700 and 1800 cm^{-1} were excluded because the information attributable to organic matter is masked by C-C stretching of the diamond ATR crystal and noise from CO_2. Similarly, the bands at about 3600–3000 cm^{-1} are strongly influenced by water content [31] and were excluded as they may vary between the analyzed soil samples. Baseline correction of the spectra was performed using OPUS (ver. 6.5, Bruker Optik GmbH, Ettlingen, Germany) spectral processing software package. As there is a strong overlap among the bands of organic functional groups, individual bands were resolved by spectral deconvolution using a series of Gaussian curves fit on the Fityk software package (version 1.2.1) [32] as described in Dhillon et al (2017) [24]. Spectral deconvolution was performed on a total of 39 samples from *RF* and *DS* ecoregions.

The curve parameters were constrained to ensure equal FWHM (full width at half maximum) of the curves. Individual spectral band identification was performed by using the available knowledge of characteristic infrared peak positions of soil organic compounds as reported in the literature, and the peaks were fitted as shown in figure 1. Peaks were excluded from further analysis when not present in ≥ 5 samples. The relative absorbance intensity (rA) of the deconvoluted bands was calculated by dividing the area of individual bands within the 1800–900 cm^{-1} wavenumber region (i.e. 1000, 1100, 1160, 1430, 1540, 1600, 1640cm^{-1}) with the sum of total area of all the bands in this region (e.g., A_{1510}=A1510/Σ A(980–1640cm^{-1})). Arithmetic means of the relative intensities of the absorption bands for three soil samples, collected from each soil pot, were calculated for each band to obtain the representative relative intensities of the bands for all samples. The relative intensity of the bands depends on the amount of absorbing functional groups, and it was used as a semi-quantitative estimate of the relative proportion of C functional group within each soil sample, such that high absorption intensity indicates high content of the corresponding functional group and vice versa [33].

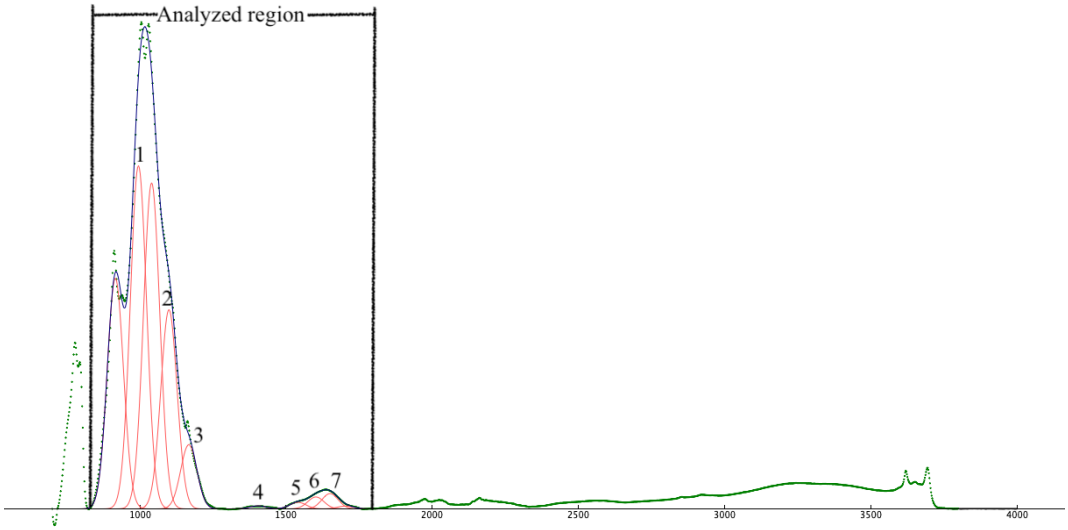

Figure 1. A representative ATR-FTIR spectra of whole soils showing the fitted Gaussian peaks representing the major C functional groups within the wavenumber range of 900 – 1800 cm^{-1}. Peaks are identified as follows: 1-1000 cm^{-1}, 2-1100 cm^{-1}, 3-1160 cm^{-1}, 4-1430 cm^{-1}, 5-1540 cm^{-1}, 6-1600 cm^{-1}, 7-1640 cm^{-1}.

2.4. Statistical Analysis

The data analysis was done using the Statistical Package of Social Sciences (SPSS Inc.). Data was checked for and met the assumption of normality, main and interaction effects was analyzed using repeated measure analysis of variance and determined significant at $p \leq 0.05$ probability level.

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3. Results

3.1. Soil pH and Vegetable Yield

Vegetable yield and soil pH for control and fertilizer treatments for the soil samples of both ecoregions are shown in figures 2a and 2b respectively. For each of the fertilizer treatments, *RF* samples had a significantly lower pH (Fig 2b; Table 2), and significantly higher vegetable yield (Fig 2a; Table 2) compared to the *DS* samples. The pH ranged from 4.7 to 5.5 in the *RF* soil samples and 4.5 to 6.1 in the *DS* soil samples for different fertilizer treatments (Fig 2b). In soils of both ecoregions, soil pH was significantly affected by treatment (Table 2). In the *RF* samples, all treatments increased soil pH compared to the control, with the increment in pH highest in the urea treatment, O80 (Fig 2b). In the *DS* samples, the M and M80 treatments significantly increased soil pH, while the treatments which received urea maintained (O80) or reduced (S80) soil pH compared to the control treatment (Fig 2b).

Soil pH was significantly reduced after harvest in the *DS* samples for all treatments except S80, while in the *RF* samples, the difference in soil pH after harvest was not statistically significant ($p = 0.243$). Yields ranged from 1753 kg/ac to 3198kg/ac in the *RF*, and 1281 kg/ac to 1951 kg/ac in the *DS* (Fig 2a). Note that S80 has no yield because no vegetable was seeded into that treatment. While the yield difference between the *RF* and *DS* ecoregions was significant (Table 2), statistical analysis showed that all treatments that received some sort of N fertilization, either manure, urea or both, yielded significantly better than the control in both ecoregions (Fig 2a). In samples from both ecoregions, vegetable yield was highest in the treatment that received only urea, O80, followed by the treatment that received both urea and manure (M80), and the treatment that received only manure (M). However, the yield differences between the O80 and M80 treatments was not significant.

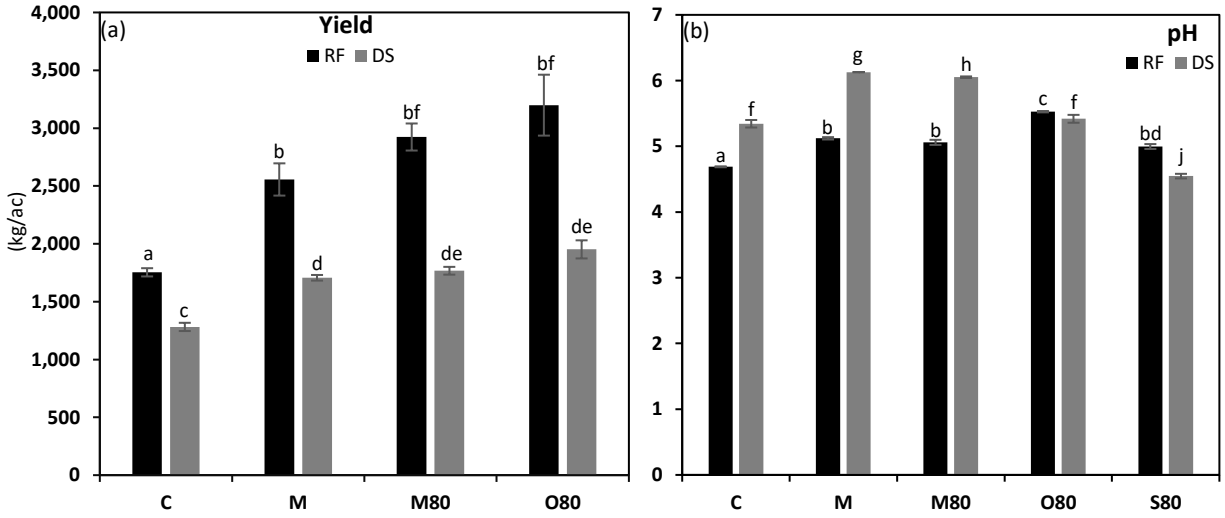

Figure 2. Treatment effect on (**a**) Yield and (**b**) soil pH of the Rainforest, RF, and Dry Savanna, DS, soils. C = control, M = manure only treatment, M80 = Manure + 80 kg N/ha, O80 = No manure + 80 kg N/ha S80 = soil pot microdosed with 80 kg N/ha without any vegetables. Bars with different letter(s) for each site are significantly different at $P < 0.05$. (n = 3).

3.2. Soil N, P and organic C content

Total N concentrations ranged from 1200.3 to 2246.8 µg/g in the *RF* samples and 697 to 1017.6 µg/g in the *DS* samples. Total P concentrations ranged from 624 to 766 µg/g in the *RF* and 405 to 563 µg/g in the *DS*, while organic C concentrations ranged from 1.66 to 1.87 % in the *RF* and 0.55 to 0.63 % in the *DS* (Table 1). The difference in the total N, P and organic C concentrations between the ecoregions is statistically significant (Table 2). Within the ecoregions, statistical analysis found no significant treatment and time

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effect on total N, P and organic C concentrations, indicating that the addition of urea and/or manure does not significantly increase N, P, and organic C concentrations in this system and at the rates applied. However, in the *DS* samples, treatment effect on organic C concentrations was significant (Table 2), with the single addition of manure, M, increasing the organic C concentration, while the combined addition of manure+urea M80, maintained the current level of organic C concentration (Table 1). The urea treatments, O80 and S80, saw a decline in organic C concentration (Table 1).

Table 1. Carbon content, total phosphorus and total nitrogen concentrations of soil samples by ecoregion and treatment. Values are Mean (standard error). (n = 3). C = control, M = manure only treatment, M80 = Manure + 80 kg N/ha, O80 = No manure + 80 kg N/ha S80 = soil pot microdosed with 80 kg N/ha without any vegetables. BP = Before planting, AH = After harvest.

	Total P (µg/g)		Organic C (%)		Total N (µg/g)		pH	
Treatment	**BP**	**AH**	**BP**	**AH**	**BP**	**AH**	**BP**	**AH**
Rainforest								
C	766 (28.1)	687 (13.5)	1.75 (0.04)	1.81 (0.05)	2039.9 (128)	1200.3 (197.1)	4.7 (0.02)	4.7 (0.02)
M	709 (16.4)	722 (22)	1.70 (0.01)	1.80 (0.08)	2053.5 (185.1)	2034.9 (269.4)	5.2 (0.02)	5.0 (0.02)
M80	650 (56.3)	715 (18)	1.66 (0.09)	1.84 (0.05)	1820.7 (102)	2021.1 (480)	4.9 (0.04)	5.2 (0.04)
O80	666 (16.7)	647 (15.6)	1.87 (0.02)	1.84 (0.02)	2246.8 (252.1)	2150.1 (200.2)	5.9 (0.06)	5.2 (0.05)
S80	624 (58.7)	675 (23.8)	1.81 (0.03)	1.71 (0.11)	1261.6 (129.5)	1571.1 (266.9)	4.8 (0.05)	5.2 (0.03)
Dry Savanna								
C	405 (4.6)	408 (18)	0.56 (0.01)	0.58 (0.02)	823.9 (33.9)	849.0 (70.8)	5.5 (0.06)	5.2 (0.05)
M	412 (4.4)	419 (5.6)	0.62 (0.01)	0.63 (0.01)	995.1 (82.6)	841.5 (21.9)	6.4 (0.04)	5.8 (0.04)
M80	406 (2.6)	411 (1.7)	0.56 (0.01)	0.56 (0.01)	919 (60)	1017.6 (235.1)	6.3 (0.007)	5.8 (0.02)
O80	402 (5.2)	405 (6)	0.62 (0.02)	0.60 (0.02)	787 (114)	959.5 (110.2)	5.6 (0.06)	5.3 (0.06)
S80	563 (61.5)	414 (10.8)	0.57 (0.01)	0.55 (0.01)	916.6 (44.2)	697.0 (71.2)	4.4 (0.06)	4.7 (0.02)

Table 2. Results of an ANOVA on organic carbon, total nitrogen and phosphorus and yield (significant differences, i.e. p ≤0.05 are in bold)

	pH	**Total P**	**Org. C**	**Total N**	**Yield**
Rainforest					
Treatments	**0.002**	0.208	0.432	0.105	**0.018**
Time	0.243	0.533	0.212	0.487	
Treatments*Time	**0.012**	0.098	0.327	0.299	
Dry Savanna					
Treatments	**0.002**	0.607	**0.006**	0.607	**0.005**
Time	**0.006**	0.816	0.996	0.816	
Treatments*Time	**0.003**	0.394	0.538	0.394	

3.3. GHG Emissions

Daily N_2O emissions were higher in the *DS*, ranging from 1.31 to 11.83 mg N/day, than in the *RF*, which ranged from 0.25 mg N/day to 2.23 mg N/day (Fig 3a). In both ecoregions, daily N_2O emissions were higher in treatments that received some form of N treatment (manure and/or urea) than in the controls, C. In the *DS*, the treatment that received only manure, M, (1.7 mg N/day), had a lower daily N_2O emission than the manure + urea, M80, treatment (3.44 mg N/day) which was twice as much as the manure treatment,

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M, emissions, while the urea treatment, O80 (4.35 mg N/day) was more than two and half times the emissions of the manure alone. In the *RF*, the manure + urea, M80, treatment (0.70 mg N/day) had a higher N_2O emissions than the urea treatment, O80, (0.59 mg N/day), and nearly tripled the emissions from the control, C, (0.25 mg N/day). However, the difference in the daily N_2O emissions between the M80 and O80 was not statistically significant (Fig 3a). N_2O emissions in both ecoregions was highest in the S80 treatment, which had no vegetable seeded, emissions from the S80 treatment was ~ 9 times that of the control, *C*, ~ 3 times that of M80, and ~ 3 and 4 times that of O80, in the *DS* and *RF* respectively (Fig 3a).

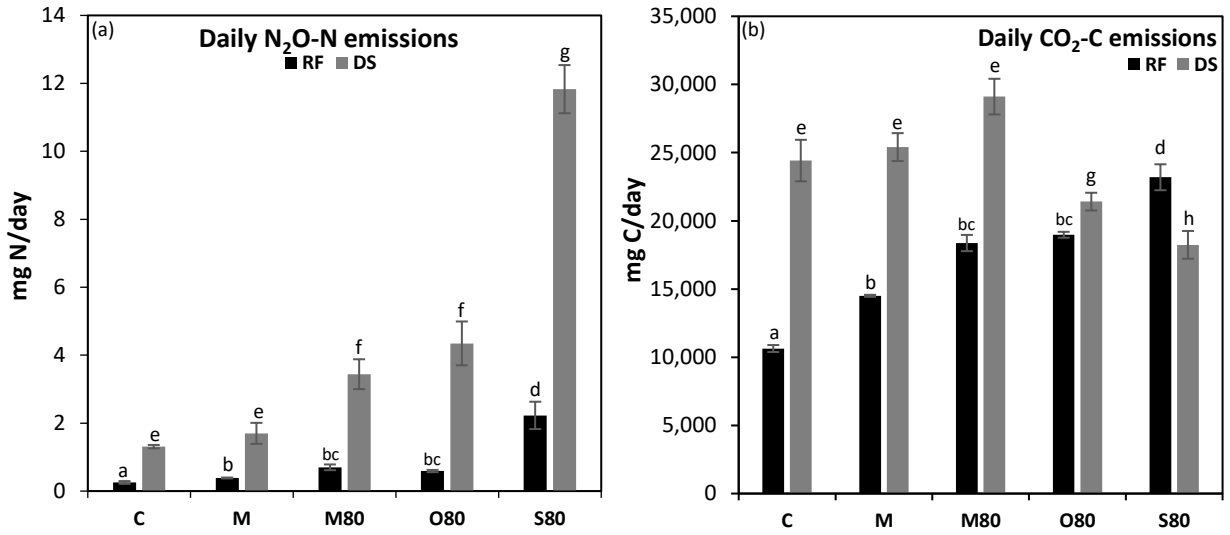

Figure 3. Mean (n = 3) daily emissions of (**a**) N_2O and (**b**) CO_2 from four cropping treatments on Rainforest, *RF*, and Dry Savanna, *DS*, soils. (n = 3). C = control, M = manure only treatment, M80 = Manure + 80 kg N/ha, O80 = No manure + 80 kg N/ha S80 = soil pot microdosed with 80 kg N/ha without any vegetables. (n = 3).

Similar to the N_2O emissions, daily CO_2 emissions were significantly higher in the *DS*, ranging from 18.24 to 29.11 g C/day, compared to the *RF*, which ranged from 10.64 g C/day to 23.20 g C/day (Fig 3b). In the *RF* soils, CO_2 emissions increased from C (10.64 g C/day) < M (14.50 g C/day) < M80 (18.37 g C/day) < O80 (18.98 g C/day) < S80 (23.20 g C/day). Although the difference between the daily CO_2 emissions from M80 and O80 was not statistically significant (Fig 3b), manure addition increased daily CO_2 emissions by ~36%, while urea addition and manure + urea increased daily CO_2 emissions by ~78% and 73% respectively (Fig 3b). In the *DS*, the S80 treatment, which had no vegetable seeded, had the least CO_2 emission (18.24 g C/day), followed by the urea treatment, O80 (21.41 g C/day). There was no significant difference between the CO_2 emissions from the manure, M, (25.41 g C/day) and control, C, (24.42 g C/day) (Fig 3b).

Cumulative N_2O emission ranged from 64.53 to 279.54 mg N in the *RF* (Fig 4a), and 24.78 to 99.08 mg N in the *DS* (Fig 4b) and was higher in the *RF* than in the *DS*. In the *RF*, cumulative N_2O emission was highest in the urea treatment, O80 (279.54 mg N), followed by the manure + urea treatment, M80 (220.86 mg N), the S80, *C* and *M* had 115.81, 81.94, and 60.53 mg N respectively (Fig 4a). In the *DS*, *M80* (99.08 mg N) had the highest cumulative N_2O emission, followed by the urea treatment, O80 (87.39 mg N), the S80, C and M had 65.89, 27.59, and 24.78 mg N respectively (Fig 4b). In both ecoregions, the manure treatment, M, had the least cumulative N_2O emission and was even lower than the controls (Figs 4a and 4b).

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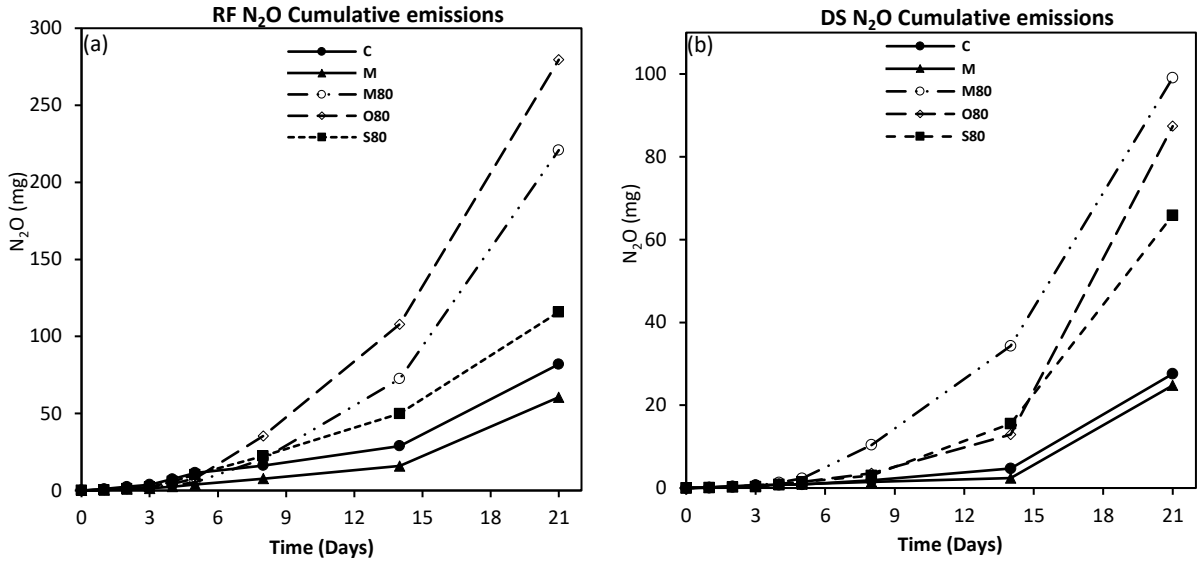

Figure 4. Cumulative N_2O emissions from four cropping treatments on (**a**) Rainforest, *RF*, and (**b**) Dry Savanna, *DS*, soils. C = control, M = manure only treatment, M80 = Manure + 80 kg N/ha, O80 = No manure + 80 kg N/ha S80 = soil pot microdosed with 80 kg N/ha without any vegetables. (n = 3).

Cumulative CO_2 emission ranged from 497.06 to 579.47 g C in the *RF* (Fig 5a), and 322.96 to 624.97 g C in the *DS* (Fig 5b). In the *RF*, cumulative emission was highest in the control, *C* (579.47 g C), followed by the manure, M (537.60 g C), manure + urea, M80 (522.91 g C), soil pot, S80 (513.12 g C) and it was least in the urea treatment, *O80* (497.06 g C) (Fig 5a). In the *DS*, cumulative CO_2 emission was highest in the manure + urea, M80 (624.97 g C), followed by the urea treatment, O80 (524.72 g C), soil pot, S80 (494.88 g C), the manure, M (411.61 g C), and the control, C (322.96 g C) which had the least cumulative (Fig 5b) CO_2 emission.

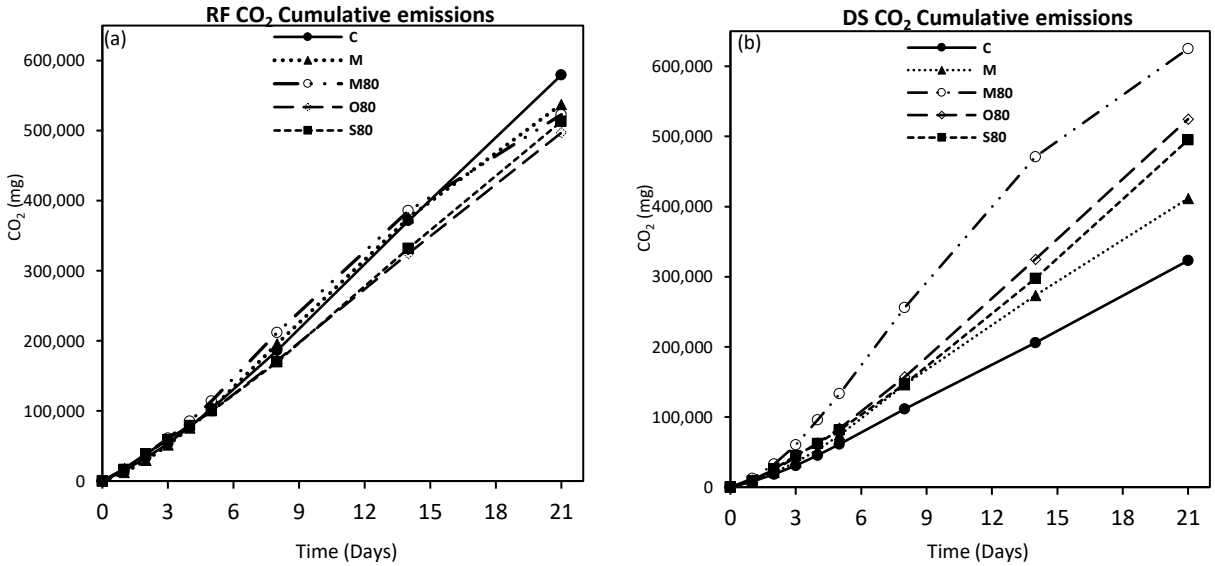

Figure 5. Cumulative CO_2 emissions from four cropping treatments on (**a**) Rainforest, *RF*, and (**b**) Dry Savanna, *DS*, soils. C = control, M = manure only treatment, M80 = Manure + 80 kg N/ha, O80 = No manure + 80 kg N/ha S80 = soil pot microdosed with 80 kg N/ha without any vegetables.

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3.4. ATR-FTIR spectroscopy

The relative abundance of different C functional groups (Tables A1 and A2) was estimated using the relative intensities of the ATR-FTIR bands. While the FTIR bands did not show statistically significant differences amongst the fertilizer treatments, there were similar trends amongst the FTIR bands of related C functional groups. In the *DS* samples, the bands at 1430, 1540, 1600 and 1640 cm^{-1} had the highest relative absorbance in the manure+urea (M80) treatment, followed by the manure (M) treatment (Fig 6). In contrast, the bands at 1000, 1100 and 1160 cm^{-1} showed higher relative absorbance in urea treated samples (S80, O80 and M80) (Fig 6c). The absorbance band near 1640 cm^{-1} is attributed to C=O stretching of carboxylates and conjugated ketones, as well as to aromatic C (C=C) stretching [24,34,35], while the band at 1430 cm^{-1} is assigned to aliphatic (C-H) bending of CH_2 and CH_3 groups [34,35]. The band around 1600 cm^{-1} is assigned to amide N-H bends and C=N stretching of amides [34]. The absorbance band at 1540 cm^{-1} is assigned to aromatic C-H and C=C vibrations [36]. The bands at 1160-1000 cm^{-1} are assigned to C-O-C and C-OH stretch of polysaccharides, polysaccharide-like compounds [35] or of other groups such as alcohols, ether and esters [37,38]. Thus, processed C forms such as aromatic-C, aliphatic-C and carboxylic-C showed higher abundance in manure-treated samples (M and M80), while the polysaccharide-derived C forms are of higher abundance in urea-treated samples (S80, O80 and M80) in the *DS* samples.

In the *RF* samples, the 1640 cm^{-1} band had the highest relative absorbance in the control, C, and the manure, M, treatments, and followed by the urea treated samples (S80 and O80), with the M80 treatment having the least relative absorbance (Fig 7a). For the 1430 cm^{-1} band, the S80 treatment had the highest relative absorbance, with the C and M, and M80 and O80 having identical absorbance (Fig 7b). For the band at 1000 cm^{-1}, the urea treated samples (S80 and O80) had the highest relative absorbance (Fig 7a), followed by the C and M treatments, which had similar relative absorbance, and the M80 treatment having the least relative absorbance. Unlike the *DS* samples, the samples in *RF* soils did not show repeatable trends between the fertilizer treatments.

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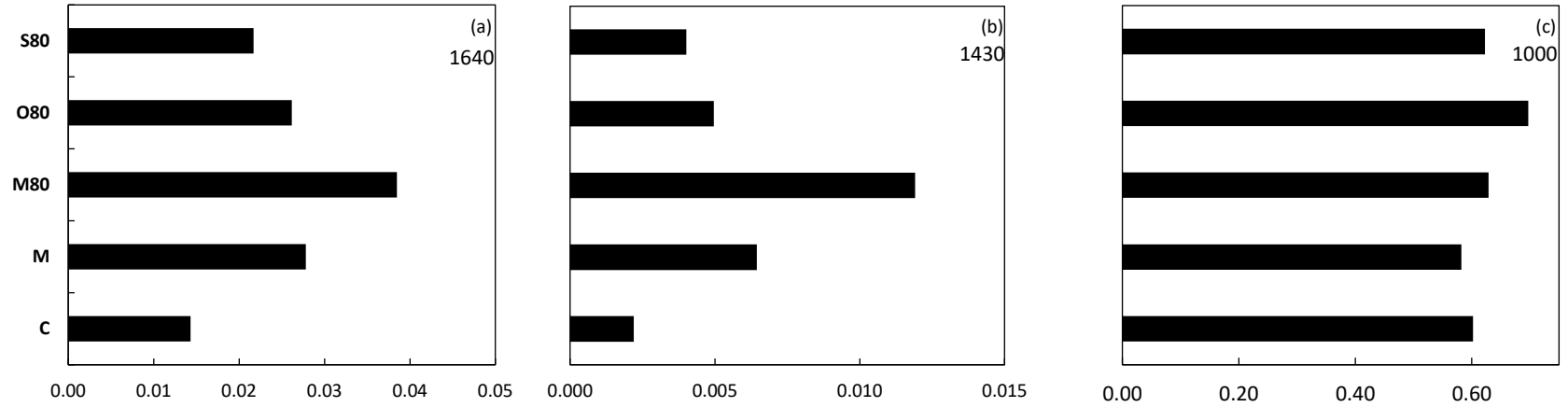

Figure 6. Mean (n = 3) relative absorbance intensities of (a) 1640 cm^{-1} (b) 1430 cm^{-1} and (c) 1000 cm^{-1} ATR-FTIR bands identified for **dry savanna** soils after harvest. C = control, M = manure only treatment, M80 = Manure + 80 kg N/ha, O80 = No manure + 80 kg N/ha S80 = soil pot with 80 kg N/ha without any vegetables.

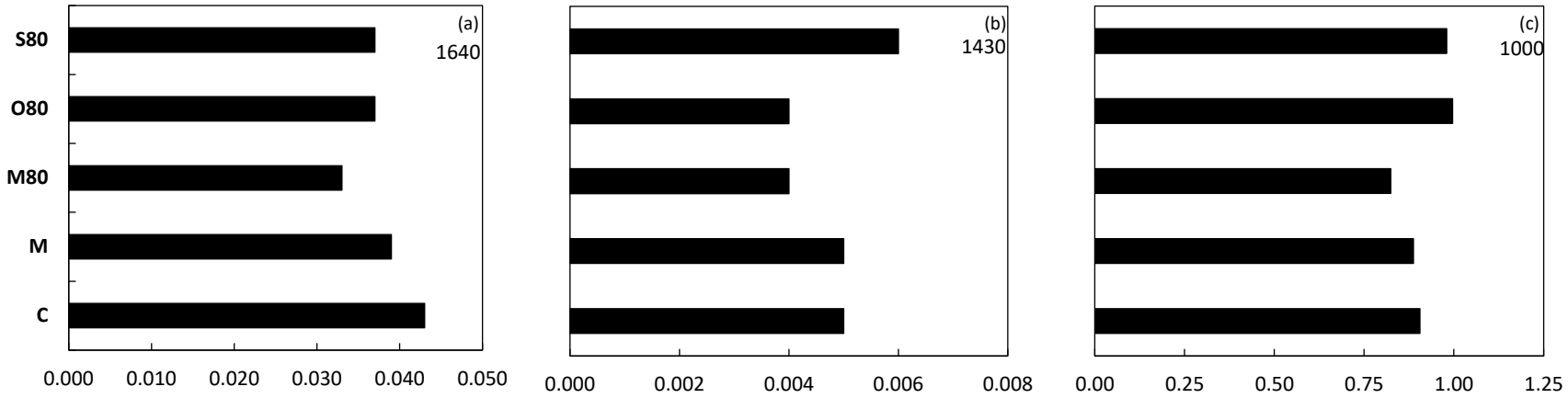

Figure 7. Mean (n = 3) relative absorbance intensities of (a) 1640 cm^{-1} (b) 1430 cm^{-1} and (c) 1000 cm^{-1} ATR-FTIR bands identified for r**ainforest soils** after harvest. C = control, M = manure only treatment, M80 = Manure + 80 kg N/ha, O80 = No manure + 80 kg N/ha S80 = soil pot with 80 kg N/ha without any vegetables.

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A correlation analysis found the bands at 1430, 1540, 1600 and 1640 cm^{-1} to be positively correlated with soil pH in the *DS*. While in the *RF*, there was a negative correlation between the bands and total N. There was also a negative correlation between the 1000cm^{-1} band with SOC in both the *DS* and *RF* (Table 3).

Table 3. Pearson correlation coefficients between the relative intensity of absorbance of ATR-FTIR bands, soil pH and nutrient concentrations for Dry Savanna and Rainforest soils. **. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

	B1000	B1100	B1160	B1430	B1540	B1600	B1640
Dry Savanna							
pH	-0.111	-0.418	-0.359	**.597***	**.644****	**.613***	**.516***
Total P	0.003	0.035	0.011	-0.044	-0.038	-0.074	0.016
Org C	**-.558***	0.114	0.146	-0.092	0.092	0.051	0.010
Total N	0.225	-0.193	-0.300	0.008	-0.123	-0.068	-0.125
Rainforest							
pH	0.067	-0.407	-0.419	-0.125	-0.267	**-.543***	-0.289
Total P	-0.059	0.019	-0.045	0.259	-0.053	0.063	0.183
Org C	**-.534***	0.340	0.389	**-.541***	-0.445	-0.264	**-.577***
Total N	-0.227	0.051	0.062	**-.528***	**-.620***	**-.625***	**-.556***

4. Discussion

4.1. Soil pH and Vegetable Yield

The lower pH of *RF* soils (Fig 2a and 2b) may be related to that soil having a higher Fe- or Al-related clay content than the *DS* soils [27,39], while the decrease in pH observed in soils of the *DS* after harvest (Table 1) may be caused by rhizosphere acidification due to organic acids or root exudates released by the vegetable. The significantly better vegetable yield measured in the *RF* soils over the *DS* (Fig 2a; Table 2) may also be attributed to the *RF* soils being able to retain nutrients better than the *DS* soils due to their higher clay content [39].

The yield response observed in all treatments receiving N, either in the form of manure and/or urea in both ecoregions (Fig 2a) strongly suggests that N is limiting in these soils. We also found that vegetables will respond to any form of N addition, and the magnitude of the yield response will be influenced by the bioavailability of N and potential soil retention (as determined by soil organic matter content and soil structural properties). The urea treatment, O80 which marginally (but not significantly) out yielded the manure+urea treatment M80, and manure only treatment M, in soils of both ecoregions (Fig 2a) provides strong evidence for this. These yield findings further continue the discussion on what the best fertility management practice might be for SSA crops. For example, Mando et al. (2005) [40] reported that manure addition led to greater sorghum yield than urea addition in the Sudano-Sahel region, Tovihoudji et al. (2017) [41] reported that maize yield increases in Northern Benin were greater for the urea treatment, and followed by manure compared to the unfertilized control, while Detchinli and Sogbedji (2015) [42] recommended a combination of mineral fertilizer and farm yard manure to sustain enhanced maize crop productivity and profitability. In this

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study, urea fertilization had no significant vegetable yield advantage over the combined use of manure and urea. Thus, our results suggest that vegetable response to mineral and/or organic fertilization may be more site (ecoregion) and crop (plant species cultivated) specific.

4.2. GHG Emissions

Daily N_2O emissions increased in response to urea and/or manure addition in soils of both ecoregions (Fig 3a). In the *DS* soils, daily N_2O emissions (Fig 3a) and cumulative N_2O emissions (Fig 4b) were lowest in the manure treatment, M, and when manure was used in combination with urea, it suppressed the daily N_2O emissions in these soils (Fig 3a), but not the cumulative N_2O emissions, which was highest in the manure+urea treatment M80 (Fig 4b) of the *DS* samples. This suggests that the combined use of manure and urea slightly amplified cumulative N_2O emissions in the *DS* samples. We know that N_2O emissions are significantly influenced by interactions with other factors such as clay content and soil structure [43,44], As such, it is possible that the low SOC (Table 1) and poor structural properties of the *DS* soils [27,39] provided minimal protection for the N content in the soil, including the manure, and resulted in high N losses. It is also possible that these soils have a threshold of N they can retain, and once N applied exceeds soil-holding capacity, N losses increases, as found by Malhi et al. (2006) [45] where N_2O emissions increased when fertilized N levels exceeded 80 kg N ha^{-1}, or by Kachanoski et al. (2003) [46] which reported increases in soil N_2O emissions at N levels above 100 kg N ha^{-1}. If this is the case, incorporating plant residue and practicing minimal or no tillage may help improve the soils ability to retain more N [45].

Although the combined application of manure+urea increased daily N_2O emissions in the *RF* soils when compared to emissions from the urea fertilization alone (Fig 3a), the cumulative N_2O emission was highest in the urea treatment, O80 and lowest in the manure, M, treatment (Fig 4a), with the combined use of manure and urea slightly suppressing cumulative N_2O emissions. This is consistent with other N_2O emissions studies from SSA such as Nyamadzawo et al. (2017) [9] in soils from Zimbabwe with very low N content cultivated with maize and wheat, which had lower N_2O emissions in plots amended with a combination of inorganic N and manure and manure alone compared to soils amended with inorganic N. Dick et al. (2008) [13] also reported that combining organic manure and urea emitted significantly less N_2O in soils of Mali cultivated in a cereal-legume rotation than urea alone. Vallejo et al. (2005) [47] in a study conducted on low organic C agricultural soil in Spain also reported that the application of organic fertilizers reduced emissions of N_2O, when compared to emissions from soils only treated with urea.

The lower cumulative N_2O emissions in soils amended with manure can be attributed to N immobilization and slow release of mineral N [48]. It could also be that the addition of manure, a low soil C input favors complete denitrification to N_2 and therefore reduces N_2O emissions, or that the simultaneous addition of easily available C and N to an already deficient soil was more efficiently immobilized by the existing microbial biomass than when N alone was applied. Also, the application of manure, a low soil C input, implies a reduced energy source for microbial processes such as denitrification, while the low soil N means there is low substrate to drive both nitrification and denitrification which are responsible for the production of N_2O [49]. Peng et al. (2011) [50] suggested that N_2O emitting pathways compete for N with assimilatory N immobilization by both microbes and plants, and that it is only when N applied to soil exceeds microbial immobilization and plant N

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demand that N_2O emissions increase. We propose that, in this study, additional N was likely taken up by the vegetable, leaving low amounts of N available for microbial use and loss as N_2O.

In the *DS* soils, there was no significant difference between the daily CO_2 emissions of C, M and M80, and the urea treatment, O80, suppressed daily CO_2 emissions in these soils (Fig 3b), but the manure+urea treatment, M80, amplified the emission of CO_2, having a higher cumulative CO_2 emission than the urea treatment, O80, and manure treatment, M (Fig 5b). This suggests that the combination of manure and urea in soils of this ecoregion increases CO_2 emissions under current vegetable production system. This increment in the cumulative CO_2 emissions in the manure+urea treatment may be linked to increase in the rate of decomposition, and is also linked to the manure+urea treatment, M80, having the highest rate of cumulative N_2O emission (Fig 4b) as additional N from the manure further lowers the C:N ratio and drives decomposition forward [13], or could also be as a result of the stimulation of the activity of heterotrophic microbes caused during the hydrolysis of urea [17] thereby producing more CO_2. In the *RF* soils, although not statistically significant, daily CO_2 emissions were higher in the urea treatment, O80, than they were in the manure+urea treatment, M80, and manure treatment, M, (Fig 3b) this suggests that any form of N addition to soils of this ecoregion may lead to an increase in CO_2 emissions by increasing the rate of decomposition, and that the magnitude of the increase is highest for mineral fertilization than for manure fertilization.

4.3. Treatment effect on SOC composition

ATR-FTIR absorbance bands in soils of both ecoregions showed identical peak positions, indicating similar molecular C composition of SOM in soils of both ecoregions. The lack of any significant main and/or treatment effects limits our ability for comparison to just the observed trends.

The highest relative abundance of 1640 and 1430 cm^{-1} bands in the manure+urea, M80, treatment of the *DS* samples (Fig 6a and 6b) suggests that the combined use of manure and urea is increasing the proportion of the processed C species including aliphatic-C, aromatic-C and carboxylic-C forms in the *DS* samples. This observation is also supported by the lower abundance of the 1000 cm^{-1} band in the manure treatment, M, and manure+urea, M80, treatment in the *DS* (Fig 6c) samples suggesting that the combined use of manure and urea leads to lower abundance of labile polysaccharide C species due to their higher rate of decomposition in this treatment. This increased rate of decomposition observed in the *DS* samples that received both manure and urea may be because the *DS* samples are more N deficient than the *RF* samples (Table 1), and as a result of the increased N input, microbial degradation rate is increased. In the *RF* samples however, we observed that the combined use of manure and urea had a lower relative absorbance at the 1640 cm^{-1} band (Fig 7c), and similar relative absorbance at the 1430 cm^{-1} band with the urea treatment, O80. This suggests that the combined use of manure and urea did not increase the rate of microbial decomposition in the *RF* samples.

5. Conclusions

This study investigated the link between vegetable yield, GHG (CO_2 and N_2O) emissions and OM speciation in SSA soils of the Rainforest ecozone of Nigeria and Dry savanna ecozone of Benin Republic under cultivation with an indigenous vegetable; local amaranth (*Amaranthus cruentus*)

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under urea fertilization and/or organic manure fertilization in a controlled environment (phytotron). We used ATR-FTIR spectroscopy to identify and quantify C species and measured and quantified GHG emissions and analyzed it against vegetable yield. Analysis of the soils of both ecoregions showed that the combined use of manure and urea acted differently in the *RF* and *DS* soils in terms of regulating microbial activity, C speciation and GHG emissions. We found that the combined use of manure and urea increased the rate of microbial decomposition in the *DS* samples, thus, increasing the proportion of the processed C species including aliphatic-C, aromatic-C and carboxylic-C forms in the *DS* samples, while no such effect on microbial decomposition was observed in the *RF* samples. We also found that the combined use of manure and urea led to an increase in cumulative CO_2 and N_2O emissions in the *DS* soils, but suppressed both emissions in the *RF*. These results agree with the trends shown in FTIR results, which showed higher abundance of processed C forms in the M80 treatment, thus indicating higher degree of microbial breakdown, which may be linked to higher cumulative CO_2 and N_2O emissions. The *RF* soils do not show any such trends in FTIR bands indicating that addition of manure+urea affected microbial decomposition differently in *RF* and *DS* soil samples. Our results also show that the *RF* soils had a higher vegetable yield than the *DS* soils, with the urea and manure+urea treatment showing no significant difference in vegetable yield.

We found that by combining manure and urea, *RF* farmers can reduce agricultural emissions without compromising productivity, dispelling any concerns that the combined use of manure and urea may result in relatively lower vegetable yields in the short-term. It is clear from this study that even the low organic matter sandy soils of SSA can be significant sources of CO_2 and N_2O, and that fertility management to optimize yield, build/maintain soil productivity and mitigate greenhouse gas emission will differ by ecoregion, soil type and maybe even crop.

Author Contributions: D.O.; P.A.; O.A.; and D.P. were responsible for the funding acquisition, project administration, provision of resources and technical information; A.O.; and D.P. conceptualized the study; A.O. was responsible for the methodology and writing of the original manuscript; A.S. and G.D. helped with the gas and FTIR analysis respectively; G.D.; A.S.; and D.P. reviewed, edited and validated the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Relative abundance of different C functional groups before planting (BP) as estimated using the relative intensities of the ATR-FTIR bands.

Band (cm^{-1})	1000	1100	1160	1430	1540	1600	1640
Dry Savanna							
C							
M	1.023	0.811	0.249	0.007	0.020	0.036	0.034
M80	0.803	1.312	0.463	0.007	0.012	0.036	0.030
O80	0.889	0.535	0.118	0.008	0.020	0.032	0.043

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S80	0.877	0.578	0.157	0.008	0.021	0.041	0.055
Rainforest							
C	0.708	0.737	0.266	0.005	0.007	0.020	0.021
M	0.689	0.719	0.245	0.003	0.006	0.017	0.017
M80	0.784	0.961	0.366	0.002	0.009	0.024	0.020
O80	1.156	0.558	0.202	0.001	0.004	0.008	0.012
S80		1.406	0.475	0.004	0.009	0.030	0.025

C = control, M = manure only treatment, M80 = Manure + 80 kg N/ha, O80 = No manure + 80 kg N/ha S80 = soil pot with 80 kg N/ha without any vegetables.

Table A2. Relative abundance of different C functional groups after harvest (AH) as estimated using the relative intensities of the ATR-FTIR bands.

Band (cm^{-1})	**1000**	**1100**	**1160**	**1430**	**1540**	**1600**	**1640**
Dry Savanna							
C	0.602	0.716	0.265	0.002	0.006	0.019	0.014
M	0.582	0.667	0.278	0.006	0.013	0.033	0.028
M80	0.629	0.738	0.288	0.012	0.017	0.046	0.038
O80	0.697	1.058	0.375	0.005	0.011	0.031	0.026
S80	0.623	1.016	0.369	0.004	0.008	0.022	0.022
Rainforest							
C	0.905	1.069	0.364	0.005	0.020	0.039	0.043
M	0.887	0.837	0.233	0.005	0.016	0.029	0.039
M80	0.824	0.772	0.252	0.004	0.014	0.030	0.033
O80	0.996	0.692	0.231	0.004	0.017	0.031	0.037
S80	0.980	0.633	0.194	0.006	0.018	0.029	0.037

C = control, M = manure only treatment, M80 = Manure + 80 kg N/ha, O80 = No manure + 80 kg N/ha S80 = soil pot with 80 kg N/ha without any vegetables.

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