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Assessment of Greenhouse Gases Emission in Smallholder Rice Paddies Converted From Anyiko Wetland, Kenya

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Rice is an important food crop in Kenya and is the third most consumed cereal crop after maize and wheat. The high demand for rice has resulted in the conversion of wetlands to rice paddies and the increased use of fertilizer, ultimately reducing the ability of wetlands to store carbon. Consequently, emissions from wetlands of three potent greenhouse gases (GHGs): methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2) have increased. This study assessed the influence of fertilizer application on GHGs emission, organic carbon and nutrient stocks in rice paddies in papyrus dominated wetlands in the Nzoia River basin in Kenya. Sampling was done on a weekly basis for the first two months, and thereafter twice per month in the Anyiko rice paddies, which is a smallholder system partly converted from the Anyiko wetland. Two replicates of three fertilization treatments (standard, control and under fertilization) were assigned randomly in six rice plots. The static chamber method was used to collect the GHGs, which were then analyzed using gas chromatography. Soil samples were collected and analyzed for nitrogen and organic carbon stocks. Statistical tests revealed no significant differences in organic carbon and nitrogen stocks among the three fertilization treatments. The mean CH_4 fluxes did not differ significantly among the three treatments where mean flux for control plots were 8.30 ± 4.79 mgm^{-2}h^{-1}; under-fertilized plots had a mean of 6.93 ± 2.42 mgm^{-2}h^{-1} and standard fertilized plots mean fluxes were 4.00 ± 6.34 mgm^{-2}h^{-1}. Similarly, CO_2 mean fluxes were insignificantly different among the three treatments, where control plots had mean of 174.80 ± 26.81 mgm^{-2}h^{-1}, under-fertilized plots mean were 208.81 ± 36.20 mgm^{-2}h^{-1} and standard fertilized plots mean fluxes were 248.29 ± 41.22 mgm^{-2}h^{-1}. However, mean N_2O fluxes were significantly different among the three treatments, control plots had a mean of -3.59 ± 2.56 μgm^{-2}h^{-1}, followed by under-fertilized with mean of -0.59 ± 0.45 μgm^{-2}h^{-1} and standard fertilized plots with mean of 4.37 ± 3.18 μgm^{-2}h^{-1}. In this study, different fertilization scenarios had significant effects on N_2O emission but no significant effect on CO_2 and CH_4 emission, organic carbon and nutrient stocks. Therefore, there is need for sustainable use of wetlands to ensure a balanced role between ecosystem management and human services.

Keywords: rice paddies, wetland, fertilizer application, GHGs, climate change

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INTRODUCTION

Wetlands occupy about 6% of the earth's surface; covering about 7% of Africa alone (Junk et al., 2013). In Kenya, wetlands cover ∼2.5% of the surface area of the country (14,000 km^2) and fluctuate up to 6% during rainy seasons (Crafter et al., 1992). Wetland drainage and land reclamation (conversion of wetlands to arable lands) for crop production, papyrus harvesting and draining of wastewater into the wetland have been reported to be the major threats leading to wetland degradation in Kenya (Morrison et al., 2012). Mironga (2005) also noted that drainage and conversion to arable land have been the key drivers to degradation of wetlands in Kenya.

Rice is one of the essential cereal crops grown globally, in Africa and in Kenya (Balasubramanian et al., 2007). The role of rice as a current and future global food security is inevitable since it is one of the three most important food crops globally after wheat and maize (FAO, 2016). The FAO (2017) predicts global rice production to reach 758.9 million tons (503.8 million tons, milled basis) by 2017. In Africa, 2016 season rice output records put the production at 30.8 million tons (20.1 million tons, milled basis) (FAO, 2017). In Kenya, rice cultivation was introduced in 1907 from Asia (Republic of Kenya, 2008). The annual rice consumption rate in Kenya is estimated at 548,000 metric tons whereas the annual production is 129,000 metric tons (Republic of Kenya, 2014). Rice is either grown in upland areas or in lowland areas where the fields are either rain fed or irrigated. Kenya's major irrigation schemes include Mwea, Yatta, Ahero, Bunyala, and west Kano. These schemes are operated by National Irrigation Board (NIB) and produce about 80% of the rice while the remaining 20% is produced from the rain fed fields (Republic of Kenya, 2008). The growing population and socioeconomic changes have stimulated the need for more agriculturally productive land in the pursuit to improve food security (Junk et al., 2013; Mitchell, 2013). To meet the high demand for rice caused by increasing population pressure, more natural wetland area is converted to rice paddies. This land conversion is coupled with an increased use of fertilizer to increase crop yield.

Global sources of GHG emissions are broadly categorized into natural (44.54%) and anthropogenic (55.46%) (Xi-Liu and Qing-Xian, 2018). Wetlands are natural sources of global GHGs emissions, and account for 17.2% of natural emissions. Olivier and Peters (2018) report that rice cultivation on flooded rice fields is the second largest anthropogenic source of CH_4 (10%) after cattle stocking. The same authors note that agricultural activities are the main source of N_2O where synthetic fertilizers (nitrogen content) account for 18% of N_2O emissions after cattle stocking (21%). Concentrations of GHGs in the atmosphere have been rising steadily since the industrial revolution (Olivier et al., 2017). Greenhouse gas emissions lead to climate change and this has been evidenced in the recent years through: rise in mean global temperature, decreasing snow and ice in the northern hemisphere, ocean warming, extreme weather conditions, and CO_2 concentration has increased by 40% since the pre-industrial era (Cubasch et al., 2013). An additional warming of 1.1°C to 6.4°C is anticipated by future climate change projections (NRC, 2010). To attain Sustainable Development Goal 13 (take urgent action to combat climate change), the 2015 Paris agreement on climate change requires member countries to reduce global warming to well below 2°C to combat climate change.

Rice paddies are an important source of GHG emission (Garthorne-Hardy, 2013). The main GHGs emitted from rice fields include CO_2, CH_4, and N_2O (Arunrat and Pumijumnong, 2017). The major processes responsible for production and emission of these GHGs are: oxic respiration (decomposition), methanogenesis, nitrification, and denitrification (Zhang et al., 2007). In paddy rice soils, CH_4 is produced through methanogenesis under anoxic conditions (Jain et al., 2004). Nitrous oxide production occurs through nitrification and denitrification processes under oxic and anoxic conditions, respectively, whereas, CO_2 production occurs when oxygen is supplied in the soil through decomposition process (Ishii et al., 2011). Rice paddy soils are usually waterlogged providing anoxic conditions; however, the soil can be supplied with oxygen at certain circumstances like when the paddy is drained, at the roots and at the soil-water interface thus providing oxic conditions.

Wetlands are usually waterlogged and therefore provide similar conditions as required in rice paddy soils. For that reason wetlands are converted to rice paddies. Draining of wetlands to convert them to agricultural land exposes soil organic matter to oxygen, leading to its oxidation and subsequent release as CO_2 to the atmosphere (Moomaw et al., 2018). Consequently, the ability of wetlands to sequester and store carbon is impaired and this leads to increased GHGs emission to the atmosphere, which contributes to climate change (Mitchell, 2013). Fertilizer application is one of the land management practices in rice paddies and is applied to increase crop yield (Singh and Singh, 2017). There are different fertilizer application management practices that influence the emission of GHGs for example: method of placement, type of fertilizer, level and form of fertilizer used (Linquist et al., 2012). Fertilizer application has been found to influence CH_4 and N_2O but have less impact on CO_2 emissions (Linquist et al., 2012). Wang et al. (2017) report that application of nitrogen fertilizer in rice paddies showed variability (increase or decrease) in CH_4 emissions but led to increase in N_2O emission. Generally, N fertilizer application increases the GWP of N_2O by 78% (Bin-feng et al., 2016). The nitrogen electron donors and acceptors can be nitrified or denitrified to N_2O when fertilizer is applied to the soil (Wang et al., 2017). Emissions of CO_2 from rice paddies is however low (<1%) since CO_2 emission is largely offset by primary productivity and atmospheric fixation by plants (Linquist et al., 2012). Apart from fertilizer application, farmers employ other management practices to increase yield production including clearing of natural wetlands to expand production area.

Studies on the effect of nitrogen fertilizer application on GHGs emissions in rice paddies have been conducted widely in other part of the world like in Asian countries (Shang et al., 2011; Cheng-Fang et al., 2012; Bin-feng et al., 2016; Arunrat and Pumijumnong, 2017). In sub-Saharan Africa, such studies remain limited despite considerable pressure on wetlands from agriculture particularly; smallholder farms (Houghton et al., 2012; Pelster et al., 2017). In Kenya for instance, area loss ranging from 34 to 55% have been reported in some wetlands in the last four to five decades majorly as a result of conversion to agriculture (Owino and Ryan, 2007; Ondiek et al., 2020). Due to alarming effect of global warming and climate change, understanding the effects of fertilizer applications in the areas of the wetland converted to agriculture is crucial. Therefore, this study assessed the effect of different fertilizer application scenarios on GHGs emissions in smallholder rice paddies converted from Anyiko natural wetlands in Kenya. The specific objectives of this study were to (1) compare soil organic carbon and nitrogen content in rice paddies under standard fertilization (basal, first and second topdressings), under-fertilization (first and second topdressings) and no fertilization (control); and (2) compare CO_2, CH_4 and N_2O emissions in rice paddies under standard fertilization, under-fertilization and no fertilization (control). We hypothesized that (1) different fertilizer application scenarios have no significant effect on the standing stocks of organic carbon and nitrogen in rice paddies; and (2) standard fertilization (basal, first and second topdressings) and under-fertilization (first and second topdressings) has no significant effect on CO_2, CH_4, and N_2O emission in rice paddies.

MATERIALS AND METHODS

Study Area

The study was carried in Anyiko irrigation scheme which is a smallholder system partly converted from Anyiko wetland located in North East Ugenya, Siaya County, Kenya (**Figure 1**). The irrigation scheme was established in 1977 by the Ministry of Agriculture and lies between longitudes 0°16', 38°56"E, 0°14', 18°66"E and latitudes 34°16', 35°55"N, 34°18', 0°57"N in Nzoia River Basin. Currently the scheme is managed by farmers. On inception, the scheme only used water diverted from the adjacent Anyiko wetland via a canal for irrigation. Over the years however, the farmers have converted parts of the wetland to rice paddies and several canals dug out for irrigation. The area of the scheme expanded as a result of conversion of the wetland is unknown. The area covered by the scheme is 120 acres with ∼100 farmers, each owning a paddy rice field of ∼¾ acres. The growing season of rice runs from April to December.

Study Design and Sample Collection

The study was carried out from September 2018 to January 2019, during rice growing season in Anyiko irrigation scheme. The experiment was completely randomized with two replicates of three treatments. The three treatments included different fertilization scenarios: standard fertilization (basal, first and second top dressings), under fertilization (first and second top dressings only), and control (no fertilization) at 50 kg of fertilizer per acre for each, excluding the control. Nitrogen Phosphorus Potassium (NPK) 23:23:0 was used for basal fertilization whereas calcium ammonium nitrate (CAN) was used for first and second top dressings. Basal application was done immediately after transplanting, first top dressing was done 21 days after transplanting (DAT) and second top dressing was done 45 DAT. The fertilizer was applied using broadcasting method. In each of the six study plots, three gas chambers were placed. Gas samples were collected and analyzed (see section Study Design and Sample Collection) as well as soil samples (see section Soil Sampling and Analyses for NH4-N, NO3-N, TN and OC).

Gas Sampling and Analysis for CO_2, CH_4 and N_2O Fluxes

Greenhouse gases, CO_2, CH_4 and N_2O fluxes were measured on a weekly basis for the first two months, and thereafter twice a month in the rice paddies using the static chamber method (Butterbach-Bahl et al., 2016). The chambers were fabricated from twenty-four 30-liter plastic buckets from which eighteen were used for bases (34 cm diameter × 15 cm height) and six as lids (34 cm diameter × 23 cm diameter × 41 cm height). The plastic buckets were used because they are inert to the gases being sampled (Collier et al., 2014). The lids were fitted with a gas sampling port, thermometer to measure chamber internal temperature and 50 cm long vent tube (2.5 mm diameter) to equilibrate pressure differences between ambient and headspace as indicated in **Plate 1** (Collier et al., 2014; Rosenstock et al., 2016; Pelster et al., 2017; Tully et al., 2017). The lids were also insulated with a reflective duct tape all round to minimize insulation. Three chamber bases per rice plot of about a quarter of an acre were inserted 10 cm into the soil 1 week before the first gas sampling. The chamber bases remained in the field for the entire sampling period to prevent collection of GHGs emitted due to soil disturbances **(Plate 2A)**.

During each sampling event, the gas samples were collected between 10 am and 12 noon since studies have shown that this gives average daily emissions estimates (Butterbach-Bahl et al., 2016). Given the six rice plots and having to conduct the sampling within the given time frame, two people (1 person per rice plot) collected the gas samples. Hence, three lids per person per rice plot were used during the gas sampling. The bases and lids were clamped together for 30 min using metallic clips and a rubber seal fitted between them to ensure airtightness (Rochette, 2011). The chambers covered at least 4 rice plants in a transplanted system with spacing of 4 by 6 inches (Butterbach-Bahl et al., 2016). Gas samples (60 ml) were collected from the headspace at 0, 10, 20 and 30 min after lids deployment using a propylene syringe fitted with Luer lock, therefore, giving a total of 4 gas samples per rice plot (**Plate 2B**). The air inside the chamber was manually mixed before gas collection at each time interval by drawing gas from the chamber then pumping it back several times. To overcome spatial heterogeneity of soil GHG fluxes, samples were pooled from the three replicate chambers at each plot to form a composite air sample of 60 ml (Arias-Navarro et al., 2013). The first 40 ml of the sample was used to flush a 10 ml sealed glass vial through a rubber septum, while the final 20 ml was pushed into the vial, leading to a slight overpressure to minimize leakage and contamination of the gas with ambient air (Rochette and Normand, 2003). The ambient air sample was collected using the same procedure in order to assess ambient GHGs concentration during sampling. The height of each chamber base was measured on each sampling date to derive the total chamber volume (volume of the lid = 30 liters plus volume of the base = base area × height). The gas samples were wrapped with parafilm over the vial's crimp seal and transported to the International Livestock Research

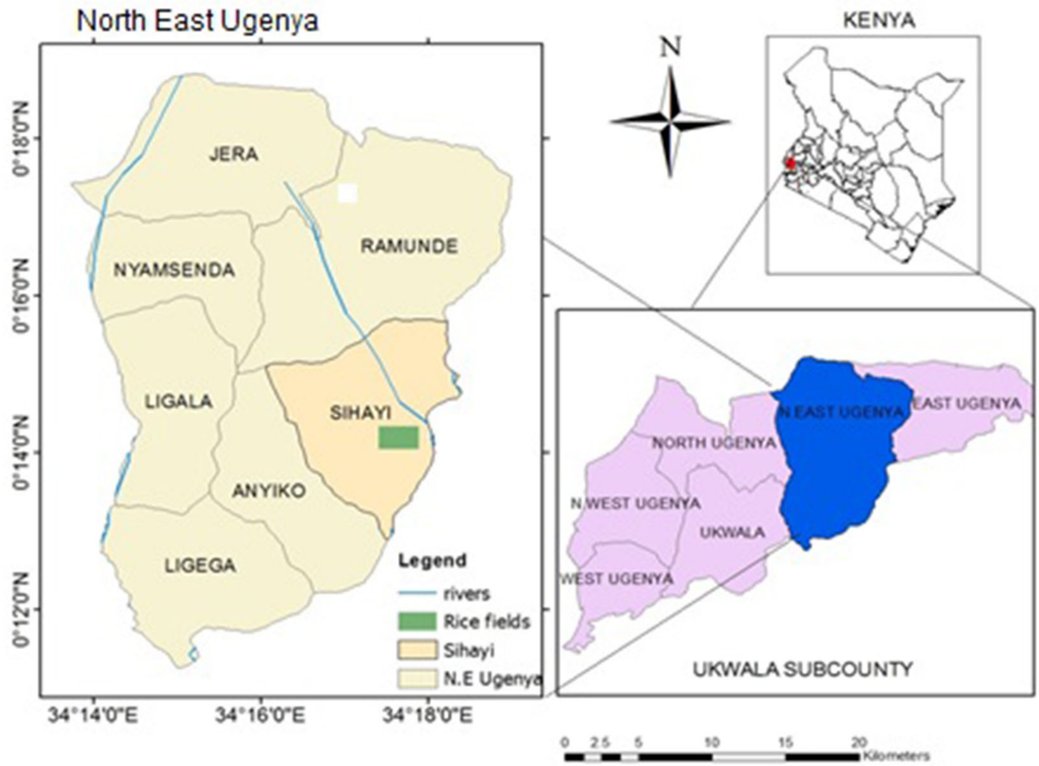

FIGURE 1 | Map of Kenya showing the location of Anyiko rice fields (Modified from Topographical map of Kenya, scale 1:50,000).

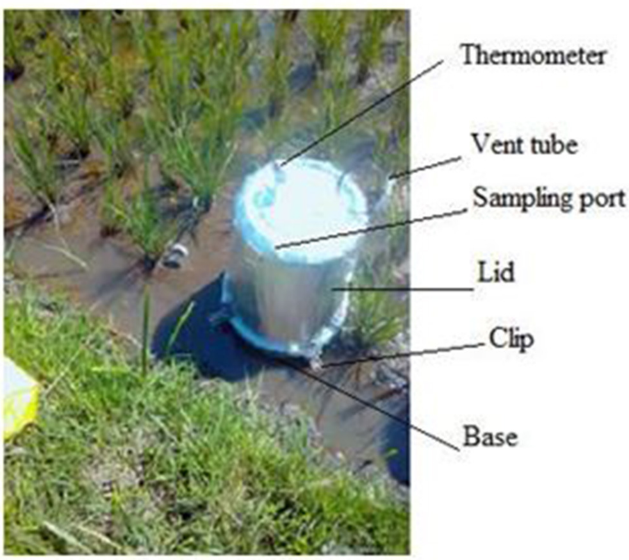

PLATE 1 | Pictorial representation of a fabricated gas chamber.

Institute (ILRI) laboratory, in Nairobi for analysis within 12 h after collection.

The gas samples were analyzed for CO_2, CH_4, and N_2O in an SRI 8610C gas chromatograph (2.74 m Hayesep-D column) fitted with a 63Ni-electron capture detector for N_2O and a flame ionization detector for CH_4 and CO_2 (after passing the CO_2 through a methanizer) (**Plate 3**). The flow rate for the carrier gas (N_2) was 20 mL min^{-1}. Gas concentrations were calculated based on the peak areas measured by the gas chromatograph relative to the peak areas measured for four standard calibration gases. The ideal gas law, atmospheric pressure, internal chamber temperature and chamber volume, measured during sampling were then used to convert the concentrations to mass per volume flux calculated using the the equation below (Butterbach-Bahl et al., 2011).

$$\text{Flux}_{\text{GHG}(\text{mgm}^{-2}\text{h}^{-1})} = \text{Ct} \times \left(\frac{\text{M}}{\text{Vm}}\right) \times \left(\frac{\text{V}_{\text{ch}}}{\text{A}_{\text{ch}}}\right) \times \left(\frac{273.15}{273.15+\text{t}}\right) \times \text{P} \times 60 \tag{1}$$

Where: Ct = slope derived from the linear regression (ppm min^{-1}) for CH_4 and CO_2 and (ppb min^{-1}) for N_2O-N, M = molar weight (g mol-1) (C = 12 for CH_4 and CO_2, and N = 28 for N_2O), Vm = molar gas volume ($m^1 mol^{-1}$), (22.41), Vch = Volume of the chamber headspace (3 0 liters), A_{ch} = Area of gas chamber, t = Chamber temperature (°C), P = Pressure at the time of sampling (atm), 60 = conversion factor of minutes to hour.

Auxiliary Measurements

During each gas sampling campaign, soil temperature at a depth of 0 to 20 cm was measured in each rice plot

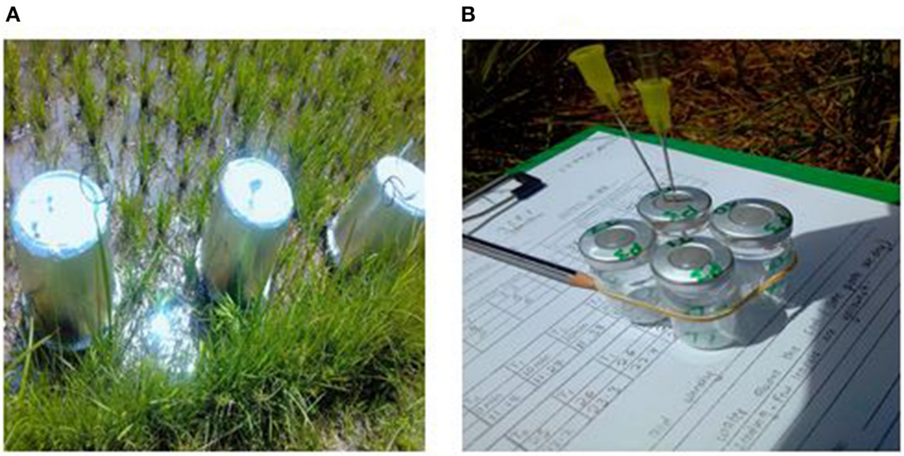

PLATE 2 | (A) Shows the three chambers installed in a plot, **(B)** pre-labeled 10 ml glass vial with crimp seal fitted with two syringes, one for evacuation and one for refilling the vial.

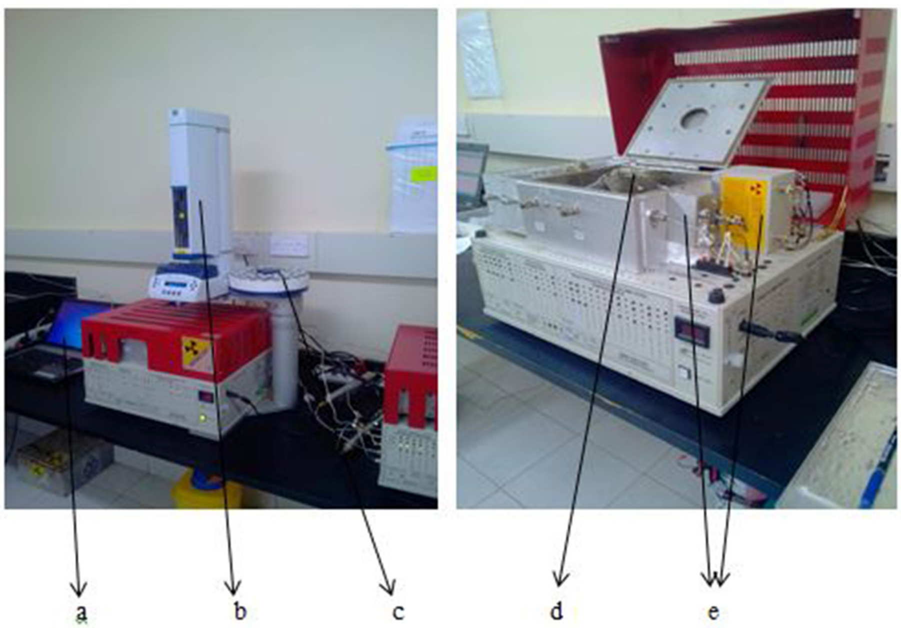

PLATE 3 | (a) A computer for recording peaks, (b) automated injector, (c) gas sample tray, (d) separation columns, (e) the detection units (FID and ECD).

using digital thermometer (Brannan). Digital thermo-hygrometer (model 1141Y43) was used to measure air temperature. Bulk density was determined on the upper 0 to 15 cm using the bulk density ring (98 cm^3). Atmospheric pressure was measured during each sampling campaign using a barometer (installed in phone), though the pressure was quite constant since altitude did not change.

Soil Sampling and Analyses for NH_4–N, NO_3-N, TN and OC

Soil samples were collected using random composite sampling technique at each rice plot using soil auger to a depth of 0 to 15 cm. The samples were collected on every sampling campaign for soil moisture, NO_3-N and NH_4-N analysis. For soil OC and TN analysis, sampling was carried out twice per month. The samples were then transferred into polythene bags and placed in a cool box containing ice packs for transportation to the laboratory for further analysis.

The standard procedure described by Okalebo et al. (2002) was followed to determine soil moisture content. Soil moisture was determined by oven-drying 250 g of soil samples from each rice plot for 48 h at 105°C and then reweighed. Soil moisture content was calculated as:

$$\text{Soil moisture content (\%)} = \frac{\text{weight of the moisture} \times 100}{\text{weight of the dry soil}} \quad (2)$$

The concentrations of NO_3-N and NH_4-N in the soil samples were determined by colorimetric method, where 10 g of fresh soil samples were extracted with 100 ml of 0.5 M K_2SO_4. The solution was shaken for 1 h on a Gallenkamp orbital shaker. The samples were filtered through Whatman GF/C filters and the supernatant analyzed for NO_3-N and NH_4-N. The concentrations of NH_4-N and NO_3-N were calculated from their respective equations generated from standard calibration curves. The concentrations were converted to soil mass as follows:

$$NH_4^+ \left(\mu g\, kg^{-1}\right) / NO_3^- \left(\mu g\, kg^{-1}\right) = \frac{\left(a - b\right) \times V \times MCF \times f \times 1000}{w} \quad (3)$$

Where a = concentration of N in the solution, b = concentration of N in the blank, v = volume of the extract; w = weight of the fresh soil; MCF = moisture correction factor; f = dilution factor.

Total nitrogen was determined by Kjeldahl method (acid digestion, followed by steam distillation and then titration). Soil was oven dried (70°C) and from the dried sample, 0.3 g was digested using 2.5 ml of digestion mixture (hydrogen peroxide, sulphuric acid, selenium, and salicylic acid) at 360°C for 2 h. Thereafter, an aliquot of 10 ml was transferred into a reaction chamber. This was followed by addition of 10 ml of 1% sodium hydroxide and immediately steam distilled for 2 min into 5 ml of 1% boric acid. The distillate was titrated with N/140 HCl until endpoint (color change from green to definite pink). Concentration of total nitrogen was calculated as follows:

$$\text{\% N in soil sample} = \frac{b - a \times 0.1 \times v \times 100}{1000 \times w \times al} \quad (4)$$

Where a = volume of the titer HCL for the blank, b = volume of titer HCL for the sample, v = final volume of the digestion, w = weight of the sample taken and al = aliquot of the solution taken for analysis.

Organic carbon was determined by Walkley–Black method [digestion by sulphuric acid and aqueous potassium dichromate ($K_2Cr_2O_7$) mixture] (Okalebo et al., 2002). Soil samples was oven dried (70°C) to a constant weight. This was followed by complete oxidation of 0.3 g using 7.5 ml sulphuric acid and 5 ml aqueous potassium dichromate ($K_2Cr_2O_7$) mixture. The unused $K_2Cr_2O_7$ was titrated against ferrous ammonium sulfate to endpoint where color changed from greenish to brown. Difference between the added and residual $K_2Cr_2O_7$ gave the measure of OC content in soil. The concentration of OC was determined according to Okalebo et al. (2002).

$$\text{Organic Carbon (\%)} = \frac{\left(0.003 \times 0.2 \left(Vb - Vs\right) \times 100\right)}{w} \quad (5)$$

Where Vb = volume in ml of 0.2 M ferrous ammonium sulfate used to titrate reagent blank solution, Vs = volume in ml of 0.2 M ferrous ammonium sulfate used to titrate sample solution and 12/4000 is the mili-equivalent weight of C in grams.

TABLE 1 | Ancillary variable affecting GHG emissions measured at the study site.

Treatment	Density (g/ml)	Moisture content (%)	Soil temperature (°C)
Control	0.95 ± 0.15^a	53.93 ± 14.97^a	23.99 ± 1.27^a
Under	1.01 ± 0.18^a	74.72 ± 18.55^b	22.95 ± 1.33^a
Standard	1.04 ± 0.17^a	69.14 ± 18.17^{bc}	23.43 ± 1.60^a

Values are presented as mean ± standard deviation. Similar letters indicate no significant difference whereas different letters indicate significant differences.

Data Analysis

Data collected were statistically analyzed using IBM SPSS statistics version 2.0 (USA). All tests were carried out at $p < 0.05$ significance level and data subjected to normality (Shapiro-Wilk) and homogeneity of variance (Levene's) tests. Data for soil organic carbon and total nitrogen were normality distributed and therefore, one-way ANOVA was used to test significant differences between means of their standing stocks in the different treatments. The data for NH_4-N, NO_3–N and fluxes of CH_4, CO_2, and N_2O were not normally distributed and therefore analyzed using the non-parametric Kruskal–Wallis test. Under different fertilizer application scenarios, only N_2O emission varied significantly and hence, Tukey's *post hoc* test was applied to separate the means. Spearman's rank correlation was conducted to determine the relationship between soil properties (C/N ratio, soil moisture content, organic carbon, total nitrogen) and GHGs.

RESULTS

Study Site Characteristics

The mean air temperature and soil temperature for the site were 27.06 ± 3.32°C and 23.46 ± 1.45°C, respectively (**Table 1**). The soil moisture content differed significantly within the plots [one-way ANOVA, $F_{(2, 57)} = 7.74$ $P = 0.001$] with control plots recording lower moisture content (53.93 ± 3.35%) compared to standard fertilized plots (69.14 ± 4.06%) and under-fertilized plots (74.72 ± 4.15%) (Tukey's *post-hoc* test $P < 0.05$) (**Table 1**). The soil bulk density showed no significant variations among the sites [one-way ANOVA $F_{(2, 57)} = 1.697$, $P = 0.192$].

Comparison Between Soil Organic Carbon and Nitrogen Content Among Fertilization Scenarios

Mean TN for the control plots was 0.70 ± 0.38%, 0.78 ± 0.43% for under-fertilized and 0.71 ± 0.35% for standard fertilized plots. The mean soil organic carbon fluxes did not differ significantly among the three treatments [one-way ANOVA, $F_{(2,33)} = 0.219$, $P = 0.804$; **Figure 2**, left]. The mean organic carbon for the control plots was 2.21 ± 0.70%, for the under-fertilized was 2.26 ± 0.68% and for the standard fertilized plots 2.08 ± 0.64%. Mean TN also did not differ significantly among the three treatments (one – way ANOVA, $F_{(2, 33)} = 0.134$, $P = 0.875$; **Figure 2**, right). Mean soil NH_4-N for control plots was 44.96 ± 9.60 µg/Kg, 63.57 ± 10.28 µg/Kg for standard fertilized plots and 68.02 ± 12.49 µg/Kg for under-fertilized plots (**Figure 3**, left). Mean soil NH_4-N however did not differ significantly among the three treatments (Kruskal–Wallis test, $P = 220$). Similarly, mean NO_3-N was also insignificant among the three treatments (Kruskal–Wallis test, $P = 0.602$). Control plots had a mean of 49.37 ± 18.82 µg/Kg, 63.64 ± 26.20 µg/Kg for under-fertilized plots and 71.66 ± 29.44 µg/Kg for standard fertilized plots (**Figure 3**, right). The C/N ratio did not differ significantly among the three treatments [one-way ANOVA, $F_{(2, 33)} = 0.399$, $P = 0.674$]. The C/N ratio for the control plots ranged from 1.2:1 to 8.0:1, under-fertilized plots ranged from 1.3:1 to 8.0:1 while that for standard fertilized plots ranged from 1.2:1 to 5.7:1.

Carbon nitrogen ratio (C/N), TN, organic carbon and soil moisture were determined as some of the drivers of GHG emissions using Spearman correlation. Carbon/nitrogen ratio affects GHGs emissions by influencing mineralization and immobilization processes of the soil. Nitrous oxide showed positive correlation with TN but negative correlation with organic carbon (OC) and C/N ratio; However, both the positive and negative correlations were statistically not significant (**Table 2**). Methane showed an insignificant positive correlation with OC, C/N ration and TN (**Table 2**). Carbon dioxide showed an insignificant positive correlation with OC and C/N ration whereas it had a negative correlation with TN which was equally not significant. Total nitrogen and C/N ratio showed a significant negative correlation ($r_s = -0.808$) whereas OC and C/N ration had a significant positive correlation ($r_s = 0.370$), as illustrated in **Table 2**. However, there was no significant correlation between the soil moisture and the GHGs as shown in **Table 2**.

Comparison of GHG Fluxes Among the Fertilization Scenarios

The mean CH_4 flux was slightly lower in the under-fertilized plots (7.80 ± 2.12 $mgm^{-2}h^{-1}$) compared to that of standard fertilized (10.68 ± 3.79 $mgm^{-2}h^{-1}$) and control (10.82 ± 3.74 $mgm^{-2}h^{-1}$) plots. No significant difference in the CH_4 fluxes was observed among the fertilization scenarios (Kruskal–Wallis test, $P = 0.964$) as shown in **Figure 4**. No significant differences in mean CO_2 flux were observed among the three fertilization scenarios (Kruskal–Wallis test, $P = 0.573$; **Figure 4**). The mean carbon dioxide (CO_2) flux was slightly higher in the standard fertilized plots (248.29 ± 41.22 $mgm^{-2}h^{-1}$) compared to that of the under fertilized plots (208.81 ± 36.20 $mgm^{-2}h^{-1}$) and control plots (174.80 ± 26.81 $mgm^{-2}h^{-1}$). The mean N_2O flux was significantly higher in standard fertilized plots (4.37 ± 3.18 $\mu gm^{-2}h^{-1}$) than in the control plots (−3.59 ± 2.56 $\mu gm^{-2}h^{-1}$), (Tukey's *post-hoc* test, $P = 0.009$). However, there was no statistical difference in the mean N_2O fluxes between standard fertilized plots and under-fertilized plots with a mean of −0.59 ± 0.45 $\mu gm^{-2}h^{-1}$, (Tukey's *post-hoc* test, $P = 0.140$; **Figure 4**). The mean N_2O fluxes in control and under-fertilized plots also had no statistical difference (Tukey's *post-hoc* test, $P = 0.260$; **Figure 4**). The mean GHG fluxes indicated that under-fertilized rice plots were a sink for N_2O (−0.59 ± 0.45 $\mu gm^{-2}h^{-1}$) and a source for CH_4 (6.93 ± 2.42 $mgm^{-2}h^{-1}$) and CO_2 (208.81 ± 36.20 $mgm^{-2}h^{-1}$). Standard-fertilized rice plots were source for N_2O (4.37 ± 3.18 $\mu gm^{-2}h^{-1}$), CO_2 (248.29 ± 41.22 $mgm^{-2}h^{-1}$) and CH_4 (4.00 ± 6.34 $mgm^{-2}h^{-1}$). The control rice plots acted as sink for N_2O (−3.59 ± 2.56 $\mu gm^{-2}h^{-1}$) and a source for CH_4 (8.30 ± 4.79 $mgm^{-2}h^{-1}$) and CO_2 (174.80 ± 26.81 $mgm^{-2}h^{-1}$).

Global warming potential of CH_4 and N_2O were estimated by multiplying their fluxes by the IPCC global warming potentials factors which are 25 and 298, respectively, Solomon et al. (2007), and thus converting into CO_2 equivalents. The combined effect of the three treatments combined on greenhouse gases emission summed up in the mg CO_2 equivalents (CO_2 E) did not show any statistical difference (**Table 3**). The total effect for the three treatments after applying the CO_2 equivalents was not significantly different (Kruskal–Wallis test, $P > 0.05$).

DISCUSSION

Carbon and Nitrogen Stocks in Rice Paddies

Wetland based rice production is an important source of GHGs (Garthorne-Hardy, 2013; Wang et al., 2017). Increased conversion of wetlands to rice paddies reduces their ability to store carbon, thus increasing amount of GHGs (Mitchell, 2013). In this study, low levels of organic carbon stocks were recorded throughout the study period as indicated by the results. Drainage of wetland and land preparation for rice plantation exposed the accumulated organic carbon to oxygen and this accelerated oxidation of organic matter to CO_2 and thus reduced carbon stocks. Kumar et al. (2014) and Ma et al. (2016) reported loss of organic carbon through cultivation and wetland drainage, which could be an explanation for the low levels of organic carbon observed in this study. Mitsch and Hernandez (2013) also noted that drainage of saturated wetland soils in addition to its natural dryness result in increased oxygen diffusion, translating to higher rates of decomposition of organic carbon, consequently an increase in CO_2 emissions. The observed low soil organic carbon can also be attributed to the high CO_2 emission in all the three fertilization scenarios. According to VandenBygaart et al. (2003), when soils in a natural state are converted to agricultural land, there is an important loss of soil organic carbon (SOC) mainly in the form of CO_2. Furthermore, rice paddies are characterized with anoxic conditions which result in methanogenesis, leading to a loss of carbon as CH_4 and hence reduce carbon stocks (Jain et al., 2004). The loss of soil organic carbon in Anyiko rice paddies can also be explained by–alternate drying and wetting conditions which favor growth of microorganisms and hence high carbon mineralization (Ma et al., 2017). Other studies have also reported an increase in soil microbial activity and carbon mineralization under alternate drying and wetting conditions in

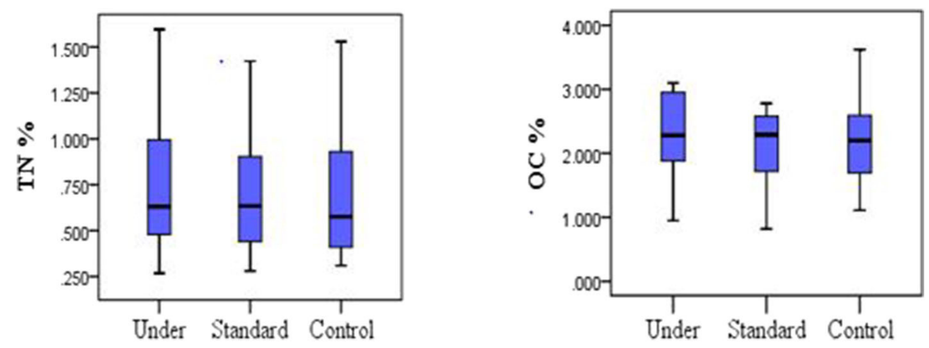

FIGURE 2 | Comparison between nitrogen and carbon stocks in the soil under different fertilizer treatments, (One-way ANOVA, $P > 0.05$).

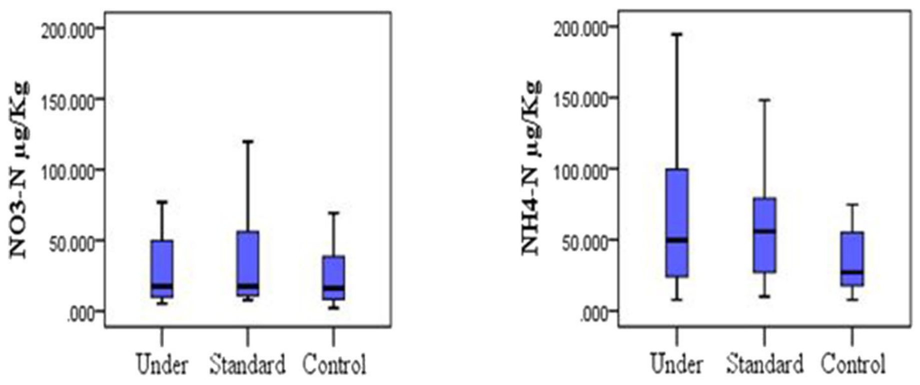

FIGURE 3 | Distribution of nitrogen species (nitrate and ammonium) under different fertilization scenarios in the soil, (Kruskal-Wallis test, $P > 0.05$).

incubation experiments (Fierer and Schimel, 2002; Zhao et al., 2011). The alternate drying and wetting season experienced during the experiment supplied more oxygen into the soil and hence increased oxidation of soil organic carbon which results to high emission of CO_2 into the atmosphere.

The two major microbial processes responsible for nitrogen transformations in soil are mineralization and assimilation by plants and microorganisms (Booth et al., 2005). In this study, the amount of total nitrogen increased from the initial value recorded in pretest (0.18 ± 0.06%) to 0.73 ± 0.38% after the experiment. Supply of nitrogen fertilizer in the soil during the experiment led to increased nitrogen stocks in under-fertilized and standard fertilized plots. Even though the amount of total nitrogen increased, the effect of the different treatments on the plots was not significant. According to Fuhrmanna et al. (2018), accumulation of nitrogen in the soil could be due to immobilization and retention of N fertilizer in the soil. The applied nitrogen fertilizer increased the available nitrogen stock but did not affect the amount of NH_4-N and NO_3-N among the three fertilizer application scenarios used. However, the standard fertilized and under-fertilized plots had high amount of NH_4-N and NO_3-N compared to control plots. This could be associated with fact that application of N fertilizer supplied more nitrogen substrate resulting to enhancement of mineralization and ammonification process (Chirinda et al., 2018). Consequently, more ammonium in the fertilized plots than in the control plots, though the impact was not substantial. Lowland rice is usually grown in waterlogged soils and this condition leads to reductive deamination (conversion of amino acid-N to ammonia via saturated acids), a process called ammonification (Sahrawat, 2010). Additionally, due to varying weather conditions at the study site, the field experienced episodic dry and wet periods. During dry periods, soils become relatively aerated and ammonium formed during mineralization got converted to nitrate via nitrite under oxic conditions (nitrification) (White and Reddy, 2001). This can explain the observed high amount of NO_3-N in the paddy soil. Also Sahrawat (2010) noted that nitrification can be supported at the rice plant's root-soil interface in wetland soils by oxygen transported through the air spaces or aerenchyma tissues of the stem and roots of the plant.

The ratio of carbon to nitrogen (C/N) in arable soils usually ranges between 8:1 and 15:1, with the median being 10:1 and 12:1 (Brady and Weil, 2008). The C/N ration in this study ranged between 1:1.2 and 8:1 which is quite low compared to the normal range of 8:1 and 15:1. Carbon nitrogen ratio in the soil is very important because it affects mineralization and immobilization processes of soil. The available carbon and nitrogen stocks in

TABLE 2 | Correlation matrix between GHGs, organic carbon, total nitrogen and carbon-nitrogen ratio.

Parameters		N_2O	CH_4	CO_2	OC	TN	C/N	MC
N_2O	r_s	1.000						
	Sig							
CH_4	r_s	**−0.395****	1.00					
	Sig	0.002						
CO_2	r_s	0.004	0.050	1.000				
	Sig	0.976	0.703					
OC	r_s	−0.153	0.159	0.054	**1.000**			
	Sig	0.374	0.354	0.755				
TN	r_s	0.046	0.016	−0.005	0.192	1.00		
	Sig	0.791	0.928	0.977	0.262			
C/N	r_s	−0.149	0.123	0.030	**0.370***	**−0.808****	1.000	
	Sig	0.387	0.476	0.860	0.027	0.000		
MC	r_s	−0.166	0.180	0.100	0.940	−0.027	0.017	1.000
	Sig	0.206	0.168	0.448	0.585	0.878	0.923	

**Correlation is significant at 0.05. **Indicate significance at 0.01. The bold values indicated where significant correlation was observed between the variables.*

soil, deposition from the atmosphere, addition of manure and application of inorganic fertilizer influences the GHGs emissions (Oertel et al., 2016). This study noted that N_2O emissions increased with decreased C/N ratio but CH_4 and CO_2 had a positive correlation with C/N ratio, though not significant. This is in agreement with the study by Oertel et al. (2016) who reported a negative correlation of N_2O emission with the C/N ratio, with the lowest emission being recorded at C/N $\geq$ 30 and highest at C/N values of 11 and a positive correlation of CO_2 and CH_4 emission with the C/N ratio. Toma and Hatano (2007) noted that, N_2O and CO_2 emissions increased as the C/N ratio decreased, but not significantly. It is worth noting that in this case, the result for CO_2 contradicts the results of the study by Oertel et al. (2016) and the results of this study. Moreover, intensive management of the peat lands has been found to alter the soil C/N balance, leading to higher variability of GHG emission (Veber et al., 2017).

Other environmental and agronomic factors like temperature, soil moisture content, water regimes, pH, C:N ratio, nutrient supply among others affect the mineralization processes in waterlogged rice soils (White and Reddy, 2001; Li et al., 2003). The observed high NO_3-N content compared to NH_4-N could be because of varying environmental factors during the experiment, like water regime. Sahrawat (2008) explained that mineralization of organic nitrogen in aerobic soils resulting to formation of NO_3-N (nitrification) is more sensitive to high temperature than ammonification. However, more studies need to be done to investigate the impact of environmental and agronomic factors to nitrification and ammonification.

Greenhouse Gas Fluxes Under Different Fertilizer Application Scenarios

Greenhouse gas fluxes for CH_4 and CO_2 were not significantly affected by fertilizer application regimes. N_2O fluxes however varied significantly among the three treatments. This suggests that whether there was fertilizer input or not, the wetlands soils had adequate carbon stocks for the production of GHGs, particularly CH_4 and CO_2. Application of NPK 23:23:0 and CAN at a rate of 50 kg per acre at planting and for top dressing, respectively, promotes release of N_2O as opposed to when fertilizer is applied only at planting or no fertilizer used at all.

Methane emissions in flooded paddy rice fields or any waterlogged soils occur due to anoxic conditions (Ma et al., 2010). The emissions of CH_4 to the atmosphere from paddy rice fields constitute a predominant source of anthropogenic CH_4 (Agnihotris et al., 1998). The three fertilization scenarios did not have an effect on the amount of CH_4 emission. This is in consistent with a study done by Linquist et al. (2012), which reported no effect of fertilizer N rate on CH_4 emissions. Even though CH_4 emission was not affected by the varying fertilization scenarios, the general CH_4 emissions from all the treatment plots were high. The consistently high soil moisture content created by the hydrologic modification to suit rice production provided favorable conditions for methanogens which proliferate methanogenesis (Veber et al., 2017). Lu et al. (2000) explained that fertilized larger plants provide more carbon substrate (roots and exudates) for methanogens thus enhances CH_4 production. Fertilization also leads to enlarged aerenchyma in rice plants and therefore enhancing the pathway for gas movement through the soil substrate and consequently facilitates CH_4 emission (Tang et al., 2018). Nitrogen fertilizer applications however, have been reported to have varying effects on CH_4 emissions. Shang et al. (2011) reported stimulation of CH_4 emission with N fertilizer application. According to Venterea et al. (2005) CH_4 emission is inhibited with N fertilizer application and in certain situations there are no significant effects of different N fertilizer application regimes on CH_4 emission (Mosier et al., 2006).

Fertilizer application regime did not affect the CO_2 emissions. Since fertilizer application had no direct effect on carbon stocks, therefore under similar humidity conditions, a difference in organic carbon based GHG emission is not expected. Carbon dioxide emissions to the atmosphere occur under oxic conditions which favors microbial decomposition of organic matter

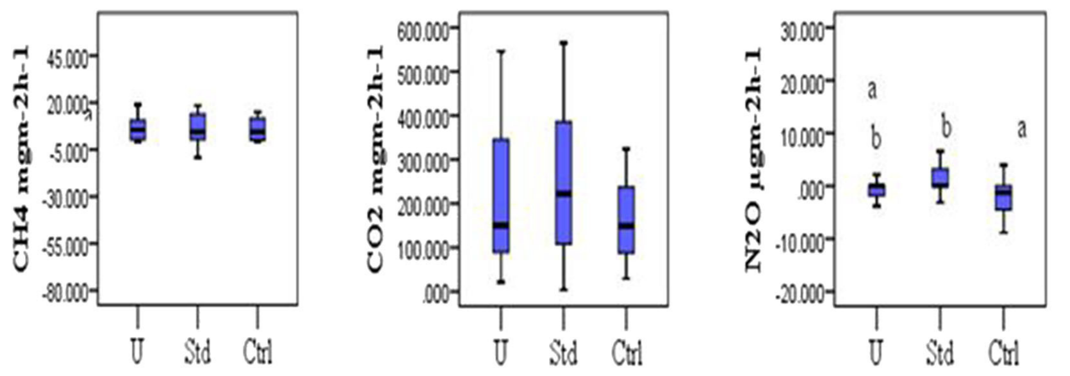

FIGURE 4 | Comparison of GHG fluxes (CH_4 $mgm^{-2}h^{-1}$, CO_2 $mgm^{-2}h^{-1}$, and N_2O $mgm^{-2}h^{-1}$) among the three fertilization scenarios (U, Under-fertilized; Std, Standard fertilized; Ctrl, Control). Different letters denote significant difference, while similar letters indicate no significant differences (Kruskal Wallis test, $P > 0.05$). Pairwise comparison indicated that the significant difference was between control and standard fertilization.

TABLE 3 | Total effect of the greenhouse gases summed up in mg CO_2 Equation.

Treatment	N_20_E (mgm^2h^{-1})	CH_4_E (mgm^2h^{-1})	CO_2 (mgm^2h^{-1})	Total (mg CO_2 E)
Control	−1.07 ± 0.76	207.54 ± 119.81	174.80 ± 124.72	381.27 ± 124.72
Under	−0.18 ± 0.13	173.19 ± 60.41	208.81 ± 36.20	381.83 ± 69.86
Standard	1.30 ± 0.94	100.09 ± 158.50	248.29 ± 41.22	349.69 ± 170.77

Values are presented as mean ± standard error. The GWP of CH_4 and N_2O calculated using the IPCC GWP factors.

(Whiting and Chanton, 2001). The dry incidents experienced during sampling could have led to oxygen supply into the soil, enhancing the aeration, and thus increased CO_2 emissions. In rice paddies, apart from drainage, oxic conditions also occur at the soil-water interface and in the roots hence increasing CO_2 emissions to the atmosphere (Boateng et al., 2017). A study done by Cheng-Fang et al. (2012) showed no significant effect of N fertilizer application on cumulative CO_2 emissions. These results are consistent with the findings of this study where CO_2 emissions within the plots treated with different fertilization scenarios did not differ significantly. However, variable results have been reported from different studies where (Xiao et al., 2005; Iqbal et al., 2009) reported increased CO_2 emissions with use of N fertilizer from rice paddy farms whereas (Burton et al., 2004) recorded a decrease in CO_2 emissions with use of N fertilizer. Long term studies are necessary to improve the understanding of the effect of fertilizer application on carbon stocks and CO_2 emissions in rice paddies.

Nitrogen fertilizer application affected the nitrogen stocks and therefore a notable difference in N_2O emission from the three treatments. Emission of N_2O is influenced by the availability of nitrogen species (NH_4-N and NO_3-N) in the soil since they are required by microbes for nitrification and denitrification processes (Cowan et al., 2015). Bin-feng et al. (2016) reported that N_2O emissions became progressively greater as the quantities of N fertilizer increased. The study noted that N inputs in the range of 52.5–300 kg N ha^{-1} per season caused a significant increment (average 145%) in N_2O emissions. When fertilizer is applied into the soil, there is increased supply of nitrogen substrate for decomposers resulting to enhanced emission of N_2O (Chirinda et al., 2018). Linquist et al. (2012) meta-analysis study also reported that N_2O emissions increased significantly with increasing N fertilizer application rates, which is in agreement with the findings of this study.

Despite the observed differences in the emission levels of the three treatments, their net N_2O emissions were still very low. The low N_2O emissions could be attributed to other environmental factors like immobilization and retention of N fertilizer in soil (Fuhrmanna et al., 2018). The low N_2O fluxes could also be due to some of the nitrogen being–lost through leaching thus reducing amount of nitrogen substrate available for N_2O emissions. Bronson et al. (1997) in their study also observed negligible N_2O emissions during rice growing season when the soil is flooded. This is probably because the strictly anoxic conditions in the flooded paddies are suitable for denitrification and the major product of this process is nitrogen gas (N_2).

The greenhouse gases have varying residence time in the atmosphere and they all have different radiative force and thus different global warming potential GWP. The global warming potential of each gas is measured over a certain period of time using CO_2 as the reference gas. Over a span of 100 years, the GWP for CO_2, CH_4, and N_2O have been found to be 1, 25, and 298, respectively (Solomon et al., 2007). To evaluate the overall effect of GHG production in this study, the GWPs was applied to the fluxes measured and then carbon dioxide equivalent (CO_2 Eq) summed up. However, the effect of the three treatments on the overall GWP was not significant. This could be probably because of the short duration of the study and the similar weather conditions experienced in all the treatment plots. Fertilizer application had no effect on the net GWP. This is in contrast with the study by Bin-feng et al. (2016) which noted that response of GWP to N addition was 3-10 folds greater for fertilization of 250–300 kg N ha^{-1} (266%) than for 50–250 kg N ha^{-1} (26 to 80%). Methane and nitrous oxide emissions from rice fields are however of great concern due to their radiative effects as well as GWP (IPCC, 1995).

Land use change is the major driver of loss of ecosystem services. Ecosystem services maybe lost or reclaimed through change in land use. Wetlands have various roles to human well-being including; provisioning (food, water, and raw materials, fuel), regulating (floods, climate change through carbon sequestration, and water purification), and cultural (aesthetic, spiritual, educational, and recreational) services (MEMR, 2012). Despite their critical role, wetlands are being degraded rapidly and they suffer the greatest transformations worldwide (Zorrilla-Miras et al., 2014). Globally, wetlands losses due to conversion to arable cropping have been the key drivers to degradation of wetlands and increased emission of greenhouse gases (Tangen et al., 2015). The major wetland function impacted by land use change is loss of ability to sequester carbon. Wetlands accumulate carbon in soil and their expansive canopy litter due to anaerobic conditions which results to slow decomposition rates of organic carbon (Batson et al., 2015). However, during land preparation and cultivation for planting rice, the soil organic matter is exposed to favorable decomposition conditions which enhances loss of soil carbon as CO_2. Use of different fertilizers (organic or inorganic) alter the chemical budget adds nutrients into the wetland and; as a result, impair the wetlands ability to purify water (nutrient sink). Cultural value of wetland is also lost when wetlands are cleared for agricultural use.

CONCLUSION

This study assessed soil organic carbon, soil nutrients stocks and greenhouse gas emissions under different treatments including: control (no fertilizer applied), under-fertilization (involved first and second topdressings fertilizer application only), and standard fertilization (involved basal, first and second fertilizer application). The various fertilization regimes did not significantly affect the soil nitrogen species (ammonium and nitrate), total nitrogen and soil organic carbon stocks. Although the fertilizer application regime did not affect the amount of available ammonium and nitrate, there was a significantly higher N_2O emission under standard fertilization compared to no fertilizer application. Fertilizer application regime however, had no effect on CH_4 and CO_2 emissions in the short time of the study. From this study, even though the effect of the three fertilizer application scenarios was not significant, we can conclude that cultivation and land preparation for planting rice increased the loss of organic carbon in the form of CO_2 and therefore the ability of the Anyiko wetland to store carbon was reduced. Use of nitrogenous fertilizer also led to impairment the of climate change regulatory function of the Anyiko wetland. Our findings suggest that the cumulative effects of such changes in the wetland land use may have negative implications on the ecosystem climate change regulating services.

LIMITATIONS OF THE STUDY

The study was conducted during rice growing period and therefore, only provides limited information on temporal variations of the GHG emissions in the rice fields. A year-round study would provide adequate information on emission levels before planting (when the land is bear), during rice growth and after planting.

The study was only conducted in the rice paddies. A concurrent study on the nutrient and organic carbon stocks, and GHG emissions in the natural wetland would provide useful information on the climate regulating ecosystem service of the wetland.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

CO designed the study, fabricated the gas chambers, collected the samples, analyzed the data, and wrote the manuscript. NK and JK gave guidance on the study design and assisted on the manuscript write up. RO gave guidance on gas chambers fabrication, study design and assisted on the manuscript write up. All authors contributed to the article and approved the submitted version.

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