



## Soil Carbon and Nitrogen Mineralization and Crop Parameters in Typical Maize-Bean Intercropping in Western Kenya

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### Authors' contributions

This research was carried out in collaboration between all authors. Authors AJO and UN conceptualized the study design, performed data collection and statistical analysis. Author DA oversaw field management, sampling and data collection and made substantial contribution to manuscript editing and formatting. Author CEO assisted in manuscript reviewing and editing. Author JBN was the principal investigator of the project who managed the funds, played a key role in the inception and conceptualization of the experiment. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work presented.

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### ABSTRACT

Smallholder farmers in western Kenya who plant maize (*Zea mays* L.) intercropped with beans (*Phaseolus vulgaris* L.) face many challenges associated with nutrient-poor soils and weather-related crop failures. In regions where temperatures are favorable, crops are grown twice per year during long and short rainy seasons and in other regions, once per year during one long growing

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season. Growing crops two times per year necessitates frequent land preparation using inversion-type tillage. Little is known about the impact of current tillage-intensive crop management on annual soil organic matter (SOM) recovery. The aim of this study was to assess changes to soil carbon (C), nitrogen (N) and crop performance in typical maize-bean production during long rainy season (LR), short rainy season (SR) and a fallow period (FP) in areas where crops are grown one time (Trans-Nzoia) and two times per year (Bungoma). The two locations were sampled three times per year for a period of three years. Soils were analyzed for potentially mineralizable nitrogen (PMN), ammonium (NH<sub>4</sub>), nitrate (NO<sub>3</sub>), water filled pore space (WFPS), nitrous oxide (N<sub>2</sub>O), methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). Results demonstrated significantly greater PMN, NH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> during SR in Bungoma compared with LR and FP suggesting high C and N mineralization caused by warmer temperatures and high frequency of tillage. Soils in Trans-Nzoia also showed elevated NH<sub>4</sub>, NO<sub>3</sub> and N<sub>2</sub>O during the same period but the magnitude of these changes was much lower compared with Bungoma. Mineralization negatively impacted annual SOM renewal and crop production as further demonstrated by low soil total C and N and cumulative crop yields in Bungoma. Planting edible cover crops, reducing tillage and using manure should become a necessity to support sustainable crop production. Particular attention should concentrate on designing appropriate management strategies for SR season in Bungoma.

**Keywords:** long rainfall season; nutrient cycling; short rainfall season; soil disturbance; Sub-Saharan Africa; sustainability of crop production; tillage disturbance.

## 1. INTRODUCTION

Smallholder farmers in Sub-Saharan Africa (SSA) face numerous challenges associated with nutrient-poor soils and high climatic variability [1]. These challenges impact agroecosystem capacity to maintain and restore soil fertility in support of continuous maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) production. Better understanding of how typical farming practices drive seasonal changes to soil organic matter (SOM) mineralization is needed to support the development of alternative cropping strategies for better soil resource restoration [2].

In much of SSA, maize and beans are intercropped and managed with intensive deep tillage by using a hand hoe and animal-drawn moldboard plow. Maize/bean intercropping allows farmers to intensify production and benefit from diverse plant life strategies [3]. For example, beans fix atmospheric nitrogen (N) that ultimately contributes to soil organic matter (SOM) and provides N to maize [4] while maize provides shading and sheltering for bean plants.

The majority of the mid-elevation SSA region where maize and beans are produced, has bimodal rainfall that delivers between 1,000 mm to 1,600 mm of rain annually. Areas below 1,500 meters of elevation experience warmer temperatures, which allow maize and beans complete their growing cycles faster than at slightly higher elevation [5]. This in combination with bimodal rainfall, permits farmers to plant

crops twice per year, during both “long” and “short” rainfall seasons. Planting during short rainfall season occurs despite high variability of rainfall and results in frequent crop losses [6].

Growing crops twice per year however, necessitates more frequent land cultivation associated with planting and weeding, which may ultimately result in limited land rest and limited annual SOM recovery [7]. Research in other parts of SSA where maize and beans are also produced, has confirmed that deep tillage contributes to poor nutrient retention of already nutrient-depleted acidic soils [8]. For example, Smalling and Fresco [9] reported an annual loss of 30kg ha<sup>-1</sup> N as nitrate (NO<sub>3</sub>) leaching due to inversion-type moldboard plowing. Other factors that contribute to soil nutrient losses from agricultural soils these regions are high soil and weather variability and sloping topography [10].

Estimates of soil labile N, in conjunction with greenhouse gas (GHG) fluxes are robust indices of soil nutrient status and soil response to disturbance and the magnitude of their emissions can be important indicators of agroecosystem health and resiliency. For example, decreasing N<sub>2</sub>O levels with time following adoption of a novel legume cover crop will indicate more efficient belowground C and N retention and improved SOM storage [11]. When coupled with soil inorganic N, measurements of potentially mineralizable N (PMN), carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) fluxes can be of particular interest because all these

compounds are biogenically produced by soil microorganisms that use SOM as their substrate during decomposition and mineralization [12]. Among many a biotic and biotic factors influencing GHG emissions to the atmosphere and SOM mineralization are temperature, moisture, SOM content and plant residue, pH, texture and mineralogy and practices such as cultivation and conservation.

The aim of this study was to assess SOM mineralization and crop performance in typical maize-bean production under double cropping and single cropping systems during long rains (LR), short rains (SR) and fallow period (FP). Our overall hypothesis was that double cropping drives much greater SOM mineralization compared with single cropping and the C and N losses are much higher during the short rains. Better understanding of the consequences of typical crop growing on soil will facilitate development of alternatives aiming to improve soil quality, crop productivity and ultimately agroecosystem health.

## 2. MATERIALS AND METHODS

### 2.1 Site Description

The experiment was carried out for three years starting in May 2011 at two research stations in western Kenya: the lowland Mabanga Farmers Training Centre in Bungoma County and highland Manor House Agricultural Centre in Trans-Nzoia County. The Bungoma study site (00°35'N, 34°34'E; 1200 mm MAP; 27°C MAT; referred to here as Bungoma) is located at 1433 meters elevation in the lower midland agro-ecological zone suitable for two crop growing seasons annually [13].

The Trans-Nzoia study site (01° 01' N, 35° 00' E; 1300 mm MAP; 20°C MAT; referred to here as Trans-Nzoia) is located at 1890 meters elevation in the upper midland agro-ecological zone suitable for one crop growing season annually [13]. Soils in both locations are clay loams or sandy clay loams classified as ferralsols formed on kaolinite clays with high iron and aluminum oxides [14,15]. Soil physical and chemical properties are presented in Table 1.

The long rainfall season typically lasts from late March until late July, and the short rainfall season occurs from August through November. On average, 60% to 70% of annual precipitation falls during the LR [16]. December through March is the fallow period (FP) when, after crop

harvest, very little rainfall occurs. Fig. 1 provides information on seasonal climate and farming practices. Daily precipitation and temperature were monitored using weather stations equipped with data loggers (Hobo® Weather Station, Onset Computer Corp, Cape Cod, Massachusetts). Cumulative monthly precipitation and average air temperatures are shown in Fig. 2.

**Table 1. Soil (0-15 cm) physical characteristics for Bungoma and Trans-Nzoia sites**

Soil properties	Bungoma	Trans-Nzoia
Bulk density (g m <sup>-3</sup> )	1.7	1.6
Clay (%)	36	28
Silt (%)	16	20
Sand (%)	48	52
Soil texture	Clay loam	Sandy clay loam

At each study site, a series of experimental plots (0.36 hectare) were managed in accordance with typical practices used by local farmers. Land preparation involved inversion-type tillage using an animal drawn moldboard plow and a hand hoe. These two tillage implements invert soil to approximately 25-cm depth and 15-cm depth, respectively. Fields were planted with maize and common bean varieties from Kenya Seed Company Ltd. Maize hybrid H513 and Rosecoco-GLP2 bean were used for SR and LR in Bungoma. Maize hybrid 614D, also intercropped with Rosecoco-GLP2 bean was used in Trans-Nzoia.

Planting for LR season in Bungoma and for the entire year in Trans-Nzoia was done in mid-April and in mid-September for SR season in Bungoma. Maize was planted at 53,500 plants per hectare and spaced 75 cm x 30 cm. Beans were planted at 89,000 plants per hectare in between maize rows and spaced at 15 cm within rows. More information on plant parameters is provided in Table 2. Weeding was done three times during each growing season by deep tillage with a hand hoe. Phosphorous (P) at a rate of 60 kg ha<sup>-1</sup> as dia-ammonium phosphate (DAP, 18% N and 46% P<sub>2</sub>O<sub>5</sub>) was applied at planting and N at a rate of 60 kg N ha<sup>-1</sup> as calcium ammonium nitrate (CAN, 27%N) was applied as top dress to maize when maize had six leaves and was 30-45 cm tall.

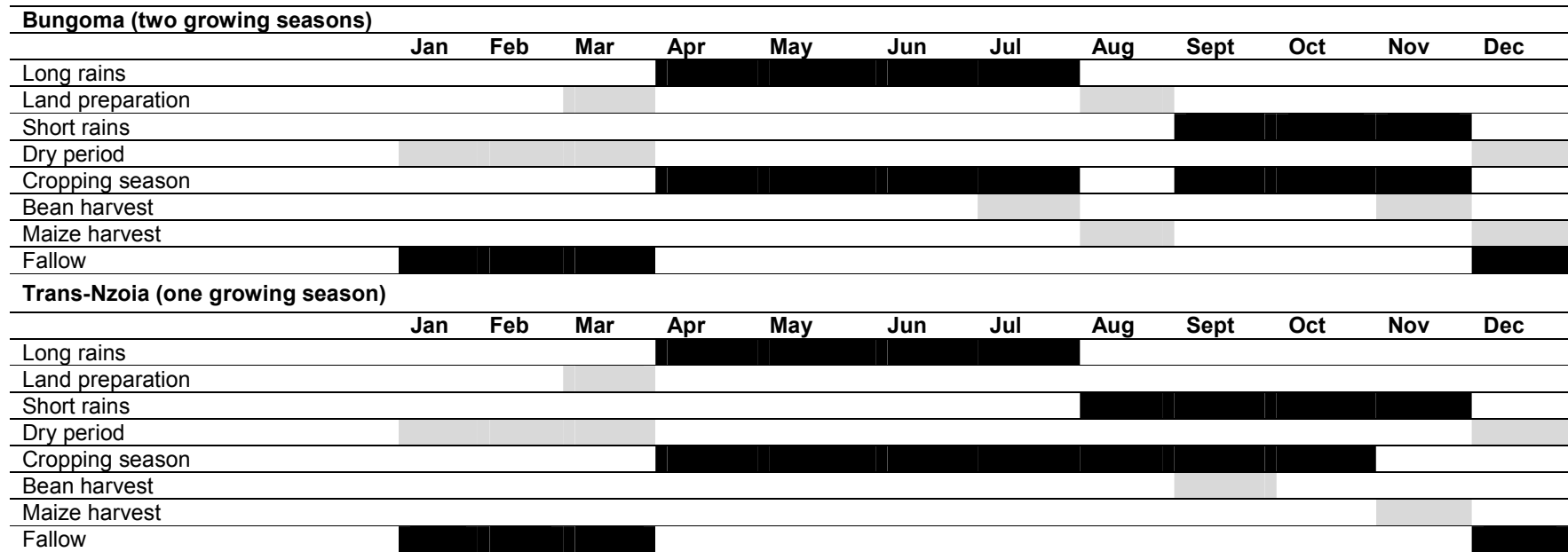
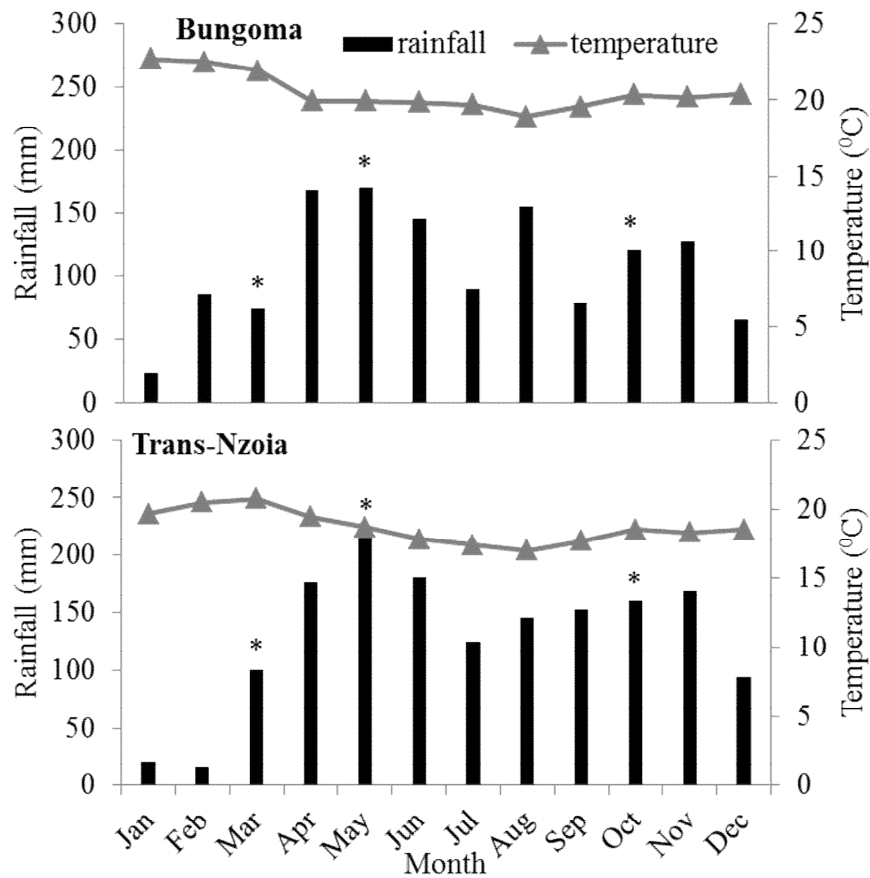


Fig. 1. Diagram representing timeframe of cropping seasons and associated management practices for Bungoma and Trans-Nzoia sites



**Fig. 2. Three-year average monthly cumulative rainfall (mm) and air temperatures (°C) during long rains (LR), short rains (SR) and fallow period (FP). Asterisks indicate sampling periods**

## 2.2 Field Sampling

Soil and gas sampling was done for three years, three times per year during periods that corresponded with LR, SR and FP seasons. Four randomly established 5- by 10-mplots were laid out within fields under typical farmers cropping practices. In each plot, two sub-plots within the vicinity of maize plants and two sub-plots within the vicinity of bean plants were established. Within each sub-plot, polyvinyl chloride (PVC) rings (10 cm high and 25-cm diameter) were installed at each point (four per plot, 16 per study site). These rings served as bases for chamber tops installed periodically for GHG sampling. Chamber tops were 10 cm high and 25 cm in diameter and were made of PVC coated with thin-walled aluminum material [17,18]. Tops were fitted with silicone septa that served as a port for gas sampling.

Soil samples (0-10 cm) were collected 20 cm from the chamber. Soil was homogenized and a

sub-sample oven dried at 105°C for 48 hours to calculate gravimetric water content at the time of sampling [19]. The remainder of each soil sample was air-dried, sieved through a 2-mm sieve, packed and shipped to USA for further analyses. Upon arrival at the laboratory, soil was brought to 23% moisture content and pre-incubated in the dark for 14 days at 30°C [20]. At the end of the 14-day period, 5 g of moist soil was oven dried at 105°C for 48 hours to calculate gravimetric water content as described above. Ammonium-N (NH<sub>4</sub>) and nitrate-N (NO<sub>3</sub>) concentrations were determined by extracting 10 g of soil with 50 ml of a 2.0 M potassium chloride (KCl) using colorimetric methods of Weatherburn [21] and Doane and Horwath [22] on a microplate spectrophotometer (BioTek, Inc., Winooski, VT).

Potentially mineralizable nitrogen (PMN) was determined using 14-day anaerobic incubation [23,24]. Specifically, 5-g samples of pre-incubated soil were placed in 50-ml plastic centrifuge tubes with 12.5 ml of deionized water.

Tube headspace was filled with dinitrogen (N<sub>2</sub>) gas to replace atmospheric air, sealed with an air-tight plastic cap and incubated in the dark at room temperature for 14 days [25]. At the end of the 14-day period, soil was extracted using 12.5 ml of a 4.0 M KCl and analyzed for NH<sub>4</sub> following the method described earlier. PMN was calculated as the difference between initial and post anaerobic incubation concentrations.

Additional soil samples were collected at the beginning and end of each experimental year for determination of soil pH, total P, total C, total N and available P at the Department of Soil Science, University of Eldoret in Kenya using methods described by Okalebo et al. [26]. Soil bulk density was determined using the volumetric core method [27]. Bulk density estimates were used to convert gravimetric soil water content to water filled pore space (WFPS).

Gas measurements were initiated by deploying chamber tops on the previously installed PVC base rings and immediately sealed with rubber gaskets. Gas samples were drawn from chamber headspace using 60-ml plastic syringes. Samples were drawn immediately after each chamber was sealed and then at 15 and 30 minutes. For each sample, a 30-ml aliquot of gas was injected into a previously evacuated 12-ml Labco® glass vial sealed with butyl rubber septa. Samples were shipped to USA within two weeks of sampling. Gas samples were analyzed for CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O concentrations using gas chromatography (Varian 38001 equipped with automatic injector, thermal conductivity, flame ionization and electron capture detectors to measure CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O, respectively). Ten samples containing internal lab standards that travelled to research sites were also analyzed. Gas fluxes were estimated from the rate of change of gas concentrations in chamber headspaces over the 30-minute time periods using Fick's Gas Law [28,29]. Air and soil temperature were recorded at the beginning and end of each sampling and used to calculate GHG fluxes.

Maize height was assessed on five randomly selected plants using a measuring tape stretched between the plant base at soil surface and the arch of the uppermost fully developed leaf. Maize and bean yields were determined at crop maturity by hand harvesting from a three-meter length of the middle row. Grain was air dried to 12% moisture content and weight recorded.

## 2.3 Data Analysis

Data was analyzed using split plot in time and completely randomized design using R [30]. Effects of site, season and site x season interaction were assessed using site as a fixed term, time of sampling as a repeated measurement and replicated plots as random terms in the PROC MIXED statistical model. For site and season comparisons, data were based on weighted values derived from individual crop planting densities. The effect of individual crops was assessed using site as a fixed term, time of sampling as a repeated measurement and crop and replicated plots as random terms in the PROC MIXED statistical model. Data was tested for normality using the Shapiro-Wilk test and log-transformed to assure normal distribution of data for further statistical analyses. Mean separations were conducted using the Fisher's protected Least Significance Difference (LSD) procedures. Treatment effects were considered significant when probability of a greater F values were equal to or lower than 0.05, unless otherwise stated. Pearson correlations and linear regressions were carried out to test the relationships among WFPS, PMN, NH<sub>4</sub>, NO<sub>3</sub> and GHG fluxes.

## 3. RESULTS AND DISCUSSION

### 3.1 Weather, Crop Performance and Soil Parameters

Air temperatures in Bungoma during the study period averaged 21°C and were two degrees higher than in Trans-Nzoia (Fig. 2). Bungoma also received a cumulative annual rainfall of 1,305 mm, which was 250 mm less than Trans-Nzoia. Seasonal distribution of rainfall showed more intense rainfall events during LR and more prolonged periods of no rainfall during SR in Bungoma.

Soils in Bungoma had 2.0 g kg<sup>-1</sup> of total C and 0.2 g kg<sup>-1</sup> of total N which amounted to 30% less than soils in Trans-Nzoia (Table 2). Both sites had low but comparable total P contents, but soils in Bungoma had 7.7 mg kg<sup>-1</sup> of available P which amounted to 50% less than soils in Trans-Nzoia. Soil pH was comparable between the locations and averaged 5.3.

Soil WFPS was significantly greater in Trans-Nzoia than Bungoma across all sampling periods (Fig. 3). Interestingly, there were no statistical differences in WFPS between sampling events in

Bungoma and the values ranged between 30.0% and 37.0%. In Trans-Nzoia, the highest soil WFPS of 49% was reported in LR and the lowest WFPS of 39% was observed in FP.

**Table 2. Soil (0-15 cm) chemical properties averaged across two years. Values that follow “±” are standard errors of a mean. Lower case letters indicate significant differences between sites at  $P \leq 0.05$**

Soil properties	Bungoma	Trans-Nzoia
pH	5.2±0.1 $ns$	5.3±0.1 $ns$
Total C (g kg <sup>-1</sup> )	2.0±0.5 $b$	3.0±0.1 $a$
Total N (g kg <sup>-1</sup> )	0.20±0.05 $b$	0.30±0.03 $a$
Total P (mg kg <sup>-1</sup> )	30.0±10 $ns$	50.0±10 $ns$
Available P(mg kg <sup>-1</sup> )	7.1±1.5 $b$	13.3±2.0 $a$

Maize plants at V-6 were between 40% to 50% shorter in Bungoma than in Trans-Nzoia (Table 3). Maize yield in Bungoma totaled 1.4 Mg ha<sup>-1</sup> per year (two growing seasons), which amounted to 40% less overall yields than from one long growing season in Trans-Nzoia. Bean yields in Bungoma totaled 0.23 Mg ha<sup>-1</sup> in LR and, no bean yields were obtained in SR due to poor crop establishment in all three years of the experiment. This amounted to 66% lower annual yields in Bungoma compared with Trans-Nzoia.

### 3.2 Soil Nitrogen

Soil PMN concentrations were up to four times greater in Bungoma than in Trans-Nzoia (Fig. 4a). In Bungoma, PMN values during SR and FP amounted to 9.1 and 6.9 mg kg<sup>-1</sup>, respectively and were almost three times greater compared with values reported for LR. In Trans-Nzoia, no differences in PMN between seasons were observed. Crop species had a significant impact on seasonal soil PMN in Trans-Nzoia only (Fig. 5a). Up to four times more PMN was observed in soils beneath maize during LR compared with 1.0 mg kg<sup>-1</sup> of PMN in soils beneath bean plant (Fig. 5a).

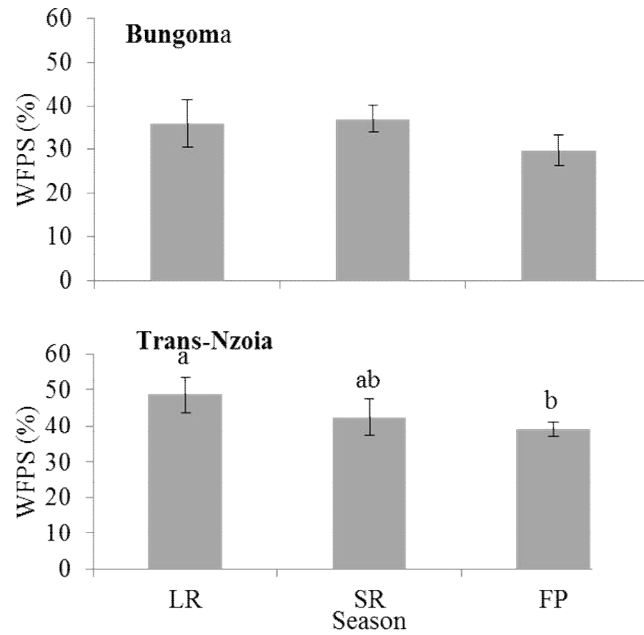
Soil NH<sub>4</sub> concentrations were also up to four times greater in Bungoma than in Trans-Nzoia (Fig. 4b). The highest NH<sub>4</sub> in Bungoma (18.5 g kg<sup>-1</sup>) was observed in SR and was five to nine times the levels reported for FP and LR, respectively. In Trans-Nzoia, NH<sub>4</sub> of 2.8 g kg<sup>-1</sup> was also significantly higher in SR and the values were only two times greater compared with FP and LR (Fig. 4b).

In comparison, soil NO<sub>3</sub> was higher in Trans-Nzoia compared with Bungoma (Fig. 4c). In Trans-Nzoia, the highest NO<sub>3</sub> of 32.5 g kg<sup>-1</sup> was observed in SR. The values in LR and FP were significantly lower and ranged between 19.7 g kg<sup>-1</sup> and 23.7 g kg<sup>-1</sup>. In Bungoma, NO<sub>3</sub> was comparable between LR and SR and ranged between 25.6 g kg<sup>-1</sup> and 26.9 g kg<sup>-1</sup>, which was about 40% more than FP.

Both locations demonstrated the highest N<sub>2</sub>O fluxes during SR and the lowest fluxes during FP with much greater fluxes in Bungoma (Fig. 4d). In Bungoma, the highest flux of 70.6 µg m<sup>-2</sup> h<sup>-1</sup> was almost twice as high as the N<sub>2</sub>O flux during SR in Trans-Nzoia. Fluxes during LR ranged between 25.8 µg m<sup>-2</sup> h<sup>-1</sup> in Bungoma and 19.0 µg m<sup>-2</sup> h<sup>-1</sup> in Trans-Nzoia and between 6.2 µg m<sup>-2</sup> h<sup>-1</sup> and 12.0 µg m<sup>-2</sup> h<sup>-1</sup> in Bungoma and Trans-Nzoia during FP. The magnitude of N<sub>2</sub>O fluxes depended on soil associations with specific crops as demonstrated by a significant season x crop interactions at both locations. Maximum N<sub>2</sub>O of 120.8 µg m<sup>-2</sup> h<sup>-1</sup> was reported in soils beneath beans during SR in Bungoma, which was twice as much as soils beneath maize plants (Fig. 5b). In Trans-Nzoia, maximum N<sub>2</sub>O of 26.5 µg m<sup>-2</sup> h<sup>-1</sup> was reported also for soils beneath bean plants but during LR which was almost three times as high compared with soils beneath maize.

### 3.3 Soil Carbon

Soil CO<sub>2</sub> fluxes were the highest during SR and the lowest during FP in both locations with values in Bungoma much higher compared with Trans-Nzoia (Fig. 6a). The highest CO<sub>2</sub> fluxes of 116.3 mg m<sup>-2</sup> h<sup>-1</sup> were observed in SR in Bungoma and the highest flux of 82.3 mg m<sup>-2</sup> h<sup>-1</sup> was observed in SR in Trans-Nzoia. Fluxes in SR were approximately 1.5 times higher than in LR. In contrast, CO<sub>2</sub> fluxes in FP declined to the lowest levels at both locations. Soil CO<sub>2</sub> fluxes also depended on soil associations with specific crops as demonstrated by a significant season x crop interactions (Fig. 7a). In Bungoma, CO<sub>2</sub> fluxes from soils beneath maize were 45% greater compared with soils beneath beans. The fluxes depended on the season and were 24%, 75% and 34% greater in soils under maize than beans during SR, LR and FP, respectively. In Trans-Nzoia, CO<sub>2</sub> fluxes from soils under bean plants were comparable to those from soils under maize except for FP, when they were 19% greater from soils under beans than from soils under maize (Fig. 7a).



**Fig. 3. Water filled pore space (WFPS %) during long rains (LR), short rains (SR) and fallow period (FP) averaged across the three years. Error bars indicate standard error of the mean. Lower case letters indicate significant differences at  $P \leq 0.05$**

**Table 3. Maize and bean growth parameters. Values that follow “±” are standard errors of a mean. Lower case letters indicate significant differences between sites at  $P \leq 0.05$**

	Bungoma	Trans-Nzoia
<b>Long rains</b>		
Maize height at V-6(cm)	70.5±10.7b	126.1±20.6a
Maize yield (Mg ha <sup>-1</sup> )	1.10±0.4b	2.00±0.1a
Bean yield (Mg ha <sup>-1</sup> )	0.20±0.1b	0.70±0.1a
<b>Short rains</b>		
Maize height at V-6(cm)	68.8±17.1	-
Maize yield (Mg ha <sup>-1</sup> )	0.30±0.1	-
Bean yield (Mg ha <sup>-1</sup> )	-	-

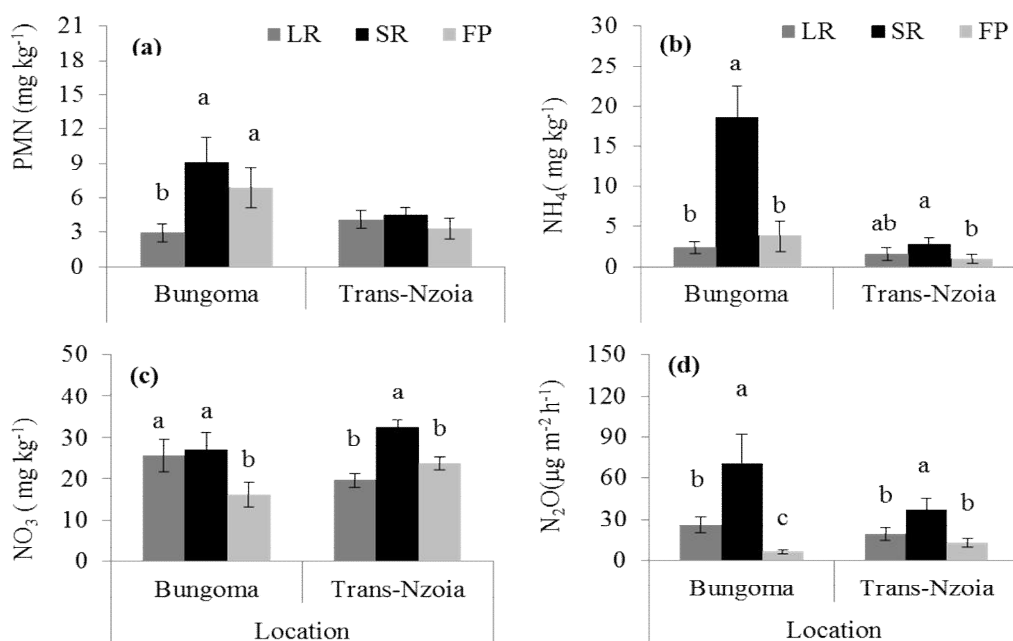
Soil CH<sub>4</sub> fluxes were the least negative during FP, intermediate during LR and the most negative during SR at both sites (Fig. 6b). The values were much lower in Bungoma than in Trans-Nzoia. Soil CH<sub>4</sub> fluxes also depended on soil associations with specific crops as demonstrated by a significant season x crop interaction. Soils under beans had over 50% more negative fluxes than soils under maize

except for CH<sub>4</sub> fluxes during FP in Trans-Nzoia, where the reverse pattern was observed (Fig. 7b). In Bungoma, CH<sub>4</sub> fluxes in soils under beans amounted to 211%, 38% and 62% greater CH<sub>4</sub> assimilation compared with soils associated with maize in FP, LR and SR, respectively. In Trans-Nzoia, CH<sub>4</sub> fluxes from soils under beans and maize were comparable during FP (Fig. 7b). In LR and SR, CH<sub>4</sub> fluxes from soils under beans were 100% and 156% more negative than from soils under maize, respectively.

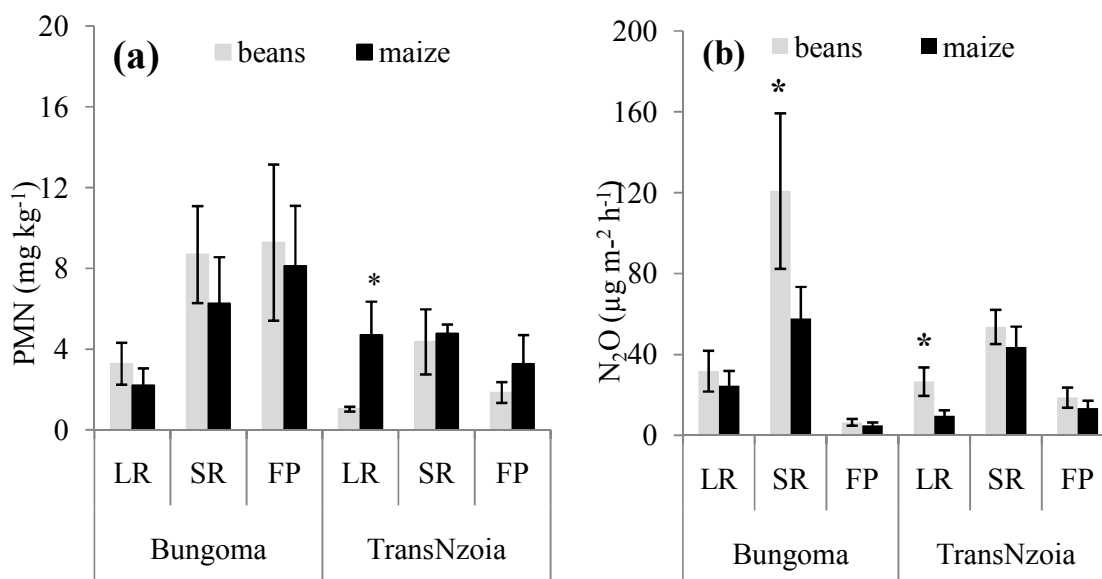
### 3.4 Relationships between C and N Mineralization

Pearson correlations showed positive relationship between N<sub>2</sub>O and WFPS in LR and SR at both locations and between N<sub>2</sub>O and CO<sub>2</sub> in Trans-Nzoia only (Table 4). However, N<sub>2</sub>O was negatively correlated with CO<sub>2</sub> in SR in Bungoma. In Trans-Nzoia, CO<sub>2</sub> was positively correlated with WFPS in both seasons and NO<sub>3</sub>-N was negatively correlated with WFPS and CO<sub>2</sub> in LR only. In Bungoma, NH<sub>4</sub>-N was negatively correlated with NO<sub>3</sub>-N in LR but positively correlated with N<sub>2</sub>O in SR.





**Fig. 4. (a) Soil potentially mineralizable nitrogen (PMN, (b) ammonium (NH<sub>4</sub>), (c) nitrate (NO<sub>3</sub>) concentrations and (d) nitrous oxide (N<sub>2</sub>O) fluxes for long rains (LR), short rains (SR) and Fallow Period (FP) for Bungoma and Trans-Nzoia locations. Lower case letters indicate least significant differences at  $P \leq 0.05$**



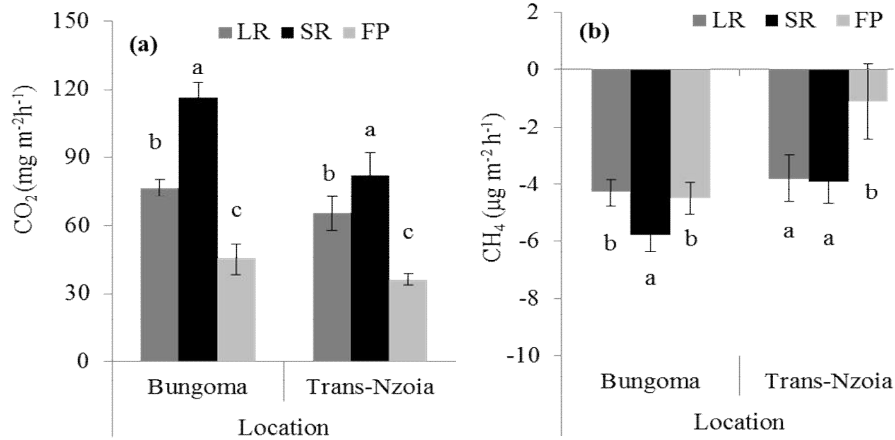
**Fig. 5. (a) Soil potentially mineralizable nitrogen (PMN) and (b) nitrous oxide (N<sub>2</sub>O) fluxes from soils beneath beans and maize plants during long rains (LR), short rains (SR) and Fallow Period (FP) at Bungoma and Trans-Nzoia. Asterisks indicate significant differences between crops within a season at  $P \leq 0.05$**

Regression analyses demonstrated that 77% to 80% of N<sub>2</sub>O fluxes in SR can be predicted based on CO<sub>2</sub> in both locations and 43% of N<sub>2</sub>O fluxes

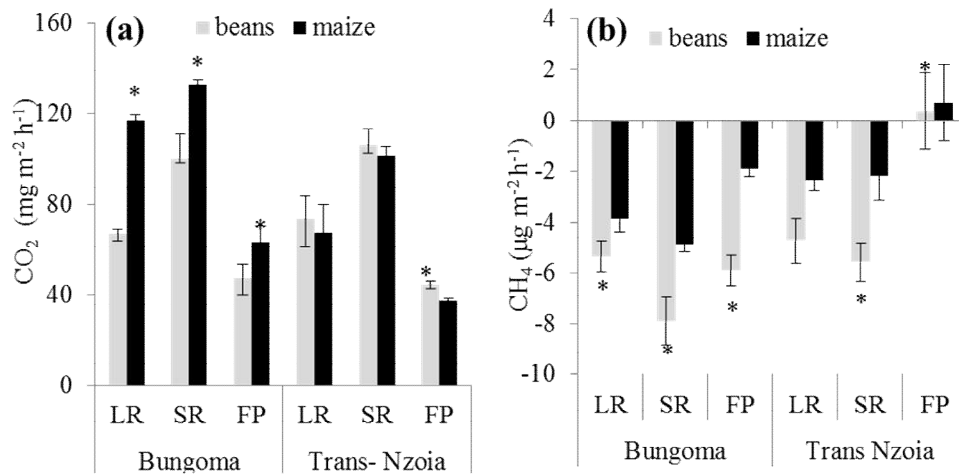
in LR can be predicted based on CO<sub>2</sub> in Trans-Nzoia only (Table 5). Values of the regression slopes for both locations however, demonstrated

differential patterns. The regression slope was negative for SR in Bungoma and positive for LR and SR in Trans-Nzoia. In addition, 61% to 65% of N<sub>2</sub>O can be predicted based on soil WFPS for both seasons in Bungoma, for SR in Trans-Nzoia and 30% of N<sub>2</sub>O for LR in Trans-Nzoia (Table 5). The regression slope representing the relationship between N<sub>2</sub>O and WFPS for LR was comparable between the two locations (1.74 and

1.46). The value for the slope during SR however, was 3.5 times greater in Bungoma (5.68) than Trans-Nzoia (1.65). Moreover, between 35% and 38% of N<sub>2</sub>O flux in Bungoma during SR can be predicted based on incubated soil NH<sub>4</sub>-N and PMN and 41% of N<sub>2</sub>O flux in Trans-Nzoia during SR can be predicted based on soil NO<sub>3</sub>-N (Table 5).



**Fig. 6. (a) Carbon dioxide (CO<sub>2</sub>) and (b) methane (CH<sub>4</sub>) fluxes during short rains (SR), fallow period (FP) and long rains (LR) averaged across the three years in Bungoma and Trans-Nzoia locations. Lower case letters indicate least significant differences at P ≤ 0.05**



**Fig. 7. (a) Carbon dioxide (CO<sub>2</sub>) and (b) methane (CH<sub>4</sub>) from soils associated with different crops (beans and maize) during long rains (LR), short rains (SR) and fallow period (FP) at Bungoma and Trans-Nzoia locations averaged across three years. Asterisks indicate a significant difference between crops at P ≤ 0.05 within each location**

**Table 4. Pearson correlations between percent water filled pore space (WFPS), carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), soil potentially mineralizable N (PMN), nitrate (NO<sub>3</sub>) and ammonia (NH<sub>4</sub>) in long rains (LR) and short rains (SR) in Bungoma and Trans-Nzoia. “\*\*\*” indicates statistical significance at  $P \leq 0.01$ , “\*\*” indicates statistical significance at  $P \leq 0.05$ , and “†” indicates statistical significance at  $P \leq 0.1$**

		LR						SR					
		WFPS	CO <sub>2</sub>	CH <sub>4</sub>	N <sub>2</sub> O	PMN	NO <sub>3</sub>	WFPS	CO <sub>2</sub>	CH <sub>4</sub>	N <sub>2</sub> O	PMN	NO <sub>3</sub>
Bungoma	WFPS	1						1					
	CO <sub>2</sub>	0.32	1					-0.49	1				
	CH <sub>4</sub>	-0.23	-0.44	1				-0.27	-0.53†	1			
	N <sub>2</sub> O	0.75**	0.11	0.03	1			0.81**	-0.87**	0.14	1		
	PMN	-0.46	0.22	0.16	0.37	1		0.46	-0.33	-0.1	0.53†	1	
	NO <sub>3</sub>	-0.19	0.14	0.3	0.35	0.19	1	-0.33	0.27	0.1	-0.39	-0.45	1
	NH <sub>4</sub>	0.02	-0.03	-0.07	-0.11	-0.1	-0.89**	0.58	-0.41	-0.1	0.63*	0.38	-0.08
Trans-Nzoia	WFPS	1						1					
	CO <sub>2</sub>	-0.13	1					0.73**	1				
	CH <sub>4</sub>	0.21	-0.47	1				-0.1	-0.33	1			
	N <sub>2</sub> O	0.63*	0.66**	0.04	1			0.79**	0.89**	-0.41	1		
	PMN	0.09	-0.57†	0.35	-0.58†	1		0.1	-0.33	0.03	-0.11	1	
	NO <sub>3</sub>	0.26	-0.33	0.49	-0.22	-0.11	1	-0.66**	-0.83**	0.27	0.21	0.47	1
	NH <sub>4</sub>	-0.51†	-0.1	-0.1	-0.23	0.18	-0.51†	-0.30	-0.44	0.13	-0.17	-0.16	0.21

**Table 5. Regression equations, R square (Rsq) and P values for the relationships between nitrous oxide (N<sub>2</sub>O) and percent soil water-filled pore space (WFPS), carbon dioxide (CO<sub>2</sub>), soil potentially mineralizable nitrogen (PMN), ammonium (NH<sub>4</sub>) and nitrate (NO<sub>3</sub>) in long rains (LR) and short rains (SR) in Bungoma and Trans-Nzoia. “\*\*\*” indicates statistical significance at P ≤ 0.01, “\*\*” indicates statistical significance at P ≤ 0.05**

Location	Equation	LR		SR		
		Rsq	P value	Equation	Rsq	P value
<b>Bungoma</b>						
WFPS	y = 1.74x - 53.71	0.61**	≤ 0.01	y = 5.68x - 98.93	0.65**	≤ 0.01
CO <sub>2</sub>			<i>ns†</i>	y = -3.1x + 451.50	0.77**	≤ 0.01
PMN			<i>ns</i>	y = 9.59x + 18.57	0.35*	≤ 0.05
NO <sub>3</sub>			<i>ns</i>			<i>ns</i>
NH <sub>4</sub>			<i>ns</i>	y = 4.90x + 28.45	0.38*	≤ 0.01
<b>Trans-Nzoia</b>						
WFPS	y = 1.46x + 105.00	0.30*	≤ 0.05	y = 1.65 x - 38.39	0.61**	≤ 0.01
CO <sub>2</sub>	y = 0.36x + 43.80	0.43*	≤ 0.05	y = 1.48x - 105.30	0.80**	≤ 0.01
PMN			<i>ns</i>			<i>ns</i>
NO <sub>3</sub>			<i>ns</i>	y = 0.66x + 6.05	0.41**	≤ 0.01
NH <sub>4</sub>			<i>ns</i>			<i>ns</i>

#### 4. DISCUSSION

The aim of this study was to assess seasonal soil C and N mineralization from typical maize-bean intercropping in two agricultural areas in SSA. Our results demonstrated significant rates of C and N mineralization in both locations, suggesting limited soil ability to restore SOM in these already low C and N containing soils. This was especially acute in Bungoma during SR where growing crops during two seasons resulted in much greater magnitude of SOM mineralization compared with Trans-Nzoia where crops were grown during one long growing season.

Soil incubation assays to determine potentially mineralizable N are robust indices often used to assess the magnitude of N mineralization during the growing season. Direct field N<sub>2</sub>O flux measurements combined with laboratory PMN assay suggest significant N mineralization across all seasons and all sites. In general, the magnitude of soil N mineralization and high N<sub>2</sub>O fluxes were comparable to those reported from maize production from tilled under agroforestry residues in Kenya [31], under comparable rates of inorganic N fertilizer in SSA [32] and in South America [33]. The magnitude of N<sub>2</sub>O fluxes in our study however, was much greater when compared with fluxes from high SOM soils in other studies [34,35]. However, the magnitude of NH<sub>4</sub>-N and NO<sub>3</sub>-N concentrations after 2-wk aerobic incubation in this study was relatively high compared with, for example, estimates of *in-situ* net N mineralization in field settings following tillage and N fertilizer application in maize [31]. This suggests that a significant portion of plant available N was likely lost to NO<sub>3</sub> leaching and N<sub>2</sub>O emissions instead of being taken up by plants as demonstrated by low overall yields.

Tillage and weed management practices associated with growing the second crop in SR played an important role in accelerating soil N mineralization in Bungoma as demonstrated by the highest rates of PMN, NH<sub>4</sub> and N<sub>2</sub>O fluxes. Tillage operations during SR triggered frequent episodes of soil drying/rewetting which likely accelerated post-harvest plant residue breakdown accumulated in soil after LR growing season. Interestingly, soil WFPS during SR was comparable to FP in Bungoma yet the seasonal pattern of rainfall precipitation suggest frequent wetting and drying cycles. Significant relationships between N<sub>2</sub>O fluxes and soil PMN and NH<sub>4</sub>-N supported the notion of high rates of

seasonal N mineralization and possible nitrification despite high soil water content and low soil pH.

Not only were the overall soil N mineralization and N<sub>2</sub>O fluxes greatest during SR in Bungoma, but also, contrary to our expectations, N<sub>2</sub>O fluxes were the greatest from soils beneath bean plants, further demonstrating limited SOM-N retention and high N turnover of N-rich bean residues also proposed by Jeuffroy et al. [36]. Elevated N<sub>2</sub>O fluxes from soils beneath beans could also be attributed to periodic soil disturbance associated with weeding operations in maize inter-rows severing Live plant roots and nodules, further confirming the negative impacts of frequent tillage on SOM recovery [37].

Tillage also results in a greater overall C mineralization as demonstrated by much lower CO<sub>2</sub> fluxes during the FP than CO<sub>2</sub> flux values reported for undisturbed grassland soils in SSA [38-40]. High rainfall during the growing seasons is also an important factor contributing to large CO<sub>2</sub> fluxes in LR and SR. Interestingly, CO<sub>2</sub> fluxes observed in this study in LR and SR were considerably greater than fluxes from similarly managed high SOM content in maize-soybean production [34,35,41,42]. Though sporadic rainfall events are known to trigger C mineralization and immediate CO<sub>2</sub> pulses [11], our data collection was always completed before the afternoon rain showers and the CO<sub>2</sub> fluxes did not correlate with WFPS except for during SR in Trans-Nzoia.

The highest CO<sub>2</sub> flux was observed during SR in Bungoma when soil WFPS was comparable to FP and the location experienced below average cumulative rainfall during the study period. Therefore, the highest CO<sub>2</sub> flux during SR in Bungoma was likely caused by additional tillage disturbance for land preparation and weeding of the second maize bean crop, which likely accelerated decomposition of fresh plant residues left behind after LR crop harvest. This was also observed by Ellert and Janzen [43]. Additional tillage operations and new crop production during the second growing season limited soil ability to sequester newly deposited plant-derived organic material and could contribute to SOM depletion of already much lower C and N soils when compared with Trans-Nzoia. Employing soil conservation practices such as reduced tillage for both locations and, in case of Bungoma, letting the land rest during SR can help regain some of the SOM over time.

Mapanda et al. [32] demonstrated that fallowing for at least 10 years and amending maize with adequate amounts of N fertilizer lowered CO<sub>2</sub> fluxes and improved SOM and maize yields in Zimbabwe.

Most agricultural soils worldwide are effective CH<sub>4</sub> sinks except for submerged agricultural soils where the anaerobic environment stimulates CH<sub>4</sub> production [44]. Soils in this study appeared to be weak sinks or even mild sources of CH<sub>4</sub> fluxes. The flux values obtained in this study were however, less negative compared with a previous study by Mapanda et al. [32] from typical maize production under different levels of N fertilizer application in Zimbabwe. Low soil ability to act as a CH<sub>4</sub> sink in our study could be due to the negative effects of tillage disturbance on SOM and possible destruction of microsites reducing the populations of methanotrophic bacteria [45].

## 5. CONCLUSION

Results from this study supported the overall hypothesis of greater SOM mineralization and lower yields in regions where crops were grown during two seasons per year compared with one long growing season. High SOM mineralization with low plant residue returns and low overall yields in this region suggest declining soil fertility and limited annual SOM replenishment. Continuing typical crop production may ultimately become more challenging and unsustainable in the long-term. Smallholder farmers in Bungoma are however, in economic need to grow crops during two growing seasons. Therefore, the alternative of fallowing the land during SR may face limited adoption. Other alternatives may include government supported incentives. These may include establishment of N fixing edible cover crops that can be introduced as a relay between maize rows after LR bean harvest. Moreover, since the incidence of crop failure during SR in Bungoma is likely to become more frequent with climate change, focusing efforts toward one-season crop production during LR may also be of a value to farmers. In general, both locations could greatly benefit from crop residue retention and transitioning to soil management systems that rely on less deep and less frequent tillage operations such as no-till. In addition many of the common farming systems could benefit from the use organic manure that encourage additional SOM build up.

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## COMPETING INTERESTS

Authors would like to declare that there were no competing interests of any nature in this research article and that each author played the stipulated roles in all the process towards the success of this manuscript.

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