1 2	Original Research Article Soil Carbon and Nitrogen Mineralization and Crop Parameters in Typical Maize-Bean
3	Intercropping in Western Kenya
4	
5	ABSTRACT
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7	Smallholder farmers in Sub-Saharan Africa face many challenges associated with nutrient-poor
8	soils and frequent weather-related crop failures. Little is known about the impact of current
9	tillage intensive crop management on seasonal changes in soil organic matter
10	(SOM)mineralization and renewal. Farmers in western Kenya intercropmaize (Zea mays L.) and
11	common beans (Phaseolus vulgaris L.) usinginversion-type tillage and low fertilizer inputs. At
12	high elevation crops are grown during one long growing season and twice per year during long
13	and short rains at low elevation. Growing crops twice necessitates frequent land preparation and
14	soil disturbance. The aim of this study was to assess SOM mineralization and crop performance
15	in typical maize-bean production under double cropping (Bungoma) and single cropping (Trans-
16	Nzoia)systems during long rains (LR), short rains (SR) and fallow period (FP). Sites in Bungoma
17	and Trans-Nzoia were sampled three times per year for three years. Soils were analyzed for
18	potentially mineralizable nitrogen (PMN), ammonium (NH ₄), nitrate (NO ₃), water filled pore
19	space (WFPS), nitrous oxide (N ₂ O), methane (CH ₄) and carbon dioxide (CO ₂). Results
20	demonstrated significant increases in PMN, NH4 N2O and CO2 during SR in Bungoma suggesting
21	that additional tillage in support of the second crop facilitated SOM mineralization and potential
22	losses. Soils in Trans-Nzoia also showed increases in NH_4 , NO_3 and N_2O during SR but the
23	magnitude of these changes were lower compared with Bungoma. High carbon (C) and nitrogen
24	(N) mineralization likely did not support annual SOM renewal and crop productivity further

25	demonstrated by low soil total C and N and low cumulative crop yields in Bungoma. Typical
26	crop production may become unsustainable in the long-term, thus considering alternatives such
27	as edible cover crops and reducing tillage should become a necessity. Particular attention should
28	concentrate on designing appropriate management strategies for growing crops during SR in
29	Bungoma.
30	
31	Keywords: long rain season; nutrient cycling; short rain season; soil disturbance; Sub-Saharan
32	Africa; Sustainability of crop production; tillage disturbance
33 34	1. INTRODUCTION
35	Smallholderfarmersin Sub-Saharan Africa (SSA) face numerouschallengesassociated
36	with nutrient-poor soils and high climatic variability[1]. These challenges can
37	impactagroecosystemcapacity to maintainand restore soil fertility in support of annual maize
38	(Zea mays L.) and common bean (Phaseolus vulgaris L.) production. Better understanding of
39	how typical, widespread farming practices driveseasonal changes to soil organic matter (SOM)
40	mineralization is needed to support the development of alternative cropping strategies for better
41	soil resource protection[2].
42	In much of SSA, maize and common beans are intercropped and managed with intensive
43	deep tillage using hand hoes and animal drawn moldboard plows. Farmers intercropmaize with
44	beans to intensify production and benefit from diverse plant life strategies of the two crops [3].
45	For example, beans fix atmospheric nitrogen (N) that ultimately contributes to soil N and
46	benefits maize [4] while maize provides shading and protection of bean plants from occasional
47	hailstorms.Majority of the area has bimodal rainfall that delivers on average 1000 to 1600 mm of

48 rain annually. Low elevation areas (below 1500 meters) experiencewarm temperatures which allow maize and beans complete their growing cycles much faster than athigh elevation [5]. This 49 in combination with bimodal rainfall, permit farmers to plant crops twice per year, during two 50 rainy seasons known as the "long" and "short" rains. Planting during short rains occurs despite 51 high variability of a rainfall that often results in frequent crop loss [6]. However, growing crops 52 twice a year necessitates more frequent land cultivation for planting and weeding, which may 53 ultimately result in limited land rest and annual SOM recovery[7]. Research in SSA has shown 54 that deep tillage contributes to low nutrient retention of already nutrient depleted acidic soils[8]. 55 For example, Smalling and Fresco [9] reported loss of 30kg ha⁻¹ N in form of NO₃annual from 56 cultivated fields in SSA. Many factors may affect high soil nutrient variability in these regions 57 and factors such as topography and management are one of the leading causes[10]. 58

Soil inorganicand labileorganic N in conjunction with greenhouse gas (GHG) fluxesare
robust indices of soil nutrient status and soil response to disturbance [11]When coupled with soil
inorganic N concentrations, the measurements of potentially mineralizable N (PMN), carbon
dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O)fluxesare of particular value becauseall
these compounds are biogenically produced by soil microorganisms that use SOM as their
substrate during decomposition and mineralization[12].

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 wasthat double cropping drives much greater SOM mineralization compared withsingle 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 ultimatelyagroecosystem health.

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2. MATERIALS AND METHODS

75 **2.1 Site Description**

76 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 77 Countyand highland Manor House Agricultural Centre in Trans-NzoiaCounty. The Bungoma 78 study site(00°35'N, 34°34'E; 1200 mm MAP; 27°C MAT; referred to here as Bungoma) is 79 located at 1433 meters elevation in the lower midland agro-ecological zone suitable for two crop 80 growing seasons annually [13]. The Trans-Nzoia study site(010 01' N, 35° 00' E; 1300 mm MAP; 81 82 20°C MAT; referred to here as Trans-Nzoia) is located at 1890 meters elevation in the upper midland agro-ecological zonesuitable for one crop growing season annually [13]. Soils in both 83 locations are clay loams or sandy clay loams classified as ferralsolsdominated by kaolinite clays 84 with high iron and aluminum oxides contents[14-15]. Soil physical and chemical properties are 85 presented in Table 1. 86

Long rains occur between late March through July, and short rains occur from August
through November. Approximately 60 to 70% of annual precipitationoccurs during the LR [16].
December through March is referred to as the fallow period (FP) and receives very little rainfall.
More information on seasonal climate and associated farming practices are shown in Fig.1. Daily
precipitation, maximum and minimum air temperatures were monitored during the experiment
using weather stations equipped with data loggers (Hobo® Weather Station, Onset Computer

- 93 Corp, Cape Cod, Massachusetts) at each location. Cumulative monthly precipitation and monthly
- 94 average air temperatures for the study periods are shown in Fig. 2.
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- 96
- **Table 1** Soil (0-15 cm) physical characteristics for Bungoma and Trans-Nzoia sites.

Soil Properties	Bungoma	Trans-Nzoia
Bulk density (g m^{-3})	1.7	1.6
Clay (%)	36	28
Silt (%)	16	20
Sand (%)	48	52
Soil texture	Clay loam	Sandy clay loam

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99 At each study site a series of experimental plots (0.36 hectares in size) were managed in 100 accordance with typical farmer practices. Land preparation involved inversion-type tillage 101 usingan animal drawn moldboard plow and a hand hoe(Trans-Nzoia) or a hand hoe only 102 (Bungoma). These two tillage implements exert comparable soil disturbance by inverting soil to 103 20 to 25 cm depth.All fields were planted with recommended maize and common bean varieties 104 sourced from Kenya Seed Company Ltd.Maize hybrid H513suitable for low elevation was 105 intercropped with Rosecoco-GLP2 bean (locally known as 'Nyayo') in Bungoma during LR and SR seasons. Maize hybrid 614D suitable for high elevation was intercropped with Rosecoco-106 GLP2 bean in Trans-Nzoia. 107



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

110 Planting for LR season in Bungoma and for the entire year in Trans-Nzoiawas done in mid-April and for SR season in Bungoma in mid-September. Maize was planted at 53,500 plants per 111 hectare and spaced 75cm x30 cm. Beans were planted at 89,000 plants per hectare in between 112 113 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 114 hand hoe.Phosphorous (P)at a rate of 60kg Pha⁻¹ asdia-ammonium phosphate (DAP, 18 % N and 115 $46\% P_2O_5$) was applied at planting and N at a rate of 60 kg N ha⁻¹ as calcium ammonium nitrate 116 (CAN, 27%N) was applied as top dress to maize when maize had six leaves and was 30-45 cm 117 118 tall.

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120 **2.2 Field Sampling**

Soil and gas sampling was done for three years, three times per year during periods that 121 corresponded with LR, SR and FP seasons in both Bungoma and Trans-Nzoia. Four randomly 122 established ten x five meterplots were laid out within fields under typical farmers cropping 123 practices. In each plot, two sub-plots within the vicinity of maize plants and two sub-plots within 124 125 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 126 site). These rings served as bases for chamber tops installed periodically for GHG sampling. 127 128 Chamber tops were 10 cm high and 25 cm in diameter and were made of PVC coated with thinwalled aluminum material following [17-18]. Tops were fitted with silicone septa that served as 129 a port for gas sampling. 130

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Soil (0-10 cm) samples were collected 20 cm from each chamber. Soil was homogenized
and a sub-sample oven dried at 105°C for 48 hours to calculate gravimetric water content at the

134 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 135 pre-incubated for 14 days in dark aerobic conditions, at 23% soil moisture content and 136 temperature maintained at 30° C [20]. At the end of the 14-day period, 5 g of moist soil was oven 137 dried at 102°C for 48 hours to calculate gravimetric water content as described above. 138 Ammonium-N (NH₄) and nitrate-N (NO₃) concentrations were determined by extracting 10 g of 139 140 soil with 50 ml of a 2.0 M potassium chloride (KCl) using colorimetric methods of Weatherburn[21] and Doane and Howarth [22] on a microplate spectrophotometer (BioTek, Inc., 141 Winooski, VT). Potentially mineralizable nitrogen (PMN) was determined using 14-day 142 anaerobic incubation [23-24]. Specifically, 5-g samples of pre-incubated soil were placed in 50 143 ml plastic centrifuge tubes with 12.5 ml of deionized water. Tube headspaces were filled with 144 145 dinitrogen (N_2) gas to replace atmospheric air, and tubes were sealed with plastic caps. All samples were incubated in the dark at room temperature for 14 days [25]. At the end of the 14-146 day period samples were extracted using 12.5 ml of a 4.0 mol L⁻¹KCl and extracts analyzed for 147 NH4following the method described earlier. PMN was calculated as the difference between 148 initial and post anaerobic incubation concentrations. 149

Additional 0-15 cm 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 chambertops 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 157 chamber was sealed and then at 15 and 30 minutes. For each sample, a30-ml aliquot of gas was 158 injected into a previously evacuated 12-ml Labco® glass vial sealed with butyl rubber septa. 159 160 Samples were shipped to USA within two weeks of sampling. Pressurized gas in vials was analyzed for CO₂, CH₄ and N₂O concentrations using gas chromatography (Varian 38001 161 equipped with automatic injector, thermal conductivity, flame ionization and electron capture 162 detectors to measure CO_2 , CH_4 and N_2O , respectively). Ten samples containing internal lab 163 standards that travelled to research sites were also analyzed. Gas fluxes were estimated from the 164 rate of change of gas concentrations in chamber headspaces over the 30-minute time periods 165 using Fick's Gas Law [28-29]. Air and soil temperature were recorded at the beginning and end 166 of each sampling and used to calculate GHG fluxes. 167

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 determinedat crop maturity by hand harvesting grain from a three-meter distance in the middle row. Grain was air dried to approximately 12% moisture content.

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1742.3 DataAnalysis

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

180	using site as a fixed term, time of sampling as a repeated measurement and crop and replicated
181	plots as random terms in the PROC MIXED statistical model. Data was tested for normality
182	using the Shapiro-Wilktest and log-transformed to assure normal distribution of data for further
183	statistical analyses. Mean separations were conducted using the Fisher's protected Least
184	Significance Difference (LSD) procedures. Treatment effects were considered significant when
185	probability of a greater F values were equal to or lower than 0.05, unless otherwise stated.
186	Pearson correlations and linear regressions analyses were developed to test the relationships
187	amongWFPS, PMN, NH ₄ , NO ₃ and GHG fluxes.
188	
189 190	3. RESULTS AND DISCUSSION
191 192	3.1 Weather, Crop Performance and Soil Parameters Air temperatures in Bungoma during the study period averaged 21°C and were two
193	degrees higher than those in Trans-Nzoia (Fig. 2). Bungoma also received a cumulative annual
194	rainfall of 1305 mm, which was 250 mm less than Trans-Nzoia. Seasonal distribution ofrainfall
195	showed moreintense rainfall events during LR and more prolongedperiods of no rainfall during
196	SR in Bungoma.



197

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.

Soils in Bungoma had 2.04 g kg⁻¹ of total C and 0.2 g kg⁻¹ 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⁻¹ of available P which amounted to 50% less than soils in
Trans-Nzoia. Soil pH was comparable between the locations and averaged 5.3.

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Table 2 Soil (0-15 cm) chemical properties averaged across two years. Values that follow "±"

208 are standard errors of a mean. Lower case letters indicate significant differences between sites at

209 $P \le 0.05$.

	Soil Properties	Bungoma	Trans-Nzoia
	pH	$5.2 \pm 0.1 \ ns$	$5.3 \pm 0.1 \ ns$
	Total C (g kg ⁻¹)	$2.04 \pm 0.5 b$	$3.04 \pm 0.1 a$
	Total N (g kg ⁻¹)	$0.20 \pm 0.05 \ b$	$0.30 \pm 0.03 \ a$
	Total P (mg kg ⁻¹)	$30.0 \pm 10 \ ns$	$50.0 \pm 10 \ ns$
	Available P (mg kg ⁻¹)	7.1 ± 1.5 <i>b</i>	$13.3 \pm 2.0 a$
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217	Soil WFPS was significantly great	er across all sampling per	iods in Trans-Nzoia
218	compared with Bungoma (Fig.3). Interesti	ingly, there were no differ	rences in WFPS between
219	sampling events in Bungoma and the valu	es ranged between 30.0%	during FP and 37.0% during
220	SR. In Trans-Nzoia, the highest soil WFP	S of 49% was reported du	ring LR and the lowest
221	WFPS of 39% was observed in FP with in	itermediate values reporte	d for SR.





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Maizeplants at V-6 werebetween 40% to 50% shorter in Bungoma compared with Trans-Nzoia(Table 3). Maize yields in Bungoma totaled 1.4Mg ha⁻¹per yearfrom both seasons, which amounted to 40% loweroverall yields ported forTrans-Nzoia from one long growing season. Bean yields in Bungoma totaled 0.23Mg ha⁻¹during LRand, no bean yields werepoor crop establishment during SRin all years of the experiment. This amounted to 66% lower annual yields in Bungoma compared to Trans-Nzoia.

233

Table 3 Maize and bean growth parameters averaged for two seasons. Values that follow "±" are

standard errors of a mean. Lower case letters indicate significant differences between sites at $P \leq$

237 *0.05*.

238		Bungoma	Trans-Nzoia
239	Long Rains		
240	Maize height at V-6 (cm)	70.5 ±10.7b	$126.1 \pm 20.6a$
241	Maize spacing (m ⁻²)	0.23	0.23
242	Maize yield (Mg ha ⁻¹)	1.10±0.4b	$2.00 \pm 0.1a$
243	Bean spacing (m ⁻²)	0.11	0.11
244	Bean yield (Mg ha ⁻¹)	$0.20 \pm 0.1b$	$0.70 \pm 0.1a$
245			
246	Short Rains		
247	Maize height at V-6 (cm)	68.8 ± 17.1	-
248	Maize spacing (m ⁻²)	0.23	-
249	Maize yield (Mg ha ⁻¹)	0.30 ± 0.1	-
250	Bean spacing (m ⁻²)	0.11	-
251	Bean yield (Mg ha ⁻¹)	-	-

252

253 **3.2.Soil Nitrogen**

Overall, 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⁻¹, respectively and were almost three times greater compared with values reported for LR. No differences in PMN between seasons were observed in Trans-Nzoia with greater but nonsignificant soil total N mineralization during SR and FP compared with LR.Crop species effect

had a significant seasonal impact on soil PMN in Trans-Nzoiaonly (Fig. 5a). Up to four times
more PMN was observed in soil associated with maize plants during LR compared with 1.0 mg
kg⁻¹ of PMN in soils associated with bean plants (Fig.5a).

Soil NH₄ concentrations were also up to four times greater in Bungoma than in Trans-

263 Nzoia (Fig.4b). The highest NH_4 in Bungoma of 18.5 g kg⁻¹ was observed in SR which amounted

to anywhere between nine to five times the levels reported for LR and FP, respectively.In Trans-

Nzoia, NH₄of only 2.8 g kg⁻¹ was also significantly higher in SR and the values were only two

times greatest compared with FPand LR (Fig.4b).

In comparison, soil NO₃was higher in Trans-Nzoia compared with Bungoma (Fig. 4c). In Trans-Nzoia, the highest NO₃ of 32.5 g kg⁻¹ was observed in SR and the values in LR and FP were significantly lower and ranged between 19.7 g kg⁻¹ and 23.7 g kg⁻¹. In Bungoma, NO₃were comparable between LR and SR and ranged between 25.6 g kg⁻¹ and 26.9 g kg⁻¹ which was about 40% more compared with FP.

Both locations demonstrated the highest N₂O fluxes during SR and the lowest fluxes 272 273 during FP with much greater N₂O fluxes in Bungoma (Fig. 4d). In Bungoma, the highest flux of 70.6 μ g m⁻² h⁻¹ was almost twice as high compared with N₂O flux during SR in Trans-Nzoia. 274 Fluxes during LR ranged between 25.8 μ g m⁻² h⁻¹in Bungoma and 19.0 μ g m⁻² h⁻¹ in Trans-Nzoia 275 and between 6.2 μ g m⁻² h⁻¹ and 12.0 μ g m⁻² h⁻¹ during FP. The magnitude of N₂Ofluxes depended 276 on soil associations with specific crops as demonstrated by a significant season x crop 277 interactions at both Bungoma and Trans-Nzoia. Maximum N₂O of 120.8 µg m⁻² h⁻¹ was reported 278 in soils associated with beans during SR in Bungoma, which was twice as much compared with 279 soils associated with maize plants (Fig 5b). In comparison, maximum N₂O of 26.5 μ g m⁻² h⁻¹ was 280

reported for soils associated with bean plants during LR in Trans-Nzoia which was almost three

times as high compared with soils associated with maize.

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Fig. 4 (a) Soil potentially mineralizable nitrogen (PMN, (b) ammonium (NH₄),(c) nitrate (NO₃) concentrations and (d) nitrous oxide (N₂O) 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 fluxes from soils associated with different crops (beans and maize) during Long Rains (LR), Short Rains (SR) and Fallow Period (FP) at Bungoma and high Trans-Nzoia locations averaged across the three years. Asterisks indicate a significant difference between crops at $P \le 0.05$ within each location.

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3033.3 Soil Carbon

Soil CO₂ fluxes were the highest during SR and the lowest during FP in both locations 304 with values in Bungoma much higher compared with Trans-Nzoia(Fig. 6a). The highest CO₂ 305 fluxes of 116.3 mg m⁻² h⁻¹ during SR in Bungoma and the highest flux of 82.3 mg m⁻² h⁻¹ during 306 SR in Trans-Nzoia were approximately 1.5 times higher that during LR. During FP, CO₂ fluxes 307 declined to significantly lowest levels at both locations. CO₂ fluxes also depended on soil 308 associations with specific crops as demonstrated by a significant season x crop interactions (Fig. 309 7a). In Bungoma, CO₂ fluxes from soils associated with maize plants were 45% greater 310 311 compared with soils associated with beans in general and specifically, 24%, 75% and 34%

greater during SR, LR and FP, respectively. In Trans-Nzoia, CO₂ fluxes from soils associated 312 with beans were comparable to those associated with maize except for FP, when they were 19%313 greater from soils associated with beans than from those associated with maize (Fig. 7a). 314 Soil CH₄ fluxes were the least negative during FP, intermediate during LR and the most 315 negative during SR at both sites and the values were much lower in Bungoma than Trans-Nzoia 316 (Fig. 6b). CH₄ fluxes depended on soil associations with specific crops as demonstrated by a 317 significant season x crop interaction. Soils associated with beans demonstrated over 50% more 318 negative fluxes than soils associated with maize except for CH₄ fluxesduring FP in Trans-Nzoia, 319 where the reverse pattern was observed (Figure 7b). In Bungoma, CH₄ fluxes associated with 320 beans amounted to 211%, 38% and 62% greater CH₄ assimilation compared with soils associated 321 with maize during FP, LR and SR, respectively. In Trans-Nzoia, CH₄ fluxesfromsoils associated 322 323 with beans and maize plants were comparable during FP (Figure 7b). During LR and SR, CH_4 fluxes from soils associated with beans were on average, 100% and 156% more negative than 324 from soils associated with maize, respectively. 325 326



Fig.7 (a) Carbon dioxide (CO₂) and (b) methane (CH₄) from soils associated with different crops (beans and maize) during Long Rains (LR), Short Rains (SR) and Fallow Period (FP) at Bungoma and high Trans-Nzoia locations averaged across the three years. Asterisks indicate a significant difference between crops at $P \le 0.05$ within each location.

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348 **3.4 Relationship Between C and N Mineralization**

Pearson correlations showed positive relationship between N₂O and WFPS during LR
and SR at both locations (Table 4). N₂O was positively correlated with CO₂ in Trans-Nzoia but
negatively correlated with CO₂ in Bungoma during SR only. CO₂ was also positively correlated
with WFPS during SR in Bungoma only. PMN was positively correlated with N2O during SR in
Bungoma only and negatively correlated with N₂O during LR in Trans-Nzoia. NH₄ was
negatively correlated with NO₃ in both locations during LR only.

Regression analyses demonstrated that 77% to 80% of N₂O fluxes can be predicted based 355 on CO₂ fluxes during SR only in both locations and 43% during LR in Trans-Nzoia (Table 5). 356 357 The slope values however, demonstrated differential patterns as the regression slope for Bungoma had a negative value while for Trans-Nzoia had a positive value. Furthermore, regression 358 analyses demonstrated that between 61% and 65% of N₂O flux can be predicted based on soil 359 WFPS for both cropping seasons in Bungoma and for SR in Trans-Nzoia (Table 5). Only 30% of 360 N₂O flux during LR in Trans-Nzoia can be predicted based on soil WFPS. The regression slope 361 for the N₂O fluxes per unit WFPS for LR was comparable between both locations and ranged 362 between 1.74 and 1.46. The slope of regression during SR however, was 5.68 for Bungoma, 363 which was approximately 3.5 times greater compared with the slope calculated for Trans-Nzoia. 364 365 Between 35% and 38% of N₂O flux in Bungoma during SR can be predicted based on incubated

soil NH_4 and PMN concentrations and 41% of N_2O flux in Trans-Nzoia during SR can be predicted based on incubated soil NO_3 concentrations (Table 5).

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369 **4. DISCUSSION**

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Soil incubation assays to determine potentially mineralizable N are robust in assessing 371 the magnitude of N mineralization under controlled enclosed conditions. Therefore, direct field 372 N₂O flux measurements and laboratory N incubation assays suggest that soil water content 373 374 played an important role in support of microbial processes contributing to N mineralization across all seasons and all sites. Our results indicate that growing crops during two growing 375 seasons resulted in very high rates of soil N mineralization as demonstrated by high PMN and 376 N_2O fluxes. Since the NH₄ and NO₃ concentrations appeared to be high compared with studies 377 378 performed in field-conditions of fertilized maize tillage treatments [31], a significant portion of inorganic N was likely lost to NO₃ leaching, N₂O production and not plant uptake as 379 demonstrated by low overall yields. In general, the magnitude of soil N mineralization indicated 380 381 by N₂O fluxes were comparable to those reported from maize production using comparable rates of inorganic N fertilizer in SSA [32] and in South America [33]. The magnitude of N₂O fluxes in 382 our study however, was much greater when compared with fluxes from loamy soils with high 383 SOM [34-35]. 384

385	Table 4 Pearson's correlations between percent water filled pore space (WFPS), greenhouse gas fluxes (CO ₂ , CH ₄ and N ₂ O) and soil
386	nitrogen mineralization (PMN, NO ₃ and NH ₄) measured during long rains (LR) and short rains (SR) seasons at low elevation

387 (Bungoma) and high elevation (Trans-Nzoia) sites.

			LR			SR							
		WFPS	CO ₂	CH ₄	N_2O	PMN	NO ₃	WFPS	CO ₂	CH ₄	N_2O	PMN	NO ₃
Bungoma	WFPS	1						1					
	CO ₂	0.32	1					-0.49	1				
	CH ₄	-0.23	-0.44	1				-0.27	-0.53†	1			
	N_2O	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 ₃	-0.19	0.14	0.3	0.35	0.19	1	-0.33	0.27	0.1	-0.39	-0.45	1
	NH ₄	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_2	-0.13	1					0.73**	1				
	CH ₄	0.21	-0.47	1				-0.1	-0.33	1			
	N_2O	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 ₃	0.26	-0.33	0.49	-0.22	-0.11	1	-0.66**	-0.83**	0.27	0.21	0.47	1
	NH ₄	-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 5Regression equations, regression R square value (Rsq) and P values of the relationship between nitrous oxide (N₂O) fluxes and
percent soil water-filled pore space (WFPS), carbon dioxide (CO₂), soil potentially mineralizable nitrogen (PMN), ammonium (NH₄)
and nitrate (NO₃) concentrations for long rains (LR) and short rains (SR) seasons at low elevation (Bungoma) and high elevation
(Trans-Nzoia) sites.

	LR			SR		
Location	Equation	Rsq	P value	Equation	Rsq	P value
Bungoma						
WFPS	y = 1.74x - 53.71	0.61**	≤0.01	y = 5.68x - 98.93	0.65**	≤0.01
CO ₂			ns†	y = -3.1x + 451.50	0.77**	≤0.01
PMN			ns	y = 9.59x + 18.57	0.35*	≤ 0.05
NO ₃			ns			ns
NH4			ns	y = 4.90x + 28.45	0.38*	≤0.01
Trans-Nzoia						
WFPS	y = 1.46x + 105.00	0.30*	≤ 0.05	y = 1.65 x - 38.39	0.61**	≤0.01
CO ₂	y = 0.36x + 43.80	0.43*	≤ 0.05	y = 1.48x - 105.30	0.80**	≤0.01
PMN			ns			ns
NO ₃			ns	y = 0.66x + 6.05	0.41**	≤0.01
\mathbf{NH}_4			ns			ns

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402 Soil disturbance associated with planting the second crop played an important role in accelerating soil N mineralization during SR in Bungoma as demonstrated by the highest rates of 403 PMN, NH₄ and N₂O fluxes. Significant relationships between N₂O fluxes and soil PMN and NH₄ 404 concentrations supported the notion of high rates of seasonal nitrification.Not only were the 405 overall soil N mineralization and N₂O fluxes greatest during SR in Bungoma, but also, contrary 406 to our expectations, N₂O fluxes were the greatest from soils associated with bean plants, further 407 demonstrating limited N retention and high Nturnover of N-rich bean residues also proposed by 408 Jeuffroy et al. [36]. However, elevated N_2O fluxes from soils associated with beans could also be 409 410 attributed to seasonal weeding tillage performed in maize inter-rows that can sever bean plant roots and root nodules [37]. 411

Typical farming practices also resulted in a greater overall C mineralization compared 412 413 with values reported for similar but unmanaged soils in SSA [38-40]. Periodic CO₂ fluxes during LR and SR were greater than fluxes from similarly managed soils with higher total C content 414 under maize-soybean production [34-35; 41-42]. Though, sporadic rainfall events are known to 415 416 trigger C mineralization and immediate CO₂ pulses [11], our data collection was always completed before the afternoon rain showers and the CO_2 fluxes did not correlate with WFPS 417 except for during SR in Trans-Nzoia. The highest CO₂ flux was observed during SR in Bungoma 418 when soil WFPS was comparable to FP and the location experienced below average cumulative 419 rainfall during the study period. Therefore the highest CO₂ flux during SR in Bungoma was 420 likely caused by additional tillage disturbance for land preparation and weeding of the second 421 maize bean crop, which likely accelerated decomposition of fresh plant residues left behind after 422 LR crop harvest. This was also observed by Ellert and Janzen [43]. Additional tillage operations 423 424 and new crop production during the second growing season limited soil ability to sequester

425 newly deposited plant-derived organic material and could contribute to SOM depletion of 426 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 427 428 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 429 N fertilizer lowered CO₂ fluxes and improved SOM and maize yields in Zimbabwe. 430 Most agricultural soils worldwide are effective CH₄ sinks except for submerged 431 agricultural soils where the anaerobic environment stimulates CH_4 production [44]. Soils in this 432 study appeared to be weak sinks or even mild sources of CH₄ fluxes. The flux values obtained in 433 this study were, however, less negative compared with the previous study by Mapanda et al.[32] 434 from typical maize production under different levels of N fertilizer application in Zimbabwe. 435 436 Low soil ability to act as a CH_4 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 437 methanotrophic bacteria [45]. 438

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440 **5. CONCLUSIONS**

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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 in this region 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 448 fallowing the land during SR may face limited success of adoption. Other alternatives may 449 include government supported incentives. These may include abstaining from staple crop 450 451 production and instead, establishment of N fixing edible cover crops that can be introduced as a relay after LR bean harvest. Moreover, since the incidence of crop failure during SR in Bungoma 452 is likely to become more frequent, focusing efforts toward one-season crop production during 453 454 LRmay 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 455 frequent tillage operations such as no-till. 456

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