International Scholars Journals

African Journal of Agriculture and Food Security ISSN: 2375-1177 Vol. 10 (1), pp. 001-006, January, 2022. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Long-term land management effects on soil properties and microbial populations in a maize-bean rotation at Kabete, Kenya

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Accepted 17 October, 2021

The effect of continued application of mineral and organic fertilizers on soil agro-properties and soil microbial populations and activity, was studied in a long-term field experiment at Kabete, in the highlands of Kenya. The area is sub-humid with an average bimodal rainfall of 980 mm and two cropping seasons per year. The main treatments were 3 rates of inorganic N and P fertilizers, farmyard manure (FYM) with or without stover retention. Maize and beans were planted during the long and short rain seasons, respectively. Total % N declined by 25% from 0.16% while soil organic carbon decreased from 2 to 1.2%. The soil pH value dropped by 1.3.units from 5.5 but the decrease in bulk density from 1.04 to 1.08 g cm^{-3} soil in the no-input control treatment was not significant. Use of FYM alone or in combination with chemical fertilizers led to higher numbers of microbes and enhanced microbial respiration than use of chemical fertilizers alone. The topsoil layer had significantly ($p = 0.05$) higher microbial activity than the sub-soil regardless of management strategy. Bacteria were more numerous {1×10^5 cfu (colonies forming units) g dry wt. soil $^{-1}$} than fungi (1×10^3 cfu g dry wt. soil $^{-1}$), which may lead to more soil organic matter (SOM) mineralization and less SOM retention in this cropping system. Integrated use of organic inputs such as farmyard manure and chemical fertilizers is recommended to maintain soil productivity under continuous cultivation.

Key words: Land management, fertilizers, farmyard manure, crop residues, soil properties, mineralization, microbial population.

INTRODUCTION

Increased food production in the sub-Saharan African region is dependent on intensive agricultural production to meet acute food deficits and overcome effect of declining agricultural land productivity. Follett and Schimel (1989) reported a more rapid mineralization of SOM when continuous cultivation was combined with residue removal and tillage. Thus continuous cropping may enhance loss of SOM stocks due to accelerated mineralization unless appropriate land management practices are put in place. However, current economic policy reforms have led to high fertilizer prices compared to crop harvests leading to low inputs : output returns and stagnation in agricultural productivity (Heerink, 2005).

Soil biota plays a major role in the decomposition and mineralization of plant residues (Pankhurst and Lynch, 1994). Microflora, including bacteria, fungi and actinomycetes, are the main agents of nutrient cycling. During the decomposition and transformation of organic substances, part of the substrate carbon is released as carbon dioxide (CO_2); another part is used for the production of new microbial tissue and population maintenance, while the remainder becomes a constituent of humus (Tesarova, 1988). Nitrogen, which is a key element for growth is also released through mineralization by the nitrifying bacteria. Other microorganisms especially fungi are important in stabilizing the soil structure. The basic unit of a fertile soil

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Table 1. Soil treatments in the study on soil properties and microbial populations in the long-term trial at Kabete, Kenya.

No.	Treatment	Organic inputs	Inorganic inputs
1	NIL	None	None
2	NIL+R	Stover retention	None
3	FYM	5 tons manure ha^{-1}	None
4	FYM+R	5 tons manure ha^{-1} + stover	None
5	NP	None	60 kg N + 26 kg P ha^{-1}
6	NP+R	Stover	60 kg N + 26 kg P ha^{-1}
7	FYM+N+P	5 tons manure ha^{-1}	60 kg N + 26 kg P ha^{-1}
8	FYM+N+P + R	5 tons manure ha^{-1} + stover	60 kg N + 26 kg P ha^{-1}

FYM = Farmyard manure; N = Nitrogen; P = Phosphorus; R = Stover; NIL = No inputs.

is the soil aggregate, which is, held together by microbial polymers while fungal hyphae bind the microaggregates into macroaggregates.

Land management alters the pattern of decomposition and nutrient release and affects the relative proportion of soil organic matter in cropped land. Changes in soil structure through continuous cultivation often lead to significant losses in soil organic matter content. The extent of this loss depends on the intensity of cultivation, the quality and quantity of fertilizers and organic residues returned to the soil. This may also affect the predominant groups of microbes involved in soil organic matter decomposition and mineralization. Thus farming practices such as crop rotation, residue retention, tillage and fertilizer use all affect the ecological niches available for occupancy by microorganisms, microfauna and mesofauna. These practices may cause a change or loss of soil biodiversity. This in turn may lead to a change in the regulation of functions from soil organisms to regulation through chemical and mechanical inputs (Giller et al., 1997). This is particularly pertinent to the small-scale farmers who depend on organic inputs such as farmyard manure and crop residues for the maintenance of their soil's fertility. The objective of this study was to determine the impact of continuous cropping with repeated applications of organic and inorganic inputs on the soil properties, soil microbial populations and activity.

MATERIALS AND METHODS

In order to assess the effect of management strategies on soil properties and microbial diversity, a study was superimposed on the on-going long-term field experiment at Kabete. The field experiment was established in 1976 to investigate the effect of continuous application of farmyard manure, crop residues and nitrogen (N) and phosphorus (P) fertilizers in a maize-bean rotation. The trial site is located at the National Agricultural Research Laboratories of the Kenya Agricultural Research Institute (KARI), 36^0 41' E and 01^0 15' S about 8 km NW of Nairobi at an altitude of 1737 m above sea level. The area is sub-humid with an average bimodal rainfall of 980 mm and two cropping seasons per year. The soil is well-drained, very deep dark reddish, friable clay classified as a Humic Nitisol (UNESCO, 1974). It is considered to be moderately fertile (Qureshi, 1987).

Experimental design

In this study eight treatments were monitored for a period of one year from March 2001 to February 2002 (Table 1) and included one maize and one bean - growing season. The plots were 4 × 7.5 m in size. Nitrogen and phosphate fertilizers were applied at three rates (0, 60 and 120 kg N ha^{-1} as Calcium ammonium nitrate 'CAN' and 0, 26.4 and 52.8 kg P ha^{-1} as Triple super-phosphate 'TSP', respectively) . Farmyard manure (FYM) was applied at three rates (0, 5 and 10 t ha^{-1}). Crop residues were returned only to half of the plots but removed in the others. Fertilizer and manure were applied only once a year during the long rain season when maize was planted. Maize hybrid variety ''512'' was planted at the onset of the long rain (LR) season (March-Sept) while beans, *Phaseolus vulgaris* cultivar "*Mwezi moja*", were planted during the following short rain (SR) season (Oct - Jan) on residual fertilizer inputs and biologically fixed N. Both crops were planted as mono-cultures.

Soil sampling

Composite soil samples were collected from four replicate plots at 0 - 15 and 15 - 30 cm soil depth at planting, at flowering and at the onset of physiological maturity of the crop. Populations of culturable microorganisms in the soils were determined by the spread plate method after mechanical shaking and serial dilution using water as the diluent (Vincent, 1970). Soil samples were normally processed on the same day they were collected. However when this was not possible the samples were stored in polythene bags overnight at 4°C and processed the following day.

Physico-chemical analysis of the soils

The soil organic content, pH, macronutrients and bulk density were determined using standard methods (Hinga et al., 1980; Anderson and Ingram, 1993).

Enumeration of soil microorganisms

Aerobic bacteria were counted on nutrient agar (NA) medium as described by Gray (1990) and fungi were enumerated using potato dextrose agar (PDA) medium at pH 5.6 (Kendrick and Parkinson, 1990). Actinomycete numbers were determined using Starch-casein agar (CSA) according to Hunter-Cevera and Eveleigh (1990) while

Table 2. Changes in some physico-chemical characteristics of 0 - 20 cm soil layer at Kabete, Kenya after cropping for 27 years with various inputs†.

Soil properties	1976†	2002			
		NIL	NP	FYM	NPFYM
pH (1:2 in H_2O)	5.5 [b]	4.2 [a]	4.3 [a]	4.5 [a]	4.4 [a]
SOC (%)	2.0 [c]	1.3 [b]	0.9 [a]	1.2 [b]	1.4 [b]
Total N (%)	0.16[b]	0.12 [a]	0.12 [a]	0.14 [a]	0.15[a]
Ca (m.e. /100 g)	3.65[b]	2.5 [a]	2.7 [a]	3.8 [b]	4.0 [b]
Mg (m.e. /100 g)	2.2[a]	2.0 [a]	2.0 [a]	1.9 [a]	1.9 [a]
K (m.e. /100 g)	0.73[a]	0.6 [a]	0.6 [a]	0.5 [a]	0.5 [a]
Available P (mg kg^{-1}	15[a]	14.0 [a]	13.0 [a]	15.0 [a]	16.0 [a]
(Mehlich)	1.04[a]	1.08 [a]	1.08 [a]	1.06 [a]	1.04 [a]
Bulk density (g cm^{-3})	67 clay: 22 silt: 11	nd	nd	nd	nd
Soil texture (%)	sand	Clay	Clay	Clay	Clay
Textural Class	Clay				

†Source of 1976 data: Qureshi, 1987.
Nil - control
FYM - farmyard manure at 5 t ha^{-1}.
NP - chemical fertilizer at 60 kg N ha^{-1} and 26.4 kg P ha^{-1}.
NPFYM - chemical fertilizer at 60 kg N ha^{-1} & 26.4 kg P ha^{-1} and farmyard manure at 5 t ha^{-1}.
Nd - not determined.

rhizobia were counted on yeast extract mannitol agar (YEMA) medium containing Congo red dye to distinguish them from other bacteria (Vincent, 1970). Nitrifying bacteria were enumerated using the most probable number (MPN) method as described by Schmidt and Belser (1982). Ammonium oxidizers were grown on Ammonium (NH_4^+) oxidizer medium while nitrite oxidizers were grown on Nitrite oxidizing medium. A freshly collected field soil sample weighing 10 g was used to prepare ten-fold serial dilutions (10^{-1} to 10^{-6}) using 1 mM Phosphate buffer. Aliquots (1.0 ml) of each dilution were used to inoculate 4 ml of the liquid nitrifier media, which was then incubated at 25 - 30°C in the dark for 3 - 6 weeks. Checks for nitrification were done visually by noting the colour change in the incubated nitrifier medium from blue-green to yellow, which is indicative of active acid production (that is, the oxidation of NH_4^+ to NO_2^-) as detailed in Schmidt and Belser (1982). The number of positive tubes was noted at the end of the 6 weeks period. Data collected from microbial counts were subjected to analysis of variance (ANOVA) and means separated by the least significance difference (LSD) test at $P < 0.05$.

Soil microbial respiration

Soil respiration, an estimate of soil microbial activity, was determined as the amount of CO_2 evolved by the soil in a given time using the method outlined by Bauer et al. (1991). Freshly collected soil (20 g) was placed in a small polyester bag (7 × 10 cm) and mounted in a 300 ml screw-capped glass bottle containing 20 ml of 0.05 N NaOH using a string. The bottle was then closed tightly and incubated for 24 h at 25^{o}C in the dark. The absorbed CO_2, which was trapped in NaOH, was precipitated as $BaCO_3$ by the addition of 1.0 M $BaCl_2$. Excess NaOH was then titrated with 0.05 N HCl. Four replicate samples including a blank without soil, were prepared for each treatment. The amount of CO_2 evolved was calculated as the mean value of the replicates minus the average blank value and expressed as:

mg CO_2 g^{-1} dried soil 24 h^{-1} = (B -V) × 1.10025 mg CO_2

where:
B = volume (ml) of acid needed to titrate the excess NaOH in the control bottle to the end-point, V = volume (ml) of acid needed to titrate the excess NaOH in the treatment bottles to the end-point, (1 ml 0.05 N HCl is equivalent to 1.10025 mg CO_2).

RESULTS

Changes in soil properties

The soil properties at the start of the experiment in 1976 (Qureshi, 1987) and 27 years later (2002) were compared. A general decline in soil organic C, total N, pH in water, the macronutrients Ca, Mg, K and P over time was noted. Soil organic carbon (SOC) in the year 2002 ranged from 0.93 - 1.44% compared to the original SOC of 2.0% at the beginning of the experiment in 1976 (Table 2). Continuous cultivation with no inputs or with sole NP fertilizers caused the greatest reduction in SOC of 37 and 53.5%, respectively (Table 2). On the other hand, application of combined fertilizer, farmyard manure and crop residues sustained the highest amount of SOC of 1.44% (Table 2). Combined analysis showed that while SOC declined significantly ($p = 0.01$) with time, the change in SOC was significantly influenced by the addition of farmyard addition ($p = 0.01$) and crop residue incorporation ($p = 0.02$). Further analysis indicated that N was significantly influenced by all the treatments applied, that is, farmyard manure ($p < 0.01\%$), NP fertilizers ($p = 0.22\%$), crop residues ($p < 0.01\%$). The decline of N was also highly significant ($p = 0.01\%$) with time. However, soil organic carbon declined at a faster rate than total

Table 3. Microbial populations in the 0 - 15 and 15 - 30 cm soil horizons under different management options in the long-term trial, Kabete, Kenya, March 2001 to January 2002.

Treatments	Microbial groups (c.f.u. g $^{-1}$ dry wt. soil)							
	Bacteria × 10^5		Fungi ×10^3		Actinomycetes × 10^3		Rhizobia × 10^3	
	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm	0 - 15 cm	15 - 30 cm
Nil	27.3	3.2	22.8	2.4	29.1	4.7	15.8	1.9
Nil+R	14.3	6.9	26.6	13.8	19.8	9.8	13.3	3.2
FYM	51.6	12.2	24.8	20.0	49.7	22.7	24.6	8.4
FYM+R	61.2	7.4	36.0	21.8	33.8	17.0	48.5	19.2
NP	28.3	7.8	21.8	3.4	21.7	5.9	16.9	4.8
NP+R	19.0	4.4	27.1	10.9	25.8	14.4	25.7	3.1
NPFYM	41.6	10.7	33.4	13.9	54.6	6.2	82.7	17.7
NPFYM+R	49.6	15.8	24.7	12.8	43.1	7.9	85.9	10.6

N - 60 kg N ha^{-1} (applied as Calcium ammonium nitrate (CAN).
P - 26.4 kg P ha^{-1} (applied as Triple superphosphate (TSP).
FYM - 5 tons ha^{-1} Farm yard manure (75 kg N and 20 kg P t^{-1} dry manure).
R - all harvested stover returned back to the experimental plot.
NIL - no-input control.
c.f.u - colony forming units.

nitrogen. Use of NP fertilizers alone led to faster decline in % SOC (54%) and % total N (25%). Other results showed that soil pH in plots where no inputs were applied had dropped from 5.5 to 4.2 over the 27-year period. Soil bulk density decreased slightly, though not significantly, from 1.04 to 1.08 g cm^{-3} (Kibunja, 2007).

Microbial diversity

The bacteria population was much higher than those of other groups of microorganisms (1 × 10^5 - 10^6 cfu g dry wt.soil^{-1}) throughout the season (Table 3). A significantly ($p < 0.05$) higher population of microbes was recorded in the upper soil horizon (0 - 15 cm) throughout the year but there were no significant differences between the treatments. Some functional groups of bacteria, for example, the N_2-fixing rhizobia were affected by the cropping system, that is, the numbers were generally low (1 x 10^3 cfu g dry wt.soil^{-1}) and continued to decline during the maize monocrop but rose during the bean crop season. The application of organic inputs led to an increase in microbial populations especially fungi and actinomycetes soon after incorporation but declined about two months later. Plots where chemical fertilizers alone were applied had the lowest microbial populations.

The population of nitrifiers was affected by the quality of the inputs as well as the sampling depth. The average numbers of ammonia oxidizers were in the range of 2.8 ± 2.4 × 10^3 to 8.0 ± 6.8 × 10^4 g^{-1} dry wt. soil in the 0 - 15 cm soil layer and 1.8 ± 1.5 × 10^3 to 8.8 ± 7.5 × 10^3 g^{-1} dry wt. soil at the 15 - 30 cm soil depth. The population of nitrite oxidizers in the 0 - 15 cm layer was in the range of 8.0 ± 7.0 × 10^2 to 17.4 ± 14.8 × 10^3 g^{-1} dry wt. soil and 0.8 ± 0.7 × 10^2 to 4.4 ± 3.7 × 10^2 g^{-1} dry wt soil in the 15 - 30 cm soil layer. The addition of both organic and inorganic inputs increased the ammonia oxidizing populations in both soil layers compared with the unamended control treatment. However, this effect was only significant ($p < 0.05$) after the application of farmyard manure either alone or in combination with inorganic inputs in the 0 - 15 cm soil layer but not at the 15 - 30 cm soil depth.

Microbial respiration

Soil microbial respiration, measured as carbon dioxide (CO_2) evolution in the 0 - 15 cm layer, varied significantly with sampling time and quality of inputs (Figure 1). The mean CO_2 evolved was in the range of 0.4 to 0.93 g CO_2 - C g^{-1} soil h^{-1} throughout the year. The rate of CO_2 evolution was significantly ($p = 0.05$) higher in plots with farmyard manure than in either the control or chemically fertilized plots and was in the following order: farmyard manure combined with chemical fertilizers > farmyard manure > chemical fertilizers combined with crop residues > crop residues > control > chemical fertilizers alone. A higher soil respiration rate was recorded in October 2001 during the bean growing period while the least respiration rate was noted in February 2001, which coincided with time of minimum precipitation (Figure 1) as the bean crop matured.

DISCUSSION

Results obtained from this study indicate that continuous cultivation had an adverse effect on both the physical and

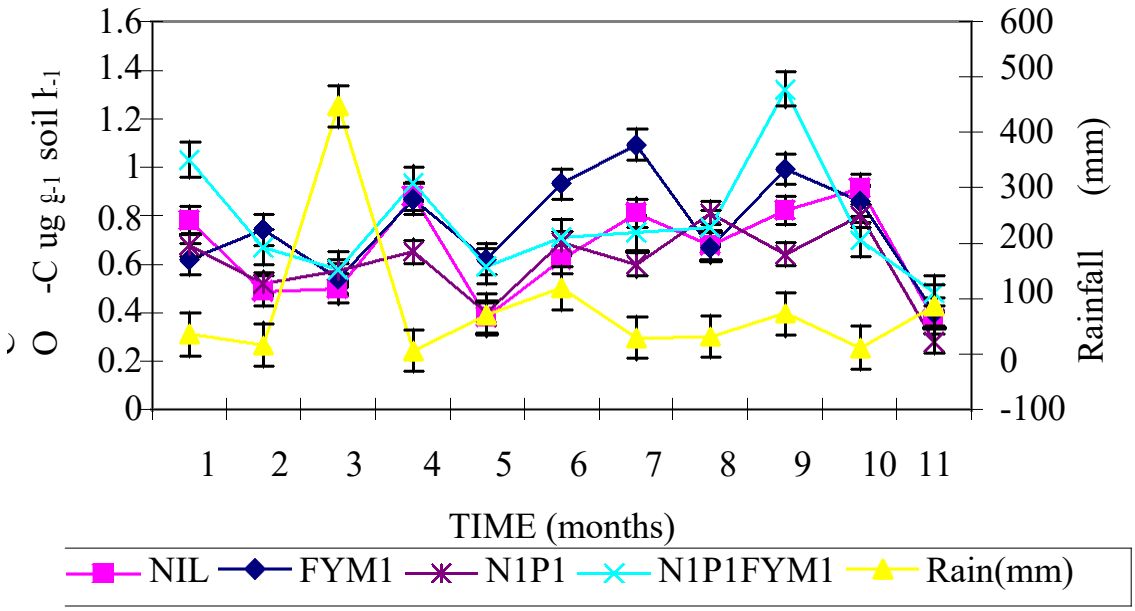

Figure 1. Changes in the carbon dioxide (CO_2 - C g g^{-1} soil h^{-1}) evolution upon application of various organic and inorganic inputs over time at the long term trial, Kabete, Kenya. Symbols: N - 60 kg N ha^{-1} (as Calcium ammonium nitrate (CAN); P - 26.4 kg P ha^{-1} as triple superphosphate (TSP); FYM - 5 tons ha^{-1} manure (with 15 kg N and 4 kg P t^{-1} dry manure); R - all harvested stover returned back; NIL - no-input control; Rainfall in mm. Bars indicate the SE.

chemical properties of the soil over time. The greatest changes were noted in the no-input control plots (Table 2), but even the application of mineral nitrogen, crop residues and farmyard manure did not totally arrest the decline of soil nitrogen and carbon content over time. The rate of mineralization of SOM was probably faster than the rate of SOM formation in this soil site. The soil type, humic Nitisol, is typical of soils frequently deficient in N and P and tend to acidify under continuous cultivation (Bekunda et al., 1997). Continuous application of chemical fertilizers in this site failed to maintain the level of SOC but led to a net loss of SOC by 54% of the original value of 2.0% (Table 2) and a drop in soil pH from 5.5 to 4.27 units. Other studies have similarly noted that use of inorganic fertilizers without any organic inputs or other corrective measures to maintain soil pH might lead to a decline in SOC and reduced crop productivity due to increased soil acidity (Wapakala, 1976; Bekunda et al., 1997). Previous work in the same site showed that use of manure, NP fertilizers and crop residue retention caused the least organic C loss from the soil and raised the total soil N significantly compared to the control (Kapkiyai et al., 1998) . Continuous cultivation without organic inputs probably led to faster soil organic matter (SOM) turnover due to repeated tillage and increased soil aeration accompanied by reduced soil cover leading to enhanced SOM losses due to erosion (Giller et al., 1997). The macronutrients Mg and K were found to have declined over time probably due to higher nutrient uptake in plots with higher inputs and leaching in plots with least SOC content. Soil acidity and bulk density had increased over the period possibly due to continuous cropping with chemical fertilizers or inadequate inputs. Repeated use of sole chemical fertilizers may have led to increased soil acidification (Giller et al., 1997) and declining SOM (Palm et al., 1997). Liming may be an important strategy to deal with rising soil acidification especially where inorganic fertilizers were applied without any organic inputs.

Studies on land use effects on microbial composition and diversity in tropical regions are relatively few, as most of the reported work has been conducted in temperate regions. Several of these studies from the temperate regions indicate that surface application of crop residues leads to higher fungal populations while incorporation in the soil gives rise to higher bacterial numbers (Hendrix et al., 1986; Pankhurst and Lynch, 1994). However, Mafongoya et al. (1997) found that under tropical conditions this was only true for low quality surface applied litter but high quality litter resulted in higher numbers of bacteria than fungi. In the present study, bacteria were more numerous than fungi even in plots with surface applied bean crop residues. Results further showed that treatments with farmyard manure supported higher numbers and activity of various groups of microorganisms probably due to higher SOC content. Chemical fertilizers on the other hand did not enhance SOC build up but instead raised the soil pH. Rhizobia are known to be very sensitive to low pH and function best at near neutral pH (Vincent, 1970), which probably explains the low numbers recovered from the

treatments with chemical fertilizers where a notable decline

in pH was recorded (Table 2). The nitrifier population was affected by both treatments and depth possibly in direct relationship to the availability of nutrients and soil moisture. According to Verhagen et al. (1995), nitrification generally takes place when ammonium is available in non-limiting amounts as nitrate acts as a sink for surplus nitrogen. Higher counts of the nitrifying bacteria were found in the treatments where both chemical fertilizers and farmyard manure were applied. These treatments most likely had unlimiting ammonium supply especially at the beginning of the planting season when there was adequate moisture in the soil with minimum competition from the developing plant roots or at the end of the season due to reduced plant uptake as the crop matured. These findings may have some important implications for nutrient cycling and soil fertility under continuously cropped lands.

Fungi have higher carbon utilization efficiency than bacteria (Holland and Coleman, 1987). Since bacteria dominated this cropping system, this may lead to faster mineralization and release of nutrients where a fungi-dominated system may immobilize nutrients. Thus changes in microbial composition through manipulation of inputs can affect the efficiency and retention of nutrients within an agro-ecosystem. The challenge now is how to manage the agents of decomposition and mineralization of SOM in order to synchronise it with plant demand (Kibunja, 2007). Fortunately, in the past decade advances in molecular techniques and other microbiological analysis have rekindled research interest and investigations in soil microbiology (Girvan et al., 2003). . Integrated use of organic inputs such as farmyard manure and chemical fertilizers is recommended to maintain soil productivity under continuous cultivation.

ACKNOWLEDGEMENTS

The authors wish to thank the Director, KARI for permission to publish this work and the EU for financial support. This work formed part of a PhD study submitted by C. N. Kibunja to the University of Nairobi, Nairobi, Kenya.

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