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Short-term dynamics of soil organic matter fractions and microbial activity in smallholder potato-legume intercropping systems

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ABSTRACT

Continuous cultivation of potato (*Solanum tuberosum* L.) in monoculture systems represents the greatest factor deteriorating soil organic matter (SOM) in smallholder farms. With an aim to breaking this norm, a 2-year field trial intercropping potato with two legumes: lima bean (*Phaseolus lunatus*) and dolichos (*Lablab purpureus*), was conducted in the upper-midland (1552 meters above sea level (masl.)), lower-highland (1854 masl.) and upper-highland (2552 masl.) agro-ecologies of Kenya. Residues from each cropping system were quantified at the end of each season and incorporated back into the soil at start of the subsequent season. A combined physical and density fractionation was used to separate the soil in macro-aggregates (> 250 μm), micro-aggregates (250–50 μm) and silt plus clay fractions (< 50 μm), while SOM was partitioned into labile (density of 1.65 to 1.85 g cm^{-3}) and stable (2.60 g cm^{-3}) fractions. Microbial biomass contents were determined by chloroform fumigation while enzymatic activities were assessed by hydrolyses of fluorescein diacetate and dehydrogenase. Compared to sole potato, intercropping increased the contents of light fraction organic matter by 12–28%, dissolved organic matter by 7–21% and microbial biomass by 15–38%, thus stimulating enzyme activities. Trends in soil microbial respiration followed those of enzyme activity and were 20–34% higher in intercropping than in sole potato. Intercropping ensured high residue returns which got short-term residence within the macro-aggregates, thus ensuring steady supply of substrates to the soil microbes. These results affirm legume intercropping as a possible entry point to restoring the impoverished soil quality in smallholder potato farming systems.

1. Introduction

Potato production in subtropical highlands is done mainly in monoculture systems yet the crop retains very little residues and therefore has limited capacity to return organic matter into the soil (Angers and Carter, 1996; Nyawade, 2015; Gitari et al., 2018a, 2018b; Nyawade et al., 2019). Even with this little residue retained, most of it is used to feed the livestock or burned during land preparation. This problem is particularly important among the smallholder farmers who lack the capacity to apply fertilizer or put in place adequate soil conservation measures to control soil erosion (Nyawade et al., 2018b; Gitari et al., 2019). As potato takes 40–45 days to establish full groundcover, and maintains this cover only for 20 days before senesces sets in, the crop leaves the soil exposed to high surface temperatures that accelerates oxidation of soil organic matter (SOM) making its supply to be imbalanced (Reicosky et al., 1995; Nyawade et al., 2018a). An imbalanced SOM lacks the capacity to supply nutrients to the microbial population and therefore restricts their growth and activity (Haynes and Tregurtha, 1999). Consequently, the average potato yields in subtropical highlands range between 3 and 15 t/ha, an observation that has been related to the low content of SOM. This factor if not checked, may compel farmers to abandon potato production.

The extent of the adverse effect of poor potato cropping systems on SOM depends largely on which size fraction is affected (Nyawade et al.,

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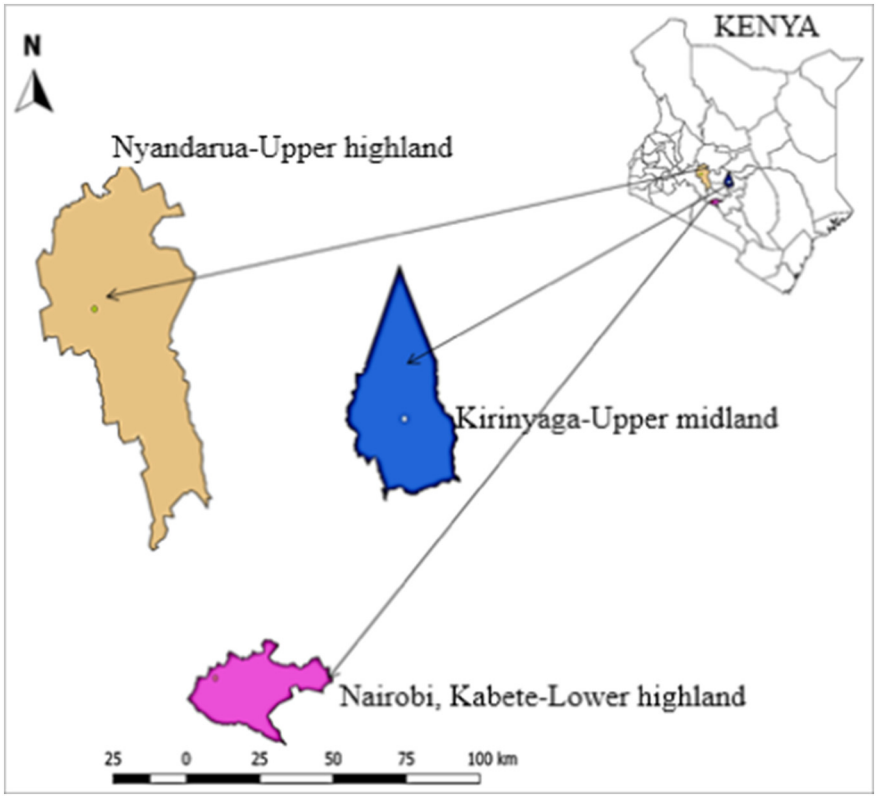

Fig. 1. Experimental site.

2018b). These fractions differ in their quality, quantity and turnover rates, and respond differently to soil management (Kapkiyai et al., 1999). The stable SOM includes well-decomposed microbial products that form mineral complexes with organic compounds and is relatively recalcitrant to microbial attack (Camberdella and Elliot, 1992; Six et al., 2002). The labile SOM fractions are derived from the newly incorporated organic materials and include the light fraction organic matter, microbial biomass and dissolved SOM (Naresh et al., 2017; Haynes, 2005). This component of SOM has rapid turnover rates and responds fast to soil management intervention compared to the total and stable SOM (Louis et al., 2016; Haynes, 2005).

The soil microbial biomass is a living fraction of SOM consisting mainly of bacteria and fungi that decompose litter and crop residues (Naresh et al., 2017). This process releases nutrients that are made available for plant uptake (Smith and Paul, 1990). Generally, the microbial biomass makes up 5–10% of the total SOM (Naresh et al., 2017), part of which is released to the soil in plant available forms upon the death of soil microbes (Louis et al., 2016). The dissolved organic matter (DOM) results from microbial degradation of SOM and is used as substrate by the soil microbes (Roper et al., 2010). The rate at which labile fractions of SOM decompose determines the release of nutrients to plants (Kapkiyai et al., 1999). Therefore, any activity that rapidly alters the contents of soil labile organic matter are regarded to be early signals of changes in soil productivity that may occur over an extended period.

Quantification of soil microbial biomass activity is based on the rate at which respiration and enzyme hydrolyses by soil microbes occur (Naresh et al., 2017). If the rate of respiration is related to the corresponding microbial biomass size, it is defined as microbial metabolic quotient (qCO_2) (Lupwayi et al., 1998). The qCO_2 is sensitive to short-term changes in SOM and therefore provides early warning to a degrading soil quality. The qCO_2 gets elevated when perturbations such as high soil temperatures and soil water deficits exacerbate SOM depletion, thus negating the functions of soil microbes (Anderson and Domsch, 1990). These processes may greatly vary with cropping systems, soil management and agro-ecologies (Lupwayi et al., 1998; Naresh et al., 2017). This is because the microbial biomass just like any other organic matter fraction, is influenced by soil particle size distributions which has great relation with the soil management and cropping systems (Camberdella and Elliot, 1992).

The macro-aggregate provides conducive micro-environment for microbial growth and also confers physical protection to the micro-aggregates thus occluding the associated SOM from physical perturbations (Six et al., 2002). It is in this regard that simple and sustainable strategies capable of increasing aggregate formation have been proposed in smallholder potato cropping systems (Nyawade, 2015; Nyawade et al., 2018b; Burke, 2017). The proposed measures highly regard technologies that retain at least 22.4 Mg ha^{-1} residue dry matter *in situ*, a value that corresponds to 1% increase in SOM (MacMillan and Buchanan, 1988). This large biomass can hardly be attained by pure potato crops (Nyawade, 2015; Angers and Carter, 1996). Thus, addition of materials with high lignin contents as mulch has been considered appropriate (Franchini et al., 2002). These materials are however characterized by nitrogen immobilization resulting in low nutrient release (Hassink, 1995).

Legume intercropping due to its ability to simultaneously integrate crop components with high nitrogen contents, offers an alternative that can reduce the nitrogen immobilizations (Naresh et al., 2017). When returned to cropland, the fresh legume residues provide nucleation useful in formation of macro-aggregates which are the favorable habitat for soil microbes (Ghani et al., 2003; Liang, 2013.).

It is in view of this background that this study was conducted with the aim to assessing the short-term effect of potato-legume intercropping on dynamics of SOM fractions (microbial biomass, dissolved SOM, light fraction SOM and heavy fraction SOM) in three agro-ecologies of Kenya (upper midland, lower highland and upper highland). Early detection of changes in SOM is important for designing sound management practices that can restore soil quality, soil health and soil productivity. Identification of intercropping systems capable of increasing

SOM contents to optimal levels under different agro-ecological conditions offers the possibility of potato integration into wide range of agro-food systems.

2. Materials and methods

2.1. Study sites

The trials were installed in the wet season of 2016 and were continued till 2018 dry season in three agro-ecologies of Kenya; upper highland (UH)-Nyandarua, lower highland (LH)-Kabete and upper midland (UM)-Kirinyaga (Fig. 1). The sites lie respectively, on elevations of 2552, 1854 and 1552 m above sea level. Nyandarua lies along latitude 0°14′39.08″S and longitude 36°17′18.99″E, Kirinyaga, 0°29′35.71″S and 37°20′55.29″E and Kabete 1°14′45.00″S and 36°44′19.51″E. The region is representative of agricultural farming systems of East Africa where inherent low soil fertility greatly limits potato production. These areas receive rains in two modes: March to June for the wet season and October to December for the dry season (Jaetzold et al., 2012). Nyandarua receives mean annual temperature of 20 °C with mean annual rainfall amount of 1500 mm, and a moisture index of 75%. The annual average temperature received in Kabete is 24 °C, with a mean annual rainfall amount of 1006 mm and a moisture index of 55%. Kirinyaga exhibits relatively lower annual rainfall amount averaging at 900 mm with moisture index of 45% and average annual temperature of 27 °C.

The soils in Kabete are clay-loam classified as Humic Nitisol while the Kirinyaga soils are well-drained, shallow to very deep, reddish brown silty loam classified as Rhodic Ferralsol (FAO, 2012). The soils in Nyandarua are dark brown to very dark red brown firm clay to silt loam clay classified as Ferric Luvisol. Details of the measured soil properties (0–40 cm depth) before the experiment are provided in Table 1.

2.2. Trial design and crop husbandry

The trials were arranged in a randomized complete block design in plots measuring 6.50 m long by 4.25 m wide. The treatments comprised of sole stands of potato (*Solanum tuberosum* L., Unica cv-CIP 392797.22), sole lima bean (*Phaseolus lunatus*), sole dolichos (*Lablab purpureus*) and intercropping of potato with the two legumes. Each treatment was replicated 4 times in each season and study site. Intercropping was done in 2 rows of potato alternating with 2 rows of legumes. Potatoes were planted in pre-hilled ridges piled 0.20 m high and 0.15 m top-width with a planting depth of 0.1 m. This practice was adopted due to its ability to moderate soil temperatures and optimize the soil moisture content (Nyawade et al., 2018a). The within and between row spacing of the tubers were 0.30 m and 0.75 m respectively. Two legume bean seeds were planted per hole at within row space of 0.20 m and inter-row space of 0.75 m between potato and legume strips and 0.50 m between two legume strips.

Fertilizer application was performed according to soil analysis and crop nutrient requirements, targeting on average a supply of 90 kg N ha^{-1} for potato crop. This activity consisted of basal application of 50 kg N ha^{-1} N, 90 kg P ha^{-1}, 100 kg K ha^{-1} and topdressing with 40 kg/ha calcium ammonium nitrate. Topdressing was done 15–25 days after potato emergence depending on the general soil moisture conditions and crop growth stage. Legumes received only basal phosphorus (46% triple super phosphate) at a constant rate of 20 kg P ha^{-1} across the three study sites. Weeding was done at 14–21 days after potato emergence by raising up the soil around the plants' main-stem base using hand hoes. Aphids were controlled at weekly interval using alternations of Duduthrin (Lambda-cyhalothrin 17.5 g L^{-1}) and Bestox (Alpha-cypermethrin 50 g L^{-1}) while potato late blight disease was controlled with alternations of Ridomil Gold MZ 68WG (Mefenoxam + Mancozeb) and Dithane-M (Mancozeb-80 w/w) at 2 weeks interval. Alternations were done to curb against the chemical resistance that could be developed by the aphids and the late blight causal agents.

Potatoes were harvested when they attained physiological maturity by rooting out the tubers using hand hoes while the legumes were left growing until they physiologically matured. The pods were plucked out retaining the residues which were incorporated into the soil at start of the subsequent season. All cultural activities followed the conventional practices of local growers in the study area, except the residue retention; farmers in these areas generally uproot the beans and carry them to the homesteads where they are threshed and the residues fed to the livestock or burned for fuel.

2.3. Estimation of shoot and root biomass

Root and shoot biomass were measured at vegetative growth of potato (60 days after planting) by sampling four inner rows of each treatment (2 strips of legumes and 2 strips of potato for intercropping and 4 strips of either legume or potato for the sole crops). For each treatment, this area corresponded to about 1.2 m^2 of the plot. Roots of each cropping system was measured for every 10 cm interval in soil profile of 0–40 cm using metal cores of 0.0015 m^3 by volume (Bohm, 1979). For the monoculture system of potato and legumes, root sampling was done between the two strips of potato (at approximately 37.5 cm from the strips) and the two strips of legumes (at approximately 25 cm from the strips) respectively. For intercropping, the cores were driven between the two potato rows in potato strips (at

Table 1
Initial soil properties: Heavy fraction organic matter (HFOM), light fraction organic matter (LFOM), dissolved organic matter (DOM) and microbial biomass carbon (MBC) in the three agro-ecologies (upper midland, lower highland and upper highland).

	Soil depth	Clay	Silt	Sand	Textural class[a]	pH	HFOM	LFOM	DOM	MBC
	(cm)	%					mg C kg^{-1}			
Upper midland	0–10	24.5	33.3	42.2	CL	5.0	453	337	271	111
	10–20	24.2	36.9	38.9	CL	5.0	451	190	208	36
	20–30	28.9	29.8	41.3	CL	4.9	414	120	111	21
	30–40	23.8	32.4	43.8	CL	4.9	388	98	108	20
Lower highland	0–10	49.7	22.5	27.8	C	5.1	610	544	418	128
	10–20	49.2	24.2	28.9	C	5.1	549	324	306	79
	20–30	50.1	24.2	25.7	C	5.2	503	203	135	64
	30–40	51.3	24.8	23.9	C	5.2	500	179	126	61
Upper highland	0–10	38.3	56.1	5.6	SC	5.2	560	575	425	216
	10–20	36.9	58.4	4.7	SC	5.2	454	324	325	110
	20–30	34.6	59.5	5.9	SCL	5.3	426	205	103	41
	30–40	33.9	57.9	8.2	SCL	5.3	421	168	104	43

[a] Clay (C), clay loam (CL), silt clay (SC) and silt clay loam (SCL).

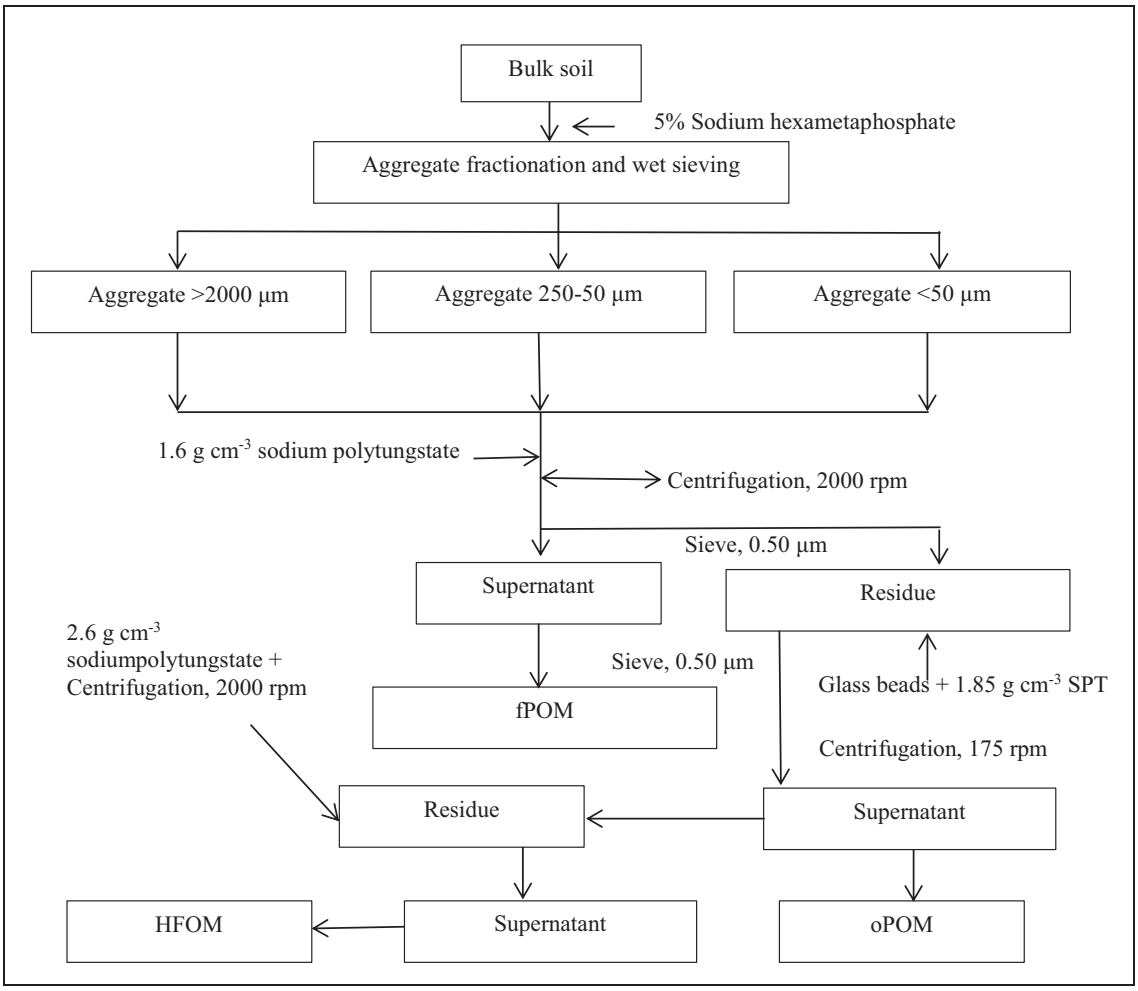

Fig. 2. Schematic diagram of the combined aggregate size and density fractionation.

approximately 37.5 cm from the strips), between two legume rows in legume strips (at approximately 25 cm from the strips) and between the potato and legumes in potato-legume strips (at approximately 37.5 cm from the strips). Additional root sampling was done directly at the legume and potato stem base (about 5 cm from the stem) to take into account the roots not extending to the inter-row space.

The samples for a given plot and sampling depth were pooled and analyzed as one. The extracted soil cores were whirled in running water to remove the embedded soil particles and dead roots and the mixture sieved through 2 mm mesh. For shoot biomass estimations, the plants were cut at about 2 cm from the soil surface using machetes. The fresh weight of the biomass was quantified using a weighing balance. The dry mass of roots and shoots were separately determined by oven-drying about 500 g samples at 65 °C to a constant mass then extrapolating to kg/ha. The rest of biomass was incorporated in respective plots at start of the subsequent season using hand hoes.

2.4. Estimations of lignin, polyphenols and C and N contents

Dry matter subsamples of roots and shoots were analyzed for C, N, lignin and polyphenol contents. For plant lignin content, about 20 g sample of the dry matter was ground and passed through 30 mm mesh (Van Soest and Wine, 1968). The sample was placed in a crucible and an acid detergent fiber prepared on 1 g sample was added. This was followed by addition of saturated potassium permanganate (5 mL) and the content stirred with a glass rod. The crucibles were allowed to stand at 25 °C for about 90 min and the content dried overnight at 100 °C and weighed. The loss in weight of the acid-detergent fiber was taken to represent the lignin content. Polyphenols were extracted according to modified Anderson and Ingram (1993) method using methanol (70%), ascorbic acid (0.05%) and formic acid (0.5%). The dry matter samples of each plant parts were digested and used for determination of total N concentrations (Bremner and Mulvaney, 1982), while a part of it was oxidized for determination of total organic carbon content (Nelson and Sommers, 1996).

2.5. Soil sampling and analyses

Sampling of soils was done within and between the crop rows using soil piston augers for every 10 cm interval along soil profile of 0–40 cm. Sampling was conducted at vegetative growth of potato (about 60 days after planting). The depth interval 0–40 cm was considered appropriate as it represented the rooting depth for potato crop (the depth at which > 70% of the root volume was found) (Mahdian and Gallichand, 1996). In each plot, 80 soil samples were taken (5 cropping systems × 4 replicates × 4 sampling depths). The samples for each treatment were composited for each depth and frozen until analysis for soil pH (by water), soil texture (hydrometric) (Gee and Bauder, 1986), total N (Keeney and Nelson, 1982) and total organic carbon (Nelson and Sommers, 1996).

2.6. Soil moisture and soil temperature

Quantification of soil moisture and soil temperature was done

between sowing and harvesting using Onset HOBO probes (UX120-006 M) installed at every 10 cm interval along soil profile of 0–40 cm and related with different SOM fractions and microbial activity. The probes were installed between the two potato rows in potato strip, between two legume rows in legume strips and between the potato and legumes in potato-legume strips. The data was recorded by automatic data-logging equipment at every 1 h common step. Prior to their installation, calibration of the moisture sensor probes was done using gravimetric soil moisture and soil mercury thermometer for temperature probes.

2.7. Particle size separation and sequential density partitioning of soil organic matter

A combined particle and density size fractionation was used to partition the soil organic matter (SOM) into size fractions (Camberdella and Elliot, 1992; Sohi et al., 2001) (Fig. 2). Fifty (50) g of the composite soil was dispersed in 50 mL of 5% sodium hexametaphosphate and subjected to overnight shaking. The content was passed through series of sieves of sizes 250–50 µm on a mechanical sieve shaker. Sand fractions were retained on sieves > 50 µm while silt and clay fractions (< 50 µm) were repeatedly siphoned off. Density separates were recovered by sodium polytungstate (SPT) following a procedure modified from Sohi et al. (2001). A deionized water was used to suspend 20 g of the soil which was then decanted to retain the organic material. The content was transferred into a centrifuge bottle (250 mL) followed by addition of 125 mL SPT solution adjusted to a density of 1.65 g cm^{-3}. The mixture was gently shaken and allowed a period of 30 min for settling and centrifuged at 2000 rpm for another 30 min. The supernatant was sieved (0.50 µm) and the residue rinsed with deionized water and referred to as light fraction organic matter (LFOM). Forty (40) ml of SPT solution of 1.85 g cm^{-3} density plus 10 glass beads were added into the settled residue and centrifuged at 175 rpm for about 18 h. The supernatant was filtered, and the retained residue rinsed to obtain occluded particulate organic matter. The material which settled was placed in SPT of density adjusted to 2.60 g cm^{-3} and shaken for 10 min before being centrifuged at 3000 rpm for about 30 min. The supernatant was decanted, the residue retained and repeatedly washed with deionized water to remove the leftover SPT. This fraction was referred to as the heavy fraction organic matter (HFOM) (stable SOM).

Soil samples obtained from the aggregate sizes and density size fractions were ground and used for determinations of total SOC and total N contents. Contents of dissolved organic matter (DOM) were estimated by incubating (at 20 °C for 3 weeks) about 10 g soil maintained at 55% water-holding capacity (Smolander and Kitunen, 2002).

2.8. Soil microbial biomass

The microbial biomass C (MBC) content of each aggregate size and density fractions were fumigated using chloroform (Vance et al., 1987). About 20 g of the samples was subjected to ethanol-free chloroform for 48 h and kept in the dark at 25 °C for 16 h. A non-fumigated sample of the same weight was subjected to similar conditions. About 50 mL of K_2SO_4 (0.5 M) was added to the two sets of samples and shaken for 30 min to obtain extracts. Carbon content of the extracts (MBC) was oxidized with Mn^{3+} and analyzed spectrophotometrically at 600 nm (Bartlett and Ross, 1988). The MBC contents were estimated using Eq. (1).

$$\text{Microbial biomass} - \text{carbon (MBC)} = \frac{SOC_{fumigated} - SOC_{non-fumigated}}{0.33} \quad (1)$$

2.9. Microbial respiration

About 10 g moist soil sample obtained from each plot at depth interval of 10 cm to soil profile 40 cm were separately measured in a vessel placed in a corked glass jar. A parafilm pricked with holes was used to cover the jars so as to allow for aeration during 7 days pre-incubation. After this period, the content was incubated for 7 days in the dark at 20 °C. The evolved CO_2 was trapped in 15 mL sodium hydroxide (0.2 N) placed into an air-tight glass jar. Precipitation of carbonate ions was done by adding excess barium chloride (1.5 M) to the sodium hydroxide solution and the excess sodium hydroxide titrated with hydrochloric acid (0.2 N) using phenolphthalein indicator. The CO_2 absorbed during handling and titration was corrected by the blank readings (Alef, 1995). The metabolic quotient (qCO_2) was calculated using Eq. (3):

$$qCO_2(\text{g biomass C})^{-1}d^{-1} = \frac{\mu gCO_2C \text{ evolved in 7 days (g soil}^{-1})}{\mu g \text{ biomass C after 7 days (g soil}^{-1})} \times 1000 \quad (3)$$

2.10. Estimation of enzyme activities

2.10.1. Fluorescein diacetate hydrolysis

Hydrolysis of fluorescein diacetate (FDA) was performed according to procedures outlined by Adam and Duncan (2001). About 2 g fresh soil (sieved, 2 mm) obtained from each experimental plot was separately transferred into a conical flask (50 mL) followed by 20 mL of K_3PO_4 buffered at pH 7.4. Addition of stock solution (FDA, 1000 µg/mL) was ensured to trigger the reaction. The flasks were stoppered, transferred to an orbital incubator subjected to temperature of 30 °C and shaken at 120 rpm for 15 min. After their removal from the incubator, about 15 mL chloroform (2:1 v/v) was added to the samples to end the reaction. The content was centrifuged at 1000 rpm for 10 min, the overlying liquid filtered through Whatman paper, No 2 and measured on a spectrophotometer at 490 nm. A calibration graph was used to extrapolate the concentration of fluorescein released during the assay.

2.10.2. Dehydrogenase activity

Dehydrogenase activity (DHA) was determined following procedures outlined by García et al. (1997). Briefly, air dry soil sample of about 1 g maintained at 60% water holding capacity (WHC) was subjected to 0.2 mL of 0.4% INT (2-p iodophenyl-3-pnitrophenyl-5-phenyltetrazolium chloride). The content was placed in distilled water maintained at 20 °C and kept off from light for about 20 h. The content was shaken vigorously for 1 min in 10 mL of methanol and filtered. The extract (iodonitrotetrazolium formazan (INTF)) was spectrophotometrically measured at 490 nm.

2.11. Data analysis

The data was analyzed using version 3.5.2 of R software. Mixed model analysis of variance was used to test the treatment effects on soil organic matter fractions and microbial activity. Cropping system and agro-ecological zones were considered as fixed factors while the variables assessed (MBC, DOM, LFOM, HFOM, DHA, FDA, SMR, and qCO_2) were considered random factors. Tukey's honest significant difference (HSD) test was used for treatment mean separations with the threshold probability level set at $p \leq 0.05$. Principal component analyses (PCA) were calculated to examine relationships between the SOM fractions (MBC, DOM, LFOM, and HFOM), microbial activity (DHA, FDA, SMR, and qCO_2), soil properties (soil temperature, soil moisture, soil texture and aggregate size), cropping systems and agro-ecologies (UM, LH and UH).

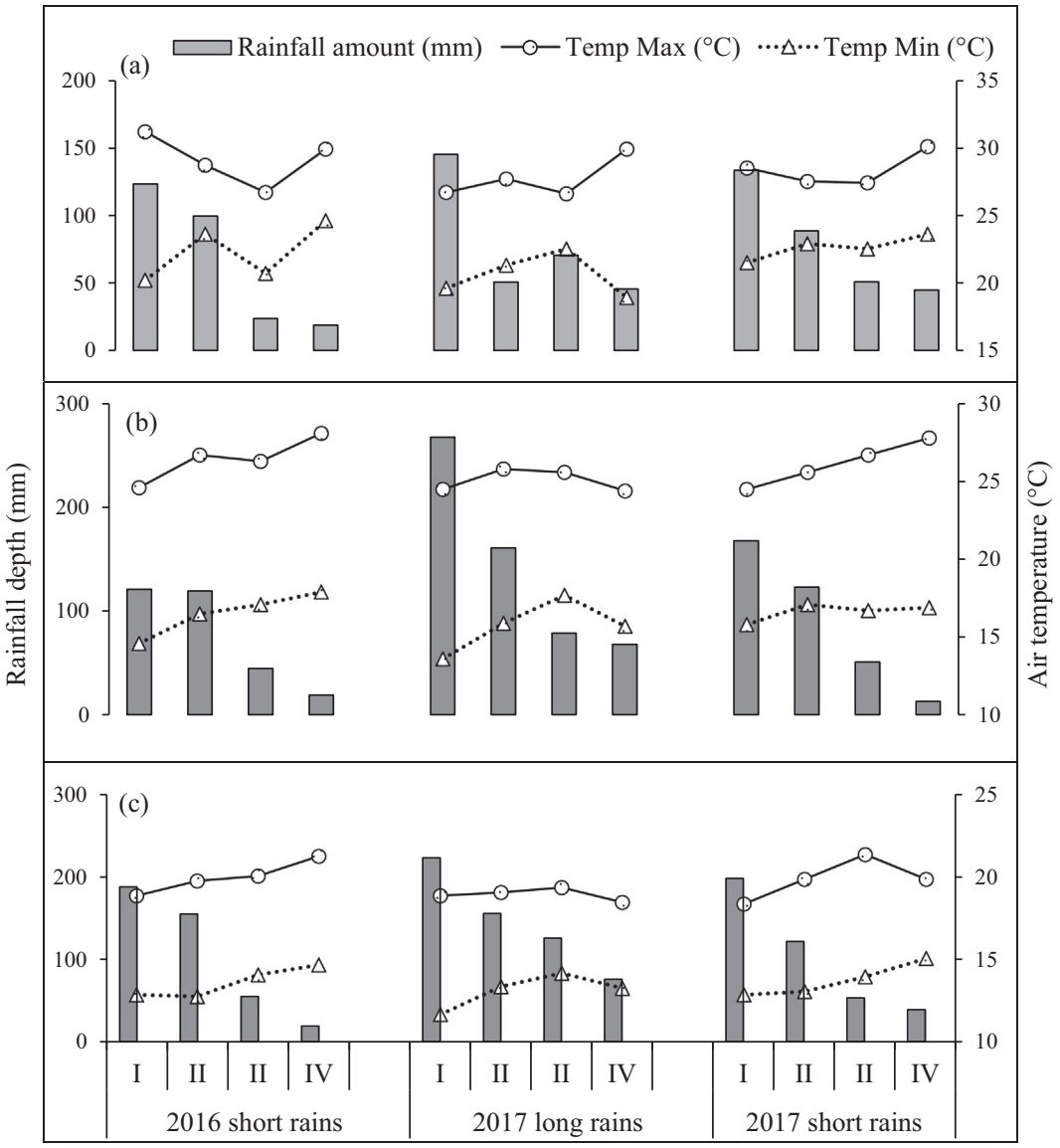

Fig. 3. Rainfall and air temperature recorded for upper midland (a), lower highland (b) and upper highland (c) agro-ecological zones at different potato development stages: sprout development (I), tuber initiation (II), tuber bulking (III) and tuber maturation (IV).

Table 2
Accumulative residue biomass, carbon and nitrogen supply, residue chemical composition and carbon to nitrogen ratio by different cropping systems.

		Root biomass	Shoot biomass	Root C	Shoot C	Root N	Shoot N	Lignin	Polyphenol	Root C/N ratio	Shoot C/N ratio
		Kg/ha									
Upper midland	Potato + dolichos	1811c	5271d	880c	699c	38.2b	38.2bc	69.4ab	19.4a	23.8a	18.9a
	Potato + lima bean	1573b	3433b	571b	592b	32.6b	26.3b	75.0bc	25.7a	24.4a	19.9a
	Sole dolichos	2432e	7502e	1125e	989e	64.1c	44.7c	52.9a	18.3a	26.3a	16.2a
	Sole lima bean	2312d	4852c	989d	769d	46.9b	38.6bc	66.2ab	23.6a	26.6a	16.9a
	Sole potato	389a	1808a	120a	311a	10.5a	2.4a	91.2c	29.1a	28.5a	25.0b
Lower highland	Potato + dolichos	2980b	6611d	1282c	933c	48.8b	52.5c	62.4b	21.3a	24.4a	19.1a
	Potato + lima bean	2880b	4840b	799b	795b	42.4b	31.3b	70.0c	24.8a	25.5a	19.7a
	Sole dolichos	3731c	8941e	1511e	1347e	80.8d	52.5c	45.4a	19.9a	28.8a	16.9a
	Sole lima bean	3611c	6290c	1339d	1065d	60.1c	49.8c	61.5b	23.6a	26.9a	17.7a
	Sole potato	746a	2850a	164a	423a	15.9a	5.5a	86.2d	27.1a	29.8a	26.5b
Upper highland	Potato + dolichos	3730c	5830c	1683c	799b	38.4b	67.3c	64.6b	21.4a	25.1a	20.1a
	Potato + lima bean	4681d	6640d	1121b	1113c	57.9c	63.3c	65.3b	23.2a	26.6a	19.2a
	Sole dolichos	1811a	4441a	2108e	678ab	35.9b	41.2b	51.4a	19.9a	26.6a	18.9a
	Sole lima bean	5411e	8091e	1947d	1491d	89.2d	75.7d	62.2b	20.6a	25.7a	16.7a
	Sole potato	2520b	5250b	228a	592a	21.4a	7.9a	83.6c	25.1a	28.8a	27.5b

Means followed by different letters within a column indicate significant differences by Tukey's $p \leq 0.05$.

3. Results

3.1. Distributions of rainfall and air temperature

The amount and distribution of rainfall and air temperature measured during this study period is shown in Fig. 3. In the short rains of 2016, total rainfall amount of 266, 312 and 416 mm was received respectively in the upper midland (UM), lower highland (LH) and upper highland (UH) agro-ecologies. This is compared to 355, 575 and 581 mm recorded respectively during the 2017 long rains. The 2017 short rains totaled to 318, 355 and 412 mm respectively in the UM, LH and UH agro-ecologies. These rains occurred mainly during the vegetative growth irrespective of the seasons and agro-ecologies. Across the seasons, the air temperatures were higher in the UM (18.9–31.2 °C), intermediate in the LH (13.6–28.1 °C) and lowest in the UH agro-ecologies (11.7–21.3 °C).

3.2. Biomass accumulation and residue composition

Significant differences between the treatments were observed for cumulative shoot biomass which were greater in intercropping (3433–6640 kg/ha) and lowest in sole potato (1808–5250 kg/ha) across the agro-ecologies (Table 2). Similar trend was observed for root biomass which ranged between 1573 and 4681 kg/ha in intercropping and 389 to 2520 kg/ha in sole potato. Lima bean exhibited significantly higher root and shoot biomass in the UH zone compared to dolichos. The reverse was true in the UM and LH agro-ecologies. The shoot and root carbon and nitrogen supplied were greatest in sole legumes, intermediate in intercropping and lowest in sole potato. Similar trend was made for total lignin, polyphenol contents and C/N ratios.

3.3. Distributions of aggregate sizes and soil organic matter fractions

Aggregate distributions differed significantly between the treatments for the macro and micro-aggregates and were significantly greater in intercropping relative to sole potato stands (Fig. 4). For silt plus clay fractions, aggregate distributions showed statistical similarities among the treatments irrespective of the agro-ecologies. Generally for a given treatment, the distributions of aggregates were proportionally greater in silt plus clay size particles (43–53%) than in the micro (18–28%) and macro-aggregates (18–36%) irrespective of treatments.

The contents of light fraction organic matter (LFOM), microbial carbon (MBC) and dissolved organic matter (DOM) were significantly greater in the macro-aggregate, intermediate in micro-aggregate and lowest in silt plus clay particles irrespective of the treatments (Fig. 5). In the UM and LH agro-ecologies, potato-dolichos intercropping recorded significantly higher contents of LFOM, MBC and DOM in the macro and micro aggregates compared to sole potato and potato-lima bean intercropping. This trend was reversed in the UH where significantly higher contents of LFOM, MBC and DOM within the micro- and macro aggregates were found in potato-lima bean intercropping. Taken together, the LFOM within the macro-aggregates accounted for 39–55% of the total SOM, this was followed by DOM within the macro-aggregates (22–38%) and MBC which comprised of 5–9% of the total SOM. For the HFOM, highest contents of organic matter were recorded in silt plus clay followed by micro-aggregate and lowest in the macro-aggregates. This aggregate fraction exhibited statistically similar organic matter content between the treatments irrespective of agro-ecologies.

3.4. Effect of cropping systems and sampling depths on distributions of SOM fractions

Labile fractions of SOM (MBC, LFOM and DOM) varied significantly among the treatments and were consistently greatest in sole legumes, intermediate in intercropping and lowest in sole potato (Table 3). Heavy fraction organic matter (HFOM) however showed statistical similarities between the treatments and ranged between 343 and 551 mg C kg^{-1} across agro-ecologies and sampling depths. General decrease in SOM contents with increasing soil depth was observed irrespective of the treatments and agro-ecologies. Cropping systems, soil depth and their interactions had highly significant ($p < 0.001$) effect on labile fractions of SOM. The HFOM was affected by sampling depth, but not by cropping system.

3.5. Microbial biomass carbon to total SOC ratio

Carbon to nitrogen ratio of microbial biomass and total SOM (MBC/SOC) were significantly influenced by cropping systems and generally decreased with increasing soil depth (Table 4). The ratios were significantly greater in intercropping relative to sole potato irrespective of the agro-ecologies. In the UM and LH agro-ecologies, dolichos recorded notably higher MBC/SOC ratio compared to lima bean, a trend that was reversed in the UH agro-ecology. The ratios generally tended to level off at 30–40 cm soil depth.

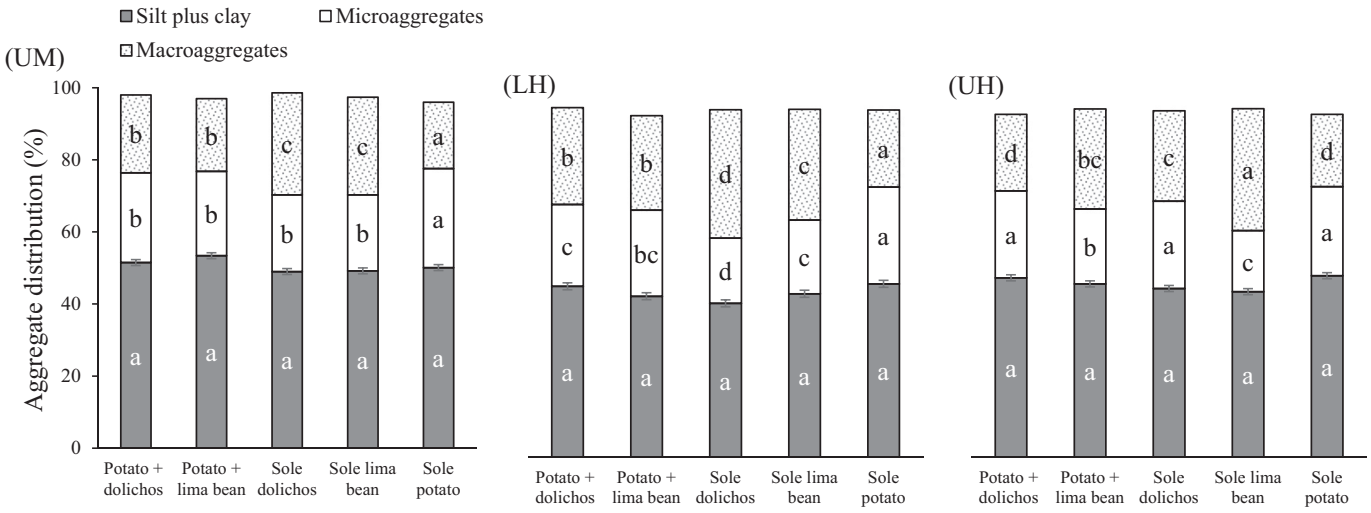

Fig. 4. Soil aggregate distribution (0–40 cm depth) at the end of the study period under different cropping systems and agro-ecologies (upper midland (UM), lower highland (LH), upper highland (UH)). Bars with different letters within an aggregate class are significantly different at Tukey's $p \leq 0.05$.

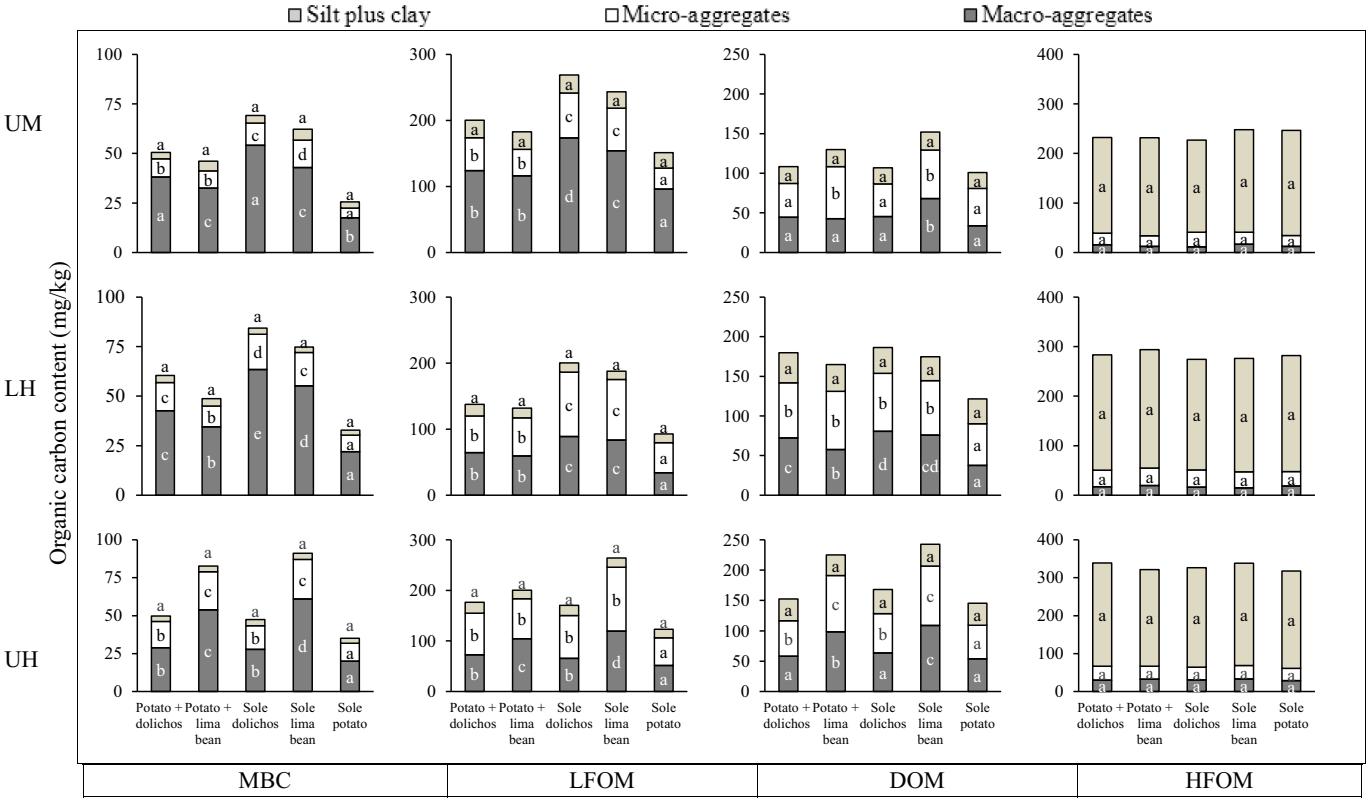

Fig. 5. Distributions of SOM fractions (Microbial biomass carbon-MBC, light fraction organic matter-LFOM, dissolved organic matter-DOM, and heavy fraction organic matter-HFOM) within aggregate classes (μm) under different cropping systems and agro-ecologies (upper midland-UM, lower highland-LH and upper highland-UH). Bars with different letters for a given aggregate-size differ significantly between treatments (Tukey's $p \leq 0.05$).

Table 3
Soil organic matter fractions: heavy fraction organic matter (HFOM), light fraction organic matter (LFOM), dissolved organic matter (DOM) and microbial biomass carbon (MBC) as measured in soil depth 0–40 cm under different treatments and agro-ecologies.

		Upper midland				Lower highland				Upper highland			
		HFOM	LFOM	DOM	MBC	HFOM	LFOM	DOM	MBC	HFOM	LFOM	DOM	MBC
Soil depth (cm)	Cropping System	mg C kg^{-1}											
0–10	Potato + dolichos	423a	412b	335b	198c	515a	682b	501b	288b	525a	606a	635b	203a
	Potato + lima bean	409a	487b	346b	106b	508a	646b	512b	279b	546a	922c	774c	309b
	Sole dolichos	432a	584d	445c	242d	525a	806c	673c	315c	520a	729b	633b	228a
	Sole lima bean	408a	509c	432c	208c	491a	813c	605c	292b	551a	983bc	750c	402c
	Sole potato	401a	298a	240a	98a	488a	435a	334a	102a	549a	564a	417a	212a
10–20	Potato + dolichos	432a	432b	301c	167bc	514b	566b	399b	222b	508a	529b	315c	115a
	Potato + lima bean	443a	420b	246b	154b	500b	542b	388b	123b	522a	795c	444b	300b
	Sole dolichos	408a	501c	330d	208d	522b	694d	482d	301d	494a	524b	434b	134a
	Sole lima bean	431a	407b	319d	174c	443a	603c	410c	254c	501a	884c	354c	339b
	Sole potato	399a	168a	184a	32a	439a	259a	245a	63a	445a	318a	319a	108a
20–30	Potato + dolichos	389a	323b	169c	102b	465b	465b	234c	157b	434b	437c	247b	108b
	Potato + lima bean	383a	312b	145b	89b	456b	471b	208b	141b	508b	591d	295b	228c
	Sole dolichos	392a	387b	189c	167c	479b	601d	279d	240c	456a	304b	105a	98b
	Sole lima bean	387a	356b	139b	153c	462b	504c	267d	209c	525b	604d	241b	293d
	Sole potato	366a	106a	98a	19a	402a	162a	108a	51a	418a	201a	101a	40a
30–40	Potato + dolichos	376a	202b	133c	76d	436a	329b	186b	102b	411a	264b	159a	52a
	Potato + lima bean	367a	189b	121c	45b	414a	301b	169b	82b	465a	275b	210b	129bc
	Sole dolichos	387a	245c	129bc	89d	442a	378b	199b	141c	432a	301c	146a	103b
	Sole lima bean	354a	234c	116b	64c	406a	342b	187b	96b	467a	424d	207b	143c
	Sole potato	343a	87a	96a	18a	400a	143a	101a	49a	448a	165a	167a	45a
Analyses of variance (p values)													
Cropping system		ns	< 0.001	0.027	< 0.001	ns	0.010	0.020	< 0.001	ns	< 0.001	0.032	< 0.001
Soil depth		0.034	< 0.001	< 0.001	< 0.001	ns	< 0.001	< 0.001	< 0.001	0.048	< 0.001	< 0.001	< 0.001
Agro-ecology		ns	< 0.001	< 0.001	< 0.001	0.003	< 0.001	< 0.001	< 0.001	0.034	< 0.001	< 0.001	< 0.001

Means followed by different letters within a column and soil depth denote significant differences (Tukey's $p \leq 0.05$); ns, not significant.

Table 4
Microbial biomass carbon (MBC) to total soil organic matter carbon (SOC) ratio as influenced by cropping systems in soil profile 0–40 cm under different agro-ecologies.

	Upper midland				Lower highland				Upper highland			
	Soil depth (cm)											
Cropping system	0–10	10–20	20–30	30–40	0–10	10–20	20–30	30–40	0–10	10–20	20–30	30–40
Potato + dolichos	4.8cd	4.1cd	3.7b	3.5c	5.2bc	4.5c	4.3b	4.1b	4.4a	3.7b	3.5b	3.3b
Potato + lima bean	4.5bc	3.8b	3.6b	3.4bc	5.0b	4.2b	4.1b	3.9b	5.6b	5.1d	4.6c	4.2c
Sole dolichos	5.2e	4.5e	4.1c	3.3b	5.5c	4.8d	4.6c	4.4c	4.2a	3.1a	3.0a	2.9a
Sole lima bean	4.9de	4.3de	3.9bc	3.2b	5.0b	4.6cd	4.3bc	4.0b	5.8b	5.1d	4.8c	4.4c
Sole potato	3.8a	3.1a	2.9a	2.5a	4.1a	3.4a	3.2a	2.9a	4.3a	4.1c	3.2a	3.0ab

Means followed by different letters within a column denote significant differences (Tukey's $p \leq 0.05$).

Table 5
Soil microbial respiration (RMS-mg kg^{-1} d^{-1}), metabolic quotient (qCO_2- mg CO_2-g biomass C^{-1} d^{-1}) and enzyme activities (fluorescein diacetate hydrolysis (FDA-g FDA/g)) and dehydrogenase (DHA-INTF/g) in response to cropping systems and soil depth under different agro-ecologies.

		Upper-midland				Lower-highland				Upper-highland			
Soil depth (cm)	Cropping System	FDA	DHA	SMR	qCO2	FDA	DHA	SMR	qCO2	FDA	DHA	SMR	qCO2
0–10	Potato + dolichos	111.8b	50.4b	20.3c	44.5b	130.8c	68.9c	25.5bc	42.2b	101.2a	50.9b	18.2a	40.1b
	Potato + lima bean	108.8b	49.2b	16.6b	44.1b	116.9b	58.6b	22.2b	41.8b	136.6b	69.9c	24.6b	38.4a
	Sole dolichos	148.9d	58.9c	22.2c	40.4a	154.8d	79.6e	28.6c	38.9a	97.2a	40.8a	17.7a	41.1bc
	Sole lima bean	133.6c	56.8c	20.3c	43.5b	137.3c	70.4d	25.8b	39.2a	148.8b	79.9d	28.9b	38.2a
	Sole potato	88.7a	35.5a	12.2a	48.9c	89.1a	42.8a	15.9a	45.6c	105.2a	53.6b	18.9a	42.2c
10–20	Potato + dolichos	98.7b	48.6c	15.5b	40.4a	120.1b	60.8b	22.3bc	40.4b	96.7a	41.1a	15.5a	39.9b
	Potato + lima bean	90.3b	44.4b	15.1b	42.4b	114.7b	55.7b	19.5b	40.1b	131.3b	65.3b	23.4b	37.7a
	Sole dolichos	133.7c	52.8d	18.9c	39.8a	144.5c	77.6d	25.2d	37.7a	93.2a	36.1a	16.6a	40.2b
	Sole lima bean	128.2c	49.7cd	17.8c	39.2a	137.8c	68.2c	23.3cd	38.7a	139.9b	73.8b	24.7b	37.4a
	Sole potato	80.5a	30.2a	9.1a	44.4c	84.8a	36.7a	10.3a	43.9c	97.8a	45.7a	16.2a	40.4b
20–30	Potato + dolichos	88.6b	44.7b	12.4c	39.1b	113.6b	52.8bc	17.4bc	37.3b	90.1a	40.5a	15.4bc	38.2b
	Potato + lima bean	82.3b	42.6b	9.4b	40.2b	99.7b	48.9b	15.5b	38.2b	128.3b	63.3b	18.8cd	38.1b
	Sole dolichos	121.5d	48.6c	13.4c	35.5a	131.9c	63.4d	20.2d	35.8a	86.7a	33.7a	13.4a	39.3bc
	Sole lima bean	110.6c	44.4b	12.1c	36.6a	108.5b	54.8c	18.8cd	35.7a	131.9b	67.9b	20.3d	35.2a
	Sole potato	70.6a	25.7a	7.2a	43.1c	73.8a	29.7a	9.9a	40.8c	88.9a	38.8a	13.9a	40.3c
30–40	Potato + dolichos	83.7c	38.8cd	11.9c	36.6b	90.3b	45.7bc	13.3b	37.1b	75.9a	37.9b	12.8a	37.7b
	Potato + lima bean	70.0b	34.6b	8.2b	37.5b	84.7b	42.4b	11.1b	37.4b	121.7c	54.7c	16.4b	37.6b
	Sole dolichos	102.8c	40.9d	11.8c	34.4a	107.7c	55.4d	16.6b	35.1a	85.8b	27.9a	12.3a	38.2b
	Sole lima bean	94.6c	36.8bc	9.1b	36.6b	103.5c	48.7c	14.4b	36.6a	127.5c	58.8c	17.9b	34.1a
	Sole potato	55.9a	22.4a	6.3a	40.4c	72.6a	25.7a	7.8b	39.4c	74.2a	28.1a	10.4a	39.5b
Analyses of variance (p values)													
Cropping system		< 0.001	< 0.001	0.003	0.042	< 0.001	< 0.001	< 0.001	0.013	< 0.001	< 0.001	< 0.001	0.021
Soil depth		0.033	0.013	0.023	0.044	0.022	0.012	0.001	0.021	0.010	0.034	0.012	0.031
Agro-ecology		ns	0.041	ns	ns	ns	ns	0.045	ns	0.041	ns	0.034	ns

Means with different letters within a column and soil depth denote significant differences (Tukey's $p \leq 0.05$), ns, not significant.

3.6. Microbial activities

Microbial activities as measured by soil microbial respiration (SMR), metabolic quotient (qCO_2), fluorescein diacetate (FDA) and dehydrogenase (DHA) hydrolyses, differed significantly ($p < 0.01$) between the treatments (Table 5). Contents of FDA and DHA in the soil were significantly greater in sole legume plots than in intercropping and lowest in sole potato plots irrespective of agro-ecologies. The enzyme activities generally decreased with increasing soil depths and were variable across agro-ecologies. In the UH and LH agro-ecologies, the FDA and DHA hydrolyses were greatest in sole dolichos and lowest in sole potato plots across the four soil depths. This trend was reversed in the UH agro-ecology where sole lima bean recorded significantly higher FDA and DHA contents relative to dolichos.

Soil microbial respiration (SMR) followed similar trend to that of FDA and DHA with sole potato plots consistently showing the lowest value across sampling depths and agro-ecologies. Metabolic quotient (qCO_2), a quotient of basal respiration to corresponding microbial biomass size, was significantly greatest in sole potato, intermediate in intercrops and lowest in sole legumes regardless of sampling depth. Across agro-ecologies, both cropping systems and soil depth had significant effect on qCO_2. All the measured microbial activity parameters were notably higher in the UH than in the LH and were lowest in the UM agro-ecology.

3.7. Principal component analyses of factors influencing soil organic matter dynamics

Principal component analyses showing factors influencing SOM dynamics is presented in Fig. 6. The two principal components explained 74% of the variability in SOM fractions with the first component accounting for 56% of the variance and the rest (18%) being accounted for by the second component. The first component was strongly correlated with 10 of the original variables ($p = 0.000$) and most strongly with the microbial biomass carbon-MBC ($r = 0.98$). This component was therefore a measure of soil microbial biomass content and increased with increasing dissolved organic matter-DOM ($r = 0.80$), fluorescein diacetate-FDA ($r = 0.80$), dehydrogenase activity-DHA ($r = 0.78$), soil water content-SWC ($r = 0.70$), light fraction organic matter-LFOM ($r = 0.65$), metabolic quotient-qCO_2 ($r = 0.61$) and microbial respiration-SMR ($r = 0.55$), but negatively with clay content ($r = -0.96$), aggregate size ($r = -0.94$), agro-ecology

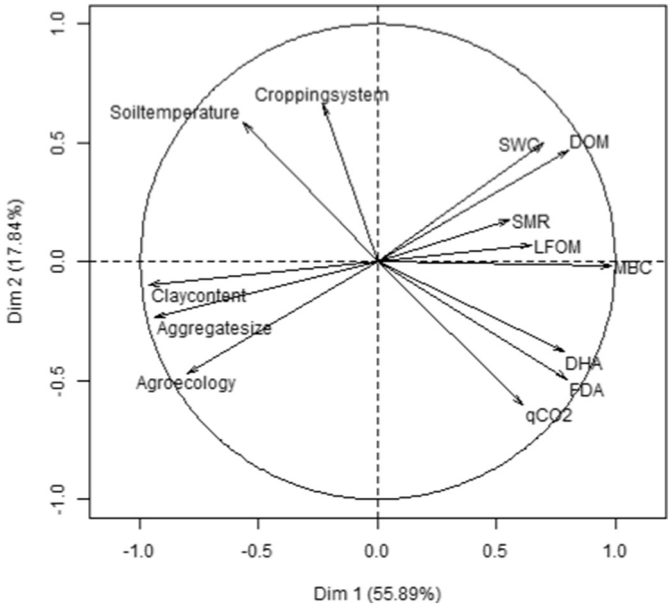

Fig. 6. Principal component analyses of factors influencing SOM dynamics in potato-legume intercropping. SWC, soil water content, DOM, dissolved organic matter, LFOM, light fraction organic matter, MBC, microbial biomass carbon, qCO_2, metabolic quotient, FDA, fluorescein diacetate, DHA, dehydrogenase activity, SMR, microbial respiration. Vectors indicate the degree of correlation between each factor and the axes.

($r = -0.80$) and soil temperature ($r = -0.57$). The second component was majorly a reflection of cropping system ($r = 0.66$), which related strongly with metabolic quotient-qCO_2 ($r = -0.60$), soil temperature ($r = -0.58$), soil water content-SWC ($r = 0.50$) but weakly with FDA ($r = -0.49$), agro-ecology ($r = -0.49$), DOM ($r = 0.47$) and DHA ($r = -0.38$).

The interrelationship of the components revealed a direct positive association between soil FDA, DHA and MBC. Soil DHA however exhibited a weaker correlation with LFOM, MBC and DOM compared to soil FDA. There was a significant positive association between qCO_2, soil DHA and FDA hydrolysis. Further, there was a positive association between the aggregate sizes with soil labile organic matter fractions (LFOM, MBC and DOM), but a negative correlation exhibited between the clay content, microbial biomass and SMR. The activity of DHA, soil FDA hydrolysis and SMR related negatively with soil temperature, but positively with soil moisture content.

4. Discussions

4.1. Distributions of aggregate sizes and soil organic matter fractions

The high variability in sizes of macro-aggregate distributions between the treatments reflected greater effect of residue retention on sand fractions relative to micro-aggregate and silt plus clay fractions. Intercropping provided readily-degradable substrates which were decomposed by the soil microbes releasing cementing agents that glued the soil particles to form micro-aggregates. The micro-aggregates were then bound with SOM and silt plus clay particles to form macro-aggregates. This effect was influenced by residue quantity that was higher in intercropping than in sole potato. When the residue supply by legume intercropping attained a threshold level, most of it remained unprotected and was highly accessible to degrading soil microbes and their enzymes. This triggered residue degradation fostering aggregate formation. This process signified improved soil physical quality such as nutrient exchange, soil water movements and aeration (Jäger et al., 2011). These conditions were favorable for microbial growth and activities.

The content of microbial biomass differed significantly among the size fractions, a reflection that different aggregate classes provided distinct microhabitats packed with organic carbon of different qualities and quantities. The macro-aggregates had the greatest contents of MBC suggesting high population of soil microorganisms. In addition, the macro-aggregates had the highest MBC/SOC ratio irrespective of the cropping system indicating high efficiency by which the soil microbes used the carbon substrates (Anderson and Domsch, 2010). A possible reason for this observation relates to the fact that the macro-aggregate size fraction is composed primarily of partially decomposed litter that provided sources of energy to the microorganisms. Intercropping increased the contents of MBC by inputting large quantity of litter and roots thus enhancing accumulation of DOM and LFOM which provided sources of nutrition to soil microorganisms. This observation is in keeping with other studies that found high diversity, functionality and spatial distribution of fungi and bacterial populations within the macro-aggregates (Mummey et al., 2006; Poly et al., 2001). They attributed it to the fact that macro-aggregates exhibit high stability of soil water potential and are physically protected from the predators.

The microbial biomass was however significantly greater in intercropping relative to sole potato for the macro-aggregate fractions. This observation is consistent with the biomass quantity and quality which was higher in intercropping than in sole potato. The residues from legume intercrops had higher nitrogen content that increased the supply of nutrients to the soil microbes. Generally, the microbial biomass is activated by continuous addition of substrates, resulting in increased production of extra-cellular enzymes that enhances the microbial activity (Loeppmann et al., 2016). This observation was depicted in our study which showed increasing fraction of MBC which had preferential use for soil labile organic carbon as source of energy. Rasmussen and Rohde (1988) showed that low residue return to the soil results in nitrogen immobilization that adversely impacts on the microbial population density and diversity. Moreover, the soil particles obtained from intercropping had greater MBC/SOC and also exhibited lower metabolic quotient (qCO_2) relative to those in the sole potato plots. This indicates that legume intercropping fostered the growth of soil microorganisms and enhanced the efficiency by which the substrates were used as energy sources. The silt plus clay fractions had the least MBC/SOC regardless of the treatments indicating the lowest carbon use efficiency. This observation was ascribed to the low SOM accessibility contributed by sorption of carbon on the clay colloids.

The high contents of light fraction organic matter (LFOM) within the macro-aggregates was a reflection of high residue transformation following addition of fresh residue to the soil. This fraction resided within the macro-aggregates which remained unprotected by physical mechanisms thus allowing accessibility of degrading microorganisms and their enzymes. Due to the fact that potato had relatively higher lignin and polyphenol concentrations and exhibited higher C/N ratios, we expected to find higher contents of partially decomposed litter (LFOM) in sole potato plots. This was not the case as intercropping generally exhibited higher LFOM contents. This observation is attributed to the low residue deposition by potato that stimulated breakdown of the more resistant litter transforming it into more refined dissolved organic matter (DOM). Intercropping however exhibited low quantity of polyphenols and lignin compounds and had lower C/N ratios making their residues to undergo rapid decomposition with increased partitioning of organic matter to the less protected macro-aggregates. Generally, the incorporation of legume intercrops with high biomass nitrogen improved crop residue quality by lowering C/N ratio.

In contrast, the LFOM and DOM content attached to clay plus silt fractions was similar between the treatments implying that these fractions were not affected by legume intercropping. For the micro-aggregates, the SOM was encapsulated within the macro-aggregates making it more protected by physical mechanisms and was thus more stabilized and resistant to further decomposition. Similarly, no response to legume intercropping was observed for the heavy fraction organic

matter (HFOM) attached to the silt plus clay fractions as these particles exhibited charged surfaces that adsorbed the SOM making it physically, chemically and biochemically protected from the microbial attack (Six et al., 2002). This fraction represented > 90% of the silt plus clay fractions reiterating the importance of these particles in carbon stabilization.

The contents of labile SOM fractions (LFOM, MBC and DOM) were significantly higher in the surface soil layer (0–10 cm) irrespective of the treatments. This observation was related to the high residue concentrations within the topsoil as the hand hoes had limited capacity to incorporate the residues beyond this depth. This is consistent with other authors who argued that the more labile SOM largely depend on the tillage depth and the quantity of organic residues incorporated (Somasundaram et al., 2018). Compared to sole potato, intercropping recorded significantly higher contents of labile SOM fractions (LFOM, MBC and DOM) in the 30 to 40 cm depth, an observation we related to the high root biomass contributed by legume intercrops. Generally, up to half of the labile SOM is found in the roots (Gregory and Atwell, 1991). The fact that roots are produced directly within the microbial habitat (Merino et al., 2015), may have triggered interactions that directly affected the structure and function of decomposer organism, potentially enhancing MBC biomass. In addition, roots exude labile soil carbon compounds that promote efficiency of microbial growth resulting in increased production of MBC (Kong et al., 2011).

Despite the general decrease in HFOM with depth across the treatments, only a slight variation was observed between treatments. This was a confirmation that this organic matter fraction is bio-chemically stabilized and resistant to further decomposition, irrespective of soil depth. Nyawade (2015) postulated that HFOM generally decreases with increasing depth due to the increasing contents of Al and Fe-oxides in soils dominated by kaolin minerals such as those in this study.

4.2. Effect of legume intercropping on soil microbial activity

The fluorescein diacetate hydrolysis (FDA) was increased by legume intercropping suggesting enhanced microbial activities. This observation relates to the high residue deposition and transformation that occurred in these plots thus providing large energy source to the soil microbes. A similar result measured for dehydrogenase (DHA) revealed a higher microbial activity which likely was contributed by the high flux of root secretions and root exudates in the plant rhizosphere (Rakshit et al., 2012). Soil DHA and FDA hydrolysis decreased under sole potato stands suggesting a negative impact of low residue quantity on soil microbiological activity. This was probably a consequence of low energy sources for the microbial activity as reflected by the low contents measured for LFOM, DOM and MBC. Reddy et al. (2003) reported greater microbial activity as measured by FDA in soils planted with crimson legume clover for three years relative to soils subjected to non-legume rye cereal. Consistent findings were reported by Sicardi et al. (2004) who suggested that the labile SOM fractions provide readily source of energy to the soil microbes leading to enhanced microbial activity. It is for this reason that this fraction of organic matter is considered the most sensitive indicator of soil quality (Nyawade et al., 2018b; Kapkiyai et al., 1999).

The high soil respiration under legume intercropping was an indication of increased rate of organic matter transformation by soil microbes. This observation was further reflected in the low metabolic quotient (qCO_2) under legume intercropping pointing to the increased microbial respiration. As soil micro-organisms breakdown residues, carbon becomes incorporated into the microbial biomass, and makes integral component of MBC, a process which is slowed by high C/N ratio (Chen et al., 2014). A mixture of high and low quality residues like in this study, enhances microbial activity, causing a larger carbon proportion to be incorporated into microbial biomass (Müller-Stöver et al., 2012). This enhances the efficiency by which the soil microbes convert the organic carbon into microbial biomass (Anderson and Domsch, 1990). In the UH agro-ecology, the unfavorable climatic conditions for dolichos growth led to lesser input of litter thus contributing to the low soil MBC content which increased the qCO_2 and lowered the microbial respiration rates.

The decrease in microbial activity with increasing soil depth was consistent with that of the substrate availability that were high in the topsoil and steeply decreased with soil depth. Topsoil represented the dynamic nexus where majority of shoot biomass was deposited. For sole potato, root biomass was found entirely in the topsoil and thus maximum plant carbon allocated to rhizodeposition was expected in this zone (Canarini and Dijkstra, 2015). In other studies, fungal evenness and richness have been shown to be high in the topsoil and drastically decrease with increasing soil depth (Jumpponen et al., 2010; Vargas-Gastelum et al., 2015). This is in line with other studies that showed that subsoils accommodate only a specific type of soil microbes that are well adapted to the low substrate conditions (Hartmann et al., 2009). Further, the content of labile SOM that readily avails the substrates to the soil microbes was high in the topsoil compared to the subsoil that was dominated by HFOM that restricted the accessibility of substrates by the soil microbes.

The significant interactive effect observed between soil depth and agro-ecology on FDA hydrolyses, DHA activity and SMR reflected the variability in soil microbial activity contributed by agro-ecological conditions and soil types. The soils in lower highland agro-ecology were clay dominating and generally exhibited increase in clay content with increasing depth. Thus sorption of SOM to the Al and Fe-oxides may have restricted the substrate availability, especially in the deeper soil layers. This environment disfavored the microbial growth, restricting their activity. In the UH agro-ecology, the high rainfall amounts repeatedly created water-saturated conditions in the subsoils that lowered the oxygen availability. This coupled with increase in Fe and Al oxides that formed mineral-organic associations with the SOM (Dietel et al., 2017), impaired the substrate availability for the soil microbes decreasing their activity. In the topsoil, the high biomass contents may have favored the activity of fungi contributing to increased microbial activity (Engelhardt et al., 2018).

4.3. Principal factors influencing dynamics of SOM fractions in potato-legume intercropping

The fluorescein diacetate hydrolysis (FDA) and dehydrogenase activity (DHA) were directly proportional to the microbial biomass, an observation that was related to the high residue quantity contribution by legume intercrops (Schnurer and Rosswall, 1982). This is consistent with previous studies that showed that production of DHA and FDA hydrolysis increases with increase in residue deposition thus reflecting the increase in microbial enzymatic activity (Elfstrand et al., 2007). On the other hand, soil DHA and FDA hydrolysis exhibited positive association with LFOM and DOM reflecting that the total activity of microorganisms highly depended on light SOM fractions as their source of nutrition. García-Gil et al. (2000) described DHA as an intracellular enzyme useful for microbial oxidoreductase metabolism. The activity of this enzyme highly depends on the microbial biomass metabolic state which increases with increase in residue quality and quantity. Soil DHA however provided a weaker correlation with LFOM, MBC and DOM compared to soil FDA suggesting that the contribution of residue to microbial biomass and activity affected greater mass of fungi as DHA does not reflect the fungal population (Kumar and Tarafdar, 2003).

The significant positive association between qCO_2, soil DHA and FDA hydrolysis reflected increase in efficiency of carbon assimilation by the soil microbes per unit amount of organic residue increase. Thus the decrease in qCO_2 observed under legume intercropping relative to sole potato plots signified a greater metabolic efficiency of the substrate contributed largely by increase in residue quality. The positive association between the aggregate sizes with soil labile organic matter fractions (LFOM, MBC and DOM), cropping systems and SMR indicated

that the enhanced residue retention promoted the macro-aggregate formation thus enhancing the microbial activity. The negative correlation exhibited between the clay content and the microbial biomass as well as with SMR showed that the high clay content impeded the SOM mineralization by physically occluding the microbes in small pores (Rutherford and Juma, 1992), thus repressing their activity.

The activity of DHA, soil FDA hydrolysis and SMR related negatively with soil temperature but positively with soil moisture content implying inhibitory and stimulatory effects of high temperatures and increased soil moisture contents on microbial activity. Under extremely dry soil conditions, soil microbes are rapidly 'starved' because a dry soil does not have enough substrate supply, and is characterized by unfavorable microenvironment for microbial growth (Murphy et al., 1998). Steinweg et al. (2012) found soil temperature to be the dominant control of enzymatic activity when soil moisture was not limiting. Chavarría et al. (2016) similarly postulated that soil moisture and soil temperature are the major regulators of soil enzymatic activity at micro-plot scale.

Agro-ecology exhibited significant association with microbial biomass, aggregate sizes and SMR reflecting spatiotemporal variability caused by fluctuating soil temperatures and soil moisture contents. Temporal changes in soil moisture contents and soil temperature affect the amount of carbon allocated to rhizodeposits and secretions of rhizosphere products that cement soil particles into macro-aggregates (Bonde and Rosswall, 1987). The quantity of residue generation and nutrient transformation by soil microbes were besides highly dependent on the prevailing soil moisture and soil temperature conditions. Dolichos due to its inability to tolerate low temperatures in the UH agro-ecology (Cook et al., 2005), was characterized by reduced biomass production. This crop however, accumulated about 2 times higher biomass compared to lima bean in the UM zone and up to 5 times higher compared to sole potato. Lima bean however, tolerated both low and high soil temperatures and accumulated high biomass across the agro-ecologies. Potato being a temperate crop performed best in the UH zone where its biomass accumulation was highest. Previous work found that agro-ecology influences crop selection for biomass production in such a way that crop varieties are acclimatized to the zones favoring growth conditions (Jaetzold et al., 2012). Van Gestel et al. (1993) observed a parallel course of MBC content when increase in ambient temperatures caused desiccation to the soil microbes thus interrupting their growth.

5. Conclusion

This study gives an insight on the potential role of legume intercropping in increasing residue return thus enhancing the formation of labile soil organic matter fractions with little impact on the stable fractions. This strategy increased the proportion of macro-aggregates that signified improved nutrient release, high aeration and increased soil water infiltrations, the conditions of which are favorable for microbial growth and activities. These benefits may only be of primary importance to the smallholder farmers if the system is designed in a manner guaranteeing high yield stability. In this way, the ultimate goal of enhanced soil productivity will be masked, but earned by the farmer as a bonus.

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