



# Land use affects soil biochemical properties in Mt. Kilimanjaro region



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## ABSTRACT

Microbial parameters have been used to monitor changes in soil quality. Soils from four land use systems common in East Africa and present in the Mt. Kilimanjaro region: (1) montane forest, (2) savannah (3) maize fields and (4) Chagga homegardens were used in laboratory incubations to assess the effects of landuse changes on soil quality. Soil organic matter mineralization and the following microbial parameters: microbial biomass C, mineralization quotient, metabolic quotient and activities of four enzymes:  $\beta$ -glucosidase, cellobiohydrolase, phosphatase and chitinase were determined. Microbial biomass C content,  $\beta$ -glucosidase, cellobiohydrolase and chitinase activities were higher in natural systems compared to agricultural soils. High phosphatase activity observed in all land use types reflected strong phosphorus limitation in andic soils of the Mt. Kilimanjaro region. Chitinase activity in montane forest soils was 3 times higher than in Chagga homegardens. Mineralization quotient and cellobiohydrolase activity best exhibited the effect of land-use changes on soil quality in the Mt. Kilimanjaro region. Cellobiohydrolase activity was up to 3 times higher under natural ecosystems compared to agroecosystems. A high percentage of microbial biomass C content in total organic C and low metabolic quotient were observed in Chagga homegarden soils. Soil enzymes (especially cellobiohydrolase) best distinguished between natural and agricultural ecosystems, and are therefore useful for monitoring changes in soil quality. In conclusion, the measured microbial parameters clearly show that the microbial organisms in traditional Chagga homegardens system have high substrate use efficiency. This demonstrates that traditional agroforestry systems promotes soil fertility and are more suitable for agricultural production in the tropics compared to monocropping systems like maize plantations.

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## 1. Introduction

Conversion of natural ecosystems for agricultural production is changing the world's landscapes in pervasive ways (Foley et al., 2005). Mountain ecosystems in the tropics are experiencing extensive land-use changes (Mugagga et al., 2012). Nearly 60% of new agricultural land in tropical Africa is derived from intact forests and 35% from disturbed forests (Gibbs et al., 2010). Encroachment for cultivation in the Mt. Elgon region in East Africa has destroyed 25,000 ha of forest land, equivalent to one fifth of Mt. Elgon's total forest cover. Virtually all of the forest cover below an elevation of 2000 m has been removed for agricultural production (Mugagga et al., 2012). Similarly, coffee plantations have expanded at the expense of forests on the southern slopes of the Mt. Kilimanjaro region (Pabst et al., 2013; Hemp, 2006).

African savannahs are also under great pressure from agricultural intensification (Grace et al., 2006). In Kenya, 8.4% of rangelands have

been subject to a decrease in vegetation cover over the last two decades (Serneels and Lambin, 2001). Cassava, millet, sorghum, beans and maize fields dominate the landscape in the foothills of Mt. Kilimanjaro. Expansion of maize plantations to marginal lands on the southern slopes of Mt. Kilimanjaro, Tanzania, has contributed to the rapid disappearance and fragmentation of the savannah woodland vegetation. Riverine woodland has been reduced to a few rows of trees (Soini, 2005).

There has been a global concern that such continued agricultural expansion and intensification could change the soil quality irreversibly. This is because agricultural intensification contributes to losses of soil organic matter (SOM) as a result of reduced input of organic matter (OM), increased decomposability of organic inputs and accelerated SOM biodegradation (Lagomarsino et al., 2011). Conversion of natural systems to croplands also contributes to changes in the composition and activities of microbial communities and biogeochemical processes affecting soil quality. Soil quality has been defined as the "continued capacity of the soil to function as a vital living system to sustain biological activity and supply of ecosystem services" (Schloter et al., 2003). Soil microorganisms regulate ecosystem processes such as nutrient cycling through the breakdown of litter and SOM and release nutrients, making them available to plants. Soil microbial biomass is the eye of the needle

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through which all OM needs to pass (Paterson et al., 2009; Jenkinson, 1977). Therefore, there is a need to investigate microbial responses to better understand the effects of land use change and ecosystem disturbances on biogeochemical processes and soil quality. Microbiological parameters of soil have often been used as an early and sensitive indicator of ecological stress in both natural and agricultural systems.

Enzymes play an essential role in catalyzing reactions necessary for SOM breakdown and nutrient cycling, hence their relevance in assessing changes in soil quality. Enzyme activities have been used as a 'soil fertility index'. For example, Badiane et al. (2001) determined soil enzyme activities ( $\beta$ -glucosidase, chitinase, amylase and xylanase) to assess the effects of fallow management practices in a western African savannah. In their study, Bossio et al. (2005) found that  $\beta$ -glucosidase, cellobiohydrolase, chitinase and phosphatase activities increased in soils under cereal fallow rotation in western Kenya.  $\beta$ -glucosidase activity is involved in the last limiting step of cellulose biodegradation to release simple sugars (Mganga et al., 2015; Sanaullah et al., 2011). Like  $\beta$ -glucosidase, cellobiohydrolase is involved in the C-cycle. However, it differs from  $\beta$ -glucosidase in that it releases dimers from the cellulose strand. This makes cellobiohydrolase more closely related to community composition (Waldrop et al., 2000). Phosphatase catalyzes the chemical breakdown of organic and inorganic phosphomonoesters and is thus important in soil P mineralization (Acosta-Martínez et al., 2007) and also reflects the general enzyme activity in soil. Chitinases, which hydrolyze chitin, are associated with a wide range of soil microorganisms, particularly fungi (Dahiya et al., 2006).

Microbial parameters (microbial biomass C, microbial biomass C to organic C ratio ( $C_{mic}:C_{org}$ ), metabolic quotient ( $qCO_2$ ), mineralization quotient ( $qM$ ) and enzyme activities) have been used as indicators of soil quality because they are sensitive to land management and are comparatively easy to measure (Schloter et al., 2003). Nsabimana et al. (2004) estimated microbial parameters  $qCO_2$ ,  $C_{mic}:C_{org}$  ratio and soil enzymes, to demonstrate how land use in Kwa-Zulu Natal, South Africa, affected the size and activity of the soil microbial community. The ratio of soil microbial biomass to total organic carbon ( $C_{mic}:C_{org}$ ) is an indicator of  $C_{org}$  available for microbial growth (Anderson, 2003). The metabolic quotient for  $CO_2$  ( $qCO_2$ ), the community respiration per unit biomass, has been criticized by some authors (Wardle and Ghani, 1995) as a poor microbial parameter. However, it is still recognized as a relative measure of efficiency in utilization of available C in soil by microorganisms (Blagodatskaya and Kuzyakov, 2013).  $qCO_2$  has been widely applied in the assessment of agricultural practices and forest ecosystems (Yan et al., 2003). Mineralization quotient ( $qM$ ) expresses the fraction of total organic C mineralized over a period of time (Moscatelli et al., 2005) and is a valuable indicator of SOM stability. However, studies investigating the impact of land-use changes on these microbial parameters in tropical Africa remain limited.

The objective of this study was to investigate the effect of land use conversions from natural ecosystems to arable land on microbial indicators of soil quality: (1) microbial biomass C (2)  $C_{mic}:C_{org}$  ratio, (3) metabolic quotient ( $qCO_2$ ), (4) mineralization quotient ( $qM$ ) and (5) enzyme activities. Soils were sampled from four ecosystems common in tropical Africa and occurring in the Mt. Kilimanjaro region at two different elevations. Lower montane forest and Chagga homegardens located at a higher elevation (between 1623 and 1648 m a.s.l.) and savannah and maize fields at a lower elevation (between 951 and 920 m a.s.l.). Both pairs reflect land use conversions from natural to agricultural systems. Mt. Kilimanjaro region provides a great opportunity to investigate the effects of agricultural expansion and land-use change because the most common ecosystems in East Africa are well represented there. Additionally, the area has witnessed extensive land-use changes over the last 100 years (Soini, 2005). We hypothesize that conversion of natural systems to arable land will decrease microbial biomass content,  $C_{mic}:C_{org}$  ratio and enzyme activities and accelerate SOM mineralization.

## 2. Materials and methods

### 2.1. Study area

Mt. Kilimanjaro is situated 300 km south of the equator in Tanzania on the border with Kenya, between 2°45' and 3°25' S and 37°00' and 37°43' E (Hemp and Hemp, 2003). Its diverse climatic conditions create a high diversity of ecosystems and vegetational zonation (Hemp, 2006). Vegetation on Mt. Kilimanjaro is described in detail by Hemp (2006). Chagga homegardens, a traditional and sustainable agroforestry system characterized by an intensive integration of numerous multipurpose trees and shrubs with food crops and animals, simultaneously on the same unit of land, has been cited as an example of a model land use system in the Mt. Kilimanjaro region (Soini, 2005).

The main rainy season lasts from March to May, and the short rains, from November to December (Axmacher et al., 2004). The southern slopes at 700 m above sea level (a.s.l.) receive an annual rainfall of 800–900 mm and slopes at 1500 m a.s.l. receive 1500–2000 mm. Mean annual temperature in the region varies between 10 and 21 °C. Soils are Andosols that have developed from the same parent materials i.e. volcanic ash (Zech, 2006).

### 2.2. Description of study sites, experimental design and soil sampling

A brief description of the sites and the soil characteristics and management are shown in Table 1. Minimal disturbance in lower montane forests is characterized by the collection of firewood for fuel. The traditional multistoried Chagga agroforestry system located at the lower montane forest borders maintain a forest-like structure and are therefore partly characterized by forest species. The tree layer consists of indigenous species from the former montane forest community e.g. *Albizia schimperi* and *Olea capensis* harvested for timber, firewood, live-stock forage and providing shade for the coffee bushes. Several banana varieties and vegetable crops e.g. beans and sweet potatoes, dominate the lower strata (Hemp, 2006). Additionally, cattle are kept for milk, while goats and pigs are kept for meat for sale and/or for home consumption. Livestock are stall-fed with fodder from shrubs/trees, banana plants and grasses grown on the homestead (Fernandes et al., 1984). Clearance of savannah *Acacia* woodlands for maize cultivation (Grace et al., 2006) best exemplifies land use change at the lower elevations. Intensively cultivated maize fields are characterized by considerable disturbances through tillage, use of pesticides and fertilization.

Soils from savannah, maize fields, montane forest and Chagga homegardens occurring on the southern slopes of Mt. Kilimanjaro region were sampled from the 0–20 cm layer. Samples were obtained from two representative plots (50 m × 50 m) for each ecosystem. The experimental plots were of the same slope and approximately 30 km apart. It was impossible to obtain samples from ecosystems located at exactly the same elevation and precipitation. In each plot, samples were collected from four corners and at the centre, giving a total of five sampling positions (measuring 10 m × 10 m), per plot. To obtain composite samples per sampling position, four soil augers were taken. This led to a total of five samples per plot and 10 samples from the two experimental plots to represent each ecosystem. Simple randomization design was conducted on the five samples to select two samples from each plot. This led to a total of four samples out of the total 10 samples to be used in this study. This was aimed at reducing the probability that all the four samples selected came from one plot. Soil was passed through a 2.0 mm mesh screen and stored under field moist conditions at 5 °C before analysis.

### 2.3. Incubation and $CO_2$ analyses

Soil (15 g) was incubated in closed glass vessels (100 ml) for 70 days at 23 °C and 50% of water holding capacity (WHC). Vials with 3 ml of 1.0 M NaOH were placed in the air-tight incubation vessels to trap

**Table 1**  
Brief site description and soil characteristics of four different ecosystems under different land use intensities in Mt. Kilimanjaro.

Land use	Elevation (m a.s.l.)	Rainfall <sup>a</sup> (mm yr <sup>-1</sup> )	Temperature <sup>b</sup> (°C)	Bulk density	Total C	Total N	pH	Fertilizer use (organic/inorganic)
Savannah	951	770	21.0 ± 0.05	0.83 ± 0.05	27.29	1.60	6.71 ± 0.01	Nil
Maize fields	920	775	20.5 ± 0.03	1.21 ± 0.03	12.56	1.03	6.61 ± 0.01	Yes
Lower montane forest	1623	1800	15.5 ± 0.09	0.34 ± 0.09	160.59	10.57	6.09 ± 0.07	Nil
Homegarden	1648	1200	19.0 ± 0.04	0.77 ± 0.04	34.43	3.12	6.68 ± 0.03	Yes

Bulk density (in g cm<sup>-3</sup>), total C and N (in mg g<sup>-1</sup> soil), pH (H<sub>2</sub>O), estimates of inorganic fertilizer use for N and P at Mt. Kilimanjaro region (ca. 40 kg N ha<sup>-1</sup>, ca. 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) (Giller et al., 1998).

<sup>a</sup> Röhr and Killingtveit (2003) as cited in Pabst et al., 2013.

<sup>b</sup> Hemp (2006) as cited in Pabst et al., 2013.

CO<sub>2</sub>. Periodically, vials containing the trapped CO<sub>2</sub> were removed and replaced with new ones containing a fresh aliquot of NaOH. Four control vessels containing only the vials with NaOH (without soil) accounted for the very small amount of CO<sub>2</sub> from air enclosed in the vessels.

The quantity of the absorbed CO<sub>2</sub> trapped in NaOH was measured by titrating the remaining NaOH solution with 0.1 M hydrochloric acid (HCl) against phenolphthalein indicator at the presence of excess 0.5 M barium chloride (BaCl<sub>2</sub>).

#### 2.4. Measurements of microbial biomass and water extractable organic carbon

Soil microbial biomass carbon (MBC) was estimated by the chloroform Fumigation-Extraction (FE) method (Vance et al., 1987). Ethanol-free chloroform was used to fumigate 5 g of moist soil for 24 h in a desiccator at room temperature. Soluble C from fumigated and non-fumigated soil samples was extracted with 30 ml of 0.05 M K<sub>2</sub>SO<sub>4</sub> via shaking on an orbital shaker (60 min, 120 rotations min<sup>-1</sup>) and filtered. Extracts were analyzed for total organic C using the 'multi N/C 2100' (Analytik Jena, Jena). C content in K<sub>2</sub>SO<sub>4</sub> extracts from unfumigated soil samples was accepted as water extractable organic C (WOC) (Blagodatskaya et al., 2009). Since not all the microbial C was extracted by K<sub>2</sub>SO<sub>4</sub>, a *k*<sub>EC</sub> factor of 0.45 (Joergensen, 1996) was used to convert microbial C flush (difference between extractable C from fumigated and non-fumigated samples) into MBC.

#### 2.5. Analyses of enzyme activity

Enzyme activities were assayed using artificial fluorogenic substrates based on 4-methylumbelliferone (MUF): 4-MUF-β-D-glucopyranoside for β-glucosidase, 4-MUF-β-D-cellobioside for cellobiohydrolase, 6,8-difluoro-4-methylumbiliferyl phosphate for phosphatase and 4-MUF-N-acetyl-β-D-glucosaminide for chitinase.

0.5 g of fresh soil was extracted with 50 ml water using low-energy sonication for 2 min. 50 μl of soil suspension was added to 150 μl of each substrate solution and 50 μl universal buffer in a 96-well microplate. Fluorescence was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm using Victor3 1420-050 Multi Label Counter. Enzymes were assayed in each soil sample at 20 °C during 2 and expressed as MUF release in nmol per g dry soil per hour (nmol g<sup>-1</sup> h<sup>-1</sup>).

#### 2.6. Microbial parameters as indicators of soil quality

We used the following soil microbial parameters:

$C_{mic}:C_{org}$  = mg of biomass C per mg total organic C (Anderson and Domsch, 1989) ratio has been used as an indicator of future changes in OM status that will occur in response to alterations in land use (Nsabimana et al., 2004).

Soil microbial respiration (*R*<sub>mic</sub>) and soil microbial biomass (*C*<sub>mic</sub>) was used to calculate the metabolic quotient (*q*CO<sub>2</sub>), the amount of CO<sub>2</sub>-C produced per unit of microbial biomass carbon

*q*CO<sub>2</sub> = mg CO<sub>2</sub> per mg biomass C (Anderson and Domsch, 1986). *q*CO<sub>2</sub> (community respiration per biomass unit) has been widely

and is based on Odum's ecological succession theory (Moscatelli et al., 2005).

$qM = \text{mg C-CO}_{2\text{cumulative}} \text{ mg C g}^{-1} \text{ soil h}^{-1}$  (Pinzari et al., 1999) at the end of the incubation period (70 days) i.e. the fraction of total organic C mineralized throughout the incubation (Moscatelli et al., 2005).

Sensitivity index (SI) related to land use conversions i.e. savannah to maize field and montane forest to Chagga homegardens, for the soil microbial parameters was calculated using Eq. (1) below modified from Liang et al. (2012):

$$SI = (MI_A - MI_N) / MI_N \cdot 100; \quad (1)$$

where *MI*<sub>N</sub> = Microbiological indicator for a natural ecosystem; *MI*<sub>A</sub> = Microbiological indicator for an agroecosystem.

#### 2.7. Statistical analysis

Microbial biomass carbon (MBC), water extractable organic carbon (WOC), *q*CO<sub>2</sub>, *q*M, *C*<sub>mic</sub>:*C*<sub>org</sub> ratio and enzymatic activity were analyzed using one-way ANOVA to test for significant differences between land uses. Duncan's *post hoc* test was used to separate the means at *P* < 0.05. Results of microbial parameters, enzyme activities and soil physicochemical properties were also analyzed by principal component analysis (PCA) and the resulting PCA scores analyzed by ANOVA.

### 3. Results

#### 3.1. Principal component analysis

Three principal components (PCs) were extracted from the microbiological parameters, enzyme activities and physicochemical properties of soils (Table 2). Highest loadings of the 1st PC (68.1% of total variance) consisted of variables that characterize the microbiological activity of soils as related to the C-cycle (microbial biomass C, water extractable organic C, β-glucosidase, cellobiohydrolase) and variables indicative of

**Table 2**  
Principal components (PC) and component loadings extracted from soil microbial parameters, enzyme activities and soil physicochemical properties; underlined component loadings were used to interpret the PC.

Variables	Principal components (PCs)		
	PC1	PC2	PC3
Microbial biomass carbon	0.9436	0.2751	0.1843
Water extractable organic carbon	<u>0.9202</u>	0.2999	-0.2516
Bulk density	-0.9726	-0.0874	-0.2154
Total carbon	0.9772	-0.0803	-0.1966
Total Nitrogen	<u>0.9774</u>	0.0187	-0.2102
Soil pH	-0.8880	0.1556	0.4328
β-glucosidase	<u>0.7048</u>	-0.6966	-0.1345
Cellobiohydrolase	<u>0.9954</u>	-0.0932	0.0201
Chitinase	0.4002	-0.4633	0.7907
Phosphatase	-0.0243	0.9889	-0.1467
Mineralization quotient ( <i>q</i> M)	-0.8699	-0.2193	-0.4417
Metabolic quotient ( <i>q</i> CO <sub>2</sub> )	-0.2878	-0.9260	-0.2640
<i>C</i> <sub>mic</sub> : <i>C</i> <sub>org</sub> ratio	-0.9935	0.0853	0.0760

SOM-related nutrient supply (total C and total N). Therefore, the 1st PC was interpreted as “SOM-related nutrient status” (Fig. 1). The 2nd PC (21.5% of total variance) was described by phosphatase, having the highest loadings on the PC. The collective term attributed to the 2nd PC was “phosphorus effect” (Fig. 1). The 3rd PC (10.4% of total variation) was characterized by chitinase and so, reflects the N cycle.

### 3.2. Microbial biomass and water extractable carbon

Conversion of natural ecosystems (savannah and montane forest) to agroecosystems (maize fields and *Chagga* homegardens) significantly ( $P < 0.05$ ) decreased microbial biomass C content. MBC and WOC content were highest in montane forest soils with  $1.15 \pm 0.10 \text{ mg C g}^{-1}$  and  $1.50 \pm 0.12 \text{ mg C g}^{-1}$ , respectively. Lowest MBC and WOC values were observed in soils under maize fields and savannah with  $0.39 \pm 0.05 \text{ mg C g}^{-1}$  and  $0.26 \pm 0.04 \text{ mg C g}^{-1}$ , respectively. The MBC/WOC ratio varied from  $0.78 \pm 0.08$  in lower montane forest to  $2.69 \pm 0.30$  under savannah soils (Fig. 2).

### 3.3. Enzyme activities

Conversion of natural ecosystems to agricultural lands lowered  $\beta$ -glucosidase and cellobiohydrolase activities.  $\beta$ -glucosidase and cellobiohydrolase activities were two times higher in savannah soils compared to maize fields. Highest activities of  $\beta$ -glucosidase ( $209.6 \pm 15.9 \text{ nmol g}^{-1} \text{ h}^{-1}$ ) and cellobiohydrolase ( $80.6 \pm 4.9 \text{ nmol g}^{-1} \text{ h}^{-1}$ ) were observed in lower montane forest soils (Fig. 3).

Similarly, chitinase activity was significantly higher in natural ecosystems compared to croplands located at the same elevation. Chitinase activity in lower montane forest soils ( $87.3 \pm 6.4 \text{ nmol g}^{-1} \text{ h}^{-1}$ ) was three times higher than in *Chagga* homegardens ( $27.5 \pm 4.1 \text{ nmol g}^{-1} \text{ h}^{-1}$ ). Conversely, agricultural practices increased phosphatase activity in arable soils compared to soils under natural vegetation located at the same elevation. Highest phosphatase activity was recorded in *Chagga*

homegarden soils ( $771 \pm 18 \text{ nmol g}^{-1} \text{ h}^{-1}$ ) while the lowest activity was observed in savannah  $258 \pm 15$ ) (Fig. 3).

### 3.4. Mineralization of soil organic matter

Conversion of natural ecosystems to crop lands lowered  $\text{CO}_2$  produced. This can be attributed to higher soil C content in natural ecosystems compared to cultivated soils. At the end of the incubation period, cumulative  $\text{CO}_2$  was highest in soils from lower montane forest ( $1.54 \pm 0.03 \text{ mg C g}^{-1}$ ) and lowest in soils from maize fields ( $0.62 \pm 0.01 \text{ mg C g}^{-1}$ ) (Fig. 4).

### 3.5. Microbiological indicators

Converting natural ecosystems to croplands increased the  $C_{\text{mic}}:C_{\text{org}}$  ratio.  $C_{\text{mic}}:C_{\text{org}}$  ratio in *Chagga* homegarden soils ( $2.6 \pm 0.3\%$ ) was three times higher than for lower montane forest ( $0.7 \pm 0.1\%$ ). Highest  $C_{\text{mic}}:C_{\text{org}}$  ratio was observed in soils under maize cultivation ( $3.12 \pm 0.46\%$ ). The sensitivity index (SI) for the  $C_{\text{mic}}:C_{\text{org}}$  ratio was high in ecosystems located at higher elevation (255%) compared to ecosystems located at lower elevation (20.5%) (Fig. 5).

Highest and lowest mineralization quotients ( $qM$ ) were observed in soils under maize fields ( $2.92 \cdot 10^{-5} \text{ mg C-CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) and lower montane forest soils ( $0.57 \cdot 10^{-5} \text{ mg C-CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ), respectively (Fig. 5). The SI for  $qM$  was higher (111%) in lower montane forest-*Chagga* homegarden land-use change compared to savannah-maize field land-use change (97%) (Fig. 6). The mineralization quotient ( $qM$ ) was the most sensitive indicator of land-use change from natural ecosystems to agroecosystems (Fig. 6).

Metabolic quotient ( $q\text{CO}_2$ ) decreased with increased elevation. Land use systems at a lower elevation (savannah and maize fields) had higher  $q\text{CO}_2$  compared to those at a higher elevation (lower montane forest and homegarden). Highest and lowest  $q\text{CO}_2$  values were in maize field and homegarden soils, with  $5.6 \cdot 10^{-4} \text{ mg CO}_2\text{-C g}^{-1} C_{\text{mic}} \text{ h}^{-1}$  and  $3.2 \cdot 10^{-4} \text{ mg CO}_2\text{-C g}^{-1} C_{\text{mic}} \text{ h}^{-1}$ , respectively (Fig. 5). The SI for

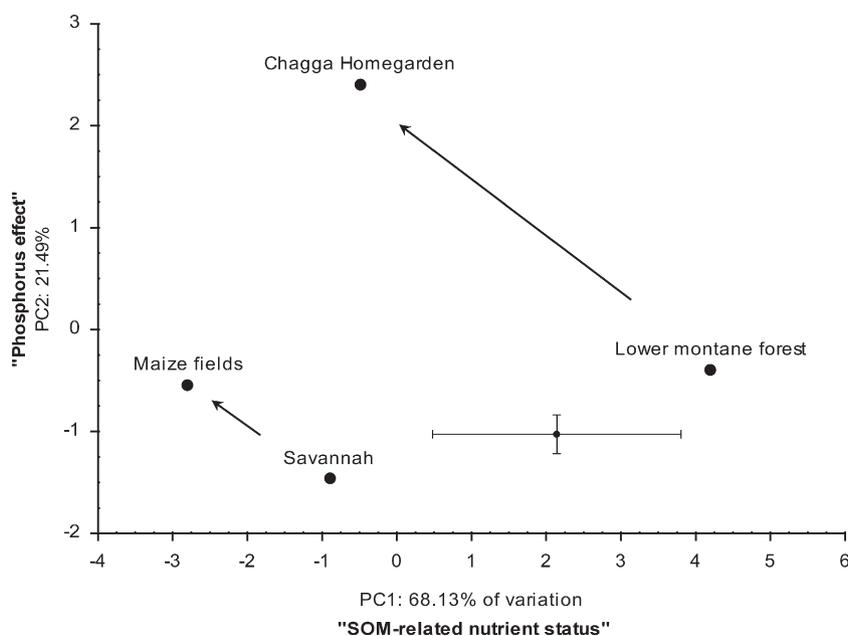
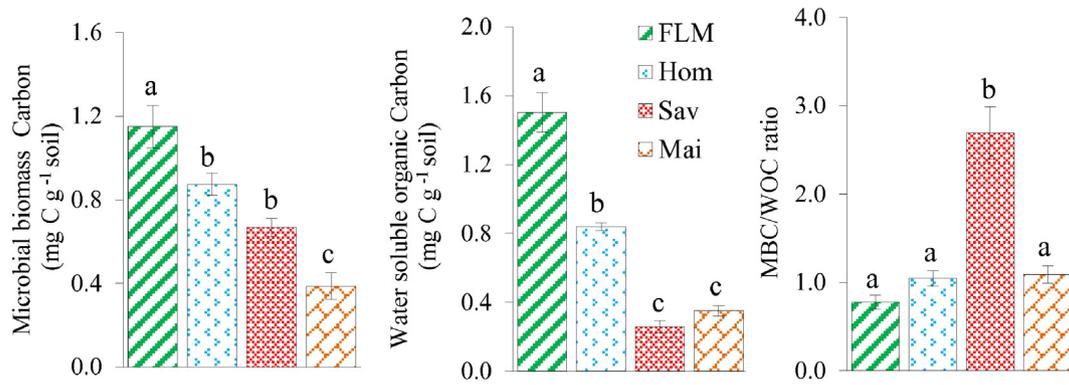


Fig. 1. Principal component plot of microbial parameters of soil quality depending on land use types at Mt. Kilimanjaro region. Arrows show direction of land use change. Bars represent least significant difference ( $P < 0.05$ ),  $n = 4$ .



**Fig. 2.** Microbial biomass C, water soluble organic C and MBC/WOC ratio for top soil layer (0–20 cm) at the end of the incubation period (70 days) depending on land use: FLM-Lower Montane Forest, Sav-Savannah, Hom-*Chagga* Homegarden and Mai-Maize fields. Error bars represent the standard error of means. Land uses followed by same letters are not significantly different at  $P = 0.05$ .

$q\text{CO}_2$  in lower montane forest-*Chagga* homegardens land-use change was  $-30\%$  compared to  $6\%$  for savannah-maize fields land-use change.

## 4. Discussion

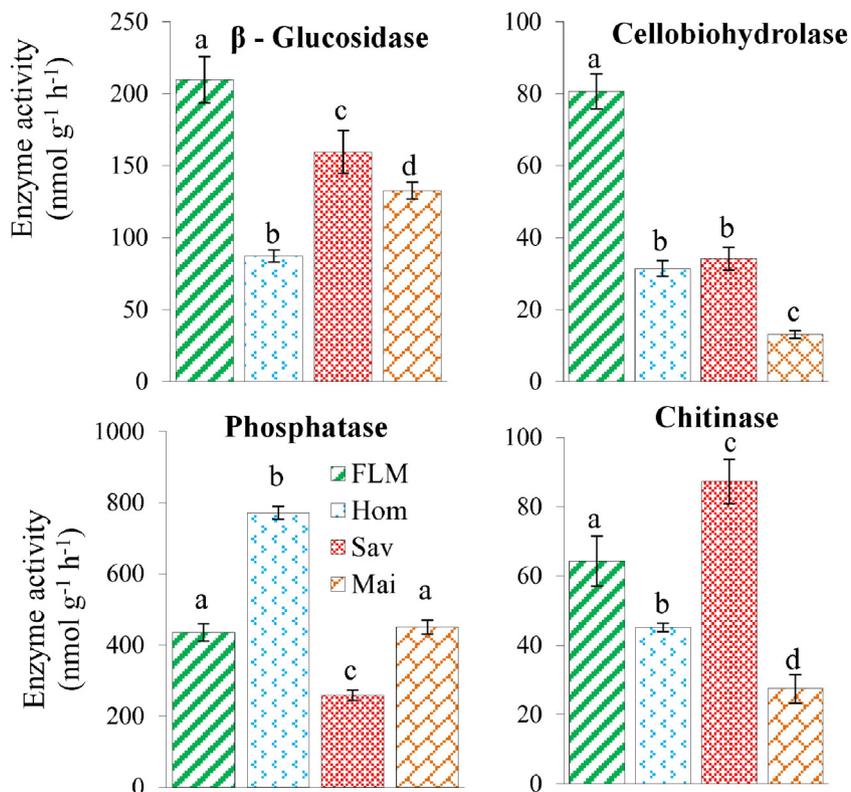
### 4.1. Principal component analysis

The XY-ordination (Fig. 1) of the factor scores of the first two PCs (89.1% of the total variance) clearly reflected differences between land uses. Based on the PCA, 'SOM-related nutrient status' was the major factor (68.1%) explaining the overall variance. Highest loadings with the 'SOM-related nutrient status' PC were provided by MBC, WOC, total C and N,  $\beta$ -glucosidase and cellobiohydrolase. This shows that the 'SOM-related nutrient status' PC and its associated microbial parameters can be used as indicators to assess soil quality. The 'SOM-related nutrient status' integrates soil chemical and biological properties and is sensitive

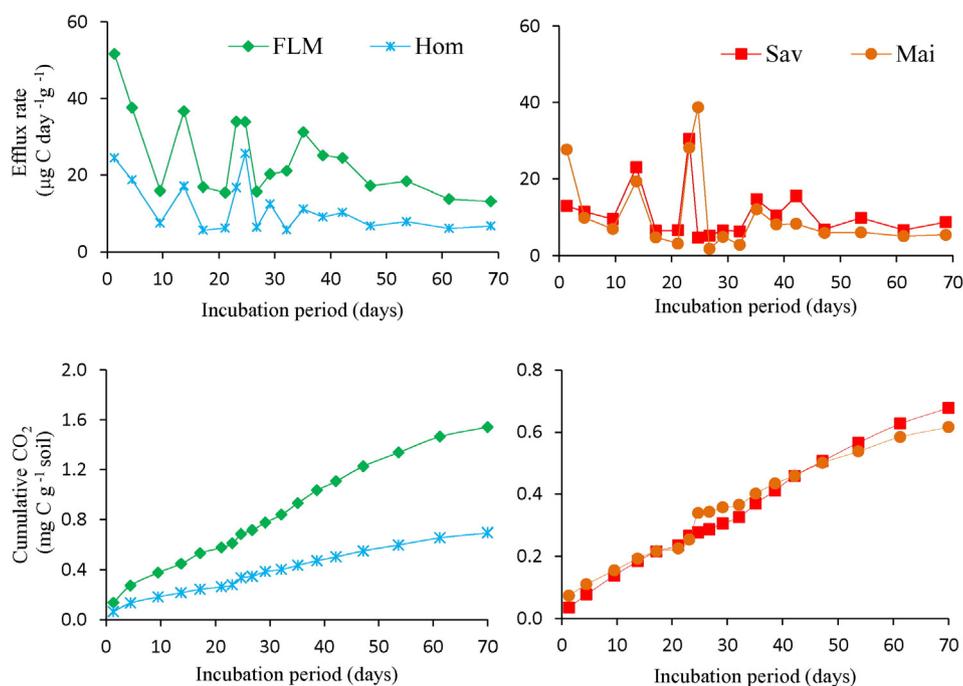
to changes in land use. Poor correlation of phosphatase with the 1st PC in our study suggests that P is limiting. Phosphorus deficiency is a very common phenomenon in the East African highlands, especially in volcanic ash soils similar to those found at Mt. Kilimanjaro region. Higher scores of the 'phosphatase effect' in agricultural compared to natural systems are linked to the effects of P fertilization in arable land. Use of organic manures as the primary source of crop nutrients is common practice in many African farming systems (Drechsel and Reck, 1998).

### 4.2. Microbial biomass carbon

Perennial natural vegetation contributes to a continuous and higher C input to soil through root exudation compared to annual crops e.g. maize (Kuzuyakov and Domanski, 2000). Root exudates stimulate microbial growth, consequently increasing the microbial biomass (Sanaullah et al., 2011; Blagodatskaya et al., 2009). Mycorrhizal fungi, a major



**Fig. 3.** Enzyme activities depending on land use. FLM-Lower Montane Forest, Sav-Savannah; Hom-*Chagga* Homegarden and Mai-Maize fields. Error bars represent the standard error of means. Land uses followed by same letters are not significantly different at  $P = 0.05$ .



**Fig. 4.** CO<sub>2</sub> efflux rates and cumulative CO<sub>2</sub> production during the incubation period from soils depending on land use. FLM-Lower Montane Forest, Sav-Savannah; Hom-Chagga Homegarden and Mai-Maize fields.

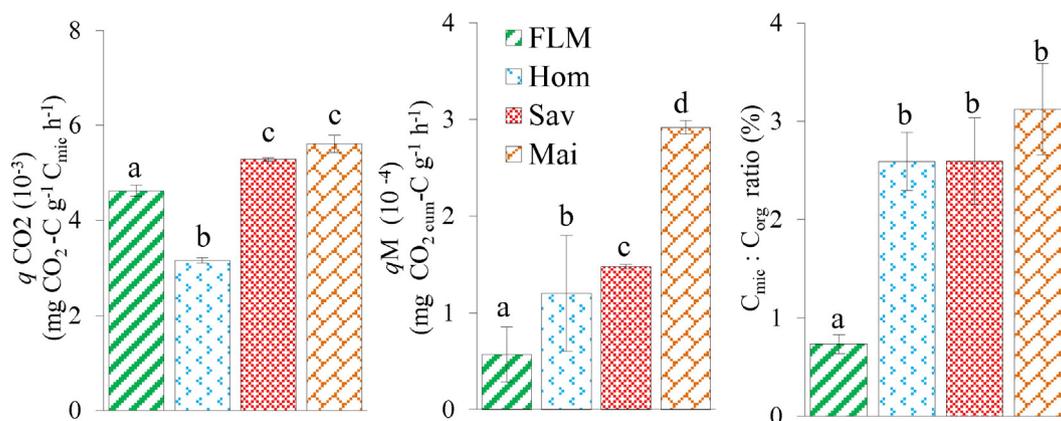
fraction of the soil microbial biomass (SMB) (~25%) are obligate biotrophs and need living roots for their growth (Spedding et al., 2004). Continuous supply and accumulation of organic C substrates in natural systems create a micro-environment suitable for microorganisms to better thrive, compared to arable lands.

Decline in microbial biomass after conversion of natural systems to arable land is also linked to tillage practices. Regular tillage in croplands changes the soil physicochemical environment, which affects microbial biomass (Borie et al., 2006). Moreover, soil compaction in agricultural soils leads to a strong surface sealing with few macropores. The strong surface sealing layer promotes the development of a rhizosphere with a poor rooting system, thus lowering the microbial biomass. Previous studies conducted in the Mt. Kilimanjaro region (e.g. Pabst et al., 2013; Mganga and Kuzyakov, 2014) have also reported lower microbial biomass in agricultural fields compared to natural systems. Similarly, Lai et al. (2014) also reported a decline in microbial biomass content with increased level of anthropogenic pressure, e.g., tillage, in different microenvironments.

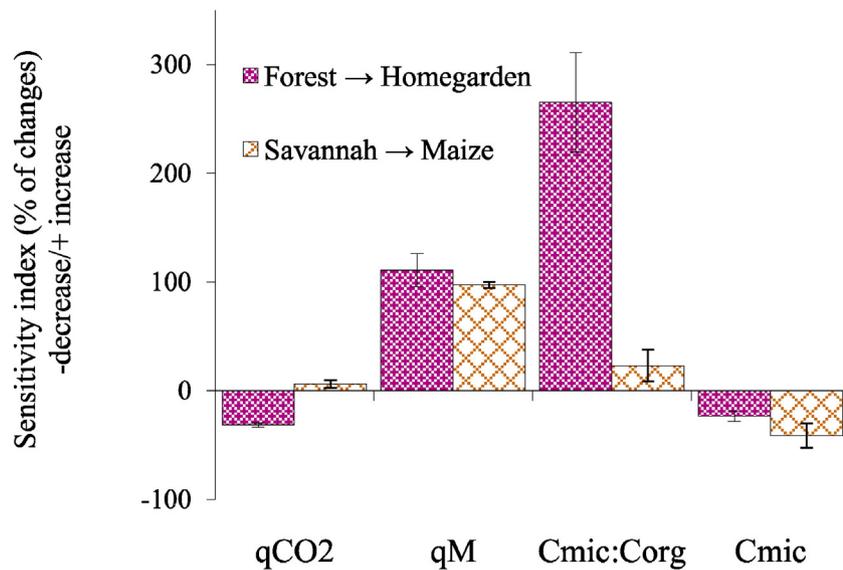
Lower microbial biomass content in savannah and maize fields (lower elevation) compared lower montane forest and Chagga homegardens (higher elevation) is attributed to differences in soil temperature and climate. Ecosystems with high soil temperatures e.g. tropical savannah have less potential to maintain high levels of OM to support soil microorganisms (Balota et al., 2003).

#### 4.3. Enzyme activities

Higher  $\beta$ -glucosidase and cellobiohydrolase activities in natural systems compared to agroecosystems located at the same elevation are linked to the positive impact of SOM, soil cover and minimal soil disturbance on these enzymes. Principal component loadings showed a positive relationship between the activities of  $\beta$ -glucosidase and cellobiohydrolase and total C, total N, water extractable organic C and microbial biomass C. The positive correlation between  $\beta$ -glucosidase and microbial biomass C and total C is logical because  $\beta$ -glucosidase is synthesized by soil microorganisms in response to the presence of suitable substrates (Turner et al.,



**Fig. 5.** Microbial parameters: metabolic quotient ( $q\text{CO}_2$ ), mineralization quotient ( $q\text{M}$ ) and  $\text{C}_{\text{mic}}:\text{C}_{\text{org}}$  ratio (%) depending on land use. FLM-Lower Montane Forest, Sav-Savannah, Hom-Chagga Homegarden and Mai-Maize field. Error bars represent the standard error of means. Land uses followed by same letters are not significantly different at  $P = 0.05$ .



**Fig. 6.** Sensitivity indices of eco-physiological indicators: metabolic quotient ( $qCO_2$ ), mineralization quotient ( $qM$ ),  $C_{mic}:C_{org}$  ratio and microbial biomass C (MBC) depending on land use change from natural to agroecosystems. FLM-Lower Montane Forest – Hom-*Chagga* Homegarden and Sav-Savannah – Mai-Maize field. Error bars represent the standard error of means.

2002). Positive correlation between activities of enzymes involved in the C-cycle and soil organic C content has also been reported in previous studies (e.g. Lai et al., 2014). Activities of  $\beta$ -glucosidase and cellobiohydrolase increase with OM content, which is why they are commonly used as sensitive biological indicators of soil quality (Badiane et al., 2001).

Chitinase activity in natural systems was significantly higher than in cultivated lands. High microbial biomass in natural ecosystems contributed to greater chitinase activity. Chitin is a common structural compound found in the cell wall of fungi (actinomycetes and basidiomycetes) (Badiane et al., 2001). Basidiomycetes are most abundant in forest systems (Sinsabaugh et al., 2008). Regular tillage practices in croplands causes changes in the soil physicochemical environment, which affects microbial biomass (Mganga and Kuzyakov, 2014; Borie et al., 2006), contributing to low chitinase activities in cultivated soils.

Fertilization with animal manure in agricultural fields to compensate for nutrient losses promoted microbial activities and P cycling in maize fields and *Chagga* homegardens. Increased phosphatase activity in croplands after addition of animal manure has been reported in previous studies (e.g. Parham et al., 2002). Croplands in the tropics that receive substantial animal manure have maintained similar or even higher enzymatic activities compared to natural ecosystems (Waldrop et al., 2000). High phosphatase activity in soils occurring in the Mt. Kilimanjaro region is attributed to the parent material, volcanic ash. Phosphatases bind onto SOM and clay surfaces which are abundant and active in volcanic ash soils (Olander and Vitousek, 2000).

#### 4.4. Soil organic matter mineralization and $CO_2$ production

Carbon-dioxide emissions from SOM mineralization varied depending on land use and elevation. Higher  $CO_2$  emissions were produced from soils under natural vegetation compared to cultivated fields. This is attributed to higher organic C content due to accumulation of SOM and minimal soil disturbance in natural systems. Minimal soil disturbance in natural ecosystems provide a steady state of organic C to support a higher microbial biomass compared to cultivated fields. Tillage in agricultural soils promote soil mixing, aeration of residues and destabilization of soil structure in the surface soil layer (Borie et al., 2006). Destruction of the physical protection exposes the soil to agents of erosion which decrease the available SOM.

Accumulation of SOM contributed to higher  $CO_2$  production in ecosystems located at higher elevation (montane forest and *Chagga* homegardens) than those located at lower elevation (savannah and

maize fields). Low temperatures and higher moisture content in ecosystems at a higher elevation reduced decomposition rates leading to the accumulation of SOM. Decreased soil temperature generally results in decreased rates of litter decay and SOM biodegradation (Griffiths et al., 2009).

#### 4.5. Microbiological indicators

The mineralization quotient ( $qM$ ) was the most sensitive microbiological indicator of soil quality. Lower values of  $qM$  in natural ecosystems than in arable soils are associated with lower ratios of easily mineralizable OM to stable OM in soils under natural vegetation.  $qM$  in natural ecosystems was lower because the labile SOM pool was used to synthesize stable humic fractions, which are more resistant to microbial decomposition. High  $C_{mic}:C_{org}$  and low  $qCO_2$  in *Chagga* homegarden soils reflect high stability (Pinzari et al., 1999) and demonstrate a more efficient use of organic substrates by the microbial biomass. Higher sensitivity of the microbial parameters ( $qM$ ,  $qCO_2$  and  $C_{mic}:C_{org}$  ratio) to the conversion of lower montane forest to *Chagga* homegardens compared to savannah to maize fields demonstrates that tropical forests are more sensitive to land use conversions compared to savannah ecosystems. Conversion of tropical forest soils to permanent agriculture decreases soil C by about 40%, whereas conversion to pasture reduces the C content by 20% (Detwiler and Hall, 1988).

### 5. Conclusions

Soil enzymes distinguished between land-use types and are therefore useful for monitoring changes in soil quality. Cellobiohydrolase was the most sensitive enzyme for land-use effects. Mineralization quotient was the most sensitive microbiological indicator of land-use change. High microbial biomass C content and low mineralization quotient and metabolic quotients observed in the *Chagga* homegardens clearly demonstrate that the microbial biomass in this traditional agroforestry system have high substrate use efficiency. Based on these microbial parameters, we conclude that traditional agroforestry systems promote soil quality and are a suitable crop production system option in the tropics.

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