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Microbial and enzymes response to nutrient additions in soils of Mt. Kilimanjaro region depending on land use

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ABSTRACT

Microbial and enzyme activities can be used to identify and assess the impacts of changes in land use management on soil quality. However, only few studies have investigated the effects of land use and nutrient additions on enzyme activities and microbial processes in tropical African soils. Glucose and nutrients (N and P) were added to soils (0–20 cm) from natural and agricultural ecosystems: (1) savannah, (2) maize fields, (3) lower montane forest, (4) coffee plantation, (5) grasslands (6) *Chagga* homegardens common at Mt. Kilimanjaro region and East Africa. Microbial biomass and activities of β-glucosidase, cellobiohydrolase, chitinase and phosphatase were monitored over 60 days incubation period. Microbial biomass content and enzyme activities were generally higher in soils under natural vegetation compared to corresponding agricultural soils. Decline in microbial biomass C content over time was higher in natural ecosystems compared to agricultural soils. However, the microbial biomass C content in *Chagga* homegarden soils was relatively stable. Land use was negatively correlated to β-glucosidase, cellobiohydrolase and chitinase activity, but positively correlated to phosphatase activity. β-glucosidase and cellobiohydrolase, involved in the C-cycle, were the most sensitive to landuse change. Chitinase activity was 2–6 times higher in soils under natural vegetation compared to corresponding arable soils. Phosphatase displayed very high activities in all land use types. This is attributed to the high P retention capacity common for andic soils similar to those occurring at Mt. Kilimanjaro region. Increased P availability stimulated enzyme activities in lower montane forest and *Chagga* homegarden soils. Overall, microbial biomass and enzyme activities showed a strong decrease with increased land use intensity and should therefore be taken into consideration in monitoring and assessing the impact of land use change at Mt. Kilimanjaro region.

1. Introduction

Land-cover change stemming from anthropogenic land uses represents a major source and component of global environmental change. During the 1980–2000 period, half of the new arable land in the tropics came at the expense of intact forests and another 28% came from disturbed forests [28]. In Africa, conversion of forests to permanent agriculture accounted for 16% of the change in forest area during the same period [24]. Savannah ecosystems in Africa are also under immense pressure and are being brought to intensive agricultural use [21]. Conversion of these natural ecosystems to agroecosystems is primarily driven by the need to feed the increasing human population and livestock. Currently, land use in East Africa is characterised by more agricultural land than natural ecosystems [32]. The population density in Africa in the next 30 years is projected to rise from 26 to 60 people km^{-2} and therefore further anthropogenic pressure will affect the natural environment [15].

Global effects of such land-use changes include the decreasing capacity of land for sustainable crop production due to loss of soil fertility. Microbial and biochemical characteristics of soil have been proposed as indicators of soil quality both in natural and agricultural systems. This is mainly attributed to their central role in cycling of C, N and other nutrients as well as sensitivity to change

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[37]. Soil enzyme activity has been suggested as an index of soil productivity and microbial activity and has been used as an accurate fertility index [6]. For example, β-glucosidase activity is the most predominant glycosidase in soil involved in the last limiting step of cellulose degradation [1]. Biodegradation of cellulose from e.g. plant litter is among the primary mechanisms transforming and recycling nutrients into forms available for plant uptake. Extracellular enzymes e.g. β-glucosidase, cellobiohydrolase, phosphatase and chitinase are important because they catalyze the rate-limited steps of decomposition and nutrient cycling [10,27].

Ecosystem processes in many tropical soils are nonetheless still constrained by low nutrient availability. In volcanic ash soils like those occurring at Mt. Kilimanjaro region, microbial biodegradation of soil organic matter (SOM) is limited by its complexation with Fe, Al and allophanes [34], as well as reduced bacterial activity and poor availability of P [12]. Poor P availability is attributed to the large P-fixing capacity of Andosols. Low soil nutrient availability limits net primary production [14]. Consequently, the use of inorganic fertilizers has widely been used to enhance soil fertility and improve crop yields. However, a large proportion of added P is adsorbed onto the surfaces of Al and Fe oxides becoming less available for plant uptake by further fixation especially in Andosols containing allophane, imogolite and ferrihydrite [46]. In addition to improving soil fertility and crop yields, fertilization significantly affects soil biochemical and biological properties [29]. Subsequently, the influence of external nutrient inputs on soil microbial ecology has been frequently emphasized [4]. However, few studies have been conducted to investigate the soil microbial dynamics and response of enzyme activities to nutrient additions in volcanic ash soils depending on land use systems especially in tropical Africa.

Here we use an incubation experiment to evaluate soil microbial biomass and enzyme activities depending on most common land use systems at Mt. Kilimanjaro region and their response to nutrient (N and P) additions. We used flourometric MUF-linked substrates [18,33] to measure soil extracellular enzyme activities (β-glucosidase, cellobiohydrolase, chitinase and phosphatase). This method has become popular in soil studies because it is very sensitive and universal. Furthermore, the use of the microplate format allows for the simultaneous activity determination of various enzymes using a small amount of soil [36] and high-throughput analysis [16]. β-glucosidase, cellobiohydrolase, phosphatase and chitinase activities were generally higher in natural ecosystems compared to corresponding intensively cultivated croplands. β-glucosidase, cellobiohydrolase and chitinase were the most sensitive to changes in land use. Chloroform fumigation-extraction method [48] was used to estimate soil microbial biomass C and water extractable organic C.

2. Materials and methods

2.1. Study area

Mt. Kilimanjaro is an ancient volcano located 300 km south of the equator in Tanzania on the border with Kenya between 2° 45′, and 3° 25′, South and 37° 0′, and 37° 43′, East, rising from the savanna plains at 700 m to the ice-capped summit of 5895 m altitude [23]. The climate at Mt. Kilimanjaro is influenced by the Intertropical Convergence Zone (ITCZ) with two rainy seasons, a weak one in November/December and a strong one in April/May [52]. Annual precipitation is strongly influenced by elevation and differs between 700 and 2000 mm annually [40]. Annual rainfall totals of more than 3500 mm have however been reported at the most humid southern slopes at 2200 m altitude [42]. Mean annual temperatures up to elevation of 3400 m vary between 10 and 21 °C [40]. Climatic factors and topography greatly affect the vegetation zonation [22]. Soils are classified as various Andosols [53] developed on layered volcanic ashes that overlay phonolites and trachytes [41].

2.2. Brief history of the study sites

Mt. Kilimanjaro region offers outstanding potential to investigate the effects of land-use change on microbiological and biochemical characteristics of African tropical soils along an elevation, vegetational and climatic gradient [22,40,42]. A brief description of the chemical and physical analysis of the study sites are given in Table 1. Naturally occurring ecosystems at Mt. Kilimanjaro region e.g. lower montane forests have been converted to coffee plantations and grasslands, while savannah ecosystems have been converted to maize plantations. Additionally, these natural ecosystems have been altered through fires, collection of building material and occasional mowing, due to high demand of building material, fuelwood and farmland. Arable soils are consequently modified by mechanisation, organic and inorganic fertilisation and intensive use of pesticides for higher crop yields and pest and disease control. The traditional *Chagga* homegardens developed through anthropogenic influence on lower montane forest have been described as a sustainable land use system model in the region and has evolved over five centuries [22]. This system has remained unchanged over the last decades compared to land uses in lower elevations [45].

2.3. Soil sampling and preparation

Soils from six land use systems at Mt. Kilimanjaro, i.e. savannah, lower montane forest, grassland, *Chagga* homegarden, maize fields and coffee plantations were sampled at the upper 0–20 cm layer using a soil auger (2 cm diameter × 60 cm depth). The sampled depth corresponds to Ah or Ap horizons. Two experimental plots of 50 m × 50 m approximately 30 km apart representing each ecosystem were sampled. Samples were taken in four corners and at the centre of each plot, giving a total of five positions per sampling. To obtain composite samples per position, four soil augers were taken per sampling position. This led to a total of 5 samples per plot and 10 samples (n) per ecosystem. Plant remains, debris and roots were removed using tweezers and the field-moist soil sieved through a 2.0 mm mesh screen and stored at 4 °C prior to analysis and incubation.

2.4. Treatments and incubation

There were five treatments: 1) soil alone, the control, 2) soil + glucose, 3) soil + glucose + N, 4) soil + glucose + P and 5) soil + glucose + (N + P). N in the form of KNO_3 (0.0645 mg g^{-1} soil) and P as KH_2PO_4 (0.025 mg g^{-1} soil) were added at the end of the pre-incubation period (10 days). Glucose (0.25 mg g^{-1} soil) was added 10 days after nutrients additions. Nutrients and glucose were added uniformly to soil (20 g) in 1 ml aqueous solution. Each treatment had six (6) replicates. Soil was incubated in 100 ml air-tight Schott bottles for 60 days and maintained at 50% water holding capacity (WHC) throughout the incubation period.

2.5. Microbial biomass and water extractable organic C

Microbial biomass C was estimated two times: (1) after 30 days and (2) 60 days, using the chloroform fumigation-extraction method [48]. Briefly, 4 g of fumigated and unfumigated soil samples were extracted with 0.05 M K_2SO_4 in 1:4 ratio. The 10-fold diluted K_2SO_4 solution (50 mM instead of 500 mM) allows the use of 'multi N/C 2100' (Analytik Jena, Jena) without dilution for C

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[a] (mm yr^{-1})	Temperature[b] (cC)	Land use type	Total C (mg C g^{-1} soil)	Total N (mg N g^{-1} soil)	Bulk density (g cm^{-3})	Cmic:Corg (%)	pH	$NO3^-$ (μg g^{-1})	$PO4^3$ (μg g^{-1})$^-$
Savannah	906	770	21.0	Natural	35.95 ± 1.04	2.16 ± 0.05	0.83 ± 0.05		5.35	–	0.15
Maize fields	886	775	20.5	Agricultural	23.87 ± 0.23	1.80 ± 0.02	1.21 ± 0.03		4.54	6.45	0.75
Lower montane forest	1623	1800	15.5	Natural	42.33 ± 0.91	3.81 ± 0.07	0.34 ± 0.09		4.32	46.55	0.35
Coffee plantation	1648	1250	19.0	Agricultural	47.98 ± 0.98	4.21 ± 0.08	1.02 ± 0.06		4.30	27.10	0.25
Grassland	1748	1650	16.5	Semi-natural	40.68 ± 0.29	3.45 ± 0.04	0.51 ± 0.01		4.57	12.85	0.25
Homegarden	1788	1200	19.0	Traditional	91.60 ± 0.27	7.23 ± 0.03	0.77 ± 0.04		5.75	30.05	0.20

[a] As cited in Ref. [40].
[b] [22] as cited in Ref. [40].

determination. C content in K_2SO_4 extracts from unfumigated soil samples was accepted as water extractable organic C [9]. Since not all the microbial C was extracted by K_2SO_4, a *k* factor of 0.45 was used to convert microbial C flush into MBC.

2.6. Enzyme assays

Enzyme activities were assayed using fluorogenically labelled substrates. Four types of artificial fluorogenic substrates based on 4-methylumbelliferone (MUF): (1) 4-MUF-β-D-glucopyranoside for β-glucosidase activity, (2) 4-MUF-β-D-cellobioside for cellobiohydrolase activity, (3) 6,8-difluoro-4-methylumbiliferyl phosphate for phosphatase activity and (4) 4-MUF-N-acetyl-β-D-glucosaminide for chitinase activity were used to assay enzyme activities in soil. All substrates and chemicals were purchased from Sigma (Germany).

The MUF-substrates were dissolved in 2-methoxyethanol. Saturation concentrations of fluorogenic substrates were determined in preliminary experiments. Half a gram of soil (dry weight equivalent) was extracted with 50 ml water using low-energy sonication (40 J s^{-1} output energy) for 60 s. 50 μl of soil suspension was added to 150 μl of each substrate solution (containing either 50 μl universal buffer) in a 96-well microplate (Puregrade, Germany).

Fluorescence was measured in microplates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, split width of 25 nm, with a Victor3 1420-050 Multi label Counter (PerkinElmer, USA). Each enzyme was assayed in each soil sample at 20 °C during 2 h. Calibration curves as well as the controls for the autofluorescence of the substrate were included in every series of enzyme measurements. Enzymes activities were measured two times; after 30 days (t1) and 60 days (t2) of the incubation period and expressed as MUF release in nmol per g dry soil per hour (nmol g^{-1} h^{-1}).

2.7. Statistical analysis

Estimated parameters of microbial biomass C content, water extractable organic C, enzyme activities were compared between landuse types and nutrient treatments using factorial ANOVA analysis (6 land use types and 5 treatments) to test for significant differences. Fischer's LSD *post hoc* test was used to separate significant differences between land use and treatments at $P < 0.05$ significance level. All displayed results represent arithmetic means of 4 replicates ± standard error (SE). Additionally, we conducted a multi-variate (PCA) of the enzyme activities and the resulting PCA scores analyzed by ANOVA.

3. Results

3.1. Microbial biomass C content depending on land use

Higher microbial biomass C content was observed in soils of natural ecosystems compared to that of agroecosystems (Table 2). Reduction in microbial biomass following conversion of natural ecosystems to croplands is attributed to reduced organic C inputs from above and below ground and the mechanical soil disturbance by ploughing.

Percent microbial C (C_{mic}) to soil organic C (C_{org}) ratio was highest (0.45%) in savannah. Higher C_{mic}:C_{org} ratio observed under savannah compared to soil under maize plantation (0.31%) can be linked to the differences in microbial biomass content. The general higher C_{mic}:C_{org} ratios in soils under natural vegetation compared to arable soils suggests that the labile C_{org} pool in natural systems is much larger and can therefore better support soil functions. This clearly demonstrates that C_{mic}:C_{org} ratio is a sensitive indicator of SOM changes.

C_{mic} represented 0.13–0.44% and 0.05–0.17% of total C in all landuse systems after 30 days and 60 days, respectively and decreased with incubation time. Decrease in microbial biomass C was generally greater in soils under natural vegetation compared to disturbed soils. Savannah soils with no amendments recorded the highest (39.23%) percent decrease in C_{mic} While the highest percent increase in C_{mic} (90.48%) was recorded in grassland soils N additions.

Table 2
Microbial biomass C after 30 days (t1) and 60 days (t2) as depending on land use, organic substrate and nutrient additions.

Land use	t1 (after 30 days)					t2 (after 60 days)				
	Ctrl	Glu	Glu + N	Glu + P	Glu+(N + P)	Ctrl	Glu	Glu + N	Glu + P	Glu+(N + P)
	μg g^{-1} soil					μg g^{-1} soil				
Savannah	$83.40^{A,a}$	$76.99^{A,ab}$	$68.73^{A,b}$	$72.32^{A,ab}$	$68.34^{A,b}$	$50.68^{A,c}$	$52.60^{A,c}$	$57.36^{A,bc}$	$50.52^{A,c}$	$60.71^{A,bc}$
Maize field	$17.07^{B,a}$	$22.83^{B,ab}$	$17.87^{B,ab}$	$20.37^{B,ab}$	$17.73^{B,a}$	$27.58^{B,bc}$	$26.94^{B,bc}$	$30.55^{B,bc}$	$30.02^{B,bc}$	$33.13^{B,c}$
Lower montane forest	$70.08^{C,ab}$	$70.37^{A,ab}$	$79.48^{C,a}$	$57.69^{C,bd}$	$54.71^{C,cd}$	$58.38^{A,bd}$	$56.68^{A,cb}$	$60.51^{A,bd}$	$59.14^{A,bd}$	$54.26^{AC,bd}$
Coffee plantation	$20.08^{A,a}$	$35.89^{C,bc}$	$29.51^{D,b}$	$34.71^{D,bc}$	$35.43^{D,bc}$	$39.74^{C,cd}$	$41.03^{C,cd}$	$43.69^{C,d}$	$37.55^{B,cd}$	$37.13^{B,cd}$
Grassland	$25.37^{A,a}$	$34.16^{C,b}$	$26.01^{BD,a}$	$33.49^{D,ab}$	$28.09^{D,a}$	$40.38^{C,bd}$	$45.39^{AC,de}$	$49.54^{C,e}$	$46.32^{CB,de}$	$45.68^{C,de}$
Homegarden	$54.55^{D,abc}$	$59.55^{D,abc}$	$53.57^{E,abc}$	$45.62^{E,a}$	$55.03^{C,abc}$	$56.51^{A,abc}$	$49.57^{A,ab}$	$65.67^{A,c}$	$47.51^{C,ab}$	$59.82^{A,bc}$

Means within each column with different capital letters indicate significant difference according to Fischer's LSD test at $P = 0.05$.
Means within each row with different small letters indicate significant difference according to Fischer's LSD test at $P = 0.05$.

3.2. Water extractable organic C depending on land use

Water extractable organic C (WOC) concentrations were generally higher in soils under natural vegetation compared to arable soils. This was attributed to the high microbial activity and high fungal abundance in soils under natural vegetation that enhanced SOM mineralization and promoted high water-soluble OM concentrations. After 30 days, WOC was highest in grassland soils with concentration of 133.92 ± 2.93 μg g^{-1} soil and lowest in soils under maize cultivation with 26.01 ± 2.33 μg g^{-1} soil.

There was a general decline in WOC concentration with incubation time. At the end of the incubation period, highest and lowest WOC concentrations were observed in grassland soils with glucose addition (112.89 ± 0.07 μg g^{-1} soil) and maize fields with P and N additions (3.27 ± 1.17 μg g^{-1} soil), respectively. Decrease in WOC content with time reflects increase in utilization of the available labile OM pool relative to supply.

3.3. Enzyme activities

3.3.1. β-glucosidase and cellobiohydrolase activity depending on land use and nutrient input

β-glucosidase activity was higher in soils under natural vegetation compared to arable soils (Table 3). Accumulation of low molecular weight and easily available organics from litter and other OM sources e.g. root exudates and necromass in soils under natural vegetation increased β-glucosidase activity. Increased P availability increased or decreased β-glucosidase activity, depending on land use. Addition of P to lower montane forest and grassland soils yielded the highest (73 ± 13.0 nmol g^{-1} h^{-1}) and lowest (19 ± 0.96 nmol g^{-1} h^{-1}) β-glucosidase activities respectively. Lower montane forest soils displayed the highest β-glucosidase activities (146 ± 9.4 nmol g^{-1} h^{-1}) and were the most responsive to P additions. In contrast, addition of inorganic N and P in soils under maize plantation decreased β-glucosidase activity (32 ± 1.06 nmol g^{-1} h^{-1}). The decrease in β-glucosidase activity with time is an indication of the gradual depletion of available organic C pool.

Similar to β-glucosidase, cellobiohydrolase activity was positively correlated to chitinase activity and negatively correlated to land-use. In contrast to β-glucosidase activity, high cellobiohydrolase activities were observed in savannah soils at day 30 after incubation starts (Table 3). Savannah soils with P amendments displayed the highest cellobiohydrolase activity (141 ± 10.87 nmol g^{-1} h^{-1}). In contrast, P additions to soils under maize plantation located at the same elevation with savannah ecosystems displayed the least cellobiohydrolase activity (14 ± 2.38 nmol g^{-1} h^{-1}). N and P additions strongly reduced cellobiohydrolase activity in soils under maize cultivation. These results display a negative feedback where cellobiohydrolase activity is inhibited by nutrient availability.

Similar to β-glucosidase, lower cellobiohydrolase activities were observed at the end of the incubation period compared to t1. Lower montane forest soils (65 ± 5.68 nmol g^{-1} h^{-1}) and maize field (14 ± 2.38 nmol g^{-1} h^{-1}) both with P additions displayed the highest and lowest cellobiohydrolase activities, respectively. These results strongly suggest that depending on landuse, P availability is the rate limiting factor for cellobiohydrolase activity, and that especially by intensive use of savannah.

3.3.2. Chitinase activity depending on land use and nutrient inputs

Chitinase activity was positively correlated with β-glucosidase and cellobiohydrolase activities and was 2–6 times higher in soils under natural vegetation compared to corresponding arable soils (Table 4). These results clearly demonstrate that soils under natural ecosystems, especially under forests, contain much more fungi than agroecosystems. This is because chitinase is mainly produced by fungi. These results show that tillage and fertilization practices strongly decrease fungi through mechanical soil disturbance which destroy fungi hyphens.

Savannah soils with no nutrient amendments (136 ± 15.09 nmol g^{-1} h^{-1}) displayed the highest chitinase activity after 30 days. At the end of the incubation period, lower montane forest with P addition (62 ± 13.63 nmol g^{-1} h^{-1}) showed the highest chitinase activity (Table 4). In contrast, grassland soils consistently assayed the lowest chitinase activities throughout the incubation period i.e. 10 ± 1.73 nmol g^{-1} h^{-1} and 4 ± 0.42 nmol g^{-1} h^{-1} after 30 and 60 days, respectively.

3.3.3. Phosphatase activity depending on land use and nutrient inputs

Phosphatase displayed extremely high activity (Table 4). Similar to other enzymes, phosphatase activity was higher in soils under natural vegetation compared to croplands (Table 4). These results

Table 3
β-glucosidase (top) and cellobiohydrolase (bottom) activities after 30 days (t1) and 60 days (t2) as depending on land use and nutrient additions.

Land use	t1 (after 30 days)					t2 (after 60 days)				
	Ctrl	Glu	Glu + N	Glu + P	Glu+(N + P)	Ctrl	Glu	Glu + N	Glu + P	Glu+(N + P)
	nmol g^{-1} h^{-1}					nmol g^{-1} h^{-1}				
Savannah	$51.99^{AB,d}$	$32.61^{A,b}$	$32.62^{A,b}$	$49.85^{AC,d}$	$44.65^{A,cd}$	$22.71^{AB,a}$	$32.37^{AB,b}$	$38.82^{BC,bc}$	$46.98^{C,d}$	$38.82^{AB,bc}$
Maize field	$42.10^{A,bc}$	$42.12^{AB,bc}$	$32.06^{A,a}$	$40.77^{A,abc}$	$31.96^{B,a}$	$45.15^{C,c}$	$35.02^{B,ab}$	$35.27^{B,ab}$	$35.26^{B,a}$	$46.42^{BC,c}$
Lower montane forest	$81.64^{C,d}$	$78.61^{D,d}$	$103.10^{B,e}$	$146.36^{E,f}$	$67.05^{C,bc}$	$73.18^{D,cd}$	$59.66^{C,b}$	$45.81^{C,a}$	$73.31^{E,cd}$	$63.68^{D,b}$
Coffee plantation	$52.85^{B,cd}$	$55.97^{C,cd}$	$66.99^{C,e}$	$49.90^{AC,bc}$	$43.56^{A,ab}$	$67.60^{D,e}$	$59.59^{C,de}$	$37.31^{B,a}$	$58.12^{D,cde}$	$40.18^{B,a}$
Grassland	$45.27^{AB,b}$	$43.72^{B,b}$	$67.04^{C,d}$	$56.50^{CD,c}$	$48.93^{A,bc}$	$20.96^{A,a}$	$26.45^{A,a}$	$25.40^{A,a}$	$18.50^{A,a}$	$52.26^{C,bc}$
Homegarden	$46.05^{AB,c}$	$42.78^{B,c}$	$44.19^{D,c}$	$63.46^{D,d}$	$43.56^{A,c}$	$29.37^{B,a}$	$26.36^{A,a}$	$39.34^{BC,bc}$	$39.58^{BC,bc}$	$30.58^{A,ab}$
Land use	t1 (after 30 days)					t2 (after 60 days)				
	Ctrl	Glu	Glu + N	Glu + P	Glu+(N + P)	Ctrl	Glu	Glu + N	Glu + P	Glu+(N + P)
	nmol g^{-1} h^{-1}					nmol g^{-1} h^{-1}				
Savannah	$129.68^{A,a}$	$98.41^{A,b}$	$69.28^{A,c}$	$141.70^{A,a}$	$126.28^{A,a}$	$14.08^{A,d}$	$20.24^{B,d}$	$13.51^{A,d}$	$13.85^{A,d}$	$17.23^{A,d}$
Maize field	$98.41^{B,a}$	$112.49^{A,b}$	$21.66^{D,b}$	$14.43^{B,bc}$	$18.06^{B,bc}$	$16.73^{A,bc}$	$15.24^{AB,bc}$	$12.61^{A,c}$	$14.54^{A,bc}$	$11.27^{A,c}$
Lower montane forest	$57.69^{D,a}$	$99.07^{A,b}$	$78.83^{AB,ab}$	$52.84^{C,ac}$	$64.53^{D,a}$	$44.76^{B,ac}$	$39.34^{C,ac}$	$28.36^{B,c}$	$65.59^{C,a}$	$62.80^{C,a}$
Coffee plantation	$84.77^{BC,ab}$	$112.49^{A,a}$	$89.05^{B,ab}$	$68.31^{C,bc}$	$42.99^{C,cd}$	$49.59^{B,cd}$	$41.93^{C,cd}$	$37.16^{B,cd}$	$36.96^{B,cd}$	$17.61^{A,d}$
Grassland	$31.92^{D,cd}$	$31.92^{B,cd}$	$38.30^{C,d}$	$21.91^{B,bc}$	$22.27^{B,bc}$	$8.33^{A,a}$	$10.26^{A,ab}$	$11.74^{A,ab}$	$7.66^{A,a}$	$18.60^{A,ab}$
Homegarden	$81.62^{C,a}$	$36.70^{B,bc}$	$39.63^{C,bc}$	$59.37^{C,b}$	$42.99^{C,bc}$	$41.65^{B,bc}$	$46.51^{C,bc}$	$43.09^{C,bc}$	$33.91^{B,c}$	$38.98^{B,bc}$

Means within each column with different capital letters indicate significant differences according to Fischer's LSD test at $P = 0.05$.
Means within each row with different small letters indicate significant differences according to Fischer's LSD test at $P = 0.05$.

suggest that the quality and quantity of SOM in soils plays an important role in protecting and maintaining phosphatase in very active form. P additions to grassland soils yielded the highest phosphatase activity after 30 days (1693 ± 293 nmol g^{-1} h^{-1}), and was therefore the most responsive to P additions. Soils under maize cultivation assayed the least phosphatase activity ranging between 117 ± 13.04 and 179 ± 15.64 nmol g^{-1} h^{-1} in all treatments.

At the end of the incubation period, phosphatase activity increased in soils under maize plantations. Combination of P and N yielded the greatest increase of more than double from 140 ± 12.88 after 30 days to 324 ± 7.95 nmol g^{-1} h^{-1} after 60 days (Table 4). Similarly, P and N added in combination increased phosphatase activity in lower montane forest soils from 269 ± 52.50 to 505 ± 113.05 nmol g^{-1} h^{-1} after 30 and 60 days, respectively. These results suggest that the addition of N and P enhanced the P mobilization potential of soil microorganisms in these soils. In contrast, soils under savannah and *Chagga* homegarden systems displayed a significant drop in phosphatase activity with incubation time.

3.3.4. Principal components analysis

The PCA is often performed to eliminate multicolinearity and to reduce the number of variables in a data set to make the data analysis and interpretation more efficient, while containing most or all of the information. β-glucosidase was positively correlated to cellobiohydrolase and chitinase activity. However, it was negatively correlated to land use. In contrast, phosphatase was positively correlated to land-use.

Similarly, applying the PCA-method three principal component (PCs) with eigenvalues of >1 were extracted (Table 5). Highest component loadings of the 1st PC (43.96% of total variance) included variables that characterize the microbiological activity of soils related to the C and N cycles (β-glucosidase, cellobiohydrolase and chitinase). Therefore, the 1st PC was interpreted as 'SOM-related nutrient' status. Phosphatase was not strongly correlated with the 1st PC and was therefore considered less important in describing it. The 2nd PC (30.19% of total variance) was described by land use and phosphatase having the highest loadings on the PC. The 2nd PC was collectively summarized as 'phosphorus component'. The relatively poor correlation of phosphatase with the first PC implies that the enzyme is not associated with the total SOM-pools (Fig. 1).

4. Discussion

4.1. Microbial biomass depending on land use and nutrient additions

Soil microbial biomass is recognized as a sensitive indicator of environmental change [31]. Sensitivity of soil microorganisms to changes in management was clearly reflected by the decline in microbial biomass after the conversion of natural ecosystems for agricultural use. Accumulation of plant litter on soil surface in natural ecosystems provides a barrier between the soil and atmosphere which reduces evaporation. Furthermore, perennial natural vegetation decrease wind speed, resulting to less rapid exchange of water and heat. These factors contribute to maintaining a suitable micro-environment for the microbial population to thrive.

Microbial biomass in agricultural soils was low both in absolute numbers and as percentage of total C. Decline in SMB with land use intensification is an early indication of a possible future decline in SOM [30]. High evaporation in tilled soils leads to more drastic drying/rewetting cycles at the upper soil layers leading to a low microbial population. Furthermore, runoff after the strong rains also lowers SMB. This is because the arable soil surface at the onset of the rainy season is not covered. Consequently, water does not penetrate into the soil, but is lost from the soil and landscape. Low microbial biomass contents both in absolute numbers and as percentage of total C in arable soils was expected. Recent studies [35,40] have shown depletion of microbial biomass under continuous cropping systems at Mt. Kilimanjaro region. Tillage and conditions associated with bare fallow in agricultural systems accelerate microbial biomass turnover.

The volcanic soils present at Mt. Kilimanjaro contributed to the exceptionally low C_{mic}:C_{org} percentages in all landuse systems. Aluminium oxides present in Andosols fix SOM especially easily available organics [34] by covalent bonds making it inaccessible to soil microorganisms. Higher C_{mic}:C_{org} percentages in natural compared to arable soils show that natural ecosystems maintain more labile organic substrates in the soil allowing for a higher MBC content per unit of soil C. Low C_{mic}:C_{org} percentages in soils under intensive cultivation is a good indicator of soil degradation after landuse change from natural to agricultural systems [30]. During soil degradation, microbial C pools decline at a faster rate than OM

Table 4
Chitinase (top) and phosphatase (bottom) activities after 30 days (t1) and 60 days (t2) as depending on land use and nutrient additions.

Land use	t1 (after 30 days)					t2 (after 60 days)				
	Ctrl	Glu	Glu + N	Glu + P	Glu+(N + P)	Ctrl	Glu	Glu + N	Glu + P	Glu+(N + P)
	nmol g^{-1} h^{-1}					nmol g^{-1} h^{-1}				
Savannah	$136.52^{A,d}$	$54.67^{A,b}$	$77.19^{A,c}$	$54.67^{A,b}$	$78.99^{A,c}$	$40.77^{C,a}$	$41.57^{C,a}$	$44.99^{D,a}$	$58.65^{D,b}$	$37.16^{D,a}$
Maize field	$22.59^{B,a}$	$50.59^{A,b}$	$14.29^{B,cd}$	$11.09^{B,c}$	$21.68^{B,ae}$	$24.03^{B,a}$	$19.10^{B,e}$	$18.20^{BC,e}$	$14.69^{B,d}$	$21.14^{B,ae}$
Lower montane forest	$103.98^{C,g}$	$125.78^{B,h}$	$67.69^{C,e}$	$85.83^{C,f}$	$50.04^{C,bc}$	$54.95^{D,cd}$	$45.63^{C,b}$	$50.46^{D,bc}$	$62.75^{D,de}$	$30.93^{CD,a}$
Coffee plantation	$22.59^{B,bc}$	$25.88^{C,c}$	$49.01^{D,d}$	$24.67^{D,bc}$	$24.93^{B,bc}$	$16.61^{B,ab}$	$26.73^{B,c}$	$13.18^{AB,a}$	$22.04^{B,bc}$	$16.69^{AB,ab}$
Grassland	$10.04^{D,abc}$	$11.21^{D,abc}$	$13.85^{B,bc}$	$14.63^{B,c}$	$10.11^{D,abc}$	$3.69^{A,a}$	$5.53^{A,ab}$	$7.77^{A,abc}$	$5.43^{A,ab}$	$11.50^{A,abc}$
Homegarden	$25.60^{B,a}$	$27.42^{C,a}$	$29.73^{E,ad}$	$35.37^{E,bd}$	$24.93^{B,a}$	$18.02^{B,c}$	$37.28^{C,b}$	$26.87^{C,a}$	$33.41^{C,bd}$	$23.65^{BC,ac}$
Land use	t1 (after 30 days)					t2 (after 60 days)				
	Ctrl	Glu	Glu + N	Glu + P	Glu+(N + P)	Ctrl	Glu	Glu + N	Glu + P	Glu+(N + P)
	nmol g^{-1} h^{-1}					nmol g^{-1} h^{-1}				
Savannah	$348.69^{A,d}$	$179.37^{A,bc}$	$141.98^{AB,ab}$	$282.23^{A,d}$	$263.49^{A,cd}$	$58.48^{A,a}$	$50.63^{A,a}$	$53.17^{A,a}$	$96.22^{A,ab}$	$80.20^{A,a}$
Maize field	$179.37^{B,bc}$	$179.37^{A,bc}$	$117.44^{A,a}$	$128.07^{B,a}$	$140.16^{C,ab}$	$244.86^{BC,d}$	$184.44^{B,bc}$	$156.18^{B,ab}$	$207.80^{B,cd}$	$324.17^{B,e}$
Lower montane forest	$539.77^{C,c}$	$755.60^{B,d}$	$264.86^{C,a}$	$506.26^{C,bc}$	$269.39^{A,a}$	$235.93^{BC,a}$	$349.18^{DE,ab}$	$278.45^{C,a}$	$297.99^{C,a}$	$505.60^{D,bc}$
Coffee plantation	$340.82^{A,a}$	$280.65^{C,a}$	$248.75^{BC,a}$	$320.85^{A,a}$	$381.70^{B,a}$	$296.52^{C,a}$	$410.00^{E,a}$	$276.39^{C,a}$	$382.31^{D,a}$	$357.94^{BC,a}$
Grassland	$585.44^{C,c}$	$785.05^{B,d}$	$434.30^{D,bc}$	$1693.39^{D,e}$	$391.72^{B,ab}$	$292.72^{C,ab}$	$253.64^{BC,ab}$	$248.62^{C,a}$	$295.71^{C,ab}$	$402.14^{C,ab}$
Homegarden	$550.28^{C,f}$	$709.17^{B,g}$	$531.86^{D,f}$	$454.41^{C,e}$	$381.70^{B,d}$	$172.61^{B,b}$	$305.99^{CD,c}$	$294.99^{C,c}$	$193.91^{B,b}$	$116.80^{A,a}$

Means within each column with different capital letters indicate significant difference according to Fischer's LSD test at $P = 0.05$.
Means within each row with different small letters indicate significant difference according to Fischer's LSD test at $P = 0.05$.

Table 5
Principal components (PC) and component loadings extracted from the variables.

Variables	Principal components (PCs)		
	1	2	3
Chitinase	0.7959	−0.4105	−0.0409
Cellobiohydrolase	0.8003	−0.1608	−0.5224
β-glucosidase	0.8065	0.1578	−0.5333
Phosphatase	0.3408	0.8769	0.4482
Land use	−0.2223	0.8573	−0.3539
Time	−0.7398	−0.2974	−0.1247

resulting to a drop in C_{mic}:C_{org} percentages [7]. Decline in microbial biomass with incubation time contributed to the decline in C_{mic}:C_{org} percentages.

Decrease in microbial biomass content was observed in savannah and lower montane forest soils compared to corresponding agricultural systems i.e. maize fields and coffee plantations, respectively. This suggests that microorganisms in the agricultural soils are better adapted to absence of fresh and readily decomposable material and can better survive on the decay of native SOM. This indirectly shows the shift of microbial community to the K-strategists [18], better adapted to hardly available organics, and having slower turnover [8]. The general increase in MBC with N additions in soils under maize plantation and grassland compared to corresponding savannah and *Chagga* homegarden soils suggest that these sites are characterized by lower N loads. Similarly, an increase in percent C_{mic} after P additions ranging from 2.49% in lower montane forest soils to 47.39% in soils under maize plantation in all ecosystems studied except savannah soils show that P availability is a rate limiting factor for microbial processes.

4.2. Water extractable organic C depending on land use and nutrient additions

The general decrease in WOC concentrations after landuse change from natural to arable cropping can be explained by the gradual depletion of SOM after conversion, especially its labile pools [38]. This is because vegetation type and amount of OM returned to the soil are major biotic factors in determining the amount and composition of water soluble organic matter.

The concentration of WOC generally declined with time. This demonstrates that solubilized OM is readily biodegradable [50] and rapidly consumed by soil microorganisms [38]. The magnitude of decrease in WOC in savannah and maize plantation soils after N and P fertilization was greater compared to control soils with no amendments. These results suggest that N and P availability enhanced consumption of WOC by microbes. In the contrary, less decrease in WOC was observed in lower montane forest, coffee plantation, grassland and *Chagga* homegarden soils after N and P addition. This may be explained by two mechanisms: 1) increased immobilization of WOC in microbial biomass after N and P fertilization and that microbial biomass is limited by nutrients, or 2) favoured SOM degradation resulting in the production of additional dissolved organic matter.

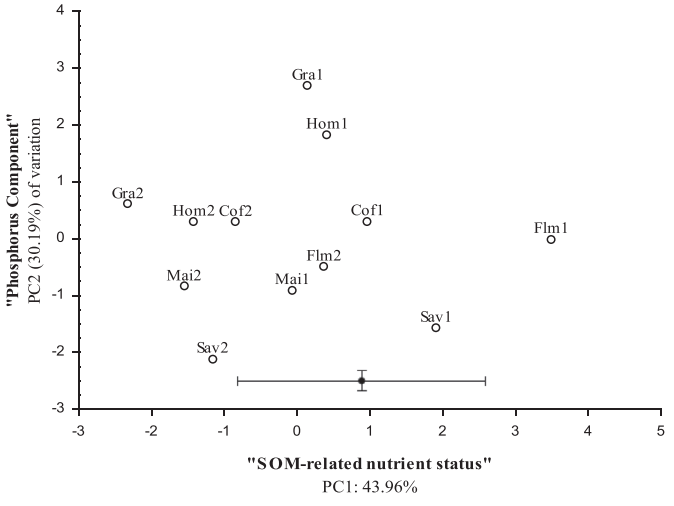

Fig. 1. Factor scores of the land use types and their assignment to the 1st and 2nd principal components (PC) at 0–20 cm soil depth. Sav = Savannah, Mai = Maize fields, Flm = Lower Montane Forest, Cof = Coffee plantations, Gra = Grasslands, Hom = Homegardens. Numbers (1, 2) after abbreviation represents sampling times where 1 (after 30 days) and 2 (after 60 days). Error bar represent least significant difference (P<0.05), n = 3.

4.3. β-glucosidase and cellobiohydrolase activity

Observed β-glucosidase and cellobiohydrolase activities as affected by land use is associated in part with C_{mic} and C_{total}. Soils under natural vegetation sustained higher enzyme activities compared to corresponding cropland because of the positive impact of surface cover, vegetation and lack of tillage on soil properties notably microbial populations and activities [1]. The positive impact of soil cover and lack of tillage on β-glucosidase and cellobiohydrolase activities shows why they are considered as very sensitive biological indicator of the effects of soil management practices.

Greater soil microbial heterotrophs diversity notably fungal decomposer groups dominating tropical forests contributed to the high β-glucosidase activity in lower montane forest soils. β-glucosidase is derived predominantly from heterotroph fungi [47]. β-glucosidase activities in maize fields were higher compared to savannah soils. However, we attribute this mainly to addition of farmyard animal manure in maize fields. Incorporation of organic manure into soil is a well-known practice to increase crop yields and maintain soil fertility in Africa. By contrast, cellobiohydrolase activity was higher in savannah soils compared to maize plantations. This can be linked to the high cellulose C input from woody trees and graminoid litter common in tropical woodland savannah ecosystems.

Glucose addition had minimal effects on β-glucosidase and cellobiohydrolase activities after 30 and 60 days. The reaction of enzymes involved in C-cycling depends on the substrate used as well as the time of incubation [11]. Low molecular weight substrates, e.g. glucose, are generally fully utilized within few hours [5,19]. Consequently, four weeks after application, glucose was completely exhausted, and therefore the soil micro-organisms reverted to attack native SOM and litter present in soil. This is well demonstrated by the decline in β-glucosidase and cellobiohydrolase activities with time of incubation. Previous studies e.g. Refs. [25] and [51] also demonstrated significance correlation between C-cycle enzymes, microbial biomass and substrate availability.

Depending on land use, the results further showed that the enzymes involved in C-cycling responded differently to N and P fertilization. After four weeks, N addition enhanced high β-glucosidase and cellobiohydrolase activities in grassland soils. Some studies [13,17,26] demonstrated that N addition stimulated the activity of C-cycling enzymes. N fertilization may increase the activity of microorganisms degrading cellulose by stimulating microbial growth with constitutive enzymes or synthesis of enzymes in microbial cells with low enzyme activity under nutrient-deficient conditions. Microorganisms can assimilate N for enzyme

production, leading to increased enzyme activity [4].

The observed trend in the C-cycle enzymes activities as affected by land use is in agreement with previous studies [2,3] which show lower activities in agricultural soils compared to the corresponding less-disturbed soils. Consequently, conservative land uses e.g. forest and savannah improve nutrient cycling and ecosystem functioning.

4.4. Chitinase activity

Chitinases are known to act against fungal pathogens by degrading chitin, a major structural component of most fungal (ascomycetes and basidiomycetes) cell walls [6]. Lower montane forest soils displayed the highest chitinase activity. This is a reflection a high abundance of fungal biomass. The number of ascomycetes and basidiomycetes forming ectomycorrhizae is tremendously high in forest soils. Previous studies (e.g. Ref. [44] have positively correlated chitinase activity to ergosterol content as a measure of fungal biomass, suggesting that chitinase activities is associated with fungal biomass and activity.

Depending on land use, nutrient availability had an inhibitory, stimulatory and neutral effect on chitinase activity. Inhibition of chitinase activity in soils under coffee plantation and stimulation in soils under grassland vegetation with N availability reflects the extent of N limitation to microorganisms. Previous studies (e.g. Ref. [39] simulating changes in N deposition have also shown decrease in chitinase activity in N poor soils.

Addition of N stimulated chitinase activity in soils under grassland. Consequently, microorganisms in soils under grassland ecosystem assimilated the additional N and invested it in chitinase production as an inexpensive means of extracting additional nutrients from the soil [4,49]. Nutrients losses notably N through regular harvesting of aboveground biomass (as livestock feed) may have degraded the grassland soils and reduced their capacity to naturally replenish depleted N pools. Contrarily, increased N availability had a neutral effect on chitinase activities in lower montane forest and savannah soils. One possible explanation for this is that chitinase activity had already been repressed and further additions of N did not result in additional suppression of its activity.

4.5. Phosphatase activity

Phosphatase displayed extremely high activity level. This reflects the ability of phosphatases to persist in soils for long periods by binding to soil organic matter and clays, which are especially abundant and active in allophanic soils [34]. High phosphatase activities in soils dominated by non-crystalline allophane and imogolite materials like ours have also been reported in previous studies (e.g. Ref. [39]. Bound phosphatases may be released during potential activity assays, stimulating their activity.

Although inorganic phosphate is known to produce a feedback inhibition for phosphatase [20,39], we did not observe inhibition of phosphatase activity in soils amended with inorganic P. One possible explanation is that the background P levels were far below the threshold and the addition of PO_4 did not raise the levels of P high enough to trigger the negative feedback mechanism. Furthermore, most of the phosphatases contributing to the overall soil enzyme activity are not suppressed by P addition because they are stabilized by the interaction with colloids [17].

In the contrary, P additions stimulated phosphatase activity in savannah, lower montane forest and grassland soils mid-way through the incubation period. Increased concentrations of phosphate in soil solution produced a positive feedback mechanism by stimulating strong microbial activity for enhanced synthesis of microbial phosphatase. These results clearly demonstrates the preferential utilization of available nutrients e.g. P, to produce enzymes. When a resource e.g. P in volcanic ash soils, is limiting, microbes may benefit from producing enzymes to obtain it. Therefore, investment in enzymes becomes an inexpensive means of extracting additional resources from the soil. After 60days, phosphatase activity dropped by almost a half in these soils. Phosphatase activities have been reported to coincide with microbial biomass [43] and general activity of microorganisms [10]. We attribute this sharp decline to the high microbial turnover observed in these ecosystems.

5. Conclusions

Soil microbial biomass and enzyme activities were affected by land management practices at Mt. Kilimanjaro. Higher soil microbial biomass in natural ecosystems compared to agricultural systems is a good indicator of decrease of soil quality after landuse change. Similarly, enzyme activities were higher in soils under natural vegetation compared to arable soils and β-glucosidase, cellobiohydrolase and chitinase activities were the most sensitive enzymes for showing differences in land use. Extremely high phosphatase in all land use types is a reflection of the ability of phosphatases to persist in soils for long periods by binding to organic matter and clays which are especially abundant and active in volcanic soils. Generally, the response of enzyme activities to nutrient (N and P) additions (i.e. stimulation, inhibition and neutral) was highly dependent on land use system and the background levels of available nutrients. The extracellular enzymes play an important role in soil biogeochemical cycles (C, N and P) involved in nutrient transformation. Therefore, significant reductions in enzyme activities and microbial biomass under agricultural compared to natural ecosystems should be considered as indicators of soil quality and landuse impacts at Mt. Kilimanjaro region.

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