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‘‘Composition, degradation and stabilization of soil organic matter along an elevation gradient of Mount Kilimanjaro”

Dissertation

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Table of contents

i. List of Tables

ii. List of Figures

iii. Abbreviations

COF – Coffee plantation

FLM – Forest lower montane

FER – Erica Forest

FOC – Forest Ocotea

FOD – Forest Ocotea Disturbed

FPD – Forest Podocarpus Disturbed

FPO – Forest Podocarpus

HEL – *Helichrysum* vegetation

HOM – Homegarden (Traditional Chagga homegarden)

KINAPA – Kilimanjaro National Park Authority

LMA – Large macro aggregates

MAI – Maize fields

MAP – Mean annual precipitation

MAT – Mean annual temperature

MiA – Micro aggregates

SAV – Savanna

SMA – Small macro aggregates

SOC – Soil organic carbon

SOM – Soil organic matter

TANAPA – Tanzania National Parks Authority

iv. Summary

Understanding the complex biotic and abiotic drivers of carbon (C) and nutrient cycles, especially in the soils of tropical mountainous ecosystems, is essential to the management of ecosystem services provided by them. Mt. Kilimanjaro contains volcanic soils with huge stocks of soil organic matter (SOM), which support the human population at its surrounding area. Increasing population, successively intensified land-use and climate changes have intensified the pressure on Mt. Kilimanjaro's soils and continuously increased degradation processes. The threat ranges from biochemical and physical to social economics factors and causes progressive deterioration of the unique mountain ecosystems. The need for understanding the interactions between biochemical and physical processes driving SOM decomposition and stabilization and its impacts on nutrient cycling in this ecosystem is the motivation for this research. The research was conducted on the southern slopes of Mount Kilimanjaro, which includes agricultural and natural ecosystems from 700-4600 meter above sea level (a.s.l). Ten ecosystems (representative for common natural vegetation and management practices) were studied along the elevation gradient. These ecosystems reached from savanna (700-1100 m), maize fields (886- 1009 m), coffee plantation (1124- 1648 m), grassland (1303- 1748 m), Chagga homegarden (1169- 1788 m), lower montane forest (1100-1800 m), *Ocotea* forest (1800- 2800 m), *Podocarpus* forest (2800- 3200 m), Erica forest (3200- 4000 m) and alpine *Helichrysum* vegetation (4000- 4600 m a.s.l.).

The first objective of this thesis was to elucidate the composition of SOM along the elevation gradient from 900 to 4200 m a.s.l. to reconstruct origin and decomposition stage of SOM. The second objective was to assess the impact of land-use change and management practices on the kinetics of four enzymes involved in decomposition of soil organic matter (SOM) in the top- (0-10 cm) and subsoil (20-30 cm) from six ecosystems, (semi-) natural and agricultural (managed) ones. The third objective was to analyze the soil aggregate size distribution for

ecosystems along the elevation and land-use gradients for the top soils (0-10 cm) to identify possible effects on SOM stabilization, degradation state, and erosion resistance..

The quality and quantity of lignin and sugars were assessed from savanna to alpine *Helichrysum* (900-4200 m a. s. l). Total lignin i.e. vanillyl, syringyl and cinnamyl (VSC) and sugars contents in topsoil (0-10 cm) both peaked at mid-elevation ecosystems (2000-3000 m), following generally the trend of SOC content in these ecosystems. The ratio of microbial-derived to plant-derived sugars, assessed by the galactose + mannose to arabinose + xylose ratio (GM/AX ratio), was similar at all mid-elevation ecosystems. The portion of microbial derived sugars significantly decreased in lowest and highest ecosystems (savannah and *Helichrysum*), where microbes were facing growth limitations e.g. by drought or temperature. In these two ecosystems, the ratio of vanillic acid to vanillyl aldehyde (Ac/Al_v), reflecting lignin decomposition status, was lowest as well. However, low Ac/Al_v ratios were also found in grassland and *Ocotea* forest, suggesting that lignin degradation in these ecosystems is hampered by additional factors e.g. nutrient limitation.

The effect of changing environmental conditions on maximum reaction rate (V_{max}) and substrate affinity constant (K_m) of four extracellular hydrolytical enzymes (β-galactosidase, cellobiohydrolase, phosphatase and chitinase) in top- and subsoil was determined by Michaelis-Menten kinetics using fluorogenic substrates. The affinity of enzymes to substrates was higher in soils of natural compared to agricultural ecosystems: i.e. higher under forests than under cropland. The maximum activity of β-galactosidase, cellobiohydrolase and chitinase enzyme were highest in lower mountain forest and grassland i.e. less disturbed ecosystems. Changes in land use and management practice did not only affect enzyme activity but also controlled enzyme kinetics (K_m and K_a) thus pointing towards the expression of different enzyme systems. The alteration of carbon and nutrients cycling affected microbial

activities and enzymes catalytic properties which were mainly related to anthropogenic effects.

Top soils from 12 representative ecosystems (5 agricultural and 7 natural) at Mt. Kilimanjaro were analyzed using dry-sieving and separated into three fractions: large macro aggregates (2-5 mm), small macro aggregates (0.25-1 mm) and micro-aggregates (<0.25 mm). Large macro aggregates were strongly affected by land-use and also changed significantly along the elevation gradient in comparison to micro aggregates. The percentage of large macro aggregates decreased with higher land-use intensity. In the colline zone, land-use change from savanna to maize fields decreased the percentage of large macro aggregates by 50%. In the lower montane zone, large macro aggregate percentage tended to decrease from 30.6% in forests to 20.3% in coffee plantations. Along the elevation gradient, large macro aggregate percentage in natural ecosystems increased up to 63.5% at mid-elevation (2000-3000 m a.s.l.) and declined towards higher elevations. Large macro aggregates were sensitive to disturbances and land-use intensification rapidly reduced their abundance. This may have severe consequences, as breakdown of large macro aggregates through land-use intensification could further reduce the potential of soils to act a carbon sink and increase degradation and erodibility of these soils. Especially at mid-elevation, where lignin and sugar data suggest high-input high-decomposition dynamic of SOM, the de-stabilization of macro-aggregates, stabilizing the fresh input of SOM, might have most severe impact on SOM losses. In contrast, at high and low elevation ecosystems, where temperature limitations and droughts have high impact, climate change might mostly endanger the SOM stocks. In the colline and lower montane zone, where anthropogenic use was studied, the impacts on biogeochemical processes were evident and enzyme data suggested shifts in microbial activity, community and enzyme systems for SOM decomposition – all indicators of a severe impact of the land use forms on the natural biogeochemical cycles which by far exceed solely the decomposition of SOM and loss of SOC. This thesis unravels the sensitive interaction of biotic actors and abiotic factors controlling biogeochemical cycles at Mt. Kilimanjaro and suggest not only the preference for less intensive management forms of these ecosystems but also points towards the need of a thorough observation of the biogeochemical cycles of these sensitive tropical mountain ecosystems facing the challenges of global change.

Zusammenfassung

Die Notwendigkeit die Komplexität von biotischen und abiotischen Treibern der Kohlenstoff- (C) und Nährstoffkreisläufen zu verstehen, besonders die von Böden montaner tropischer Ökosysteme, ist essentiell für das Management von Ökosystemdienstleistungen. Der Kilimandscharo ist eines der bedeutendsten Naturdenkmäler des afrikanischen Kontinents und speichert eine große Menge organischer Substanz in seinen vulkanischen Böden. Diese spielen eine große Rolle für die nachhaltige menschliche Nutzung und Kohlenstoffhaushalt. Die steigende Bevölkerungsanzahl, sukzessive intensivierte Landnutzung und der Klimawandel haben den Druck unter dem die Böden stehen erhöht und die fortlaufende Bodendegradation weiter verstärkt. Die Bedrohung betrifft sowohl biochemische und physikalische, als auch soziale und wirtschaftliche Faktoren und verursacht eine progressive Zerstörung dieser montanen Ökosysteme. Die Notwendigkeit die Interaktion zwischen biochemischen und physikalischen Prozessen die Abbau und Stabilisation des organischen Materials verursachen und seine Auswirkungen auf den Nährstoffkreislauf in Ökosystemen zu verstehen ist die grundsätzliche Motivation dieser Arbeit. Die Studien wurde an den Südhängen des Kilimandscharo durchgeführt. Diese beinhaltete agrarisch-genutzte Ökosysteme und Waldökosystem, die sich auf 700 bis 4600 m über dem Meeresspiegel befinden. Zehn Ökosysteme wurden entlang des Höhen- und Vegetationsgradienten

untersucht, variierend von Savanne (700-1100m), Maisfelder (1009- 886m), Kaffeeplantagen (1124- 1648m), Grasland (1303- 1748 m), Hausgärten der Chagga (1169- 1788 m), niedriger gelegene montane Wälder (1100- 1800 m), Ocotea Wälder (1800- 2800 m), Podocarpus Wälder (2800-3200 m), Erica Wälder (3200- 4000 m) und alpine *HelichrysumHelichrysum* Vegetation (4000- 4600 m ü. N.0).

Das erste Gegenstand dieser Dissertation war es die Zusammensetzung der organischen Bodensubstanz entlang eines Höhengradienten von 900 bis 4200 m über dem Meeresspiegel zu bestimmen um Rückschlüsse auf Ursprung und den Degradationsgrad zu ziehen. Als zweites wurden die Auswirkungen von Landnutzungsänderung auf die Kinetik von vier Enzymen, die dem Abbau von organischer Substanz dienen, im Ober- (0-10 cm) und Unterboden (20-30 cm) von sechs Ökosystemen (natürliche sowie anthropogen beeinflusste) untersucht. Der dritte Recherchegegenstand war die Analyse der Größenverteilung von Bodenaggregaten im Oberboden (0-10 cm) von natürlichen und Landnutzungssystemen zur Identifizierung von Höhen- und Landnutzungseffekten auf Mechanismen der Bodenkohlenstoffsequestrierung, dem Degradationsstatus und dem potentiellem Erosionswiederstand.

Die Qualität und Häufigkeit von Lignin und Zucker in der Savanne bis zum alpinen *HelichrysumHelichrysum* (900-4200 m überm Meeresspiegel) wurden untersucht. Die Gesamtmenge Lignin, bestehend aus Vanillyl, Syringly und Cinnamyl (VSC) und Zucker im Oberboden (0-10 cm), hatte ihr Maximum in den Ökosystemen mittlerer Höhenstufen (2000-3000 m). Dies entsprach dem Trend des Gesamtgehalts an Bodenkohlenstoff in den untersuchten Ökosystmen. Das Verhältnis von mikrobiellen und pflanzenbasierten Zuckern, untersucht durch das Galactose +Mannose zu Adrabinose und Xylose Verhältnis (GM/AX Ratio), war ähnlich in Ökosystemen mittlerer Höhenlage. Die Menge mikrobieller Zuckern sank in den tiefer- und höher gelegenen Ökosystemen (Savamme und

HelichrysumHelichrysum) stark ab. In diesen Ökosystemen wird mikrobielles Wachstum und Aktivität durch z.B. geringe Temperatur oder periodische Dürre eingeschränkt. In diesen zwei Ökosystemen war das Verhältnis von Vanillinsäure zu Vanillinaldehyd (Ac/Al_v), als Indikator der Lignin-Degradation, am geringsten. Jedoch wurden geringe Ac/Al_v Ratios auch im Grasland und Octoea Wald gefunden. Dies lässt vermuten, dass die Lignin-Degradation in diesen Ökosystem durch weitere Faktoren wie z.B. Nährstoffmangel limitiert wird.

Der Einfluss veränderter Umweltbedingung auf die maximale Reaktionsrate (Vmax) und Michaelis-Menten Konstante (Km) von vier extrazellulären hydrolytischen Enzymen (ß-Galactosidase, Cellobiohydroloase, Phosphatase und Chitinase) in Ober- und Unterboden, wurden anhand der Michaelis-Menten Kinetik mittels flurogener Substrate untersucht. Die Affinität von Enzymen war höher in natürlichen Böden im Vergleich zu agrarisch genutzen Böden und höher unter Wald als unter Ackerland. Das Aktivitätsmaximum von ß-Galactosidase, Cellobiohydrolase und Chitinase war am höchsten in tiefer gelegenen montanen Wäldern und Grasland, also den weniger stark anthropogen beeinflussen Ökosystemen. Landnutzungsänderungen und in Management-Praktiken betrafen nicht nur die Enzymaktivität, sondern kontrollierten auch die Enzymkintetik (K_m und K_a), was für unterschiedliche Enzym-Systeme spricht. Die Veränderung im Kohlenstoff- und Nährstoffkreislauf (vornehmlich durch anthropogenen Einfluss) beeinflussten die mikrobiellen Aktivitäten und die katalytischen Eigenschaften der Enzyme.

Oberbodenproben aus 12 repräsentativen Ökosystemen (5 agrarische und 7 natürliche) des Kilimandscharo wurden mittels Trockensieben hinsichtlich dreier Aggregatgößenfraktionen untersucht: große Makroaggregate (2-5mm), kleine Makroaggregate (0,25-1mm) und Mikroaggregate (<0,25 mm). Der Anteil großer Makroaggregate wurden stark durch Landnutzung beeinflusst und zeigte eine Veränderungen entlang des Höhengradienten. Der Prozentsatz großer Mikroaggregate verringerte sich mit intensiverer Landnutzung. In der kollinen Zone reduzierte Landnutzung den Prozentanteil großer Makroaggregate um 50%. In tiefergelegenen montanen Zonen sanken der Prozentsatz der großen Makroaggregate von 30,6% in natürlichen Wäldern um 20,3% auf Kaffeeplantagen. Entlang des Höhengradienten erhöhte sich der Prozentanteil der großen Mikroaggregate um bis zu 63,5% in natürlichen Ökosystemen in mittlerer Höhenlage (2000-3000 m) und sank wieder in höher gelegenen Regionen. Große Makroaggregate reagierten empfindlich auf Störungen und intensivierte Landnutzung. Das kann gravierende Konsequenzen mit sich ziehen, da eine Zerstörung großer Makroaggregate durch intensivierte Landnutzung die Fähigkeit des Bodens als Kohlenstoffsenke zu agieren beeinträchtigt und die Erosionsanfälligkeit erhöht.

Besonders in mittleren Höhenlage, wo die Lignin- und Zucker-Daten auf eine Dynamik aus hohem Input und schnellen Abbauraten hindeuten, sorgt die Destabilisierung der Makroaggregate, die sonst eine wesentliche Rolle für die Stabilisierung neuen organischen Materials spielen, für einen potentiellen Verlust organischen Kohlenstoffs. Im Gegensatz dazu sind hoch und tief gelegene Zonen stark Temperatur und Dürre limitiert, was den Kohlenstoffhaushalt in diesen Ökosystem besonders anfällig für klimatische Veränderungen macht. In kollinen und tiefer gelegenen montanen Zonen, wo der Einfluss anthropogener Nutzung untersucht wurde, ließen sich deutliche Veränderungen des Kohlenstoffkreislaufs erkennen, welche deutliche weitreichendere Konsequenzen mit sich ziehen als lediglich veränderte Zersetzung organischen Materials und Verlust an Bodenkohlenstoff. Die vorliegende Arbeit deckt die sensiblen Interaktionen zwischen abiotischen und biotischen Faktoren auf, die den biogeochemischen Kreislauf am Kilimandscharo kontrollieren, und legt nicht nur weniger intensivere Bewirtschaftung dieser Systeme nahe, sondern zeigt auch die Notwendigkeit zur genauen Analyse biogeochemischer Kreisläufe in diesen sensiblen tropischen Bergökosysteme, welche stark vom Klimawandel betroffen sind.

1. Extended summary

1.1. General introduction

1.1.1. Tropical Afromontane ecosystems

Tropical forests are key to challenging effects of global change because they are important carbon (C) sinks, support large human populations, and are considered a global hotspot of biodiversity (Fisher et al. 2011; Heywood, Watson, and United Nations Environment Programme. 1995; Lewis, Malhi, and Phillips 2004a; Pimm and Raven 2000). Over half of the global net primary production (NPP), including above and below ground productivity, occurs in the tropics (Aragão et al. 2009; Melillo et al. 1993). Therefore tropical ecosystems are extremely important for global biogeochemical cycles. This is especially true for montane areas. These have a large potential for belowground C sequestration (Wilcke et al. 2008) and are particularly threatened by raising global temperatures (IPCC 2013). Despite Africa hosts one third of the tropical forest area and has a C sequestration potential comparable to the Amazonian rain forest, the understanding of C cycles and storage functions in African ecosystems is still limited (Lewis, 2009; Malhi et al., 2012). Mount Kilimanjaro, records extremely high endemic plants and vertebrates species per area (Myers et al. 2000) and acts as a water tower which and regulates water supply in the surrounding semi-arid areas (Hemp, 2009; Hemp & Hemp, 2018,). Despite that, Afromontane forests are deteriorating at alarming rate due to human settlements and related subsistence agriculture and wood-based fuel use (Fisher et al. 2011) which also impacts biogeochemical cycles. To understand and mitigate the consequences of these changes and evaluate drawbacks for ecosystem services, it is fundamental to understand the functioning of biogeochemical and ecological processes in these ecosystems. Therefore, a lot of research has been conducted in the Kilimanjaro region over the recent years (Mganga, Razavi, and Kuzyakov 2016a; Pabst et al. 2016; Peters et al.

2019a, 2019b)). Yet, there are still some important pieces missing in understanding the whole complexity of ecosystem C and nutrient cycles at Mt. Kilimanjaro (Becker 2017).

1.1.2. Impacts of land-use intensification in Kilimanjaro.

The intensification of agricultural practices will remain a major factor to meet food demands for the growing population, especially in Africa (Dietrich, Schmitz, Muller et al. 2012). Kilimanjaro region experiences an annual population growth rate of 1.8% and arable land density of 0.8 ha per capita which is great than national average (Sangeda et al. 2014). This triggers changes and intensification of land use. However, land-use intensification is usually associated with soil degradation (Salome B. Misana 2012). In the recent decades, the tropical mountainous area's physical, chemical and biological environment has evidently changed with increasingly human influences (Lewis et al. 2004a). These changes have resulted in shifting biodiversity and ecosystem functions that affect C and nutrient storage, as well as water supplies which are essential for supporting human livelihood (Hartemink 2010; Peters et al. 2019a). For example, the conversion of forests into cropland has led to a decrease in soil C stock of up to 50% globally (Don, Schumacher, and Freibauer 2011; Lal 2016:20). Most of tropical agroecosystem expansion is the result of conversion of natural ecosystem (forests) to agriculture which reduced organic matter inputs and reduced physical protection of SOC (Chibsa and Ta' 2009). Because tropical soils have usually high mineralization rates, the negative impact of land-use change on soil C storage can be fast and severe – much more than in temperate region (West et al. 2010). Therefore, effects of land-use change on soil organic C in the tropics has been in the focus of several studies (Don et al. 2011; Pabst et al. 2016; Walker 2004) Despite SOM content and composition is an important factor for sustainable productivity of agricultural systems, soil qualities have been impacted and production potential has been reduced severely (Chibsa and Ta' 2009). It is therefore imperative to strike a balance between agricultural intensification, biodiversity management on the natural ecosystems to enhanced ecosystem function and its biogeochemical cycles.

1.1.3. Relationship between Soil organic matter, enzymes and soil aggregate.

Physical and chemical processes responsible for the stabilization of SOM remains a major determinant of C and nutrients cycles in the terrestrial ecosystems (Mganga et al., 2016). The persistence of soil organic matter as an ecosystem property depends on a wide set of influencing factors, e.g. the microbial community structure, the abiotic environmental processes or the vegetation-related factors such as quality of organic matter input. Within the heterogeneity driven by the soil structure, there are plenty of interactions between solid, liquid, gaseous and biological phase of the soil, which control the SOM dynamics (Schmidt et al. 2011). Since organic matter is the source of substrate and energy for enzyme-producing micro-organisms in soil, the biological availability of SOM is strongly related to the soil enzymatic activities (Salam, Katayama, and Kimura 1998). Vice versa, the hydrolytic enzymes of the soil are actively degrading complex SOM increasing its accessibility for microbial decomposition. This interaction between SOM and enzyme activity is strongly affected by physical protection mechanisms, which actively decrease the accessibility of SOM irrespective of its chemical structure (Schmidt et al. 2011). One of these factors is the formation of soil aggregates. Soil aggregates protect SOM from microbial decomposition (Tisdall and Oades 1982) via physical protection of organic matter from enzymatic attack (Six et al. 2004) and thus have massive implications on the turnover of SOM in the ecosystem (Six et al. 2004; Wang et al. 2017). However, we also observe feedback mechanisms related to the soil aggregate – SOM interaction. The protection of SOM from the soil enzymes' attacks may result in higher C content and which in turn increases the formation of large soil aggregates, "macro-aggregates". This accumulation of organic matter, frequently fresh organic matter in these large aggregates, leads to the effect that enzyme activities are higher in macro-aggregates compared to micro-aggregates (Dorodnikov et al. 2009). Hence, there are many feedbacks between the sources and quality of the organic matter, the enzymatic activities decomposing this SOM and the physical mechanisms protecting SOM. These multi-dimensional feedback interactions make it impossible to predict based on the present studies performed at Mt. Kilimanjaro, how the change in abiotic controlling factors along the elevation gradient as well as how land-use affect SOM composition, its degradation and its stabilization.

1.2. Objectives

The general objective of this thesis was to understand the effects of the changing climatic conditions in the different vegetation zones along the elevation gradient of Mt. Kilimanjaro, and of the land-use change at lower elevation zones, on SOM composition and origin, hydrolytic enzyme activity decomposing SOM, as well as soil aggregate structure protecting SOM. The studies therefore had the following specific objectives:

i. The first objective was to use lignin and neutral sugar monomer composition in topsoil (0-10 cm) to unravel the impact of vegetation type, precipitation and temperature on SOM composition, origin and degradation status along the 3300 m elevation gradient.

ii. The second objective aimed at gaining insights into the response of microbial SOM decomposition on the various impacts of land-use in two different elevation zone. Therefore, the kinetics of four key hydrolytic enzymes involved in decomposition of SOM in top- and subsoils as affected by land-use at two elevation zones (savanna (SAV) and lower mountain forest (FLM)) on Mt. Kilimanjaro.

iii. The third objective is to unravel the impact of land-use and changing climatic conditions along the elevation gradient on soil aggregate size distribution and stability in Mt. Kilimanjaro's topsoils to evaluate the role of aggregates in stabilizing SOM in these ecosystems.

1.3. Hypothesis

In this thesis, it was hypothesized that

(H1) soil biophysical and chemical processes at Mount Kilimanjaro are highly influenced by drought and/or temperature limitations

(H2) anthropogenic activities induce changes in carbon and nutrient cycles in all zones of the elevation gradient.

1.4. Materials and Methods

1.4.1. Study area description

Mount Kilimanjaro is the highest free standing mountain on earth and is located 300 km south of equator in the northern part of Tanzania, close to the border with Kenya (3°4'33"S; 37°21'12"E). The mountain covers about 90 km in diameter from the western slope to the eastern areas near Lake Chala. The study sites covered an elevation gradient ranging from 700 to 4200 m. a. s. l. This covers a broad range of diverse ecosystems along a large climatic gradient, and provides a great opportunity to study the effects of land-use changes because it contains most common land-use types in the region. Kilimanjaro ecosystem with various land uses in different altitudes (Hemp, 2006a) provides holistic sites for global change research in tropical Africa.

1.4.2. Soils of Kilimanjaro

The soils of Mt. Kilimanjaro developed on volcanic substrate. In the colline zone Vertisols are dominant (Pabst et al. 2016; Zech et al. 2014a), while in the lower montane and cloud forest zones soil were classified as Andosols with folic, histic or umbric top-soil horizons (Becker & Kuzyakov, 2018). The upper zone at alpine zones contains Leptosols and vitric Andosols, with high SOC content at the upper layer (Dawson 1992a; Zech et al. 2014a). The similar parent materials allow controlling for geological effects on soil characteristics and functions when assessing climate and land-use effects at Mt. Kilimanjaro.

1.4.3. Land-use and climatic zonation

The study sites were divided based on vegetation type whereby the lowest elevation ecosystems are savanna (SAV) and maize (MAI) located at colline zone between 700-1200 m. a. s. l (Hemp, 2006b). The colline savanna zone which is dominated by farms especially maize, beans, sunflower and sorghums and savanna vegetation or pasture land between (Hemp 2009:2009). The clearance of the Savanna to maize and sorghum cultivation involves tillage, pesticide and fertilizer usage (Mganga et al. 2016b).

Lower montane zone, between 1200 and 1800 m a.s.l. includes agricultural ecosystems such as coffee (COF), grassland (GRA), Chagga homegardens (HOM), and natural lower montane forests (FLM) (Becker & Kuzyakov, 2018; Hemp, 2006a). The mid–elevation zone contains lower and upper cloud forests; Ocotea (FOC) and Podocarpus (FPO). The sub-alpine areas above 3000 m a.s.l. are covered by *Erica* forests (FER) until the *Helichrysum* dominated alpine heathland (HEL) at above 4000 m a.s.l. (Hemp, 2006b).

1.4.4. Climatic condition

Kilimanjaro ecosystem experiences bimodal rainfall with rainy season between October to December while heavy and longer rainy season is between March- May (Hemp, 2006b). Mean annual precipitation ranges between 663 mm to 2500 mm pear year and mean annual temperature of 4.5°C to 23.7°C (Appelhans et al., 2016) (Table 1.4.4). Increasing temperature and decreasing precipitation recorded in the last 120 years in Kilimanjaro has substantially influenced the land-use trend on this ecosystem.

Table 1.4.4: Selected soil and climatic characteristics (0-10 cm) for Kilimanjaro ecosystem arranged in the order of increasing elevation gradient. The C, N, pH, mean annual precipitation (MAP), means annual temperature (MAT) adopted from (Appelhans et al., 2016; Becker & Kuzyakov, 2018; Gütlein et al., 2017)

Plot ID	**Land-use type** [c]	**Elevation (m. a. s. l.)**[c]	**MAP (mm)** [c]	**MAT (˚C)** [c]	**Total C (mg C g^{-1})**[a]	**Total N (mg g^{-1})** [b]	**Soil (pH)**[b]
Savanna (SAV)	Natural, disturbed	871-1155	663	23.7	27.5	2.0	5.4
Maize (MAI)	Agricultural, intensive	886-1009	744	22.6	14.5	1.2	4.6
Homegarden (HOM)	Agricultural, traditional	1169-1788	1267	20.8	38.4	3.5	5.4
Coffee (COF)	Agricultural, intensive	1124-1648	1250	20.1	18.9	1.8	4.3
Grassland (GRA)	Natural, disturbed	1303-1748	2257	16.5	148.6		4.6
Lower montane forest (FLM)	Natural, disturbed	1623-2040	2500	15.3	134.8	9.2	4.3
Ocotea forest (FOC)	Natural	2120-2750	2063	12.1	214.	12.4	3.5
Podocarpus forest (FPO)	Natural	2720-2970	1389	9.4	205.9	10.0	3.8
Erica Forest (FER)	Natural	3500-3880	1417	5.3	137.5	7.6	5.0
Helichrysum Helichrysum (HEL)	Natural	3880-4550	1308	4.5	131.3	8.8	5.2

1.5.Research design

The studies for this research project were conducted on the selected sites of German Research Foundation (DFG) Research-Unit 1246 Kilimanjaro Ecosystems under Global Change (KiLi). The study sites along the elevation gradient are indicated in the diagram below (Figure 1.2.2).

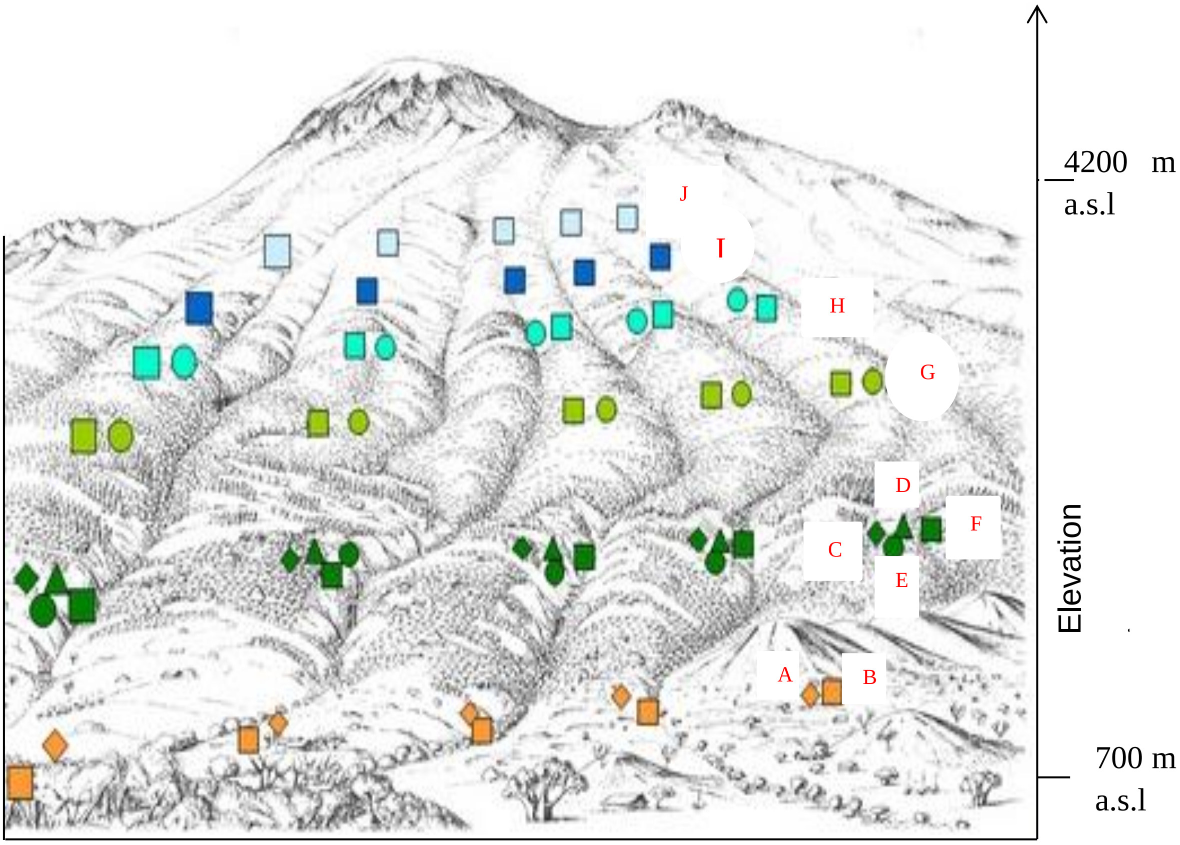

Figure 1.5: Kilimanjaro ecosystem research study sites along the elevation gradient map. Alphabet in red indicates; A- savanna, B-maize, C- Coffee plantation, D- Chagga homegarden, E- grassland, F- Lower montane forest, G- Ocotea forest, H- Podocarpus forest, I- Erica forest and J- *Helichrysum* vegetation. Adopted from (Hemp, 2006b).

1.5.1. Soil lignin and sugar composition study-sampling

The quantity and quality of lignin and sugars were assessed in seven natural ecosystems with increasing elevation: savanna, grassland and lower montane forest, cloud forests (*Ocotea* and *Podocarpus* dominated), sub-alpine *Erica* forest and alpine *Helichrysum.* The ecosystem ranged from savanna to *Helichrysum* (900- 4200 m a.s.l. elevations with semi natural to natural ecosystems. In each plot area, we established four sub-plots (5 x 5 m) in the plot corners and took mixed samples from the topsoil layer (0-10 cm) at each sub-plot. All samples were mixed thoroughly and all visible debris and coarse fragments were removed, followed by air drying, sieving to less (2 mm sieve size) and ground. Three soil samples replicates for each ecosystem were analysed under laboratory condition for seven ecosystems.

1.5.2. Enzymes kinetics

Samples were collected from 6 ecosystems (semi-) natural and agricultural (intensive and extensive) ecosystems at 0-10 cm and 20-30 cm depths. Ecosystems were savanna (SAV) and maize fields (MAI) on colline zone and the lower montane zone ecosystems in order of increasing land-use intensification i.e. lower montane forests (FLM), Chagga homegardens (HOM), grasslands (GRA) and coffee plantations (COF) ecosystems. The soils were frozen (-20 °C) and thawed at 4 °C for 1 day and then were pre-incubated at 20 °C (corresponding to the average mean annual temperature of all sites) for 2 weeks before the enzyme assay.

1.5.3. Soil aggregate distribution

Soil samples were collected from 60 sites (0-10 cm soil depth) with 5 replicates for each site which represent sub-ecosystem. Rigid containers were used which reduced mechanical stress while carrying soil samples to the laboratory in Kilimanjaro, Tanzania. Avoiding potential aggregate breakdown before analysis and consistency, soils were processed a few days after sampling.

1.6. Analytical methods

1.6.1. Lignin and sugar analysis

The origin, degradation and decomposition of SOM matter was analyzed by using plant based biomarkers i.e. lignin and sugars in the natural ecosystems along 3300 m a.s.l. The sum of vanillyl, S: syringyl and C: cinnamyl V+S+C (ΣVSC) and Acid-to Aldehyde ratios (Ad-to-AL) together with C- or S- to V ratios was calculated from CuO oxidation single phenol compounds. The ratios of (galactose + mannose) / (arabinose + xylose) and (rhamnose + fucose) / (arabinose + xylose) i.e. (GM/AX and RF/AX) was calculated. Further, Non-metric multidimensional scaling (NMDS) ordination of VSC-lignin monomers and extracted sugars was used to separate ecosystems in a two-dimensional vector space. The non-linear trends of VSC-lignin monomers and extracted sugars with elevation were assessed by fitting generalized additive models (GAM).

1.6.2. Analysis of enzyme activities and kinetics

Fluorogenic substrates based on 4-methylumbelliferone (MUF) were used to assess enzymatic activities of β-galactosidase, cellobiohydrolase, phosphatase and chitinase enzymes. Enzymes activities were determined over a range of substrate concentration from low to high (0, 20, 40, 60, 80, 100, 120 μmol g^{-1} soil). The activity of each enzyme was determined in each soil sample at constant room temperature (20 °C) over 2 hours and expressed as MUF release in nmol per g dry soil per hour (nmol g^{-1} h^{-1}). Suspensions of 1 g soil (dry weight equivalent) with 50 mL water were prepared separately for each of the three replicates by using low-energy sonication (40 J s^{-1} output energy) for 2 min [58]. Fifty microliters of soil suspension were added to 50 µL buffer (pH: 6.5) and 100 µL of each substrate solution in a 96-well micro plate. Fluorescence was measured in microplates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm at a slit width of 25 nm, with a Victor3 1420-050 multi label counter (Perkin Elmer, USA). The activity of each enzyme was determined in each soil sample at constant room temperature (20 °C) over 2 hours.

1.6.3. Analysis of stability and aggregate size distribution

Soil samples were collected at 60 sites from top-soil horizons (0-10 cm soil depth) with 5 replicates per site. The ecosystems included savanna, maize, coffee plantation, Chagga homegarden, grassland, lower montane forest, *Ocotea* forest, *Podocarpus* forest, *Erica* forest and *Helichrysum* vegetation.

Dry-sieving method was used for aggregate size distribution analysis. The samples were weighed prior and after oven drying. The soil was pre-heated at 60 ºC for 3 days. Soil Aggregate size distribution was analyzed by using a dry sieve machine (vibratory shaker, Retsch, Vibro GmbH & Co, Germany). The sieve shaker was set at 5 minutes time with 50Hz rpm shaking intensity. Afterwards, the remaining soils on each sieve were weighed and recorded. The aggregates were classified into large macro aggregates (>5-2 mm), small macro aggregates (1-0.25 mm) and as micro-aggregates (<0.25 mm).

1.7. Results and discussion

1.7.1. Effects of land-use change on SOM, enzymes and soil aggregates

The physical, chemical and biological environment of tropical ecosystems have become contentious issue especially with increasing population and is expected to cause unprecedented impacts (Lewis 2006; Lewis, Malhi, and Phillips 2004b). In Kilimanjaro, the population have increased in recent decades for about ten folds (Peters et al. 2019a) and exerted pressure on land resources especially soil (Maro 2013; Salome B. Misana 2012; Soini 2005).

The colline zone of Mt. Kilimanjaro at 700-1000 m a.s.l. and lower montane zones (1000-1800 m a.s.l) experiences strong anthropogenic impacts. Large macro aggregate percentage tended to decrease from 30.6 % in forests to 20.3% in coffee plantations for the lower montane zone. The land-use conversion from coffee-banana plantation at lower montane zone was also recorded recently (Hemp 2009:2009). Soil perturbation through tillage may have an impact on SOM decomposition by exposing the organic matter that was protected physically in micro aggregates to biodegradation (Balesdent, Chenu, and Balabane 2000). This conforms to our findings, where large soil macro aggregates decreased with land-use intensification, and lowest lignin and sugars content occurred in the ecosystems at this elevation zone.

In the colline zone, C and N cycle enzymes of SAV (natural) top- and subsoils had higher maximal activities compared to the hydrolytic enzymes at MAI (agricultural) except phosphatase activity (Figure 3. 1. 4-1). Although the response of land use was enzyme specific, land-use practices controlled the enzyme activities, substrate affinities and catalytic efficiencies topsoils. However, in the subsoils, most of the tested enzymes were not affected by the land-use practice. Higher enzyme activities were observed in top- than subsoils (Figure 3. 1 .4-3). Higher phosphatase activity accompanied with lower K_m values in topsoil of MAI than of SAV may indicate a shortage of available P in cropland soil. Phosphorus is the second most limiting nutrient for plant growth which is commonly deficient, especially in tropical soils due to its immobilization by precipitation. This P limitation is especially pronounced in allophanic soils as such of Mt. Kilimanjaro, whose parent material originates largely from volcanic eruptions (Zech et al. 2014b). Therefore, phosphatase within the colline zone demonstrated that agricultural practices in comparison to the natural ecosystem may cause higher enzymatic efficiency (low K_m values). The changes in enzyme kinetics in response to land-use intensification indicated altered enzyme systems and a possible shift in microbial community composition (Buyer and Drinkwater 1997; Lipson, Schadt, and Schmidt 2002).

Total lignin vanilyl i.e. syringyl and cinnamyl (VSC) and sugars contents in topsoil (0-10 cm) both peaked at mid-elevation ecosystems (2000-3000 m), following the general trend of soil organic C content. The primary source of SOM matter is plant materials incorporated into the various fractions through decomposition. At mid-elevation elevation (~1900-2200 m a.s.l.), climatic condition such as sufficient moisture and optimum temperature with highest plant biomass and net primary productivity of litter inputs were recorded (Becker and Kuzyakov 2018a; Peters et al. 2019). Since SOM forming materials have different turnover rates (Amelung et al. 1999), water-soluble C compounds such as sugar degrade first, followed by lignin (Paul et al. 2019). The physical aspect of SOM is responsible for stabilizing soil aggregate, chemical aspect promotes the binding of the organic matter and maintaining nutrients availability to soil minerals and for microorganisms (Bandick and Dick 1999). Therefore, the labile fraction of SOM forms aggregate especially large soil aggregates (Tisdall and Oades 1982) which have higher enzyme activities than micro aggregate (Dorodnikov et al. 2009). Agricultural land-use intensification at Mount Kilimanjaro such as coffee plantations plant debris is removed during pruning coffee bushes and in maize fields the available litter is normally removed as feeder for livestock (Pabst, Kühnel, and Kuzyakov 2013) and reducing biomass inputs. Future intensification of farming is expected to shape soil aggregate, SOM rate and change in enzyme activities to adapt to the changing environmental conditions.

1.7.2. Effects of elevation on SOM, enzymes and aggregate size distribution

The analysis of soil aggregate size distribution at Mount Kilimanjaro from colline (savanna at 700 m a.s.l) to Alpine ecosystem (*Helicrysum* at 4600 m. a.s.l) showed a bell-shaped trend. Microbial derived sugars significantly decreased at lowest and highest elevation, indicating microbes facing growth limitations e.g. by drought or temperature. The savanna ecosystem experiences drought and increased temperature (Altmann et al. 2002) and *Helichrysum* was also previously shown to be limited by temperature in its biotic activity (Becker and

Kuzyakov 2018a).The ratio of vanillic acid to vanillyl aldehyde (Ac/Al_v), reflecting lignin decomposition status, was lowest at the colline zone. However, low Ac/Al_v ratios were also found in grassland and *Ocotea* forest, suggesting that lignin degradation in these ecosystems is hampered by additional factors other than temperature and drought limitations, e.g. nutrient limitation.

At mid-elevation again, total lignin (vanilyl i.e. syringyl and cinnamyl (VSC)), sugars contents and large macro-aggregate size in topsoil (0-10 cm) displayed their maximum (2000-3000 m), following the general trend of soil organic C content (Figure 2.1.4.2.1). The ratio of microbial-derived to plant-derived sugars, assessed by the (galactose + mannose) to (arabinose + xylose) ratio (GM/AX ratio), was similar at all mid-elevation ecosystems and had a ratio below 1 of the savanna and alpine *Helichrysum* ecosystem. This suggests that both ecosystems are limited in their microbial decomposition and microbial residue accumulation (Figure 2.1.4.3.2**)**. It is know that plant derived sugars are characterized by a GM/AX ratio <0.5, while a GM/AX ratio >2 indicates microbial origin (Gunina and Kuzyakov 2015a; Tisdall and Oades 1982).

The relative increase in enzyme activities with elevation was highest for phosphatase (> 4fold in topsoil) (Fig. 6). The activity of C cycle-related enzymes showed significant differences in top- and subsoil of SAV, but no differences between top- and subsoils in FLM. The higher aboveground biomass on FLM as compared with SAV (Ensslin et al. 2015a), maximum primary productivity and litter decomposition rates in natural ecosystems contributed to the gradual increase of biomass from colline to lower montane zone (Becker and Kuzyakov, 2018). The higher vegetation biomass and C input in turn may have stimulated activity of soil microorganisms resulting in higher enzyme activities in soils of forests comparing to savanna (Balota et al. 2004) at higher and lower elevation, respectively.

1.7.3. Summary of objectives, main conclusions of the studies

Table 1.7.3: Summarized table of objectives and main conclusion of the studies for the PhD project.

Study	Objectives	Main conclusion
Study 1 Soil lignin and sugar composition along a 3300 m elevation gradient of Mount Kilimanjaro	• To analyze lignin and neutral sugar monomers in topsoil (0-10 cm) to unravel the impact of vegetation type, precipitation and temperature on SOM origin and degradation status along a 3300 m elevation gradient.	▪ Different compositions of SOM in Kilimanjaro ecosystem can be used to elucidate the sequence of processes of SOM transformation occurring from the savannah towards the alpine *Helichrysum* ecosystems. ▪ Neutral sugars, which represent a mixed substance class sugars of predominantly plant origin and such of predominantly microbial origin are suitable indicators to identify abiotic limitations in SOM transformations. ▪ The GM/AX ratio below 1 of the savanna and alpine *Helichrysum* ecosystem suggest that both ecosystems are limited in their microbial decomposition and microbial

		residue accumulation ▪ The very low $(Ac/Al)_v$ ratio of the FOC ecosystem showed that the lower fungal abundance in this ecosystem is likely to cause a strongly reduced oxidation of the central vanillyl aldehyde monomers
Study 2 Effects of land-use and elevation on the functional characteristics of soil enzymes at Mt. Kilimanjaro	• To test how the catalytic properties of four enzymes involved in the C, N and P cycles vary between a set of land use and management practices and to assess under which land use intensification these enzymes' Vmax and Km exhibit maximal changes	✓ Land-use practices control the enzyme activities, substrate affinities and catalytic efficiencies in two soil depths (0-10 and 20-30 cm). ✓ Catalytic properties of enzymes vary along a land-use gradient where maximal enzyme activity and substrate affinity exhibit severe changes. ✓ Impact of land use on enzyme activities decreases with soil depth. ✓ Constant or smaller K_m accompanied with higher catalytic efficiency could be an indicator of altered enzyme expression due to a major shift in species dominance

		under anthropogenic activities ✓ The soil microorganisms are capable to maintain their full range of biological functions and ecosystem services (e.g. alter enzyme systems with low, high or constant substrate affinity within wide range of land-uses to ensure efficient enzymatic functioning under varying condition.
Study 3: Effects of land-use change and elevation gradient on soil aggregate size distribution on Mount Kilimanjaro.	• To analyze the impact of land-use and changing climatic conditions as factor of elevation gradient on soil aggregate size distribution on top soils of Mt. Kilimanjaro.	✓ Large macro aggregates were less affected by both land-use and elevation gradient in comparison to micro aggregates in all vegetation zones ✓ The anthropogenic factors were amplified on the colline zones while at lower montane zones, the effects of abiotic factors predominated. ✓ Large macro aggregates were sensitive to disturbances and reduced the productivity of the soil for agricultural use and increased possible shift of land use from natural to agricultural use.

1.8.Conclusions

Soil organic matter is formed in soils from either plants, microbes or animals residues. They undergo decomposition by soil micro fauna and micro flora which involves biochemical processes such as exoenzymatic decomposition controlled by biological, chemical and physical factors. These factors such as moisture, temperature and nutrients supply plays role in supporting enzyme activities which break down the organic matter and aggregate formation. In this study, total lignin (VSC) and sugars contents in topsoil (0-10 cm) both peaked at mid-elevation ecosystems (2000-3000 m) and corresponded to the general trend of soil organic C. This trend was shaped by drought and temperature limitation on colline (SAV & MAI) and alpine *Helichrysum* (HEL), respectively, and resulted in lowest lignin and sugar contents in these ecosystems. The land-use intensification and the inhibition effects of enzymes activities and efficiencies were vividly shown again in the colline zone compared to other ecosystems. Besides, the aggregates stability (large macro-aggregates) was affected by land-use intensification due to lower organic matter contained in arable soils than natural ecosystem. Therefore, less organic matter inputs due to agricultural practices, inhibition of enzymes activities and reduced soil aggregation reduces the quality and functions, especially of managed soils of Mount Kilimanjaro’s ecosystem. As climatic factors especially temperature and drought controlled SOM degradation and decomposition, enzymes activities and kinetics and aggregates size and stabilization, it is most likely that climate change will have a massive impact on many of these processes affecting SOM dynamics. Besides the climate change impact on the biochemical cycles and soil fertility, there is a high risk the Mt. Kilimanjaro’s soils will lose their function as carbon sink.

1.8.1. Contribution to studies

The studies involved various co-authors who contributed at different capacities, below is their contributions for each of the studies included in this thesis;

Study 1: Soil lignin and sugar composition along a 3300 m elevation gradient of Mount Kilimanjaro

Status: Manuscript. In preparation

2. Authors	3. Contribution
Emanueli Ndossi	Field work, laboratory work, interpretation of data, writing
Sandra Spielvogel	Study design, commenting
Joscha Becker	Field work, data analysis, commenting
Michaela Dippold	Commenting, interpretation
Yakov Kuzyakov	Commenting, study design

Study 2: Effects of land-use and elevation on the functional characteristics of soil enzymes at Mt. Kilimanjaro

Status: Submitted, under review since 28.02.2019.

Authors	contribution
Emanueli Ndossi	Field work, laboratory work, data analysis, and writing.
Joscha Becker	Data analysis, commenting
Andreas Hemp	Commenting
Michaela Dippold	Data analysis, Commenting.
Yakov Kuzyakov	Commenting, study design

Bahar Razavi	Study design, laboratory work, commenting

Study 3: Effects of land-use change and elevation gradient on soil aggregate size distribution on Mount Kilimanjaro

Status: Manuscript in preparation

Authors	Contribution
Emanueli Ndossi	Field work, laboratory work, data analysis, writing
Joscha Becker	Data analysis, commenting.
Michaela Dippold	Commenting.
Yakov Kuzyakov	Study design, commenting.

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2. Publications and Manuscripts

2.1. Study 1: Soil lignin and sugar composition along a 3300 m elevation gradient of Mount Kilimanjaro

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2.1.1. Abstract

The chemical composition of soil organic matter (SOM) is a key factor affecting SOM stabilization and decomposition. Especially along altitudinal gradients, complex interactions of changing litter input and abiotic conditions (e.g. temperature and water availability) as well as changing microbial communities and their residues are affecting SOM dynamics and consequently ecosystem C and nutrient cycling.

We used a biomarker-based approach to elucidate the origin and decomposition stage of SOM along the unique elevation gradient (from 900 to 4200 m a.s.l) of Mt. Kilimanjaro. The quantity and quality of lignin and sugars were assessed in seven natural ecosystems with increasing elevation: savanna, grassland and lower montane forest, cloud forests (*Ocotea* and *Podocarpus* dominated), sub-alpine *Erica* forest and alpine *Helichrysum*. Total lignin vanilyl i.e. syringyl and cinnamyl (VSC) and sugars contents in topsoil (0-10 cm) both peaked at mid-elevation ecosystems (2000-3000 m), following the general trend of soil organic C content.

The ratio of microbial-derived to plant-derived sugars, assessed by the (galactose + mannose) to (arabinose + xylose) ratio (GM/AX ratio), was similar at all mid-elevation ecosystems. The share of microbial derived sugars significantly decreased in lowest and highest ecosystems (savannah and *Helichrysum*), where microbes are facing growth limitations e.g. by drought or temperature. In these two ecosystems, the ratio of vanillic acid to vanillyl aldehyde (Ac/Al_v), reflecting lignin decomposition status, was lowest as well. However, low Ac/Al_v ratios were also found in grassland and *Ocotea* forest, suggesting that lignin degradation in these ecosystems is hampered by additional factors e.g. nutrient limitation.

In summary, this study assessed the impacts of temperature and precipitation change on SOM stocks and composition along an Afromontane elevation gradient to provide a profound understanding of abiotic and biotic factors affecting SOM stabilization and turnover processes.

Key word: soil organic matter, lignin monomers, plant and microbial neutral sugar monomers, biomarker composition, mountain ecosystems.

2.1.2. Introduction

Soil organic matter (SOM) contains a complex and heterogeneous mixture of carbon (C) compounds. These organic matter inputs originate largely from plants, microbes and partly from animal residues (Creamer et al. 2016). The SOM is central for many functions related to soil fertility, i.e. it hosts large proportion of the cation exchange capacity, it controls and buffers the soil pH, it forms and stabilizes soil structure and thus increases the water holdging capacity and permeability. Moreover, it contains a high portion of nutrients, released and cycled by the microorganisms (Balaria and Johnson 2013). SOM plays an ecosystem overarching role by its potential for C sequestration (Trumbore 1997). Many biotic and abiotic factors control the quantity and quality of SOM, such as vegetation type, temperature, water availability as well as soil texture and mineralogy. Specific soil characteristics (e.g.

andic properties), can further control SOM composition by incorporating incompletely degraded litter inputs into stabile organo-mineral complexes and thus promote SOM stabilization across ecosystem scales. Therefore, SOM composition can strongly vary between ecosystems, reflecting specific input, turnover and stabilization mechanisms (Becker, Dippold, Hemp, & Kuzyakov, 2019).

In order to identify the sources and transformation products of SOM, biomarkers became a highly popular tool (e.g. Amelung et al. 2008; Balaria and Johnson 2013). Biomarkers are organic compounds with defined structures which can be directly linked to their biotic origin (Amelung et al. 2008). Classical plant-derived biomarker are free and bound lignin derived phenols both carrying information about rather hardly degradable plant-derived input into soils (Spielvogel et al. 2014). In the biosphere, lignin, a rather persistent component of plant input, is the second most abundant polymer following cellulose (Abiven et al. 2011; Otto et al. 2005; Thevenot et al. 2010). Lignin monomers originate from higher plants i.e. angiosperms and gymnosperms with woody, lignified tissue (Duboc et al. 2014; Hedges and Ertel 1982) and thus roots frequently have a higher lignin content than shoots and leaves (Amelung et al. 1999; Gunina and Kuzyakov 2015; Thevenot et al. 2010). By alkaline copper oxide (CuO) oxidation, phenols are released from the reactive sites of the lignin macromolecule. Besides using these phenols, the lignin monomers, to trace SOM input, the acid to aldehydes ratio (Ad/Al) of vanilyls, syringyls and cinnamyls explains the degradation stage of the lignin (Duboc et al. 2014; Hedges and Ertel 1982b; Otto et al. 2005).

Sugars are the basic component of all polysaccharides and thus make up for 50-70% of plant dry mass (Gunina and Kuzyakov 2015b). Therefore, plant litter and root exudates are the primary source for sugars in SOM followed by microbial synthesizes as secondary source such as extracellular polysaccharides forming microbial biofilms (Gunina and Kuzyakov 2015b). Although this separation is not highly specific, plant-derived sugars are arabinose and xylose dominated whereas microbial sugars show a dominance of galactose and mannose, leading to the use of the GM/AX ratio as a proxy for primary plant-derived input to microbial transformation products (Prietzel et al. 2013).

We, used the GM/AX ratio and acid to aldehydes ratio (Ad/Al) of vanilyls as indicators to elucidate the driving factors (i.e. input vs. degradation) of SOM composition along an altitudinal ecosystem gradient at Mt. Kilimanjaro. All soils along this transect developed on similar parent material (Dawson 1992b) and cover a wide range of climatic conditions and vegetation types, providing a unique potential to use Mt. Kilimanjaro as a model location for comparing elevation effects on SOM dynamics in Afromontane ecosystems. The area experience bimodal rainfall pattern with mean annual precipitation between 663 mm to 2500 mm per year and mean annual temperature from 4.5 °C to 23.7 °C (Appelhans et al. 2016).

Our objectives were to analyze lignin and neutral sugar monomers in topsoil (0-10 cm) to unravel the impact of vegetation type, precipitation and temperature on SOM origin and degradation status along a 3300 m elevation gradient. We hypothesized that; (i) elevation determines SOM origin and degradation status in correspondence to climatic factors i.e. precipitation and temperature. (ii) Abiotic limitations for microbial activity and growth (i.e. water scarcity and temperature) are key regulators on the transformation of lignin as well as on the microbial contribution to the SOM pools.

2.2. Methods

2.2.1. Study area

The study was conducted at the southern slope of Mt. Kilimanjaro in northeastern Tanzania (3°4’33’’S; 37°21’12 E). Seven plots were selected along an elevation gradient from 951 to 4190 m a.s.l. The colline savanna (SAV) is located below 1200 m a.s.l and is characterized by a co-occurrence of *Acacia* spp. trees and shrubs in a C4 grassland (Becker et al. 2016).

Grasslands (GRA), located on the lower montane zone (1660 m a.s.l.), are formerly forest areas that are now used for grazing and mowing (O'kting'ati et al. 1985). The lower montane forest (FLM) at 1920 m a.s.l. is dominated by *Macaranga kilimandscharica, Agauria salicifolia* and some *Ocotea usambarensis* (Hemp 2006). The *Ocotea usambarensis* and *Cyathea manniana* are common at 2120 m a.s.l forest ocotea (FOC). At an elevation of 2850 m a.s.l, (FPO) *Podocarpus latifolius*, *Prunus Africana* and *Hagenia abyssinica* dominate (Hemp, 2006). The sub-alpine erica forest (FER) is located at around 4000 m a.s.l. In the alpine area (HEL) at around 4200 m a.s.l. *Helichrysum cussion* vegetation occurs in combination with tussock grasses (Ensslin et al. 2015b). All ecosystems above 1800 m (i. e. FLM, FOC, FPO, FER and HEL) are located within the Kilimanjaro National Park (KINAPA) and under protection. Further details on vegetation characteristics in the area were described by (Hemp 2006).

Mount Kilimanjaro soils were classified as Andosols with folic, histic or umbric topsoil horizons with wide and thick organic layer (Schrumpf et al. 2006) for the lower montane zone of the ecosystem, while on the colline zone the soil is classified as Vertisol (Becker et al. 2019).

2.2.2. Soil sampling

The samples were taken from previously established research sites of the German Research Foundation (DFG) Project '*Kilimanjaro ecosystem under global change'*. The size of the plot squares ranged between 0.25 and 1 ha. In each plot area, we established four sub-plots (5 x 5 m) in the plot corners and took mixed samples from the topsoil layer (0-10 cm) at each sub-plot. All samples were mixed thoroughly and all visible debris and coarse fragments were removed, followed by air drying, sieving to less (2 mm sieve size) and ground.

2.2.3. Laboratory analyses

Analysis of lignin phenols by use of alkaline CuO oxidation followed the procedure of (Hedges and Ertel 1982). In summary, soil polyphenols and lignin polymers are split into monomers and quantified by gas chromatography (Hedges and Ertel 1982). Soil samples were mixed with 15 ml 2 M NaOH, 250 mg CuO, 50 mg $Fe(NH_4)_2(-SO_4)_2.6H_2O$ and 50 mg glucose and digested in pressure bomb at 172 °C for 2 hours in a N_2 atmosphere to convert soil polyphenols and lignin to monomers. To control for losses during preparation, Ethylvanillin was added and the solution was adjusted to pH 2 by using 6 M HCl. The acidified solution was extracted for lignin phenols using conditioned C_{18} column (Spielvogel et al. 2007). Agilent 7820A GC system equipped with a flame ionization detector and Optima® 17 MS column (Macherey Nagel, Dueren, Germany; phenylmethyl polysiloxane, 50% phenyl, 30 m × 0.25 mm inner diameter with 0.5 µm film thickness) was used. A sample volume of 1 µl was injected with split ratio of 33:1 and the injector temperature was set to 250 °C. The temperature programme started at 100 °C (isothermal for 0.5 min) and increased to 160 °C at 10 °C min^{-1}, then held for 6 min. Subsequently, the oven temperature was increased by 20 °C min^{-1} to 250 °C, and again by 50 °C min^{-1} to the final temperature of 300 °C, which was held for 5 minutes. Further details regarding the method and general set up for the system is available in (Spielvogel et al. 2007).

Non-cellulosic neutral sugars concentration of arabinose, fucose, galactose, glucose, mannose, rhamnose, ribose, and xylose were analyzed by gas chromatography (7820A GC, Agilent) following the setting of Prietzel et al. (2013). The sum of V+S+C and the acid-to aldehyde ratio of vanilyl units (Ad-to-AL) was calculated from the single phenol compounds i.e.: vanillyl, S: syringly and C: cinnamyly products. The ratios of (galactose + mannose)/ (arabinose + xylose) and (rhamnose + fucose)/(arabinose + xylose) i.e. (GM/AX and RF/AX) was calculated from the neutral sugar monomers.

2.2.4. Statistical analysis

Our statistical analyses followed the approach for Kilimanjaro ecosystems by (Peters et al. 2019b). To account for possible non-linear relationships we used a non-metric multidimensional scaling (NMDS) ordination of vanilyl, syringyl and cinnamyl (VSC-lignin monomers) and extracted sugars to separate ecosystems in a two-dimensional vector space. In this ordination method, closer ecosystems share a larger percentage of compound species and species loadings on each axis represent their effect direction.

The non-linear trends of VSC-lignin monomers and extracted sugars with elevation were assessed by fitting generalized additive models (GAM).

Ecosystem specific differences in Ac/Al_v and GM/AX ratios were assessed by one-way analysis of variance (ANOVA). Variance homogeneity between groups was tested prior to analysis (Levene's test at p-level = 0.05) and for each model normality of residues was tested by Shapiro-Wilk-Test (p-level = 0.05). Additional relationships between single variables were assessed by regression analysis and Pearson correlation (p level < 0.05).

All statistical analyses were conducted in R v3.5.0 (R Core Team 2018) using 'mgcv' package for GAM computing (Wood 2017), 'vegan' packages for NMDS (Oksanen et al. 2018) and 'ggplot2' for data visualization (Hadley 2016).

2.3. Results

2.3.1. Lignin and sugar composition

Non-metric multidimensional scaling (NMDS) of all ecosystems was performed for sugar and lignin monomers. Both biomarker compounds enabled separation of most of the ecosystems but with differences in distances between ecosystems. The ecosystems without dominance of woody plants (SAV, GRA and HEL) could not be separated based on their lignin monomer composition. However, the four forest ecosystems FLM, FOC, FPO and FER were completely separated based on their lignin monomer composition (Figure 1). Whereas this separation occurred along the 2nd axis, the 1st axis mainly separated forests from non-woody ecosystems. Neutral sugar monomers, however, enabled a partial separation of HEL, SAV and GRA but no precise separation of grasslands and forests ecosystems. Although the forest ecosystems FLM and FER differ distinctly with respect to their elevation, could not be separated based on their neutral sugar monomer composition. In contrast, all grasslands, FLM and FER separated well along the 1st axis from FOC and FPO and this separation is mainly based on the microbial sugars (Rhamnose, Fucose, Ribose) (Figure 1).

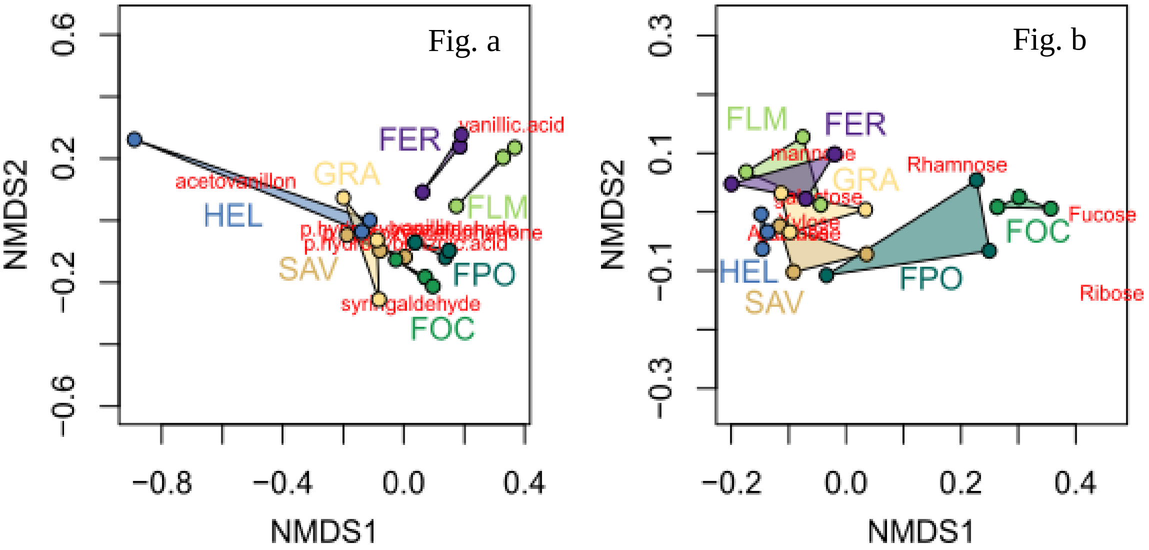

Figure 2. 3.1: Non-metric multidimensional scaling (NMDS) of VSC-lignin monomers (a) and extracted sugars (b). Ordination of ecosystems (points) indicates the range of compound (red) composition. Closer ecosystems share a larger percentage of compounds. Ecosystem are indicated as: SAV, savannah; GRA, grassland; FLM, submontane rainforest; FOC, *Ocotea* forest; FPO, *Podocarpus* forest; FER, *Erica* forest; HEL, alpine *Helichrysum*.

2.3.2. Effects of elevation gradient on lignin contents and composition

The lignin monomer content of vanilyl, syringyl and cinnamyl (VSC) increased from lower elevation to mid elevation (900 to 2000 m a.s.l.) and then decreased again (Figure 2. 3.1-1a), thus reflecting the tree biomass abundance along this ecosystem gradient. The maximum VSC content was observed in the soils of the FOC ecosystem at an elevation of 2120 m. The soils of the highest ecosystem (alpine *Helichrysum* (HEL) and the lowest ecosystem (savanna (SAV)) were characterized by the smallest amounts of VSC (Figure 2. 3. 1-1a). This pattern was inverse when related to soil organic carbon (SOC) content (Figure 2. 3. 1-2b). This results from a strong correlation of the lignin contents with the SOC contents (pearson r = 0.96, $p < 0.001$). High input of woody plant material at mid-elevation ecosystems was not reflected by a high relative proportion of lignin in its SOC and rather vice versa by rather low proportion of lignin monomers in its SOC.

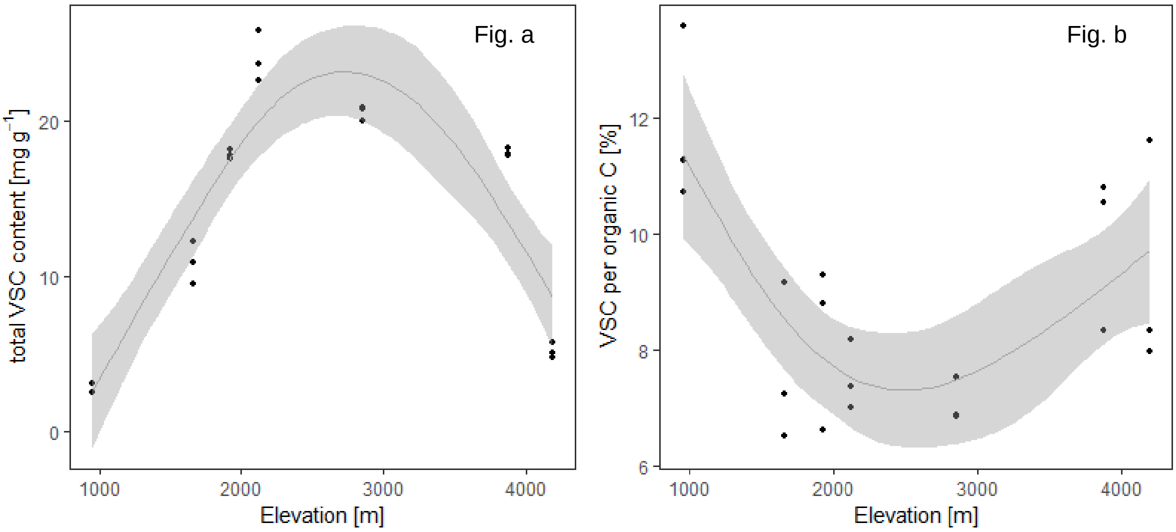

Figure 1. 3. 1-1. Total VSC-lignin content vs. elevation (a) total VSC-lignin per SOC vs. elevation (b), with generalized additive model (GAM) fits. Grey areas indicate 95% confidence intervals.

The lowest acid to aldehyde ratios of vannillyl (Ac/Al_v) is in the lowest and highest ecosystems SAV, GRA and HEL, despite these ecosystems are not forested and thus have a relatively low input of woody material but also highest relative proportion of lignin monomers relative to SOC. In general, forest ecosystems displayed much higher Ac/Al_v ratios than grassland ecosystems, ranging from 2-4, which imply much higher oxidation of the lignin side groups. FOC has the clear exception with an Ac/Al_v of close to 1 in the range of non-woody ecosystems.

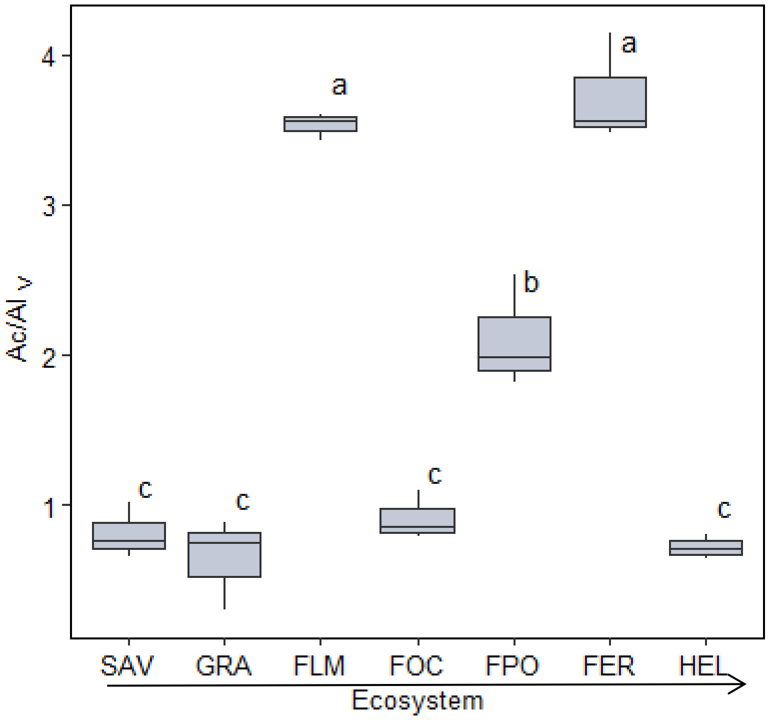

Figure 2. 2. 2-2. Boxplot of acid-to-aldehyde ratios. Ecosystems are ordered with increasing elevation (left to right), Savanna-Grassland-Lower montane Forest-Cloud forest (Ocotea)-Cloud forest (Podocarpus)-Sub-alpine Forest (Erica)-alpine heather (*Helichrysum*).

2.3.3. Effect of elevation gradient on soil sugars

Elevation caused differences in temperature and precipitation had a strong impact on the total neutral sugar content: neutral sugar contents gradually increased up to mid-elevation and then stepwise decreased again at higher elevation. Both, total lignin and sugar contents peaked at FLM, following the trend of SOC content. The soils of all mid-elevation ecosystems, of the forests but also of the grassland, had comparable GM/AX ratios of 1.2. Only the soils of the ecosystem with the lowest and the ecosystem with highest elevation, SAV and HEL, had significantly lower GM/AX ratios of 1.0 (Figure 2. 3. 3-1). Highest relative neutral sugar contents were found at SAV for the lowest elevation ecosystem, but also FOC and HEL displayed relatively high neutral sugar contents (Figure 2. 3. 3-2).

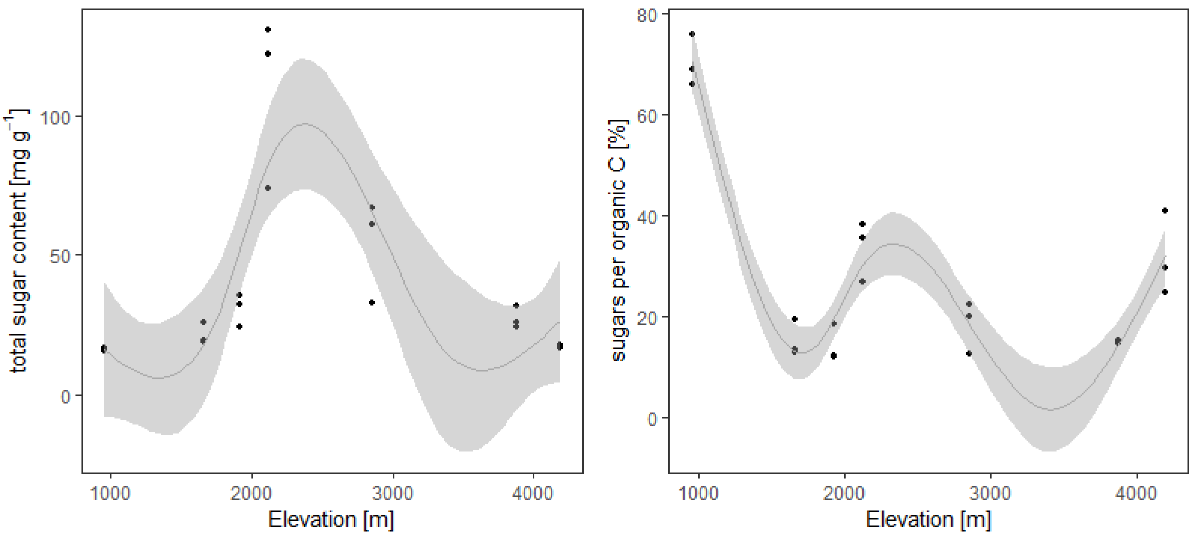

Figure 2. 3. 3-1. Total sugar content vs. elevation (a) total sugar per SOC vs. elevation (b) with polynomial regression, and with generalized additive model (GAM) fits. Grey areas indicate 95% confidence intervalls.

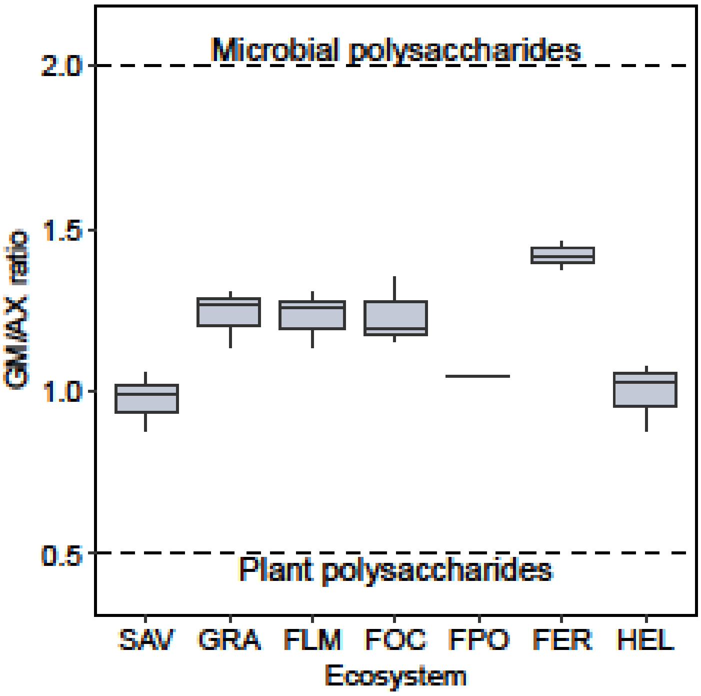

Figure 2. 3. 3-2. Galactose and Mannose to Arabinose and Xylose ratios (GM/AX) in seven Kilimanjaro ecosystems. Ecosystems are ordered with increasing elevation (left to right). Savanna-Grassland-Lower montane Forest-Cloud forest (Ocotea)-Cloud forest (Podocarpus)-Sub-alpine Forest (Erica)-alpine heather (*Helichrysum*)

2.4. Discussion

2.4.1. Lignin content and degradation status along elevation gradient

The total VSC content in soil peaked in the mid-elevation forest ecosystems (Figure 2. 3. 1-1a). This is similar to previously reported trends for SOC contents (Becker et al. 2019; Pabst et al. 2016) and follows the general trend of aboveground biomass and litter inputs at Mt. Kilimanjaro (Becker et al. 2015; Ensslin et al. 2015b). Such pattern reflects the direct control of climatic variables on Kilimanjaro ecosystems. The mid-elevation forests are located within the altitudinal zone of optimal temperature and moisture conditions, with the highest productivity and fast SOM turnover rates (Becker and Kuzyakov 2018). These conditions lead to an overall accumulation of SOC, which is linked to higher total VSC content.

However, SOC and lignin were not linearly related, and VSC contribution to SOC decreased at mid-elevation while VSC contribution to SOC was significantly higher in soils of the lowest (SAV) and highest elevation ecosystems (HEL) (Figure 2. 3. 1-1b). Lignin content in soil represents the balance between above- and belowground lignin input by litter, and its decomposition (Wang et al. 2018). The low and high elevation ecosystems are not forested, thus likely having a lower overall lignin input into their soils. Nevertheless, these two ecosystems showed highest lignin contents in soil, hence, we concluded that that these high lignin contents are rather driven by very low lignin decomposition rates than by high annual lignin input rates. At low elevation (SAV), the decomposition of fresh litter is limited by seasonal drought, thus slowing down lignin degradation. At mid-elevation, high decomposition rates reduce the relative contribution of lignin to SOC and, in consequence, lead to a relative enrichment of microbial compounds, e.g. aliphatic compounds (Becker et al. 2019). At high elevation (FER and HEL), microbial lignin degradation is limited by low temperatures. The low VSC portion in SOC may additionally be related to higher N content (12.4 mg g^{-1}), C content and litter quality at mid-elevation (FOC) (Becker and Kuzyakov 2018).

The $(Ac/Al)_v$ ratios on SAV, GRA, FOC and HEL were similar, despite covering a large variety of ecosystems (non-woody ecosystems and forests) at various elevations along the slope of Mt. Kilimanjaro. Compared to other tropical or alpine elevation transects (Wilcke et al. 2008, Duboc et al. 2014) the forest ecosystems FLM, FPO and FER displayed comparatively high $(Ac/Al)_v$ ratios. Such high vanillyl oxidation ratios were previously reported rather for forest ecosystems of temperate latitudes (Herold et al. 2014; Thevenot et al. 2010). Thevenot et al. (2010) explained the high forest lignin degradation status in these forest ecosystems by more favorable conditions for fungal activity e.g. due to low pH values. Indeed, all forest ecosystems along the elevation gradient display relatively low pH values (Pabst et al. 2013), as potentially explanatory abiotic factor for favorable lignin decomposition. However, this does not explain the specific role of the FOC within the forest ecosystems, displaying an Ac/Al_v ratio similar to the non-woody ecosystems. FOC is the boundary between lower and upper forests, it has high MAP and it is predominated by filmy and epiphytic ferns (Hemp 2001). FOC has the highest C and N contents of the studied ecosystems (12.4 mg g^{-1} and 214 mg g^{-1}, respectively) (Becker and Kuzyakov 2018). Moreover, it is characterized by a significantly lower fine root biomass (Ensslin et al. 2015b). The resulting different nutrient stoichiometry compared to the other studied ecosystems might be a decisive factors leading to an altered decomposer community in the soils of these forests, with a probably lower fungi to bacteria ratio (Becker et al. 2017). Fungi are the key lignin decomposers, especially white rot fungi (Thevenot et al. 2010), and their increased abundance induces high $(Ac/Al)_v$ ratios as found solely in forest ecosystems. Consequently, a relation between a shifts in decomposer community towards bacteria inducing lower lignin degradations in FOC ecosystems seems to be the most probable explanation for the observed phenomenon.

2.4.2. Neutral sugar content and origin along elevation gradient

As ongoing degradation and SOM transformation leads to an increasing formation of microbial-derived compounds in soils (Ludwing et al. 2014), such compound classes that do not solely reflect the transformation of plant-derived compounds (e.g. lignin), but which are of mixed origin allow to reconstruct the ongoing accumulation of microbial transformation products in soils.

Total content of neutral sugars in soil, followed a bell shaped distribution with a peak in soils at the mid-elevation and at least 50% lower contents in soils at the lower and higher elevations. However, similar to lignin the relative contribution of neutral sugars to SOM is inverse to its total content being lowest at the mid elevations. Within the forest ecosystems it is again FOC representing an increased relative proportion of sugars the SOC, an observation which can be attributed to very similar processes as for the lignin content (reduced root litter input, different microbial community composition).

Generally, plant derived sugars are characterized by a GM/AX ratio <0.5, while a GM/AX ratio >2 indicates microbial origin (Oades 1984, Gunina and Kuzyakov 2015). Although soils on SAV and HEL ecosystems had the lowest overall GM/AX ratios, they remain dominated by microbial-derived sugars. In comparison with other high-elevation transects, which show GM/AX ratio of 1.2 to 1.4, GRA, FLM, FOC and FPO reflect a comparatively high contribution of microbial sugars to the Kilimanjaro ecosystem in all elevations. Even if hemicellulose decomposition might be partially slowed down by temperature limitations at high elevations or by drought in savanna ecosystems, these highly fertile and microbial active andosols at the inner tropics of Mt. Kilimanjaro host a highly active decomposer community which contributes to the decomposition of easily accessible substrates and induces an accumulation of microbial-derived polysaccharides. Such high accumulation of microbial polysaccharides was also described by previous studies on Andosols, whose allophones seems to specifically promote the stabilization and accumulation of secondary, microbial polysaccharides (Nierop et al. 2005, Tonneijck et al. 2010).

2.5. Conclusions

This study revealed that the ecosystems along the elevation gradient of Mount Kilimanjaro contain very different compositions of SOM which can be used to elucidate the sequence of processes of SOM transformation occurring from the savannah towards the alpine *Helichrysum* ecosystems. Neutral sugars, which represent a mixed substance class sugars of predominantly plant origin and such of predominantly microbial origin are suitable indicators to identify abiotic limitations in SOM transformations. The GM/AX ratio below 1 of the savanna and alpine *Helichrysum* ecosystem suggest that both ecosystems are limited in their microbial decomposition and microbial residue accumulation.

The very low $(Ac/Al)_v$ ratio of the FOC ecosystem showed that the lower fungal abundance in this ecosystem is likely to cause a strongly reduced oxidation of the central vanillyl aldehyde monomers. However, such reduced fungal abundance within the community structure in FOC does not strongly affect transformation of easily-decomposable substance classes like hemicelluloses, as reflected by similar GM/AX values and similar monomer composition (rhamnose, fucose and ribose dominated) between FOC and FPO forests. Therefore, a combination of biomarker classes, as applied here, allows disentangling shifts in SOM composition along the elevation transect of Mt. Kilimanjaro. This further enhances our understanding of biogeochemical functioning and processes in Afromontane ecosystems, and their potential resilience and reactions to global change. Further biomarkers, such as amino sugars representing microbial residue accumulation, or free and bound lipids representing plant-derived markers of various persistence in ecosystems, provide further potential to deepen these insights into the SOM formation and transformation processes.

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2.8. Appendix A

Table 2. 8: Mean annual precipitation (MAP), Mean annual temperature (MAT) and carbon, nitrogen and pH for the ecosystem in Kilimanjaro. Adopted from (Appelhans et al. 204)[1], ((Gutlein et al 2016)[2], (Pabst et al 2013)[3]

Ecosystem	**Land use type**	**Elevation**	**MAP (mm)** [1]	**MAT**	**Soil pH**	**Soil C (Mg g^{-1})**	**Soil N (Mg g^{-1})**
Savanna (SAV 5)	Natural, disturbed	951	663	23.7	5.38	27.5	2.0
Grassland (GRA 1)[3]	Natural-disturbed	1485	1650	16.5	4.59	148.56	10.05
Lower montane forest (FLM) 1	Natural, disturbed	1920	2257	15.3	4.34	134.8	9.2
Forest Ocotea (FOC 1)	Natural	2120	2500	12.1	3.49	214.6	12.4
Forest Poducarpus (FPO 1)	Natural	2850	2063	9.4	3.83	205.9	10
Forest Erica (FER 0)	Natural	3980	1389	4.5	4.5	137.5	7.6
Hellycrusum (HEL 2)	Natural	4190	1308	4.5	5.2	4.5	2.4

3. Study 2: Effects of land-use and elevation on the functional characteristics of soil enzymes at Mt. Kilimanjaro

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3.1. Abstract

The mobilization of soil nutrients bound in organic matter is largely mediated by enzymes derived from plants, soil microorganisms and animal residues. Land-use change alters important soil characteristics that may affect the activities of soil enzymes. However, mechanistic understanding of how land use and management practices influence the catalytic properties of enzymes in top- and subsoil are still scarce, especially in African ecosystems. We linked catalytic properties i.e. substrate affinity constant (K_m) and maximum reaction rate (V_{max}), determined by Michaelis–Menten kinetics, and to a set of environmental and microbial variables in the soils of a land-use sequence (6 ecosystems) ranging from natural forests to agricultural fields at Mt. Kilimanjaro. The sensitivity of K_m and V_{max} of four extracellular hydrolytical enzymes, β-galactosidase, cellobiohydrolase, phosphatase and chitinase to changing environmental conditions was tested by fluorogenic substrates in topsoils and subsoils. The β-galactosidase activity increased with increasing soil depth. Other extracellular enzyme (cellobiohydrolase, phosphatase and chitinase) activities decreased with depth. The affinity of enzymes to substrates was higher in soils of natural compared to agricultural ecosystems: i.e. higher under forests than under cropland. The activity of β-galactosidase, cellobiohydrolase and chitinase enzyme were highest in lower mountain forest and grassland (less disturbed ecosystems). This indicated that changes in land use and management practice not only affects enzyme activity but also controls enzyme kinetics (K_m and K_a) thus pointing towards the expression of different enzyme systems. Therefore, we conclude that anthropogenic activities result in alteration of C and nutrient cycling by affecting microbial activities and enzymes catalytic properties.

Key words: soil organic matter, microbial activity, natural ecosystems, agricultural practices, subsoil, catalytic efficiency.

3.2. Introduction

Soil enzymes are the mediators of the most biochemical processes that are critical for soil functioning such as nutrient mobilization, element cycling and organic matter decomposition. The assessment of the activities of hydrolases provides information on the status of key reactions that participate in rate-limiting steps of the decomposition and transformation of organic matter [1,2]. Enzyme activities are sensitive to the land-use practice [3] such as tillage [4], fertilization [5] and cropping [6–8]. Generally, land-use conversion alters important soil characteristics that may directly or indirectly affect the activities of soil enzymes, i.e. soil pH, moisture, organic matter content [9,10], nutrient availability [11,12] and the composition of the microbial community [13]. However, mechanistic understanding of how land use and management practices affect the catalytic properties of enzymes in top- and subsoil are still scarce an we are especially lacking data from the tropics.

Changes in substrate availability may indirectly affect the kinetic properties of individual enzymes and the relative abundances of microbes [14]. Climatic conditions, vegetation, crop rotation, cultivation techniques and edaphic factors also contribute significantly to changing substrate availabilities and microbial activities [15]. Any combination of these factors may influence enzyme activities under a given land- use. As the availability of fresh and degradable soil organic matter (SOM) decreases with soil depths, enzyme activities and nutrient mobilization will decline [16]. Thus, enzyme activities in topsoil differ from that in subsoil as a result of scarce resources [17,18]. Removal of crop residues especially from agricultural ecosystems influences the community of both, above- and belowground biomass [19] and enzyme activities [20]. However, how land-use practices control vertical gradients of enzyme systems and efficiency of catalytic reaction steps remains unclear.

Human impact on ecosystems has clearly affected biogeochemical processes [21]. Apart from anthropogenic impacts, enzymes are also affected by abiotic factors especially temperature and soil moisture. Generally, temperature decreases with increasing elevation leading to reduced enzyme activity with elevation [22]. A study by Zheng et al. [16] in montane forest ecosystems in China demonstrated that enzyme activities increased with elevation up to 2000 m a.s.l and afterward decreases from 2000 - 4100 m a.s.l. as affected by mean annual temperature (MAT). Another study in Southern Ecuador related increasing phosphatase activities between 1000 m and 3000 m to an increase in root surface [23].

K_m, a key parameter of enzyme kinetics, determines the binding affinity of the enzyme to substrate. It can also be used as an indicator of the enzyme system [24]. At low temperatures, enzyme structures can ensure a fast rate of catalytic reactions at high binding affinity (i.e. low K_m) [25,26]. With temperature increase, the ability of enzymes to maintain binding conformations decreases, reducing the affinity to the substrate. This can be measured as an increase of K_m with an increase in temperature [27]. Therefore, colder temperatures are favorable for enzymes with higher substrate binding affinity [28]. Thus, if the ability of an enzyme to bind a substrate in appropriate geometry is perturbed, this can be measured as K_m increase [29]. However, this concept was established in short-term controlled systems and needs to be tested under *in situ* conditions, considering the great functional redundancy of microorganisms and their enzyme systems at the ecosystem scale. Due to functional redundancy [30], high catalytic efficiency (determined as V_{max}/K_m) is maintained in soils by numerous enzymes with different temperature optima mediating similar functions [31].

Despite intensive discussions on mechanisms of the enzyme sensitivity or controlling factors, it remains unclear how the functional characteristics of enzymes in soil will be altered with a gradual elevation increase (going along with a temperature decrease) depending on land use. Besides, there is a lack of studies on the affinity and catalytic efficiency of soil enzymes as affected by land use and management practices.

Here, we used Mt. Kilimanjaro with a very broad range of natural and agricultural ecosystems at elevation gradient of approximately 950 - 2000 m a. s. l. to study the impact of temperature and water availability for various land uses on the enzymes kinetics as a key regulator of nutrient cycling. In Kilimanjaro, agricultural land-use intensification has increased in the past 50 years as a result of increasing population and food demand [32]. Arable soils are consequently modified by mechanization, mineral fertilization and intensive use of pesticides for higher crop yields and pest and disease control [33]. Converting natural to agricultural ecosystems or increasing the intensity of land use results in microbial community shifts and consequently leads to alteration in microbial functions.

For instance, a study in Kilimanjaro [34] showed a general trend of reduced enzyme activity under intensive land use or after converting natural ecosystem to arable land due to fertilizer and xenobiotic (i.e., pesticides) input [35]. This is widely interpreted as a negative anthropogenic effect on microbial activity and as an index for soil health. Here, based on prior studies on digestive enzymes [36], we hypothesized that agricultural land-use intensification leads to the microbial production of enzymes with higher efficiencies (low K_m values) instead of higher enzyme expression, i.e. reducing metabolic costs for a certain catalytic activity. This study was designed to test how the catalytic properties of four enzymes involved in the C, N and P cycles vary between a set of land use and management practices and to assess under which land use intensification these enzymes’ Vmax and Km exhibit maximal changes.

We hypothesized that i) agricultural practices in comparison to natural ecosystems will cause lower activity (V_{max} decreases) of enzymes with higher affinity (K_m decrease) independent of elevation; this effect will be stronger in top- than in subsoils. ii) enzyme activity declines and enzyme systems will be altered towards higher affinity (K_m decrease) with increasing soil depth independent of land-use practice; and iii) K_m values will decrease with elevation indicating a fast rate of conformation change when converting a substrate to a product at higher elevation.

To test our hypotheses, we determined the kinetics of four enzymes involved in decomposition of soil organic matter in the top- and subsoil in a range of land-use practices at two elevation zones (between 950 and 2000 m. a. s. l) on Mt. Kilimanjaro: (1) cellobiohydrolase which is commonly measured as enzyme responsible for consecutive stages of cellulose degradation [37]; (2) β-galactosidase which is responsible for breaking down complexes organic compounds with polyols such as glycerol, pectins or mannitol; (3) acid phosphatase (phosphomonoesterase) catalyzing the hydrolysis of O-P bonds into phosphate under acidic conditions [38,39]; and (4) poly-N-acetyl-glucosaminidase (chitinase) that accomplishes the decomposition of chitin to low molecular weight chitooligomers [18] in top- and subsoils in a range of land-use practices at two elevations in Mt. Kilimanjaro.

3. 3. Material and method

3.3.1. Study area

The study was conducted at the southeastern slope of Mt. Kilimanjaro (3°4′33″S, 37°21′12″E), Tanzania. Samples were collected from six ecosystems, (semi-) natural and agricultural (intensive and extensive) ones. Soils at Mt. Kilimanjaro were classified as Andosols with folic, histic or umbric top horizons in the lower montane zone and Vertisols in the colline zone. All these soils developed from similar parental material: volcanic ashes and their erosion deposits.

The sites were divided into two categories depending on their elevation. First, colline zone ecosystems; semi-arid savanna (SAV) and maize fields (MAI) between 700-1100 m a.s.l. Natural savanna woodlands (SAV) are characterized by a sparse tree and shrub layer consisting of *Ozoroa insignis, Sclerocarya birrera, Boswellia neglecta*, several *Grewia, Combretum* and *Acacia* species and a herb layer dominated by grasses such as *Heteropogon contortus, Themeda triandra* and *Hyparrhenia* species [40] and experienced irregular grazing by livestock (mainly goats) and cutting [41]. Maize fields (MAI) are characterized by the application of fertilizers, herbicides and intensive ploughing [42].

The second elevation class was the lower montane zone (1275 - 1920 m a.s.l.); including lower montane forests (FLM), Chagga home gardens (HOM), grasslands (GRA) and coffee plantations (COF) ecosystems. The FLM area (above 1800 m) is protected by Kilimanjaro National Park (KINAPA) and experienced minor disturbance by firewood collection [34]. The dominant tree species include *Macaranga kilimandscharica, Agauria salicifolia* and some *Ocotea usambarensis* [43]. Chagga HOM is a special type of smallholder agroforestry owned and managed by the Chagga people [41]. HOM are an extensive land-use form with a multi-layered use of several strata including some tree species i.e. *Cordia africana, Albizia schimperiana, Persea americana, Grevillea robusta,* as well as coffee (*Coffea arabica*) and bananas (*Musa spp*) in the shrub layer and e.g. sweet potatoes (*Ipomoea batatas*), maize (*Zea mays*) and beans (*Phaseolus culgaris*) on the ground [34,44]. Grasslands (GRA) are formerly forested areas that are kept open for livestock feeding (grazing or cutting) [45,46]. Commercial coffee plantations (COF) are an intensive monoculture practice involving mechanization, fertilizer and pesticides application [47]. Additionally, COF ecosystems also involved removal of pruned coffee bushes and the most intensive application of agrochemicals. In general, the MAI and COF are intensively used ecosystems; SAV, GRA and FLM are semi natural while HOM is a sustainably managed agroforestry ecosystem [48].

To generalize the intensity of management practices, we used a previously proposed land-use index (including annual biomass removal, input of fertilizers and pesticides, vegetation structures and surrounding land-use type), and ranked our sites accordingly [49,50]. Further information about study site history and other relevant information can be found in [42, 48, 51–53].

The heavy rainfall season at Mt. Kilimanjaro is between March to May with short seasonal rainfall during October to December, so generally showing a bimodal pattern [43, 49]. The temperature and rainfall is influenced by altitude following the intertropical convergence zone. Mean annual precipitation is 663 m to 2,500 mm and mean temperature between 15.3 to 23.7 C°. Site specific details for climate and soil characteristics are highlighted in Table 2.8.

3.3.2. Soil sampling and preparation for experimentation

Soil sampling was conducted on the established research sites of the German Research Foundation (DFG) Project *Kilimanjaro Ecosystems under Global Change*. The size of the plot squares was between 0.25 and 1 ha. We established sub-plots (5 x 5 m) in three plot corners and took mixed samples from each sub-plot. Therefore, we collected 5 samples from 3 corners of the plot, mixed the sample to obtain 3 samples per each plot in two depths. The sampling depths were 0-10 cm and 20-30 cm with three replicates each. After sampling, the soils were frozen (-20 °C) and transported to the University of Gottingen.

The frozen samples were thawed at 4 °C for 1 day and then were pre-incubated at 20 °C (corresponding to the average mean annual temperature of all sites) for 2 weeks before the enzyme assay. Because freezing may affect enzyme activities [54,55], all samples were frozen similarly, and they were pre-conditioned after thawing. We therefore assumed that this pretreatment made freezing effects negligible or at least similar after the pre-incubation [27,54]. Furthermore, we assume that microbial communities established under a certain temperature regime adapt their enzyme systems to the temperature regime of their ecosystem – and thus still reflect an impact of their ecosystem MAT even if pre-incubated at identical temperature. In combination with original enzymes still present in soil from natural conditions, we expect a remaining ecosystem MAT effect on our enzyme kinetics, although we might underestimate it.

3.3.3 Enzyme Kinetics

Fluorogenic substrates based on 4-methylumbelliferone (MUF) were used to assess enzymatic activities to detect β-galactosidase, cellobiohydrolase, phosphatase and chitinase activity. Saturation concentrations of fluorogenic substrates were determined in preliminary experiments. We determined enzymes activities over a range of substrate concentration from low to high (0, 20, 40, 60, 80, 100, 120 μmol g^{-1} soil). Following Marx et al. and Razavi et al. [56,57], suspensions of 1 g soil (dry weight equivalent) with 50 mL water were prepared separately for each of the three replicates by using low-energy sonication (40 J s^{-1} output energy) for 2 min [58]. Fifty microliters of soil suspension were added to 50 µL buffer (pH: 6.5) and 100 µL of each substrate solution in a 96-well micro plate (PureGrade, Germany).

Fluorescence was measured in microplates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm at a slit width of 25 nm, with a Victor3 1420-050 multi label counter (Perkin Elmer, USA). The activity of each enzyme was determined in each soil sample at constant room temperature (20 °C) over 2 hours. Enzyme activities were expressed as MUF release in nmol per g dry soil per hour (nmol g^{-1} h^{-1}). The assays of each enzyme at each substrate concentration were performed in three analytical replicates (12 wells in the micro plate).

3.3.4. Calculations and statistical analyses

The Michaelis-Menten equation was used to define the key parameters of enzyme activity:

$$V = V_{max} \times [S] / K_m + [S]$$

Where, V_{max} is the maximum rate of enzymatic activity at a given temperature; K_m is the half saturation constant, or the substrate concentration at $\frac{1}{2}V_{max}$, and [S] is the substrate concentration [59]. Kinetics constants for the four enzymes were estimated by using nonlinear mode on Sigma Plot 12.5 (Systat Software Inc.). Katalytic efficiency K_a of the enzymes was calculated dividing V_{max} by K_m.

We used a linear mixed effect model (LME) to test for significant differences of V_{max} and K_m between ecosystems and depths ($p<0.05$), with Tukey HSD post-hoc correction. ‘Ecosystem’ and ‘Depth’ were included as interacting fixed factors, with ‘Depth’ being nested in ‘sampling location’ (random factor) to account for the spatial dependency of depth effects. For all comparisons, variances between groups were homogeneous (Levene’s test, $p<0.05$). All results are given as means and standard errors (± SE). All statistical tests were conducted in R 3.5.0 [60] using ‘nlme’ package for LME computing [61] and ‘ggplot2’ for data visualization [62].

3.4. Results

3.4.1. Effect of land use and depth on enzyme activity (V_{max})

Patterns of enzyme activity in response to land-use practices were enzyme specific: In the colline zone, C and N cycle enzymes of SAV (natural) top- and subsoils had higher maximal activities compared to the hydrolytic enzymes at MAI (agricultural) (Fig. 1). The exception was phosphatase activity, where MAI showed up to four times higher activities than SAV. Generally, we observed higher enzyme activities in top- than subsoils. Only under maize cultivation cellobiohydrolyse had similar activities in top- and subsoil. For phosphatase in SAV the relation was even inverse with twice the activity in sub- than topsoil. Chitinase and cellobiohydrolase, both enzymes breaking down central SOM pools with high contents in natural ecosystems (cellulose and chitin) had significantly lower activities in the MAI compared to the SAV topsoils (Figure 3. 4. 1-1).

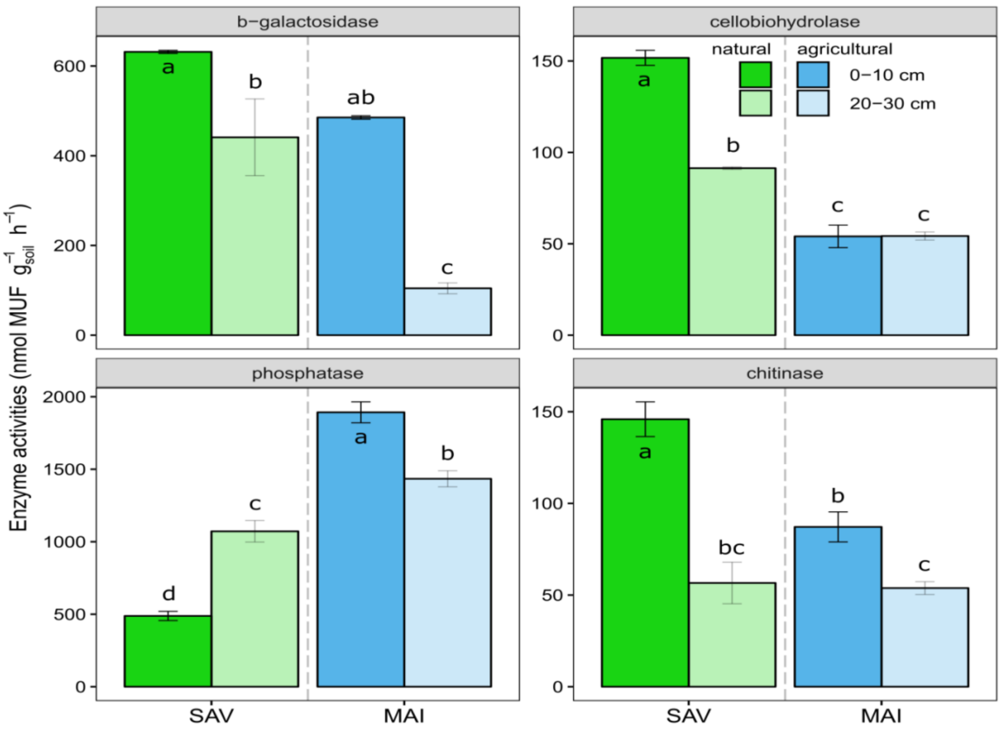

Figure 3. 4. 1-1: Enzymes activities (V_{max}) for β-galactosidase, cellobiohyrolase, phosphatase and chitinase in order of increasing land use intensification from savanna (SAV) to Maize (MAI) on colline zone. The green coloured bars denotes natural ecosystem and blue for agricultural ecosystems, solid colour refers to topsoil (0-10 cm) and the light-coloured for subsoil (10-30 cm) depth. Standard deviation of the mean is indicated by error bars. The significant correlation indicated by * ($P<0.05$) and letter (a-d) indicates significance levels for topsoil and subsoils.

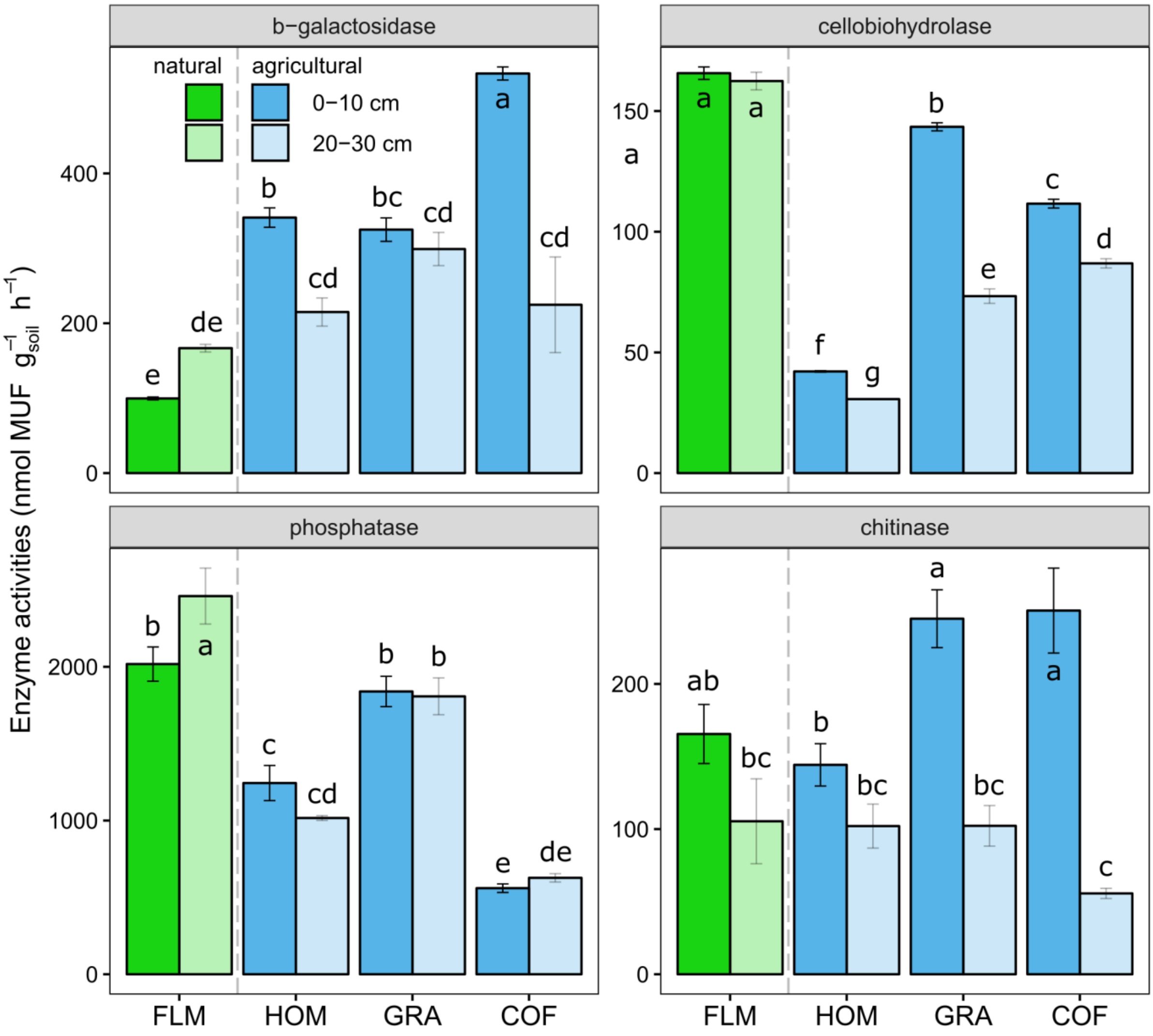

Figure 3. 4. 1-2: Enzymes activities (V_{max}) for β-galactosidase, cellobiohydrolase, phosphatase and chitinase in order of increasing land use intensification from forest lower mountain forest (FLM), Chagga homegarden (HOM), grassland (GRA) and coffee plantation (COF). The solid colored bars refer to topsoil (0-10 cm) and the light-colored bars for subsoil (10-30 cm) depth, green color denotes natural ecosystem, blue for agricultural ecosystems. The standard deviation of the mean is indicated by error bars and significance level shown by * ($P<0.05$) and letters (a-g) indicates significance levels for topsoil and subsoils.

In the lower montane zone, for almost all land-use practices enzyme activities were higher or at least similar in top- compared to subsoil (with again the exception of phosphatase) (Fig. 3. 4. 1-1). The activity of β-galactosidase in topsoil increased by land-use intensification (Fig. 3. 4. 1-2). Chitinase was the only enzyme which remained unaffected by land use in top- and subsoil. Subsoil β-galactosidase activities remained unaffected while topsoil activities increased with land use (Fig. 3. 4. 1-2). In contrast, phosphatase and cellobiohydrolyse activities decreased in top- and subsoils by land use. Thus, the impact of land-use practices on enzyme activities was highly enzyme specific.

3.4.2. Effect of depth and land use on catalytic properties (K_m and K_a)

In the colline zone, phosphatase had higher K_m in SAV compared to agricultural soils (Fig. 3. 4. 1-2). Chitinase was the only enzyme which showed lower K_m values in SAV than in MAI, both, in top- and subsoil. In contrast, the K_m values of C cycle enzymes remained constant with depth. In lower montane zone, all enzymes showed a gradual increase of K_m (decrease in substrate affinity) with land-use intensification, i.e. from FLM towards HOM, GRA and finally COF (Figure 3. 4. 2-1).

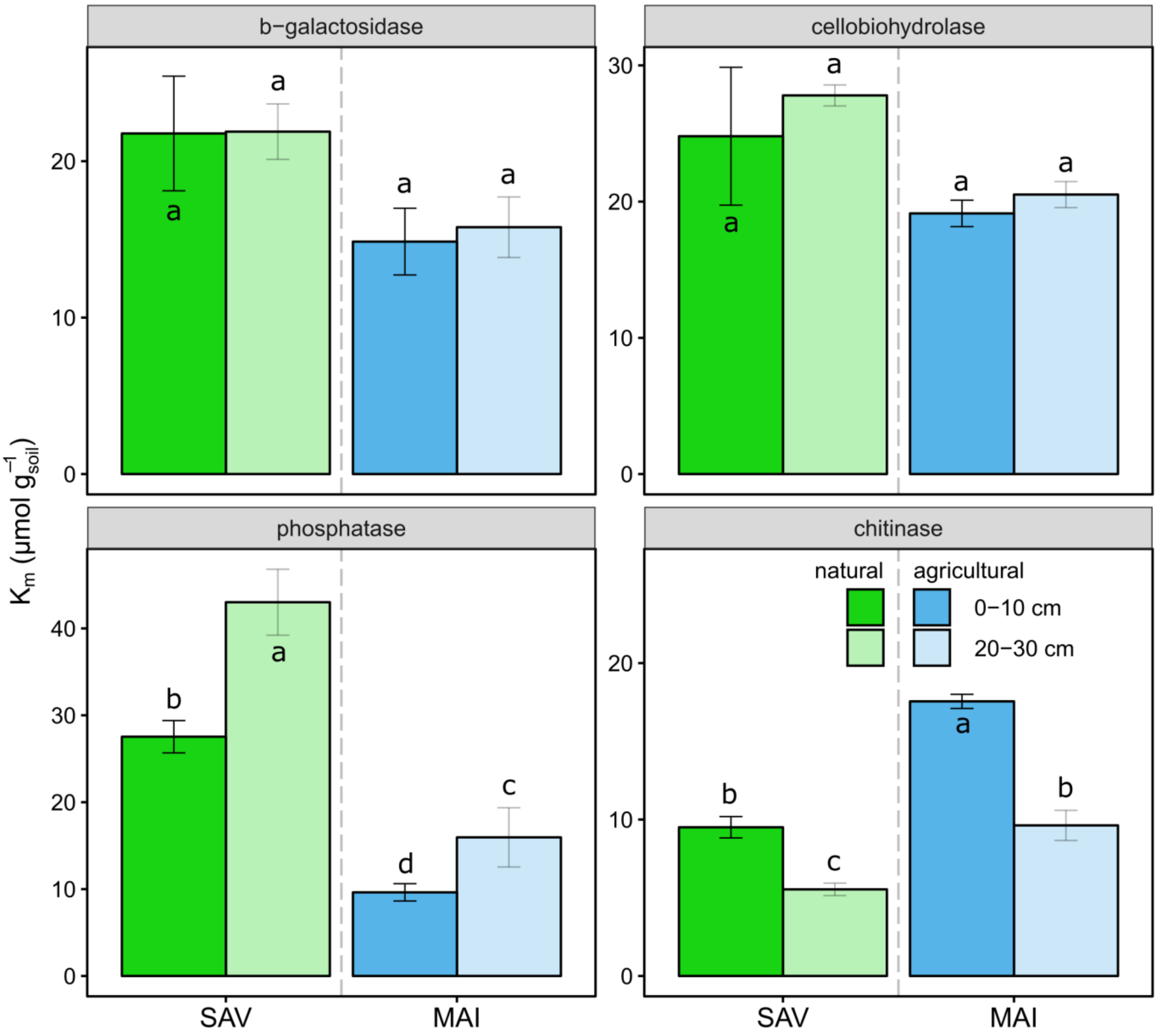

Figure 3. 4. 2-1: Substrate affinity (K_m) for β-galactosidase, cellobiohydrolase, phosphatase and chitinase in soils between savanna (SAV) and maize fields (MAI). Standard deviation is indicated by error bars at significant level ($P<0.05$). The alphabet (a-d) indicates significance levels. The solid colored bars refer to topsoil (0-10 cm) and the striped bars for subsoil (10-30 cm) depth, green color denotes natural ecosystem, blue for agricultural ecosystems. The standard deviation of the mean is indicated by error bars and significance level shown by * ($P<0.05$). Alphabets (a-d) indicate significance levels for topsoil and subsoils.

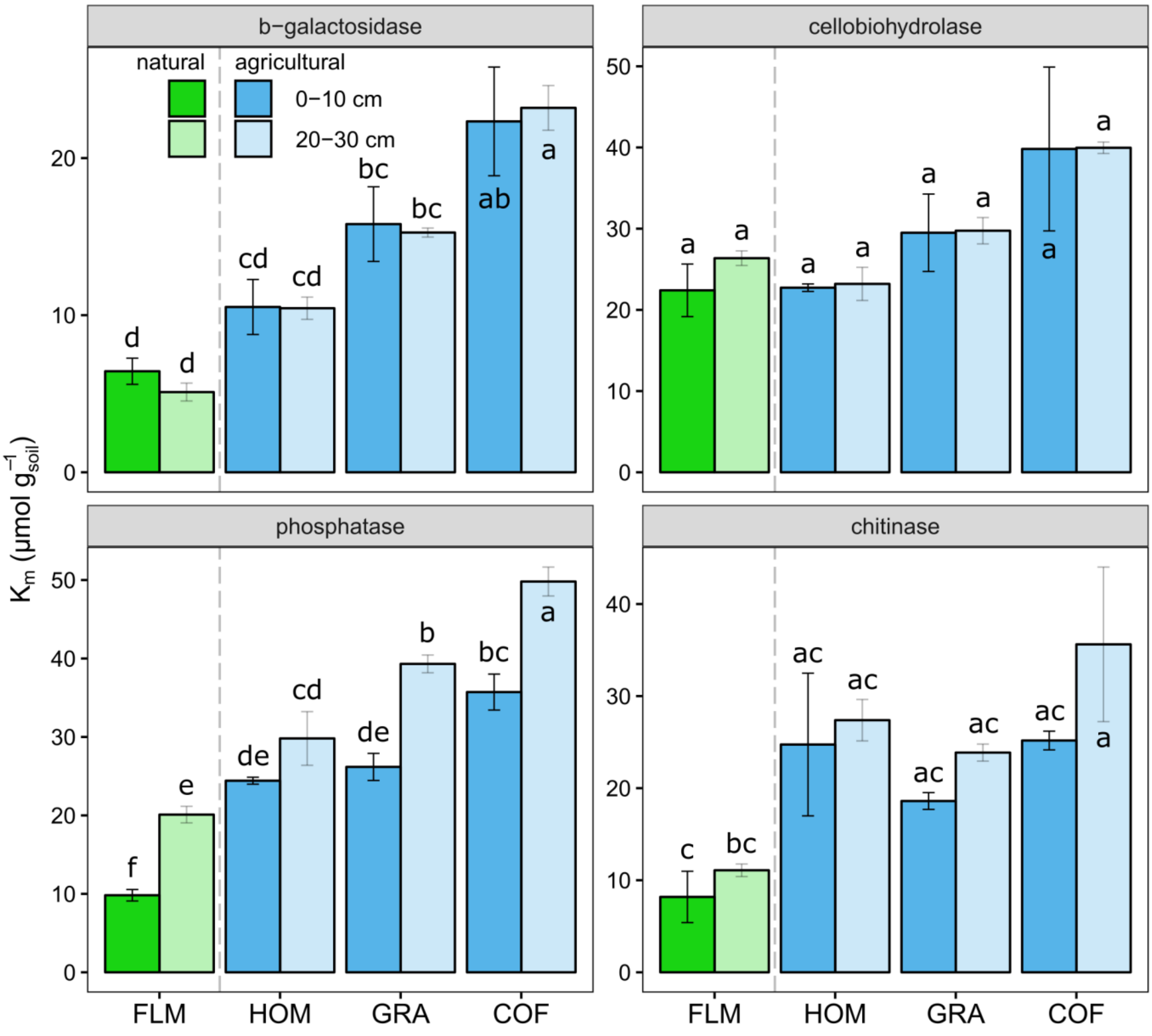

Figure 3. 4. 2-2: Substrate affinity (K_m) in order of increasing land use intensification from forest lower mountain forest (FLM), Chagga homegarden (HOM), grassland (GRA) and coffee plantation (COF). The green bars denote natural ecosystems and blue for agricultural ecosystems. Strong colored bars referring to topsoil (0-10 cm) and the light-colored bars represent the subsoil (10-30cm) depth. The standard deviation of the mean indicated by error bars and at ($P<0.05$) significant level. Alphabets letters (a-e) indicates significance level for topsoil and capital letters (A, B) for subsoils.

Land-use types in the colline zone i. e. SAV and MAI, catalytic efficiency (K_a) of C cycle enzymes remained constant both in top- and subsoil. In contrast, P and N cycle enzymes showed lower K_a values in SAV than in MAI (Fig. 5) in top- and subsoil. Relative changes in catalytic efficiency (K_a) of chitinase in lower montane zone in top- and subsoil were higher in MAI, COF and HOM than in FLM (Fig. 3. 2. 1-3). The K_a of phosphatase was the lowest in

COF (the cropland with highest intensity) among all ecosystems in this zone. However, K_a of cellobiohydrolase and β-galactosidase in lower montane zone stayed almost constant between different ecosystems i. e SAV, GRA and FLM (natural) and agroecosystems (MAI, COF and HOM) in top- and subsoil.

Overall, the trend of substrate affinity in response to land-use intensification or depth was enzyme specific. The catalytic efficiency of C cycle enzymes remained mostly constant, while it varied for N and P cycle enzymes between top- and subsoil.

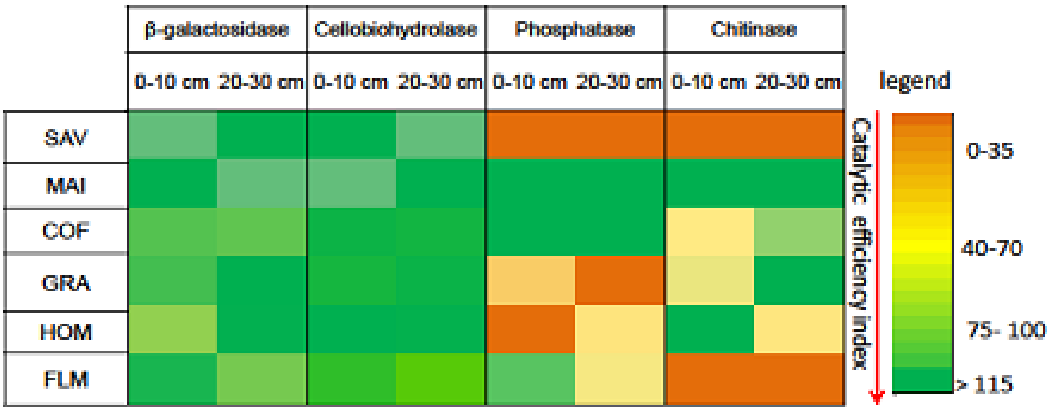

Figure 3. 4. 2-3: Relative catalytic efficiency in top- and subsoil in the colline and lower montane zones of Kilimanjaro ecosystems. Ecosystems are arranged in the order of increasing land use intensification from savanna (SAV), Maize fields (MAI) forest lower mountain forest (FLM), Chagga homegarden (HOM), grassland (GRA) and coffee plantation (COF).

Overall, the trend of substrate affinity in response to land-use intensification or depth was enzyme specific. The catalytic efficiency of C cycle enzymes remained mostly constant, while it varied for N and P cycle enzymes in top- and subsoil.

3.4. 3. Response of enzyme kinetics to elevation

We compared elevation effects only between the two natural ecosystems SAV at 950 m and FLM at 1920 m. The activities of β-galactosidase in top- and subsoils decreased with higher elevation (i.e. with decreasing temperature), whereas phosphatase and cellobiohydrolyse activities increased with higher elevation. The relative increase in enzyme activities with elevation was highest for phosphatase (> 4fold in topsoil) (Fig. 6). The activity of C cycle-related enzymes showed significant differences in top- and subsoil of SAV, but no differences between top- and subsoils in FLM. The β-galactosidase and phosphatase substrate affinity (K_m) was higher in SAV than in FLM, while for cellobiohydrolase and chitinase, enzymes responsible for polymer decomposition, it remained constant in topsoil (Figure 3. 4. 3).

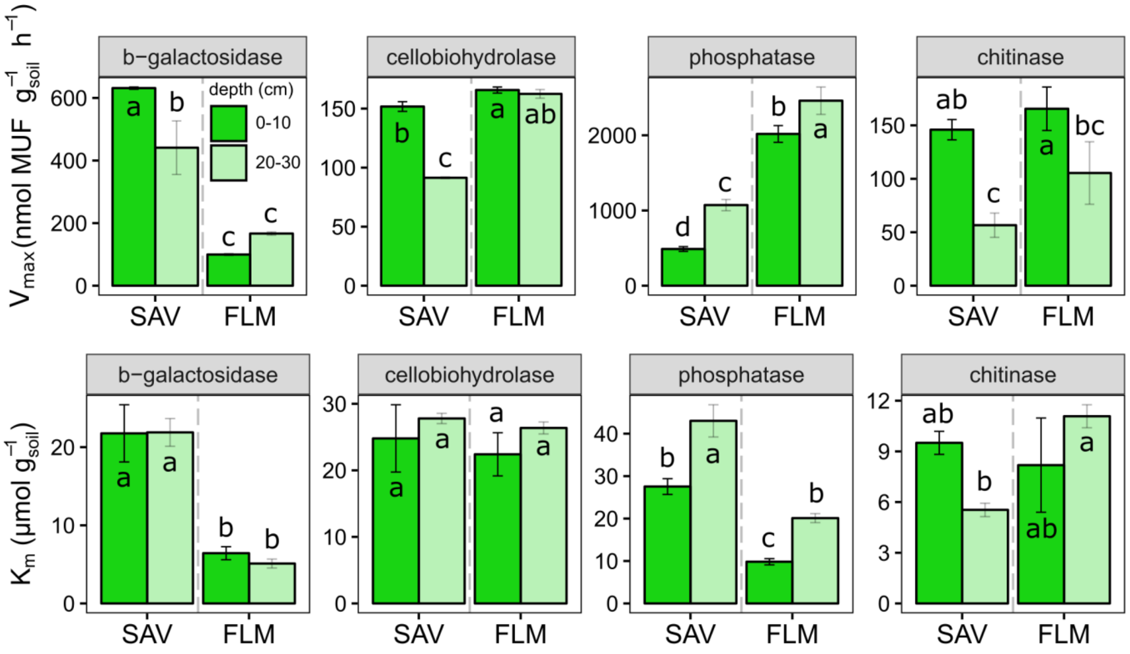

Figure 3. 4. 3: Enzymes activities (V_{max}) and substrate affinity (K_m) along temperature gradient for savanna (SAV) and forest lower mountain (FLM). Standard deviation of the mean is indicated by error bars (n=3). The strong colored bars referring to topsoil (0-10 cm) and the striped bars represent the subsoil (10-30 cm) depth. The significant correlation indicated by * ($P<0.05$). Small letter (a, b) indicates significance level for topsoil and capital letters (A, B) for subsoils.

3.5. Discussion

Higher phosphatase activity accompanied with lower K_m values in topsoil of MAI than of SAV may indicate a shortage of available P in cropland soil. Phosphorus is the second most limiting nutrient for plant growth which is commonly deficient, especially in tropical soils due to its immobilization by precipitation. This P limitation is especially pronounced in allophanic soils as such of Mt. Kilimanjaro, whose parent material originates largely from volcanic eruptions. Organic phosphate compounds as phytates, phospholipids and nucleic acids can be decomposed by phosphate-solubilizing bacteria, for example *Pseudomonas, Bacillus and Rhizobium* and increase P uptake by plants and increase crop yield [63]. In agreement with H1, SAV showed higher activity of depolymerizing C and N cycle enzymes than MAI. Such a trend suggested that fertilization reduced microbial demand to express C-degrading and N-mobilizing enzymes. A significant land-use effect even on SOC-normalized enzyme activities for cellobiohydrolyse and phosphatase (and for "land use X depth" interaction for β-galactosidase) supports the interpretation that this land-use effect is not solely driven by substrate supply, i.e. SOC (Table S3 and S4). Instead, other management practices such as fertilization caused the observed decrease in enzyme activities with increased land-use intensity.

In addition, maintaining the equal binding affinity to the substrate (constant K_m) for C cycling enzymes ensured efficient enzyme conformation within a broad range of land uses in top- and sub-soil. This demonstrated that expression of static enzymes is a preferred microbial strategy in the studied soils [27, 29]. On the other hand, phosphatase within the colline zone was the only enzyme which supported the second part of hypothesis (H1) that agricultural practices in comparison to the natural ecosystem may cause higher enzymatic efficiency (low K_m values). Empirical evidence and existing models support the idea that microbes and plant roots produce enzymes according to principles of resource supply and demand optimization [31, 64, 65]. Cost reductions can be achieved through regulatory mechanisms, such as expression of enzymes with higher substrate binding affinity and efficiency (expressed by lower K_m values). In the lower montane zone, the response to land use was enzyme specific: maximal cellobiohydrolase and phosphatase activity decreased with land-use intensification in top- and subsoil whereas β-galactosidase and chitinase showed an opposite pattern. As these significant changes with land-use remain prominent even if normalized on SOC (Suppl Table

S3 and S4) our observation from the colline zone was confirmed that the land-use effect is by far exceed a pure effect of altered SOC stocks.

In contradiction with H1, K_m of all enzymes increased stepwise with higher land-use intensity resulting in a decline of enzyme affinity. Β-galactosidase, and chitinase which catalyzes decomposition of complexes organic compounds with polyols such as pectins or chitin, showed an increase in enzyme activity with land-use intensification in topsoils. The major benefit of enzyme production is the release of organic monomers or nutrients that microbes and plant roots can take up. Thus, the excessive fertilization in agricultural system may have stimulated the production of respective C-degrading enzymes, even if the chitinase is additionally mobilizing N. However, the expression of P-acquiring enzymes may have been suppressed. Although, fertilization can provide a sufficient nutrient supply for microorganisms in tropical andic soil, there might still be a scarcity of certain elements depending on the type of fertilizer used [66–68]. In particular, the trend of increasing activity and decreasing affinity of cellobiohydrolase with increasing land-use intensification indicated that different carbon sources were increasingly depleted in different fertilization treatments which affected the activity of enzymes of the C cycle [69–71]. A gradual increase of activity and reduction of substrate affinity in the lower montane zone from natural towards agricultural ecosystems suggested that enzymes select and convert their substrate less efficiently under anthropogenic disturbances (e.g. fertilization, pesticide input and tillage). The relative changes of catalytic efficiency of phosphatase between top- and subsoils revealed that K_a of FLM was higher in top- than in subsoils probably due to higher substrate availability.

At both elevations in the subsoils, most of the tested enzymes were not affected by the land-use practice. This demonstrated that land-use practices have minor effects on subsoil in comparison to topsoil. The reduction of substrate affinity for most tested enzymes in the subsoil means maximum reaction rates at very high substrate concentrations [5]. Indeed, the activities of extracellular enzymes are mainly a function of the amount of available substrate and of the microbial biomass present to potentially synthesize them [22]. Due to generally reduced substrate availability in deeper layers the competition for easily degradable C sources increase, resulting in increased K_m in the subsoil. Microbial biomass and water extractable organic C also may decrease with depth (up to 50 cm) and leaching of dissolved organic matter is one major source supplying some substrate.

In most cases (except for phosphatase in natural ecosystems) changes of enzyme activity with depth became marginal if normalized on SOC (Table S4). This suggests that in contrast to our land-use effect, the depth effect is largely driven by substrate supply, (Table S4). Apparently, topsoil enzyme activities were regulated by various factors such as nutrient availability, moisture, etc. while subsoil on the other hand are more robust against such changes related to land use. Instead C supply driven by leaching input, root biomass, and its exudates [18] are key drivers of subsoil enzyme systems and control the increase of substrate affinity down the soil profile. Shifts in catalytic efficiency of chitinase and phosphatase from top- to subsoils also revealed the possible shift in microbial community structure due to the changes in substrate availability.

The impact of elevation on enzyme activity from SAV to FLM (lower to higher elevations) was clearly expressed. With exception of β-galactosidase, activity of all other enzymes increased at higher elevation. A previous study showed higher aboveground biomass on FLM when compared with SAV [52]. Ecosystem productivity and litter decomposition rates in natural ecosystems also increased gradually from colline to lower montane zone [72]. The higher vegetation biomass and C input in turn may have stimulated activity of soil microorganisms resulting in higher enzyme activities in soils of forests comparing to savanna [73]. In line with our third hypothesis (H3), at higher elevation K_m values of phosphatase and β-galactosidase were smaller indicating a fast rate of conformation change when converting a substrate to a product at an enzyme active site. However, the substrate affinity of cellobiohydrolase and chitinase remained unaffected by elevation. If catalytic properties are to be maintained under a particular regime, the "goal" of a microbial community is always the expression of enzymes with similar K_m values [29]. Thus, maintaining a high binding affinity to substrate (constant or even decreasing K_m) may have ensured efficient enzyme conformation in the colline and lower montane zone of Mt. Kilimanjaro.

3.6. Conclusions

Using six ecosystems, we demonstrated that land-use practices control the enzyme activities, substrate affinities and catalytic efficiencies in two soil depths. The phosphatase kinetics of the colline zone supported our hypothesis that agricultural land-use intensification leads to microbial production of enzymes with higher efficiencies (low K_m values) instead of higher enzyme expression, enabling metabolic needs to be met at lower costs. Thus, this study was the first attempt to show how the catalytic properties of enzymes vary along a land-use gradient where maximal enzyme activity and substrate affinity exhibit severe changes. We provide evidences that the impact of land use on enzyme activities decreases with soil depth. Whereas the land-use effect cannot solely be related to changes in SOC stocks, i.e. substrate supply, the depth effect is in most cases clearly driven by the decrease in organic C from top- to subsoils. Overall, the changes in enzyme kinetics in response to land-use intensification indicated altered enzyme systems and a possible shift in microbial community composition [74,75]. Further, constant or smaller K_m accompanied with higher catalytic efficiency could be an indicator of altered enzyme expression [76] due to a major shift in species dominance under anthropogenic activities. The soil microorganisms are capable to maintain their full range of biological functions and ecosystem services (e.g. alter enzyme systems with low, high or constant substrate affinity within wide range of land-uses to ensure efficient enzymatic functioning under varying condition). This ensures the easier adaptation of microbial driven decomposition to changing climate but also other abiotic factors. Static substrate affinities in subsoils proved that limitations (most likely C but also possibly nutrient and oxygen) are involved in the expression of more stable enzymes in subsoils, frequently with a similar K_m. Thus, this study calls on further *in situ* [77] and mechanistic research on the role of intensive land-use practices on microbial functions in soils and whether the microbiome will be able to provide their ecosystem services at any time point under such practices.

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3.9. Supplementary

Table 3. 9: Levene’s test results for the ecosystems at colline and lower montane zones on both depths (0-10 and 20-30 cm) at Kilimanjaro (supplementary table (S2)

Levene's test (ANOVA)						
Zones	Depth	Depth	β-galactosidase	cellobiodase	phosphatase	chitinase
Lower montane zone	Vmax	10 cm	0.126378	0.536707	0.226941	0.248043
		30 cm	0.130724	0.061194	0.31138	0.061602
	Km	10 cm	0.219389	0.300533	0.208599	0.519918
		30 cm	0.09516	0.160671	0.701877	0.6033
Colline zone	Vmax	10 cm	0.848083	0.416	0.560417	0.167498
		30 cm	0.631395	0.125029	0.325694	0.14525
	km	10 cm	0.233574	0.115776	0.189072	0.122501
		30 cm	0.082546	0.164499	0.720801	0.982254
elevation gradient	Vmax	10 cm	0.396866	0.711135	0.194828	0.139903
		30 cm	0.153761	0.120024	0.316312	0.117421
	Km	10 cm	0.09198	0.884592	0.155869	0.16899
		30 cm	0.072853	0.164857	0.859893	0.67062

4. Study 3: Effects of land-use change and elevation gradient on soil aggregate size distribution on Mount Kilimanjaro

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4.1. Abstract

Aggregate formation is affected by the interaction of several abiotic and biotic processes in soil, and can be altered by land-use practices as well as climatic conditions. In this study we aim to (1) determine the effect of land-use intensification on aggregates sizes distribution on Mt. Kilimanjaro, and (2) identify changes in aggregates size distributions along the ~4000 m elevation gradient. Soil samples (0-10 cm depth) from 12 representative ecosystems (5 agricultural and 7 natural) at Mt. Kilimanjaro were analyzed using dry-sieving. The soil aggregates were separated into three fractions: large macro aggregates (2-5 mm), small macro aggregates (0.25-1 mm) and micro-aggregates (<0.25 mm).

Large macro aggregates were less affected by both land-use and elevation gradient in comparison to micro aggregates in all vegetation zones. The percentage of large macro aggregates decreased with higher land-use intensity. In the colline zone, land-use change from savanna to maize fields decreased the percentage of large macro aggregates by 50% (p-level = 0.05). In the lower montane zone, large macro aggregate percentage tended to decrease (p-level < 0.1) from 30.6 % in forests to 20.3% in coffee plantations (p-level < 0.1). Along the elevation gradient, large macro aggregate percentage in natural ecosystems increased up to 63.5% at mid-elevation (2000-3000 m a.s.l.) and decline towards higher elevations.

Large macro aggregates were sensitive to disturbances and given the rapid land-use change in the region, the productivity of the agricultural soil has continued deteriorating. Besides that, breakdown of large macro aggregates through land-use intensification could further reduce the potential of soils to act a carbon sink and increase degradation and erodibility of these soils.

Keywords

Aggregate fractionation, East Africa, soil quality, dry-sieving

4.2. Introduction

The rising food demand of the increasing population in Africa has led to an vast expansion of agricultural areas (Pagliai, Vignozzi, and Pellegrini 2004). In Tanzania, particularly lowland areas and highland forests experience degradation at an alarming rate (Salome B. Misana 2012). The cultivation practices affect soil physical, chemical and biological properties and commonly land-use intensification is associated with soil degradation (Salome B. Misana 2012). Especially tillage, vegetation cover changes and biomass removal have a strong effect on soil stability and aggregate formation (Angers, Samson, and Légère 1993; Balesdent et al. 2000; Lawal, Ogunwole, and Uyovbisere 2009; Pagliai et al. 2004).

Separation of soil aggregates is an essential process in determining size distribution and stability (Boogar, Jahansouz, and Reza 2014). The aggregates are important in understanding greenhouse gases activities (soil-atmosphere exchange component) as they act as biogeochemical reactor (Wang et al. 2019) used as evolutionary compartments whereby enclosed microbes released interact with other microbial community and enlarge the interactions and diversification of soil biodiversity (Rillig, Muller, and Lehmann 2017). The distribution of soil aggregate determines the pore space, which is essential for aeration, water penetration, growth and distribution of plant roots including soil microbiota (Tisdall and

Oades 1982). Therefore, there is an interplay between aggregate distribution and stability whereby the greater the size the stable the aggregate (Nimmo and Park 2004). When disruption effect rate is greater than formation of aggregates, less SOM is stabilized and therefore the percentage of macro aggregates decreased because of human-induced activities and physical conditions that continue to mold soil aggregates size (Six, Elliott, and Paustian 1999). Long term disturbance of soil aggregates therefore will have an implication in the soil structure and SOC held in the micro aggregates especially on intensively-use Afrotropical highlands.

Soil macro-aggregates are bound together by primarily polysaccharides, roots and hyphae (Chang et al. 2016). In particular, large macro aggregates formation depends on agents like the plants roots, hyphae, microbial and plant related polysaccharides (Tisdall and Oades 1982). Micro-aggregates, on the other hand, are mainly bound by aromatic organic humic materials which are associated with amorphous iron and aluminum (Fe & Al) compounds (Schrumpf et al. 2007; Tisdall and Oades 1982) held by persistent binding agent such as organic polymers, oxides and polyvalent cations (Chang et al. 2016).

Several factors together influence the aggregation, some other agents in combination are important in distinguishing soil aggregate size distribution such as soil type, aggregation agents, management practices and environmental conditions (Tisdall and Oades 1982). In land degradation processes however, decrease of soil structure associated removal of micro aggregates and often times caused by various human activities (Kasper et al. 2009) and its size measurement provides quantitative indicator of structure (Six et al. 2002).

Currently, there is little information on the effects land-use and elevation zones on soil stability in Kilimanjaro ecosystems. Many studies on this area focused on microbial biomass (Mganga, Razavi, and Kuzyakov 2015; Pabst et al. 2013b) soil organic matter composition (Becker et al., 2019; Ndossi et al., under review) and decomposition (Becker & Kuzyakov, 2018). However, data on soil stability and soil physical protection are still missing. We analyzed the impact of land-use and changing climatic conditions as factor of elevation gradient on soil aggregate size distribution and stability on top soils. We expected that, (1) large soil aggregate sizes and its structure indices will decrease with land-use intensity and (2) strongly related to the overall elevation gradient and ecosystem productivity.

4.2. Materials and methods

4.2.1. Study area

The study was conducted on Mount Kilimanjaro, Northeastern Tanzania (3°4'33"S; 37°21'12"E). The study area was located on the southern slope, between Machame area in West and the Lake Chala in the East. Seven (semi-)natural and five agricultural ecosystems were selected to represent the 12 major ecosystems and land-use types of the region. The study sites cover an elevation range of ~800 to 4600 m a.s.l. These ecosystems offer a unique potential to investigate the effects of elevation (i.e. changes in temperature and precipitation) and land use on soil aggregate size distribution with varying vegetation cover. The elevation gradient is characterized by a strong ecological zonation (Hemp, 2006a). In the colline zone (800-1100 m a.s.l.) there are two distinct ecosystems which dominate: i.e. semi-natural savanna (SAV) and agricultural maize fields (MAI) (Hemp, 2006a). The SAV is co-dominated by *Acacia* trees (*Acacia nilotica, Acacia senegal and Acacia tortilis*) and *Balanites aegyptiaca,* and grass species including *Heteropon contortus, Eragrostis superba and Bothriochloa insculpta* (Becker et al., 2017*).* The common agricultural practice in maize fields included intercropping of groundnuts and millet, tillage and partial addition of fertilizers. The lower montane area (1200-2000 m a.s.l.) is covered by forest patches (FLM), representing the natural vegetation and three other land-use types; homegardens (HOM), grasslands (GRA) and large-scale coffee plantations (COF). Coffee plantation (COF) is an intensive type of agriculture which involves use of fertilizers, herbicides and removal of plant debris of pruned coffee branches (Becker & Kuzyakov, 2018; Pabst et al., 2013). Grassland (GRA) ecosystem is dominated by dense grasses covers which are frequently cut for livestock feeding; these ecosystems were partly replaced by commercial coffee plantations (Pabst et al. 2013b). Grasslands are former forested areas that are kept open for livestock feeding (grazing or cutting) (O'kting'ati et al. 1985). Chagga homegardens are a traditional form of multi-layered agroforestry, tilled and little amount of fertilization together with farmyard manure (Lawal et al. 2009). Chagga homegardens are owned and managed by Chagga community hence the name Chagga (Soini 2005). Some of trees species common in Chagga homegardens includes; *Cardia africana, Albizia schimperiana, Persea americana, Grevillea robusta,* coffee (*Coffea arabica*), bananas (*Musa spp*), sweet potatoes (*Ipomoea batatas*), maize (*Zea mays*) and beans (*Phaseolus culgaris*) on the lower strata (Hemp, 2006c; Mganga et al., 2015). The forest lower montane (FLM) ecosystem (above 1800m) is protected by Kilimanjaro National Park (KINAPA) and experienced minor disturbance by firewood collection (Mganga et al., 2015). The dominant tree species include *Macaranga kilimandscharica*, *Agauria salicifolia* and some *Ocotea usambarensis* (Hemp, 2006a).

At mid-elevation, Ocotea forest situated around 2130 m. a. s. l. comprised of *O. usambarensis*, fern i.e. *Cyathea manniana* (Becker & Kuzyakov, 2018). Upper mountaine zone follows i.e. *Podocarpus* forest (FPO) at 2,850 m a. s. l also known as 'cloud forest' dominated by *Podocarpus latifolius, Prunus africana, and Hagenia abyssinica* (Becker & Kuzyakov, 2018). Some forest disturbance through logging and recorded wildfires was represented by disturbed sites of Ocotea (FOD) in 2270-2560 m. a. s. l and Podocarpus forests (FPD) between 2270-3060 m a.s.l. Sub-alpine forest i.e. Erica forest (FER) at 4000m is covered with *Erica trimera* followed by upper afro-alpine (HEL) with *Helichrysum* cussion vegetation and tussock grasses at about 4500 m a. s. l. Further detailed descriptions of all sites and vegetation including climatic characteristics are available (Ensslin et al. 2015b; Hemp

2006c). The mean annual precipitation reaches from 530 to 2900. Mean annual temperatures range between 2.9 and 25 °C. The soils of Kilimanjaro region were generally described as highly weathered Andosols (Schrumpf et al. 2007; Zech et al. 2014a).

4.2.2. Land use intensity (LUI)

The land use intensity (LUI) for each ecosystem and site was estimated by considering measure of biomass removal, the input of chemicals agro-inputs such as fertilizers and herbicides, vegetation structure and surrounding type of land use applied. The (LUI was standardized by calculation of disturbance index by measure of individual measure from mean of all measures divided by measure of all measurement based on scale of 0 (lowest) and 1 (highest) which was calculated empirically (Table 4.1.3.2). A further detail about the calculation of the index is available (Peters et al., 2019; Schellenberger Costa et al., 2017).

Table 4. 2. 2. Land use index for savanna (SAV), maize (MAI), coffee plantation (COF) and homegarden (HOM). The index with higher value indicates intensive disturbances at (0-1 scale). Adopted from Classen et al. 2015; Schellenger-Costa et al. 2017)

Ecosystem	Category	Land use index	Disturbance index
SAV	Natural disturbed	0.246	0.333
MAI	Agricultural intensive	0.692	0.909
HOM	Agricultural traditional	0.523	0.634
COF	Agricultural intensive	0.865	0.998

4.2.3. Aggregate size fractionation

Dry-sieving method was used for aggregate size distribution analysis. The samples were weighed prior and after oven drying. The soil was pre-heated at 60 ºC for 3 days. Soil Aggregate size distribution was analyzed by using a dry sieve machine (vibratory shaker, Retsch, Vibro GmbH & Co, Germany). The sieve shaker was set at 5 minutes time with 50Hz rpm shaking intensity. Afterwards, the remaining soils on each sieve were weighed and recorded. The aggregates were classified into large macro aggregates (>5-2 mm), small macro aggregates (1-0.25 mm) and as micro-aggregates (<0.25 mm).

4.3. Statistical analysis

Effects of land-use classes on aggregate sizes distribution were assessed using mixed-effects models for nested ANOVA, with site replicates as the random factor (p-level ≤ 0.05). Turkey's (HSD) post hoc test was used to separate effects of single land-use types. Additionally, we used the Land Use Index (LUI) as a measure of site-specific land-use intensity (Schellenberger Costa et al. 2017) in a linear regression model versus aggregate size classes (p-level ≤ 0.05).

Correlations between soil and environmental variables were analyzed by Pearson product-moment correlation (p-level ≤ 0.05). All statistical analysis and data visualization were conducted in Statistica 13 and R 3.4.2 (R Core Team 2017).

4.4. Results

4.4.1. Effect of land use on aggregate size distribution

Large macro aggregate (LMA) percentages decreased with increasing land-use intensity in the colline and the lower montane zone. In the colline zone, mean LMA percentages decreased from 22.5% in SAV to 11.4% in MAI (Figure 4. 4. 1-1).

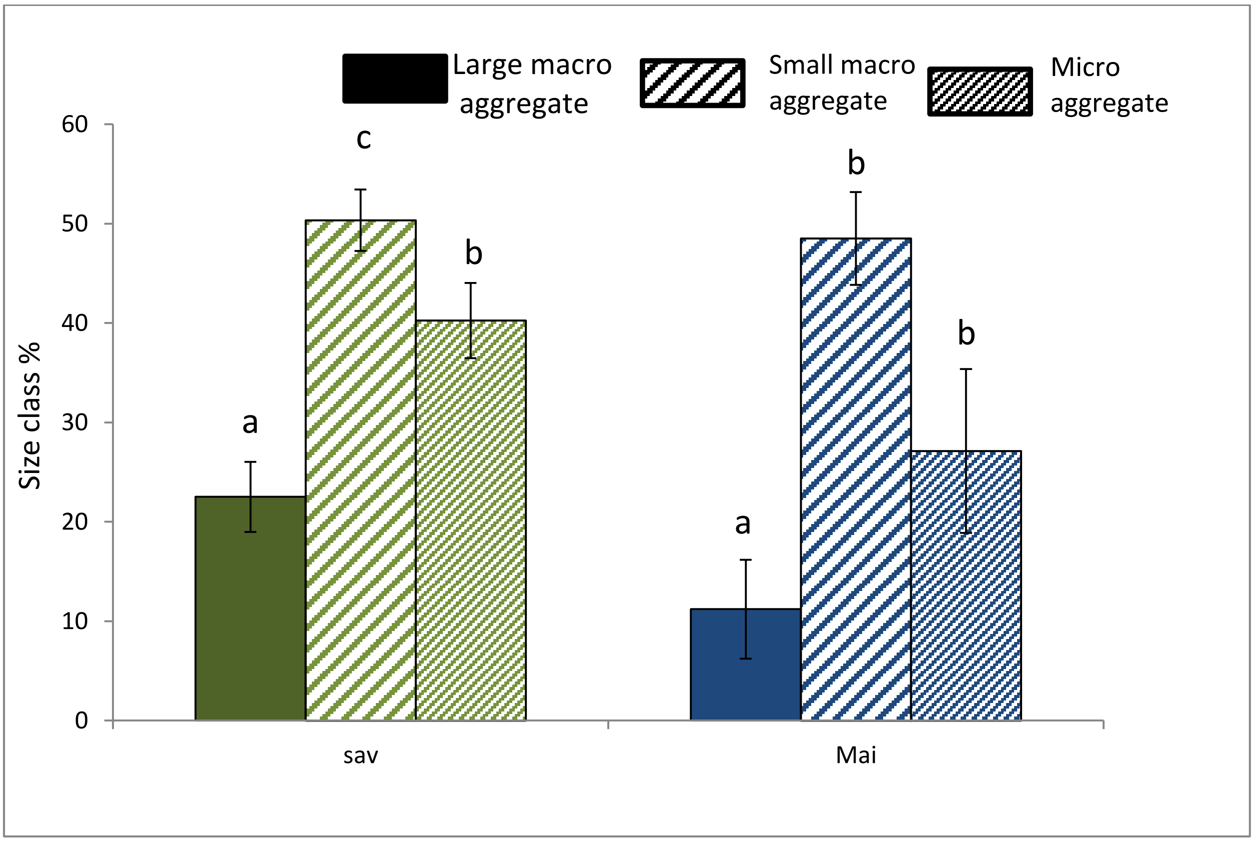

Figure 4.4. 1-1: Aggregate size distribution for the colline zone (savanna and maize fields) for large macro aggregate (LMA), small macro aggregates (SMA) and micro aggregate (MiA). Letters a, b and c indicate the significance levels at $p<0.05$, error bars indicate the standard error. Green bars refer to semi-natural while blue for agricultural-use ecosystems.

In the lower montane zone, large macro aggregate decreased in the order; FLM (30.6%) > HOM (23.6%) > GRA 22.3% > COF (20.3%) (Figure 4. 4. 1-2). Although there was not significant difference between the ecosystem aggregates percentages, the general trend corresponded to the land-use intensity (Figure 4. 4. 1-2).

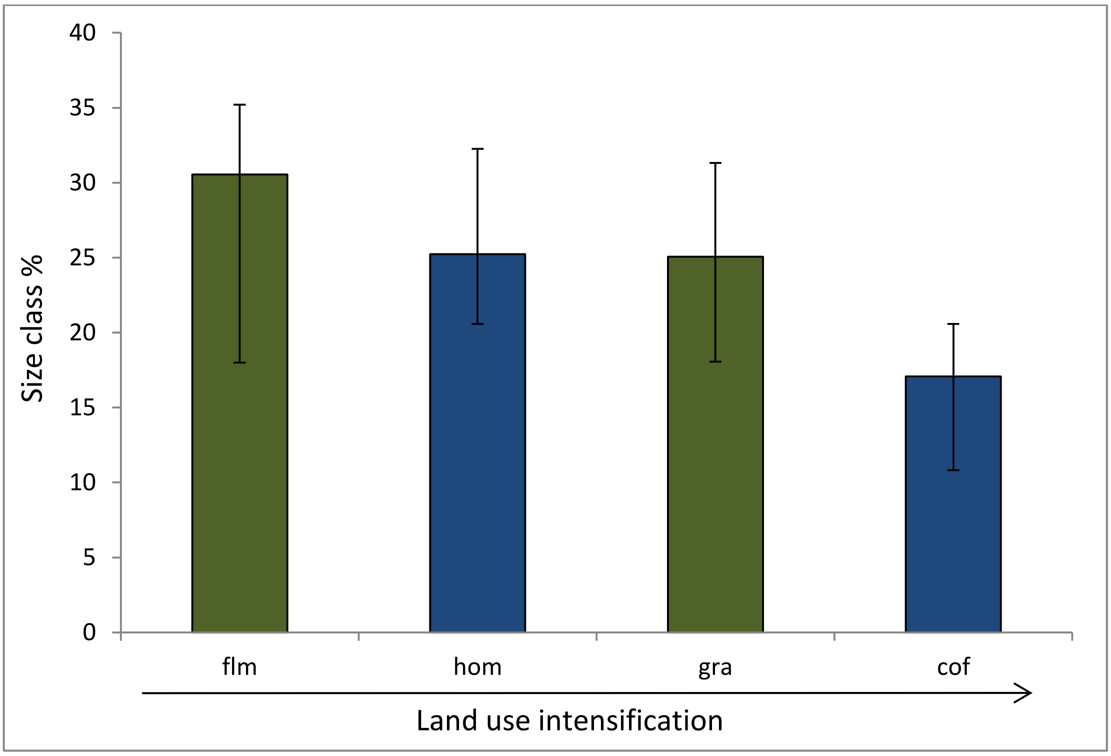

Figure 4.4. 1-2: Large macro aggregate percentages on the lower montane zone with ecosystems arranged in increasing land-use intensification from lower montane forest (FLM), Chagga homegardens (HOM), grasslands (GRA) and coffee plantation (COF). The error bar indicate standard error, green bars refers to natural/semi-natural ecosystem while blue indicates agricultural-use ecosystem.

Large macro aggregate percentages decreased, while micro aggregate percentage increased with increasing land-use index (R^2=0.08, $P < 0.05$). Small macro aggregates did not respond to changes in land-use intensity (R^2=0.02, at P=0.15) (Figure 4. 1. 4-3).

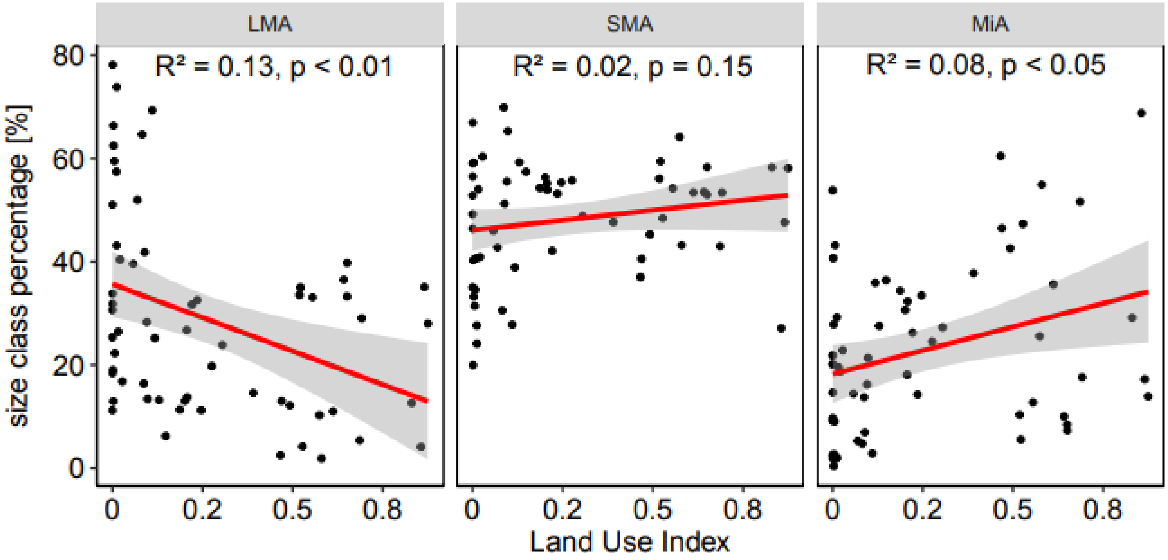

Figure 4.4. 1-3: Aggregates sizes percentage across Kilimanjaro ecosystems land use index (adopted from see (Schellenberger Costa et al. 2017). LMA refers to large macro aggregate, SMA- small macro aggregate and MiA as Micro aggregates.

4.4.2. Effect of elevation on aggregate size distribution

The LMA percentages increased substantially until the *Ocotea* forest zone at mid-elevation (2000- 3000 m a.s.l.), where it reached a maximum percentage of 63% (Supplementary Figure 4. 9-1) At higher elevation, macro aggregates decreased again towards the alpine *Helichrysum* ecosystems >4000 m. a. s. l. (Figure 4. 4. 1-4).

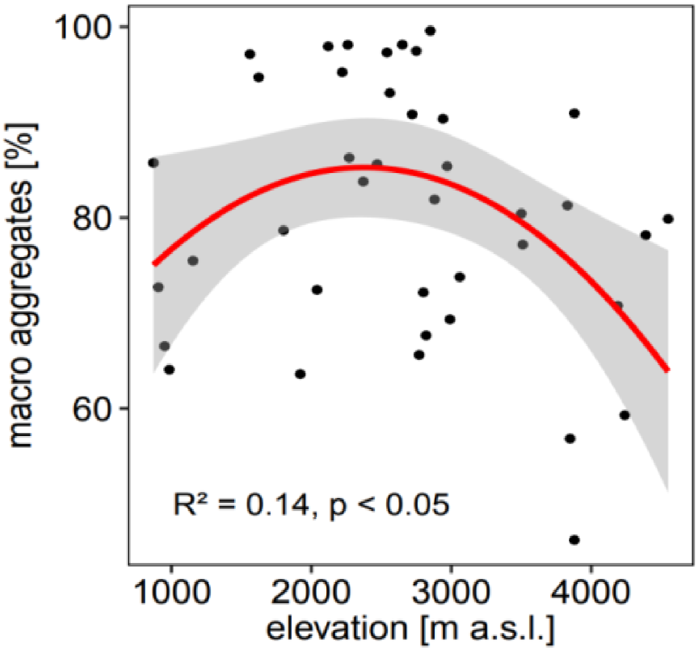

Figure 4.4. 2: Macro aggregates for natural ecosystems vs elevation of Kilimanjaro including savanna ecosystem.

4.5. Discussion

4.5.1. Effect of land use on aggregate size distribution

The average percentage of large macro-aggregate (LMA) decreased for about 50% with higher land-use intensity (i.e. from SAV to MAI). Since aggregates distributions on agriculture differ from that in natural ecosystems, we expected more LMA on SAV than counterpart MAI. In the ploughed soil, aggregates are less stable than soil without or minimum tillage (Pagliai et al., 2004). This can affect soil structure, SOC and N (Six et al 1998), which are important binding agents for soil aggregates and are responsible for macro-aggregate stability and formation (Tisdall & Oades, 1982; Wagner, Cattle, & Scholten, 2007). In our study sites, MAI is an intensive system with application of chemicals in fertilizers and the harvesting includes the complete removal of maize residues, thus reducing inputs of straw and consequently particulate organic matter (Pabst et al., 2013). This practice therefore limit the particulate organic matter (POC) input (plant and fauna debris) which are pertinent in large macro aggregate formation (Totsche et al. 2018). Furthermore, the increasing percentage of micro-aggregates makes the soil more susceptible to wind and splash erosion, since MAI (maize field) is left bare after harvest and the drought nature of the soil enhances erosion potential (Pabst et al., 2013; Wagner et al., 2007).

Soils on SAV have relatively suitable physical characteristics such as stable structure, good infiltration rate and permeability under natural state (Sanchez and Salinas, 1981). However, large macro aggregates are vulnerable to land-use disturbance because of their temporal weak binding agents of roots and mycelia (Tisdall and Oades 1982; Xiao et al. 2017).

In the lower montane zone (1200-2000 m), the decreasing trend of aggregate sizes with land-use intensity was apparent but not statically significant. The decrease was in the order; FLM > HOM > GRA > COF. Forest ecosystem showed higher percentages of LMA followed by other agricultural based ecosystems. Forests soils usually have better aggregation than soils under agricultural land (Ciric et al. 2012). Also their perennial vegetation, healthy soil biota communities and litter layers promote aggregate formation and soil physical properties in general (Ontl et al. 2015, 2015)(Liu et al., 2017). In comparison, grasslands experience no physical disturbance (i.e. tillage) but lower litter inputs and high biomass removal; coffee plantations are intensively used, with applications of fertilizers (N. P, K) as well as herbicides and fungicides; and homegardens are regularly ploughed with sporadic application of household waste. Particularly the latter disturbances cause physical disturbances of roots binding materials and on microbes producing mucilage gum and results in aggregates disintegration (Amézketa 1999; Tisdall and Oades 1982).

4.5.2. Effects of elevation on aggregate size distribution

The LMA percentage followed a unimodal pattern along the elevation gradient of Mt. Kilimanjaro. In turn, MiA contributed to a larger percentage at lower elevation and in the colline zone. This unimodal pattern is typical for a broad variety of ecological properties along montane elevation gradients (Kluge et al., 2006; Mölg et al. 2009). Similar patterns were found for MAP, litter decomposition, litter quality above ground biomass, NPP and SOM composition in other studies at Mt. Kilimanjaro (Ensslin et al.,2015, Becker et al. 2015, Becker et al. 2019, Becker & Kuzyakov, 2018; Pabst et al., 2013). We could further relate the aggregate size distribution to soil organic matter content along the elevation gradient. Ecosystems with the highest soil C contents (Pabst et al. 2013) have shown higher LMA percentages.

4.6. Conclusions

The impacts of land use practices (such as tillage and biomass removal) on soil aggregate formation occurred particularly in the colline savanna zone. Under permanent vegetation cover and in agroforestry systems of the lower montane zone, land-use effects were less prominent, but apparent when considering LUI. Climatic changes along the elevation, and the associated patterns of NPP, litterfall and SOM accumulation led to a maximum of large macro aggregates at mid-elevation ecosystems (mainly *Ocotea* forests). The higher elevation ecosystems were affected by limitation of low temperature while colline zones affected by drought. Land-use changes contribution in shaping the soil parameters including soil aggregates and other biochemical properties of the soils. The continued climate change will likely affect the biotic factors which will affect SOC content by releasing of protected SOC in aggregates and increased turnover of SOM matter decomposition and microbial processes.

4.7. Acknowledgments

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4.9. Supplementary figures- study 3

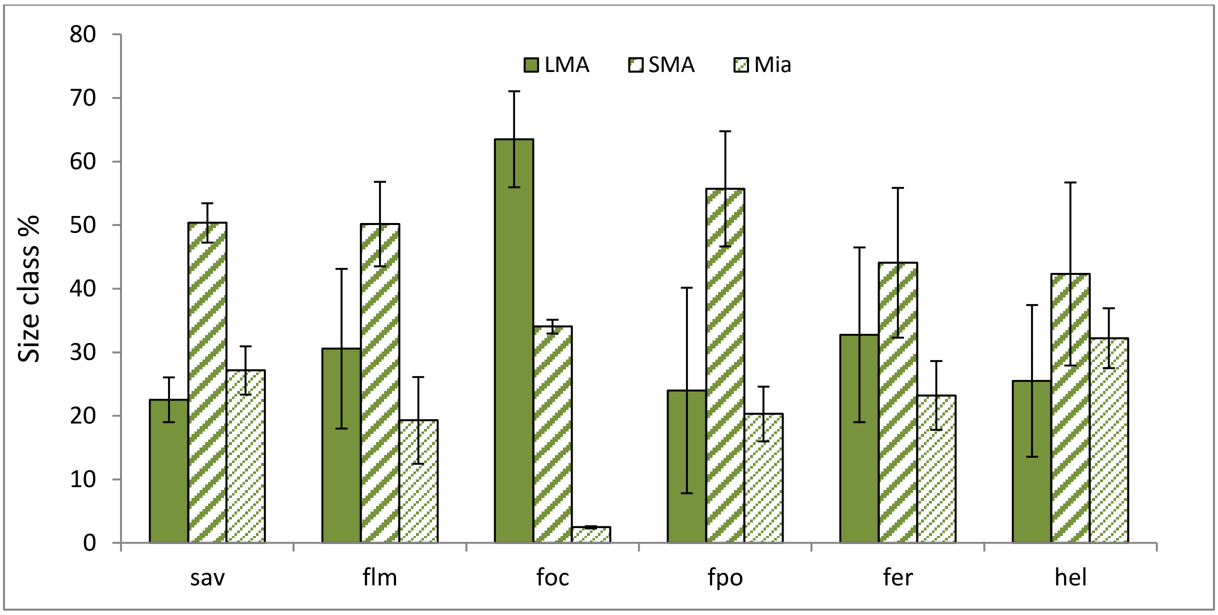

Figure 4.9-1: Aggregates size percentages for forest *Ocotea* (FOC), forest *Podocarpus* (FPO), forest *Erica* (FER) and *Helichrysum* vegetation (HEL) ecosystems arranged in increasing elevation gradient. Standard error is indicated by error bars at p <0.05 significance level.

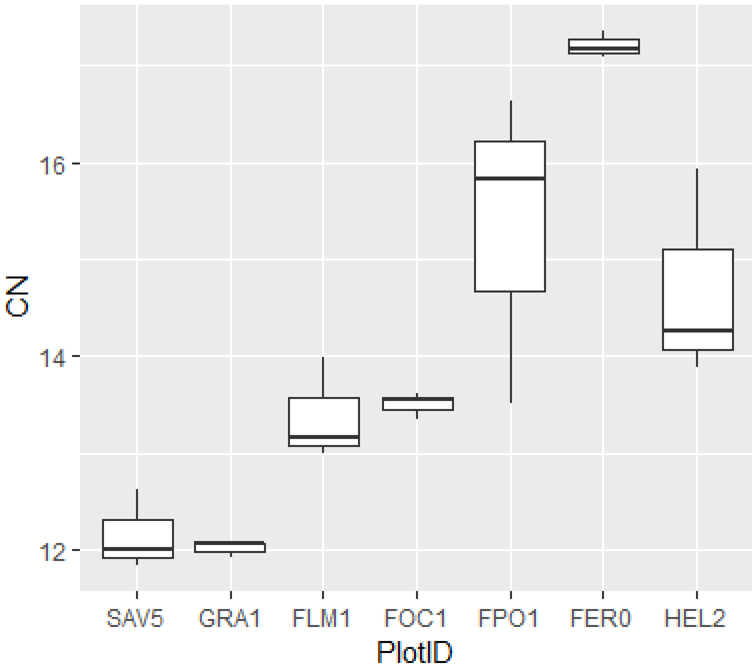

Figure 4.9-2: The CN ratios for the Kilimanjaro ecosystem from the savanna (SAV), Grassland (GRA), lower montane forest (FLM), Ocotea (FOC), Podocarpus (FPO), Erica (FER) and *Helichrysum* vegetation (HEL). The ecosystems are arranged in order of increasing elevation gradient from lowest SAV to highest HEL.

v. Legal statement

I hereby declare that this PhD dissertation has not been submitted to any other examination body either in its present or in a similar form.

Furthermore, I confirm that I have not applied for PhD at any other higher school of education. This dissertation was written independently without any unauthorized aid.

Gottingen,

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(Signature)

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