Carbon and nutrient cycles depending on climate and land use along the elevation gradient of Mount Kilimanjaro

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Summary

Ecosystem functions of tropical mountain ecosystems and their ability to provide ecosystem services are particularly threatened by the combined impact of climate and land-use change. Carbon and nutrient cycling are fundamental ecosystem function that control C storage and pools, provide plant nutrients and regulate microbial and faunal activity. Soils, as the linkage between abiotic and biotic components of an ecosystem, are strongly affected by changes in these cycles. To understand the impacts of climate and land-use changes on biodiversity and associated ecosystem services and stability on Mt. Kilimanjaro, detailed understanding and description of the current biotic and abiotic controls on ecosystem soil C and nutrient fluxes are needed. Therefore, this research described and quantified cycles of C and major nutrients (N, P, K, Ca, Mg, Mn, Na, S and Si) on pedon and stand scale along a 3400 m elevation gradient and across three stages of land-use intensity. The first objective was to assess the effects of land-use change and climatic variation along the elevation gradient, on litter fall, litter quality, litter decomposition, and C stabilization in soil. The second objective was to use qualitative indicators (composition of soil organic matter and microbial communities) to relate pool changes to the underlying processes. The third objective was to link spatial variability and characteristics of the aboveground biomass to belowground pools and processes under contrasting climatic conditions in alpine and colline ecosystems.

Twelve research sites (0.25 - 1 ha) were selected between 800 and 4200 m a.s.l., representing natural forests, savanna and alpine vegetation as well as traditional subsistence and plantation farming. Litterfall was measured every two weeks over one year and inputs of C, macro and micronutrients was calculated for a subset of these sites. Decomposition rates of native and standardized (TBI) litter were quantified and TBI indices for decomposition and C stabilization were used to assess seasonal variabilities. Annual patterns of litterfall and decomposition were closely related to rainfall seasonality and temperature. Leaf litterfall contributed 60-75% to total litterfall and decreased from 1900 to 2900 m a.s.l. Within the same elevation range, annual litter decomposition decreased by about 25%. Further decrease of decomposition rates in (sub-) alpine ecosystems indicated a strong decline of productivity and turnover at 2900 m and above. Maxima of decomposition rates occurred between 1900 and 2500 m and were linked to the seasonal homogeneity of temperature and moisture availability. At this elevation, litterfall, decomposition rates and C stabilization showed the least seasonal variation. Ecosystems below 1900 m were subjected to pronounced seasonal moisture limitation. Particularly C stabilization in savanna (950 m) was up to 23 times higher during the rainy season compared to the dry season. Above 2900 m, seasonality increased again with lower annual precipitation and greater temperature limitation during cold seasons. Land-use change from natural forests to agroforestry systems increased litter macronutrient content and deposition (N, P, K), thus enhancing

biogeochemical cycles. Carbon stabilization in these ecosystems and in the colline zone was reduced by about 30% by land-use intensification. Soil microbes in these ecosystems were less efficient in soil organic matter (SOM) decomposition but at the same time more demanding for new C sources.

Topsoil samples (0-10 cm) were analyzed for C and N content, pH, microbial biomarkers and soil organic matter chemical composition (py-GC/MS). Total phospholipid-derived fatty acids (PLFA) content increased with elevation until *Ocotea* forest (2100 m), reaching a maximum of 2100 nmol g^{-1} soil, followed by a decrease in (sub-) alpine ecosystems. Gram-negative bacteria abundance, accounting for 25-40% of total PLFAs, mainly determined this trend. Changes in the composition of microbial communities along the slopes of Mt. Kilimanjaro are a result of this climatic optimum and the consequent niche differentiation of certain groups. With increasing elevation and the harsh environmental conditions in the alpine zone above 4000 m (low temperature, low soil C and N contents), gram-positive bacteria are replaced by fungi. These variations were indirectly dependent on climatic factors, and mainly explained by changes in vegetation composition and soil parameters. Pyrolysis fractions (>280°C) quantitatively dominated the soil organic matter composition. The contribution of volatile compounds in SOM increased with elevation, indicating an increase of easily available SOM components. However, the increase of total SOM content at mid elevation is mainly determined by a more stable C pool (i.e. bound alkanes/-enes/-ols).

Two intensive research campaigns were conducted in alpine *Helichrysum* and colline savanna ecosystems. Three different vegetation cover types in *Helichrysum* were characterized. For each cover type, soil C and N pools, gross N turnover and diurnal greenhouse gas fluxes were measured, On the savanna plain, six trees were selected (legume *Acacia nilotica* and non-legume *Balanites aegyptiaca*) and crown area was distinguished from open area. Carbon, N and $\delta^{13}C$ in plant biomass and soil, soil C and N pools, water content, available nutrients, cation exchange capacity, temperature, pH, as well as root biomass and greenhouse-gas exchange were measured for each cover type. Shrub-covered patches in *Helichrysum* ecosystem had between 60% and 170% higher soil C and N compared to low-vegetation patches. Higher amounts of aboveground litter promoted microbial growth, soil C stabilization and competition for N. This led to higher substrate availability and microbial biomass, and consequently higher respiration rates. Under savanna trees, soil C and N content, microbial biomass and N availability were about 40% higher than in open area. $\delta^{13}C$ values in soil under the crown shifted towards the signal of tree leaves, suggesting that tree litterfall contributes 15% to SOM. These inputs increased microbial carbon use efficiency under the trees due to narrower C:N ratios compared to C_4-grass litter. Wide C:N ratios require microorganisms to dispose of the C surplus via increased respiration to achieve their optimum C:N stoichiometry. Therefore, CO_2 efflux was 15% higher in grassland than under the trees.

Ecosystems at mid elevation (~2000 m) represent the interception zone of optimal moisture and temperature conditions throughout the year. High litter inputs and fast turnover control the C sequestration in these ecosystems, while climatic restraints on decomposition limit the C turnover in soils at lower (drought) and higher elevation (low temperatures). Soil organic matter chemistry in Mt. Kilimanjaro forests is strongly dependent on a precipitation and temperature equilibrium. High ecosystem productivity at mid-elevations leads to increased amounts of volatile compounds but at the same time increases stabile carbon pools. Land-use intensification decreases stabilization of new C inputs through higher microbial C demand and turnover. This increases C and nutrient cycles in agricultural compared to natural ecosystems. The variability of vegetation cover types controls substrate availability in *Helichrysum* and savanna ecosystems. Two contrasting processes control the effects on CO2 fluxes in both ecosystems: Carbon mineralization at *Helichrysum* sites is enhanced by higher substrate availability under vegetated patches. In contrast, dry season C fluxes in savanna are related to the litter substrate quality and microbial C-use efficiency.

1 Extended Summary

1.1 Introduction

1.1.1 Background and Motivation

Tropical climate and land-use change are two of the major issues mankind is facing in the 21st century (Alcamo 2012; Steffen 2004). Related processes are rapidly ongoing and occur in many dimensions. For the terrestrial ecosystems, this includes changes in the biogeochemical cycles of carbon (C), water and nutrients. Those changes have far reaching implications for sustainability, biodiversity, and ecosystem services, such as provision of water, food and biomass, erosion control and carbon storage (Kremen 2005; Chan *et al.* 2006). Effects of land use and climate on biogeochemical cycles and ecosystem properties in turn feed back on global changes (Bardgett *et al.* 2008). Such feedbacks to the climate system depend on the response of the natural vegetation and its ability to adapt and migrate, since shifts in vegetation strongly affect the biophysical and biogeochemical characteristics of the land surface (Higgins & Harte 2006; Gonzalez *et al.* 2010). Understanding those effects and feedback mechanism is crucial to predict future scenarios and mitigate negative impacts of climate and land-use change, especially in tropical ecosystems and montane areas (Pounds *et al.* 1999; Lambin *et al.* 2003).

Tropical forests are among the ecologically most diverse and richest areas on Earth. They cover only about 13 % of the land surface but harbor more than half of the terrestrial species (Groombridge & Jenkins 2002). Also, they account for one third of the terrestrial net primary productivity (Saugier *et al.* 2001) and store roughly 25% of the terrestrial biosphere carbon (C) (Bonan 2008). This makes them a biome of major importance for research on biodiversity, ecosystem functioning and global C cycling (Brown 1993; Sayer *et al.* 2011). With their high belowground C sequestration potential, this is particularly true for mountain areas (Wilcke *et al.* 2008). Tropical mountains are exceptional ecosystems with huge climatic gradients and variations, and a large percentage of endemic species, which is why they are considered global hotspots of biodiversity (Gradstein *et al.* 2008). They are characterized by the frequent envelopment in orographic clouds, mists and related convective rainfall (Still *et al.* 1999). Especially cloud forests are an accumulation zone for the montane water tower, supplying lower elevation ecosystems with water in dry season and regulating floods and erosion in rainy seasons (Hamilton *et al.* 1995). An effect that is particularly important for the semi-arid East Africa. However, most research on ecosystem cycles and soil feedbacks has been focused on the Neotropics and South East Asia (Fisher *et al.* 2013), while Africa has received much less attention (Martin *et al.* 2012).

Recently efforts increased to close this knowledge gap (Dawoe *et al.* 2010; Pabst *et al.* 2016; Pabst *et al.* 2013; Mganga & Kuzyakov 2014; Mganga *et al.* 2015; Nyirambangutse *et al.* 2016). These studies

significantly helped to increase our knowledge on specific above or belowground processes in Afromontane ecosystems. However, most of these studies concentrated on single factors or processes and studies including feedback mechanisms, e.g. Schrumpf *et al.* (2006), are still scarce. It is crucial to understand functioning and interaction of C and nutrient cycles in these ecosystems as a whole to estimate their vulnerability and predict future effects from climate and land-use change (Stuart Chapin III *et al.* 2009).

1.1.2 Climatic control of ecosystem cycles

Carbon and nutrient cycling are major processes that define ecosystem functioning, control C storage and pools, provide plant nutrition and regulate microbial activity (Marschner 2012; Kuzyakov & Blagodatskaya 2015). These functions are affected by climate variables (moisture and temperature) and geological properties (Schulze & Mooney 1993; Doetterl *et al.* 2015). While climate is the factor determining biome distribution on a large scale, ecosystem specific structure and cycles are additionally controlled by geogenic nutrient supply. Mt Kilimanjaro as a stratovolcano offers the chance to exclude one of these covariates and study climate effects on soils that developed from similar parent material and have a similar age.

The elevation gradient of a mountain provides an ideal condition to investigate the response of biogeochemical cycles to climatic changes (Wang *et al.* 2016). Large variations of moisture availability and temperature occur successively along the slope, shaping ecosystem structure and affecting ecosystem cycles (Silver 1998; Hemp 2006a). Precipitation generally controls soil moisture and thus drought or water stress for plants and microorganisms (Boyer 1982; Manzoni *et al.* 2012). Higher temperatures can increase NPP (Pounds *et al.* 1999) and directly increase organic matter decomposition in soil (Davidson & Janssens 2006; Razavi *et al.* 2017). This again triggers feedback mechanisms that additionally accelerate C turnover processes – such as increased litterfall and root exudation (Uselman *et al.* 2000; Chave *et al.* 2010). Plant communities react to these changes through adaption – altered molecular structure of plant tissues (Aerts 1997), or investment in above or belowground productivity – affecting decomposability and recycling of organic matter in soil (Puget & Drinkwater 2001; Leuschner *et al.* 2007).

Soils, as the linkage between abiotic and biotic components of an ecosystem, are particularly affected by climatic changes. Soils are the largest terrestrial Carbon storage and account for more than 2500 GT C of which more than 60% is part of soil organic matter (SOM) (Lal 2008). Soil organic matter is defined as the total sum of all substances in the soil containing organic carbon, this comprises of a mixture of plant and animal residues in various stages of decomposition, substances synthesized microbiologically and/or chemically from the breakdown products, and the bodies of living and dead microorganisms and their decomposing remains (Schnitzer & Khan 1972). The amount of organic C

that is stored in soil depends on the interaction of climate variables, soil mineralogy, input from vegetation and decomposer organisms (Vitousek & Sanford 1986; Doetterl *et al.* 2015; Blagodatskaya *et al.* 2014b). As long as these processes are balanced, soil C storage remains stable. However, effects of global change will eventually unbalance the steady state, leading to either accumulation or losses of soil C (Davidson & Janssens 2006). Microbial mineralization of plant residues and organic matter in soil is a major flux in global C cycling, and releases about 58 Pg C $year^{-1}$ to the atmosphere (Houghton 2007). This flux is depending on the activity and community structure (heterotrophic vs. autotrophic) of soil microbes (Kuzyakov 2006; Blagodatskaya & Kuzyakov 2013) and their ability to effectively utilize the available substrate (Blagodatskaya *et al.* 2014a). While microbial communities govern the allocation of soil C (Schimel & Schaeffer 2012), they are directly dependent on the chemical composition of litter and SOM substrates. Hence, the interaction of these components are strongly related to the stability and turnover of C in soil (Allison & Vitousek 2004; Ng *et al.* 2014; Chen *et al.* 2014). A lot is known about quantitative effects on soil C (Jones *et al.* 2005), in contrast the variation of SOM chemistry across ecosystem scales and its relation to climate, vegetation and abiotic factors remains poorly understood (Vancampenhout *et al.* 2010). SOM chemistry is strongly varying on ecosystem scale (Vancampenhout *et al.* 2009; Yassir & Buurman 2012; Plante *et al.* 2009) and can easily change with vegetation and climatic boundary conditions (Andersen & White 2006; Stewart *et al.* 2011; Carr *et al.* 2013). These previous results indicate that local conditions cannot be easily applied to other regions and ecosystem specific fingerprints are necessary for global estimations (Schmidt *et al.* 2011).

1.1.3 Land-Use Change at Mt. Kilimanjaro

The montane areas of East Africa are an ecological and biodiversity hotspot (Mittermeier 2004). However, deforestation and the conversion of natural sites into arable land are rapidly ongoing processes (Lewis 2006). Between 2000 and 2005 the total area of forest cover losses in Africa amounted to about 11.5 Mio ha (Hansen *et al.* 2010). With its large deforestation rates, Tanzania is one of the areas most affected by land-cover change (Fisher 2010). Driven by large increases of population density, the slopes of Mt. Kilimanjaro experienced considerable agricultural land-use intensification within the last 50 years (Sébastien 2010; Misana *et al.* 2012).

Mt. Kilimanjaro ecosystems, in close vicinity to the 'cradle of mankind' (Leakey 1987), probably have been affected by human activities for millions of years. Early traces of civilization date back to more than 2200 years BP (Odner 2010) and within this time of continuous settlement, the forests below 1700m were largely transformed into agricultural land (Mwasaga 1991). The Chagga tribe, inhabiting Mt. Kilimanjaro region for more than five centuries (Odner 2010; Maro 1974), has established a form of subsistence agroforestry that is used until today: the Chagga homegardens. Homegardens are a sustainable, multilayered agroforestry system with a large variety of crops and high floral and faunal

diversity (Fernandes *et al.* 1986; Hemp 2006b). Fertilization mainly occurs in the form of livestock and household wastes while pest control is realized through a variety of anti-pest plant species (Fernandes *et al.* 1986). In 2005, one third of all homegardens were cultivated without using any fertilizers at all (Soini 2005). However, recently the usage of fertilizers and pesticides started to increase. With the introduction of cash crops (mainly *Coffea arabica*) in the late 19th century, homegardens were largely transformed into coffee plantations (Maghimbi 2007). This trend ended in the 1960th but intensification of the existing plantations is ongoing (Hemp 2006b). Increasing population pressure and cash-crop farming led to the expansion of agriculture to the down slope savanna zone (Maro 1974). The area of savanna shrub land decreased by 85% between 1961 and 2000 as it was turned into fields for maize (*Zea mays*), millet (*Eleusine coracanaarea*) and bean (*Phaseolus vulgaris*) production (Soini 2005).

These land-use changes already have had a strong negative impact on various ecosystem services and biodiversity parameters (Sébastien 2010; Winowiecki *et al.* 2016; Classen *et al.* 2014). However, from a scientific perspective this offers valuable possibilities to study the effects of anthropogenic disturbances on ecosystem C cycling in Afromontane ecosystems. Land-use change alters numerous ecological factors, which in turn, affect ecosystem functions and lead to high complexity and unpredictability of these changes (Groffman *et al.* 2001). It is especially important to assess the anthropogenic impacts on C sequestration, nutrient cycling and related ecosystem services, and to understand the underlying mechanisms of organic matter turnover and C incorporation in soil. Converting tropical forests to agricultural systems can lead to soil organic matter losses of up to 30% (Don *et al.* 2011), mainly from topsoil layers (Guo & Gifford 2002). Soil C losses from land-use change are particularly large in tropical regions (Ogle *et al.* 2005) and current estimates might still underrepresent these effects (Blécourt *et al.* 2013). Yet it remains unclear how agricultural land use affects carbon and nutrient balances and its interrelation to above- and belowground element cycles in Afromontane (agro-) ecosystems.

1.1.4 Spatial interaction of above and belowground processes

A major factor controlling the inter-ecosystem dynamic of carbon and nutrient cycles is the spatial distribution of aboveground biomass (Uriarte *et al.* 2015; Rascher *et al.* 2012). Above and belowground patterns are strongly linked especially when spatial diversity is high (Hooper *et al.* 2000). The characterization of spatial patterns in natural environments are essential to understand ecological processes and to initiate sustainable management techniques that aim to minimize degradation and alteration of ecosystem dynamics (Meyers 2012). Spatial variations are particularly large in the tropics (Houghton *et al.* 2009) and most interactions of above and belowground processes change with the climatic boundary conditions of each ecosystem. Therefore, it is important to understand these

interactions under various environmental limitations. Tropical alpine *Helichrysum* and East African savanna ecosystems each occur at one end of the vegetated slopes of Mt. Kilimanjaro (Hemp 2006a). Both ecosystems are exposed to strong, yet contrasting, climatic seasonality and are characterized by a distinctly heterogeneous vegetation cover. While *Helichrysum* sites are affected by large diurnal temperature fluctuations, savanna undergoes a pronounced seasonal dry-wet-season cycle throughout the year:

Helichrysum vegetation cover is sparse and reaches from open gravel and eroded patches, over tussock grass and herb communities, to Erica shrub patches. These vegetation patterns may feed back on soil C and N cycling through plant litter quality, root exudation of labile organic compounds and via competition for organic and mineral nutrients (Chapman *et al.* 2006; Rennenberg *et al.* 2009). Despite the important role in constraining potential changes to the C balance, soil N turnover and plant availability in high latitude and high altitude ecosystems are still poorly understood (Weintraub and Schimel 2005). Tropical alpine ecosystems are generally considered one of the least investigated ecosystems in the world (Buytaert *et al.* 2011). It is important to distinguish them from temperate alpine ecosystems, which are subjected to seasonal climatic variations with a distinct vegetation period and increased biogeochemical soil processes in summer (Schmidt *et al.* 2009). Tropical alpine ecosystems generally have lower atmospheric pressure, higher UV irradiance and variations, different rainfall regimes as well as extreme diurnal temperature changes. Particularly temperature variations are important for regulating C and N cycling. While metabolic activity increases with temperatures up to 37°C, microbes are still active under low soil temperatures (<5°C), and in particular during freeze-thaw events, and contribute significantly to gross soil N turnover and CO_2 fluxes (Schütt *et al.* 2014; Bore *et al.* 2017).

Co-dominance of trees and grasses is one of the main attributes that defines the savanna biome (Scholes & Archer 1997). Ecological interactions due to this contrasting vegetation cover have been a major research topic (Huntley & Walker 1982). Several studies have shown positive effects of trees on soil fertility, N availability, understory growth and C pools compared to open grassland. The term ‘islands of fertility’ was introduced to describe these patchy areas of distinctly altered biogeochemical conditions (Garcia-Moya & McKell 1970). It is assumed that N-fixation, whether by *Acacia* trees or by undergrowth species, is responsible for increased soil fertility of tree patches (Sitters *et al.* 2015). However, previous results are ambiguous (Bernhard-Reversat 1982; Belsky *et al.* 1989), and to date little is known about the interaction between affected soil properties and C cycle feedbacks, especially under water-limited conditions. While savannas are considered active or potential C sinks (Grace *et al.* 2006), they act as a net source of CO_2 during the dry season (Miranda *et al.* 1997). It remains unclear which factors regulate these C losses and how the vegetation cover affects them.

1.1.5 Objectives

The main objective of this research was to investigate climatic and land-use effects on soil nutrient and carbon pools, turnover, availability, and losses in natural and agricultural ecosystems along the elevation gradient of Mt. Kilimanjaro. The knowledge on land use and climate driven effects on nutrient cycles in these ecosystems is a prerequisite to predict future changes in biodiversity, ecosystem stability, productivity, and services in the Eastern Afromontane region. The specific objectives were:

- First, to assess the effects of land-use change and climatic conditions along the elevation gradient on litterfall (Study 1), litter quality (Study 1 & 3), litter decomposition and C stabilization in ecosystems with similar soil parent material (Study 2 & 3).
- Second, to identify the response of SOM pools to the highly variable climatic conditions along a 3500 m elevation gradient of Mt. Kilimanjaro by investigating:
    - the composition and abundance of microbial groups in topsoil and separating direct and indirect climatic (i.e. altered edaphic conditions) effects (Study 4)
    - the chemical composition of SOM compounds and evaluating quantitative changes in the specific SOM fractions in relation to ecosystem productivity and carbon turnover (Study 5)
- Third, to link spatial patterns of soil parameters and greenhouse gas emissions to the spatial variability and characteristics of aboveground biomass and to compare these relationships in ecosystems with very contrasting climate regimes and 3000m difference in elevation:
    - Alpine *Helichrysum* cushion vegetation with a diurnal freeze-thaw cycle (Study 6)
    - Lowland savanna with seasonal droughts (Study 7)

1.2 Material and Methods

1.2.1 Study area

The studies were conducted on the southern slope of Mt. Kilimanjaro (3°4'33"S, 37°21'12"E) Tanzania, along an elevation gradient from 770 to 4200 m a.s.l. The research sites were provided and maintained by the German Research Foundation Project: *Kilimanjaro ecosystems under global change* (KiLi-FOR 1246).

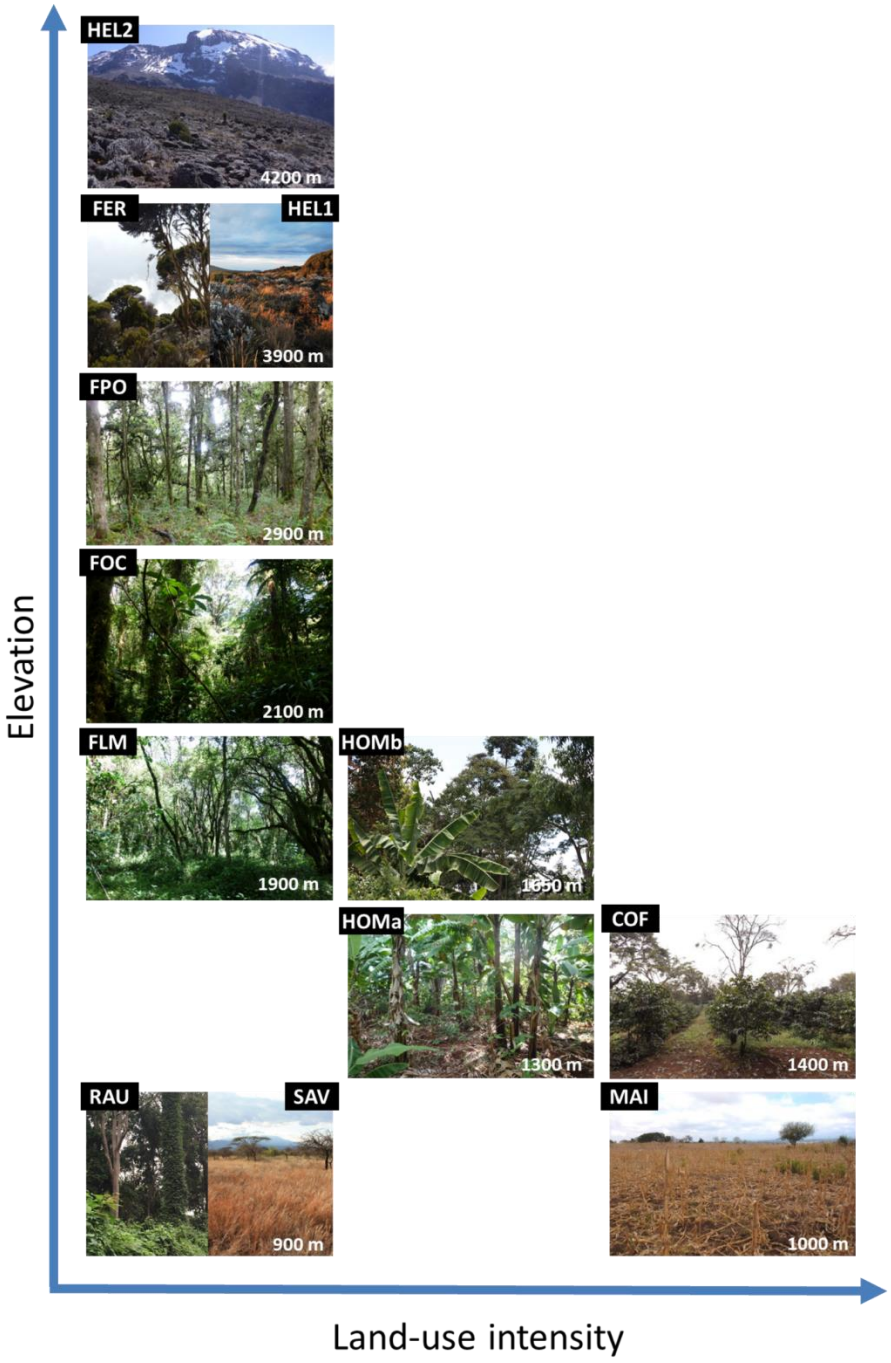

Figure 1.2-1: Research sites along the elevation and land-use gradients of Mt. Kilimanjaro. Labels are equivalent to abbreviations in the text: SAV – savanna, RAU – dry broadleaf forest, FLM – lower montane forest, FOC – *Ocotea* forest, FPO – *Podocarpus* forest, FER – *Erica* forest, HEL – *Helichrysum*, HOM – Chagga homegardens, MAI – maize fields, COF – coffee plantations.

Twelve plots (0.25 to 1 ha) were selected, representing typical natural and agricultural ecosystems of the region (Figure 1.2-1). The savanna woodland (SAV) with *Acacia* and C_4-grass species is represented by the least disturbed site within the Lake Chala Game Reserve. Remnants of lowland dry-broadleaf forest (RAU) can be found in the Rau Forest Reserve, near Moshi town (770 m). This forest is dominated by *Milicia excelsa, Macaranga capensis* and *Albizia gummifera* in the upper tree layer. Effects of transforming of these natural vegetation types into arable land (below 1200 m) are assessed by comparison to maize fields (MAI). To represent land-use change in the densely populated area between 1200 m and 1800 m, two Chagga homegardens (HOM) and one Coffee Plantations (COF) were selected. The homegardens are mainly used for smallholder crop production (*Musa* ssp. and *Coffea* ssp.) under cultivated fruit trees (e.g. *Persea Americana, Grevillea robusta*) and remnant forest trees (e.g. *Albizia schimperiana, Cordia africana*) (Hemp 2006b). They are traditionally managed with sporadic addition of organic fertilizers and household waste and a strongly variable species composition (Fernandes et al. 1986). The shaded coffee (COF) represents intensively managed plantations, with regular application of mineral fertilizers and pesticides. Natural forests and montane ecosystems above 1800 m are located inside the Kilimanjaro National Park along the Machame and Umbwe ridges. These ecosystems were thoroughly described by (Hemp 2006a). In short, with increasing elevation: Lower montane forest (FLM) at 1920 m is dominated by *Macaranga kilimandscharica, Agauria salicifolia* and partly *Ocotea usambarensis*. In *Ocotea* forest (FOC) at 2100 m, *O. usambarensis* dominate and is accompanied by large tree fern (*Cyathea manniana*). The *Podocarpus* forest (FPO) above 2800 m is dominated by *Podocarpus latifolius* together with *Prunus africana* and *Hagenia abyssinica*. In the subalpine *Erica* forest around 4000 m (FER), *Erica trimera* is dominating and reaches up to 10 m growth height. Between 4000 and 4500 m (HEL), the alpine forest is displaced by *Helichrysum* cushion vegetation with only a few specimens of *E. trimera, Dendrosenecio kilimanjari* and *Euryops dacrydioides* reaching over one meter height. The herb layer covers about 30% and is dominated by *Helichrysum newii*, *H. citrispinum* and *H. forskahlii* as well as *Haplosciadium abyssinicum* and tussock grasses (Ensslin et al. 2015). Two additional sites (~2 ha) were selected to study spatial heterogeneity in severe environments. One is representing the *Helichrysum* ecosystem located close to Shira 2 hut (3°05’36’’S; 37°27’68’’E). The other is located in the Lake Chala Game Reserve (3°18’39”S, 37°41’8”E), representing savanna shrubland vegetation.

In the colline zone, soils developed on erosion deposits from Mt. Kilimanjaro and were classified as Vertisols (Kühnel 2015). Soils in the forest zone were classified as Andosols with folic, histic or umbric topsoil horizons and accordingly high C contents in the upper horizons (Zech 2006). In the alpine zone, soils are mainly Leptosols and Vitric Andosols (WRB 2014). These soils developed from volcanic rocks, such as basalt, trachyte and olivine basalts over the last 0.2 to 2.3 Mio years (Dawson 1992). The similar parent material throughout the elevation gradient makes the comparison of ecosystems on Mt.

Kilimanjaro especially beneficial, because soil conditions are solely a function of local ecosystem characteristics.

The climate at Mt. Kilimanjaro follows a bimodal rainfall regime with long rains from March to May and a shorter rainy season between October and December (Appelhans *et al.* 2016). Mean annual precipitation (MAP) varies between 750 mm and about 3000 mm, dependent of elevation and exposition (Table 1.2-1). Mean annual temperature (MAT) ranges from 2.5 °C to 20.9 °C and monthly means vary around ±3 °C.

Table 1.2-1: Site characteristic and C and N contents in 0-10 cm soil depth for twelve ecosystems on the southern slope of Mt. Kilimanjaro*

Ecosystem	ID	Land-use class	Elevation [m]	MAT [ºC]	MAP [mm]	C [%]	N [g kg^{-1}]	pH
Colline forest	RAU	Natural, disturbed	767	23.7	845	9.5	7.4	7.5
Savanna	SAV	Natural, disturbed	951	23.7	536	2.8	2.0	5.4
Maize field	MAI	Agricultural, intensive	1009	22.6	693	1.5	1.2	4.6
Chagga homegarden (a)	HOMa	Agricultural, traditional	1275	20.8	1336	3.8	3.4	5.4
Chagga homegarden (b)	HOMb	Agricultural, traditional	1647	17.0	2616	8.5	6.7	4.8
Coffee plantation	COF	Agricultural, intensive	1305	20.1	1485	1.9	1.8	4.3
Lower montane forest	FLM	Natural, disturbed	1920	15.3	2378	17.3	11.7	4.0
Ocotea forest	FOC	Natural	2120	12.1	2998	24.2	15.1	3.8
Podocarpus forest	FPO	Natural	2850	9.4	1773	26.6	13.9	3.9
Erica forest	FER	Natural	3880	4.5	1188	15.0	8.2	4.9
Helichrysum cushion 1	HEL1	Natural	3880	5.3	778	13.1	8.8	n.d.
Helichrysum cushion 2	HEL2	Natural	4190	4.5	962	3.6	2.6	5.2

*site average may differ from individual sampling values in each study

1.2.2 Research approaches

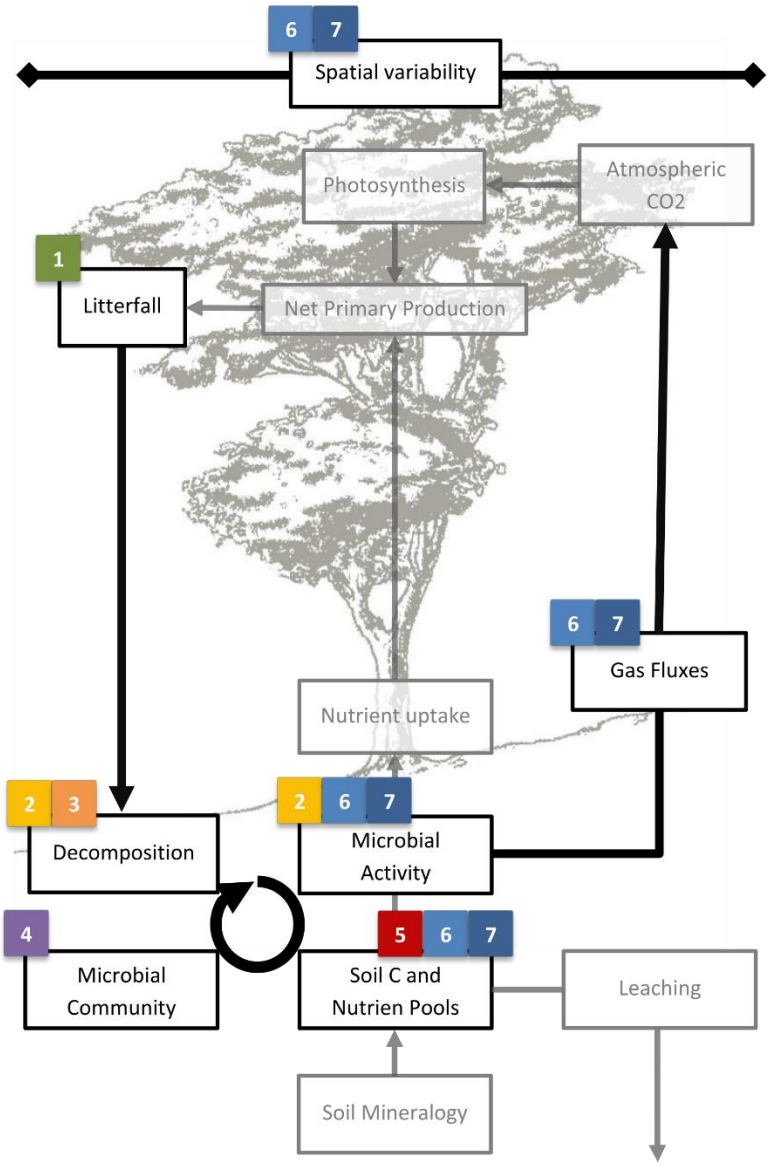

Figure 1.2-2: Scheme of steps and processes in ecosystem C and nutrient cycles. Numbers and colors indicate related studies in this PhD project.

1.2.2.1 Litterfall and decomposition studies

Annual patterns of C and nutrient input via litterfall and subsequent litter decomposition were analyzed and quantified in natural forests and agroforestry systems. Carbon and nutrient depositions were quantified and related to seasonal variations in decomposition and C stabilization. Tree litter in four natural (lower montane, *Ocotea* forest, *Podocarpus* forest and *Erica* forest), two sustainably used (homegardens) and one intensively managed (shaded coffee plantation) ecosystems was collected on a biweekly basis from May 2012 to July 2013 (Study 1). Leaves, branches and remaining residues were separated and analyzed for C and nutrient contents. The collected leaf litter was exposed for three, six and twelve months, in the natural forests sites covering an elevation gradient from 1920 to 3880 m (*Study 3*). Microcosm were covered with mesh of three different sizes (0.25 mm, 2 mm and 5 mm) to

selectively exclude decomposer fauna (Makkonen *et al.* 2012). Initial and final contents of C, N and major nutrient cations were measured. To assess the effects of climate and land-use on decomposition of standardized litter substrate Tea Bag Indices (decomposition rate constant k and stabilization factor S) were used (Study 2). Nine pairs of litterbags were exposed in ten ecosystems (adding savanna, maize fields, homegarden, coffee plantation and *Helichrysum*) during the warm-wet, warm-dry, cold-wet and cold-dry season 2015. Land-use effects were considered under the assumption that elevation related variability is neglectable when compared on the same altitudinal zone (i.e. colline and lower montane) (Hemp 2006a; Ensslin *et al.* 2015).

1.2.2.2 Soil sampling and analysis

Soil samples were collected from six research sites, representing natural forest and alpine ecosystems along the elevation gradient from 767 to 4190 m: RAU, FLM, FOC, FPO, FER and HEL2. At each site, four subplots (5x5 m) were selected. Five topsoil samples (0-10 cm depth) per subplot were taken randomly and pooled to reflect ecosystem heterogeneity. The samples were sieved (2 mm), and roots and plant materials were removed. Field samples were split and stored dry (60 °C and 104 °C) as well as frozen (-20 °C) until analysis. Basic characteristics, such as C and N contents, pH and water content were measured. Microbial composition was determined on frozen samples using phospholipid fatty acid biomarkers (PLFAs) following Frostegard & Baath (1996) (Study 4). Soil organic matter composition and stability was determined from dry samples by a combination of thermal combustion methods (Study 5).

1.2.2.3 Spatial interaction of above and belowground processes

Spatial patterns of soil parameters and greenhouse gas emissions were investigated in two ecosystems with very contrasting climate regimes (Savanna and *Helichrysum*). The spatial variability of belowground parameters was related to aboveground biomass and vegetation characteristics.

The tropical alpine *Helichrysum* site at ~4000 m a.s.l. was investigated over a 6-day period in December 2014 (Study 6). Soil characteristics in 0-5 cm and 5-10 cm depth, as well as CO_2, N_2O and CH_4 fluxes from soils were measured depending on vegetation cover (low, medium and high). Major gross N turnover rates on these patches were investigated by $\delta^{15}N$ pool dilution.

An intensive research campaign was conducted in September 2014 at the savanna site close to Lake Challa (Study 7). Three trees were selected from each of the two most dominant species: the legume *Acacia nilotica* and the non-legume *Balanites aegyptiaca*. For each tree, one transect was selected with nine sampling intervals depending on crown radius. Greenhouse gas (GHG) fluxes were measured once. Soil cores were taken from 0-10 cm and 10-30 cm depth. A broad range of soil parameters, GHG exchange, plant properties, as well as soil and biomass $\delta^{13}C$ signature were compared between tree crown area and open area.

1.2.2.4 Analytical Methods

Phospholipid fatty acid analysis

PLFAs were determined according to Frostegard & Baath (1996). Polar lipids were extracted and separated into neutral, glycol, and phospholipids. Phospholipids were then purified by liquid-liquid and solid phase extraction chromatography, and derivatized to their fatty acid methyl esters (FAMEs). Gas chromatography–mass spectrometry (GC–MS) was then used to analyze FAMEs against an internal standard (13:0). PLFAs were classified according to available reference datasets (Leckie 2005; Lewandowski *et al.* 2015) and grouped into gram negative and gram positive bacteria, actinomycetes as well as fungi and arbuscular mycorrhiza fungi. Quantification of PLFAs was based on an external standard containing 28 PLFAs as described by (Gunina *et al.* 2014).

Analytical pyrolysis

Analytical double-shot pyrolysis gas chromatography mass spectrometry (Py/GC-MS) was used to chemically characterize SOM composition (Leinweber & Schulten 1999). Double-Shot analysis was performed to increased resolution in MS spectra by separating the release of chemically sorbed compounds (thermal desorption 100-280 °C) and cracking of covalent bounds (pyrolysis: 280-600 °C). Evolving gas analysis mass spectrometry (EGA-MS) was used to quantitatively assess the results of Py/GC-MS and estimate the compound's chemical stability (Plante *et al.* 2009).

Greenhouse gas fluxes

Gas samples were collected using a static chamber approach. At each sampling location, collars for GHG measurements were installed (383 cm^2). Opaque polypropylene chambers (25.2 x 15.2 x 14.7 cm) were fixed gas tight to the collars and gas samples were taken with a 60ml gas tight syringe. Headspace gas was sampled five times at 0, 15, 30, 45 and 60 min after chamber closure. Gas samples were analyzed using a gas chromatograph equipped with an electron capture detector (ECD N_2O) and a flame ionization detector/methanizer (FID: CH_4 and CO_2). Flux rates were calculated with R version 3.2.0 including HMR package 0.3.1 for calculation of GHG flux rates by linear increase or decrease in gas concentration over time (n = 5).

Soil chemical characteristics

Carbon and N contents were measured in an automated dry combustion C:N analyzer. Inorganic C content was found neglectable on the sites and total C content was considered as equal to organic C (Becker, unpublished data; Kuehnel, unpublished data). Microbial biomass C (MBC) and microbial biomass N (MBN) were estimated by fumigation extraction (Vance *et al.* 1987) using correction factors of 0.45 (MBC) and 0.54 (MBN) (Joergensen 1996; Joergensen & Mueller 1996). Carbon that was extractable by K_2SO_4 was used as approximation of dissolved organic C. Available N (NH_4^+ and NO_3^- concentrations) in the extracts were measured by continuous flow injection colorimetry. Availability

of major nutrient cations (Al^{3+}, Ca^{2+}, Fe^{2+}, H^{+}, K^{+}, Na^{+}, Mg^{2+}, Mn^{2+}) was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) following a preparative extraction in unbuffered salt solution (1 mol l^{-1} NH_4Cl). Total cation exchange capacity (CEC) and base saturation were calculated as described by Chesworth (2008). Soil pH was measured in H_2O as well as $CaCl_2$ or KCl solution.

Soil physical properties

Bulk density (BD) was calculated from oven dried (72 h at 105°C) undisturbed soil cores (100 cm^3) taken at the center of the respective soil depth. Stone fraction (>2 mm) was measured as displaced water volume and subtracted from total core volume. Soil temperature was measured electronically at 5 and 10 cm depth.

Fine root biomass

Macroscopically visible roots (>10 mm length) were extracted by hand and were separated as belonging to shrubs, grasses, herbs and mosses (*Helichrysum*) and trees and grasses (Savanna) under the stereomicroscope. Root elasticity and degree of cohesion of cortex, periderm and stele was used to distinguished between live roots (biomass) and dead roots (necromass) (Leuschner *et al.* 2001). Fine root biomass and necromass samples were dried at 70 °C (48 h) and weighed.

Stable isotope measurements

Natural abundance of ^{13}C isotopes was analyzed by an elemental analyzer (EA) coupled to an isotope ratio mass spectrometry (IRMS). Delta values ($\delta^{13}C$) were calculated as the divergence from the standard reference for ^{13}C to ^{12}C ratio (Vienna-PDB). Gross N mineralization and nitrification rates were measured using isotope labeling of sieved soil (Dannenmann *et al.* 2009). Diffusion of ^{15}N on acid traps, and the analysis of isotopic signatures were measured by EA-IRMS.

1.3 Results and Discussion

1.3.1 Overview of Main Results

Table 1.3-1: Summary of main objectives and results

Study	Objective	Main Conclusion
Study 1: *Annual litterfall dynamics and nutrient deposition depending on elevation and land use at Mt. Kilimanjaro*	• Investigating annual dynamics • Quantification of annual C and nutrient inputs • Comparing natural and managed ecosystems and address implications for the ecosystem nutrient cycle	• Annual leaf litter production peaks at the end of dry season and decreased at higher elevations due to lower temperatures and reduced net primary production • Nutrient cycles in agroforestry ecosystems were accelerated by fertilization and the associated changes in dominant tree species
Study 2: *Teatime on Mount Kilimanjaro: Seasonal variation in standardized litter decomposition and effects of elevation and land use*	• Evaluating effects of climatic seasonality on decomposition rates of standardized litter • Assessing effects of land-use intensification on decomposition rates and C sequestration.	• Decomposition rates were reduced though seasonal moisture limitation below 1900 m and annual temperature limitation above 2850 m. • Due to their temperature sensitivity, alpine Afromontane ecosystems must be considered future CO_2 sources • Land-use intensification decreases stabilization of new C inputs
Study 3: *Climatic and decomposer community effects of leaf-litter decomposition along the elevation gradient of Mt. Kilimanjaro*	• Quantify annual decomposition of native leaf litter • Quantification of annual C and nutrient release through decomposition • Asses effects of accessibility for decomposer communities	• Climatic variables are more important than litter nutrients and complexity of decomposer communities for controlling litter decomposition along the gradient of Mt. Kilimanjaro. • Annual release rates vary considerably between ecosystems and indicate high demand for litter recycling.
Study 4: *Soil microbial community structure in forest soils along the elevation gradient of Mount Kilimanjaro*	• Evaluating the distribution of total microbial biomass (obtained by PLFA analysis) and particular microbial groups along the Mt. Kilimanjaro climosequence • Reveal effects of climatic (MAT and MAP) and edaphic factors (C, N and pH) on soil microbial communities	• Gram-negative biomarkers dominated PLFAs composition, accounting 25-40%, thus regulating the trend of PLFA distribution with elevation • Gram-positive biomarkers decreased with elevation, due to the harsh environmental conditions in the alpine zone • Fungal biomarkers increased with elevation gradient, showing resistance to the low MAT, and decrease in nutrient contents

Study 5: *Thermal and Structural Characterization of Soil Organic Matter Composition at Mount Kilimanjaro*	• Identifying changes in SOM composition along a 3500 m elevation gradient • Quantifying changes in specific C fractions and relate these changes to ecosystem carbon turnover processes	• EGA curves do not reflect the chemical composition derived from py-GC/MS. • High productivity at mid-elevation increased the amounts of volatile compounds but at the same time increases stabile carbon pools.
Study 6: *Nitrogen turnover and greenhouse gas emissions in a tropical alpine ecosystem, Mt. Kilimanjaro, Tanzania*	• Quantification and characterization of key gross N turnover rates and soil greenhouse gas (CO_2, N_2O, CH_4) exchange under different vegetation cover types • Investigating effects of precipitation and freeze thaw cycles on biogeochemical processes	• Carbon input from the vegetation and root exudates increase C and N substrate availability, and thus, increase microbial biomass and CO_2 fluxes in vegetated patches • N cycle is tight and dominated by closely coupled ammonification and NH_4^+-immobilization, which is little prone to N losses • Warming could increase vegetation cover and thus, N turnover, but only more narrow C:N ratios due to atmospheric N deposition may open the N cycle of *Helichrysum* ecosystems
Study 7: *Legume and non-legume trees increase soil carbon sequestration in Savanna*	• Determine patterns of soil properties and soil-greenhouse-gas fluxes, depending on the spatial variability and characteristics of the vegetation (legume vs. non-legume tree) • Quantifying effects of trees on soil C and nutrient contents and identify controlling mechanisms	• The spatial structure of aboveground biomass in savanna ecosystems leads to a spatial redistribution of nutrients • Lower litter quality of C_4 grasses reduces microbial C use efficiency and thus increases mineralization rates • The capability of savanna ecosystems to act as C sinks is both directly and indirectly dependent on the abundance of trees, regardless of their N-fixing abilities

1.3.2 Effects of elevation and land use on C and nutrient cycling

1.3.2.1 Effects of elevation

Within the natural forests of Mt. Kilimanjaro, between 1900 and 2900 m a.s.l., leaf litterfall decreased with elevation (Figure 2.1-1). Leaf litter production depends on net primary production and temperature, thus usually decreases at higher elevations (Girardin *et al.* 2010). Sporadic sampling at sub-alpine *Erica* forest (data not included) indicated that this trend would be further strengthened in ecosystems above 3000 m a.s.l. The effect of elevation is less clear across ecosystems (Röderstein *et al.* 2005) and by including branches and other residues the trend disappears within the Mt. Kilimanjaro forest belt (Figure 2.1-1). Nonetheless, litter decomposition experiments along the extended elevation gradient (i.e. including *Erica* forest) indicated a decline of productivity at 2900 m and above (Figure 2.2-3). Decomposition maxima occurred in FLM and FOC, between 2000 and 2500 m and can be directly linked to temperature and precipitation patterns (Figure 1.3-1). In upper montane and alpine environments (≥2850 m), the decomposition was strongly limited by temperature and increased during the warm seasons. This is commonly expected because temperature sensitivity of decomposition is generally higher at low temperatures (Davidson & Janssens 2006) and at higher elevation (Schindlbacher *et al.* 2010; Blagodatskaya *et al.* 2016). Another factor that might reduce decomposition specifically in *Podocarpus* forest (2850 m) is the regular water logging of soil due to clouds inhibiting evaporation of the perennial rainfall water (Bruijnzeel & Veneklaas 1998). However, neither negative nor positive effects of precipitation were found during the seasons (Figure 2.2-5). Strong seasonality of decomposition rates in *Erica* and *Helichrysum* ecosystems implies strong dependency on climate variables and low potential to adapt to fast climate changes compared to lower elevation forests (Hemp & Beck 2001). The projected increase of surface temperature (Bradley *et al.* 2006) will reduce C stocks. Therefore, future C losses into the atmosphere might be considerably large and fast in east African mountain ecosystems.

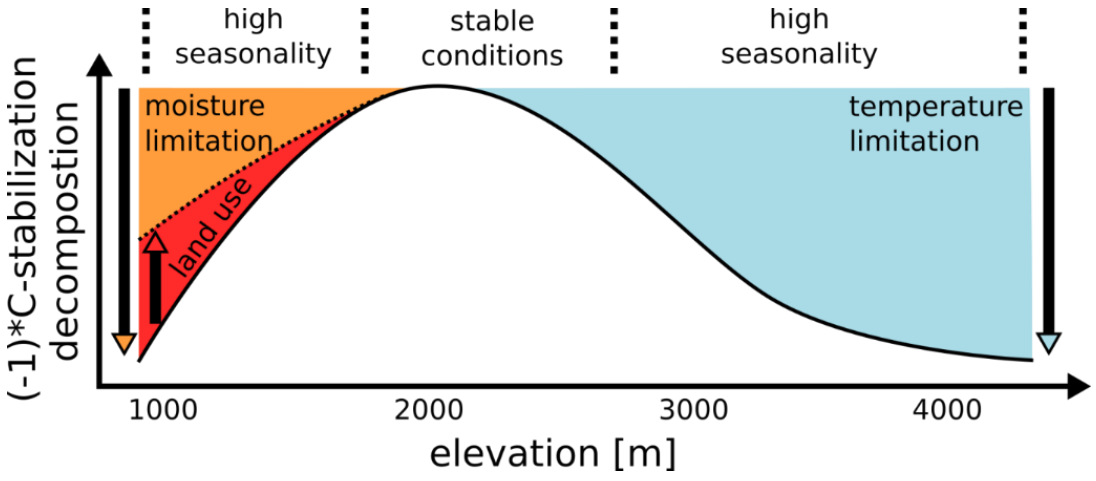

Figure 1.3-1: Climate and land-use effects on standardized litter decomposition at Mt. Kilimanjaro.

The elevation pattern was the same for native and standardized litter substrate (Study 2, Study 3): Resembling trends for litter substrates indicate that in Mt. Kilimanjaro forests - along the elevation gradient of 1900 to 3900 m - climatic drivers are more important for controlling litter decomposition rates between ecosystems than changes in leaf litter quality. Generally, native and standardized litter react similarly to environmental changes (Didion *et al.* 2016) and trends along the montane elevation zones can be regarded equivalent to longitudinal biome zonation (Stevens 1992). Decomposition patterns between biomes are usually controlled by climatic factors (Berg *et al.* 1993). Including ecosystems below 1900 m further reinforced the importance of climatic effects on C and nutrient cycles at Mt. Kilimanjaro (Figure 1.3-1). Tea Bag indices *k* and *S* had their critical values at mid elevation: the decomposition rate k – its maximum, and the stabilization factor S – its minimum (Figure 2.2-3). Ecosystems at lower elevation are highly subjected to seasonal moisture limitation (Appelhans *et al.* 2016). During the rainy season, soil microbial activity in Savanna strongly increases (Otieno *et al.* 2010) and the turnover is less selective regarding OM quality (Davidson & Janssens 2006). This effect is only present in semi-arid elevation zones (i.e. colline and sub montane). FLM and FOC (i.e. mid-elevation forests) represent the interception zone between sufficient moisture availability and temperature. This indicates that C sequestration in these ecosystems is mainly driven by amounts of litter input and productivity. At lower and higher elevation, decomposition is reduced by climatic restrictions.

Seasonal variability of leaf litterfall in natural forests on Mt. Kilimanjaro followed a U shaped pattern with increasing elevation (Figure 2.1-2). In tropical montane forests, the seasonality of litterfall is generally low compared to tropical lowland forests (Chave *et al.* 2010). The weakest seasonal variation was observed in *Ocotea* forest in 2190 m a.s.l., featuring the highest annual precipitation and least varying soil moisture conditions (Table 1.3-1). At FPO (2850 m a.s.l.), seasonality increased again with lower MAP and an increasing temperature limitation. Litter production at higher elevation was distributed over the warmer period between October and May when canopy productivity is usually higher (Girardin *et al.* 2010). This pattern is based on the dependency of litterfall seasonality on rainfall intensities as well as temperatures (Zhou *et al.* 2006; Chave *et al.* 2010). Litterfall peaks during the dry season are well documented in tropical forests and plantation systems and mainly reflect drought stress (Okeke & Omaliko 1994; Barlow *et al.* 2007; Selva *et al.* 2007). A recent meta-analysis by Zhang *et al.* (2014) has shown that this connection is a characteristic feature of tropical ecosystems. Leaf aging, caused by photo inhibition, stomatal closure and subsequent leaf overheating, might lead to leaf shedding at the end of the dry season (Röderstein *et al.* 2005).

Litterfall peaks at the end of the dry season promote an accumulation of particulate organic matter on the surface soil. This accumulation entails increased microbial activity and mobilization of C and nutrients during the following wet season (Sayer *et al.* 2007). Particularly at lower elevation,

decomposition was additionally enhanced during the wet season. Therefore, peaks of freshly mobilized C and nutrients just before the early wet season increase the possibility of leaching or translocation to deeper soil layers (Qiu *et al.* 2005; Pabst *et al.* 2013). As a consequence, an increased nutrient deposition via litterfall might not necessarily result in higher nutrient availability, but may actually increase nutrient losses. The investigated agricultural ecosystems at Mt. Kilimanjaro experienced distinct climatic seasonality and accumulated large amounts of litter at the end of dry season (Figure 2.1-2). This implies that the nutrient cycles in these ecosystems are especially vulnerable to changes in vegetation structure and species composition.

1.3.2.2 Land-use effects

Land-use intensification affected C and nutrient cycles at various levels: First, litter macronutrient content (N, P, K) in agroforestry systems increased (Figure 2.1-4), enhancing biogeochemical cycles in these ecosystems compared to natural forests. Second, C and macro nutrient deposition (N, P, K) further increased with the transformation of traditional (HOM) to plantation agriculture (COF) (Table 1.3-1). Third, C stabilization in these ecosystem and in the colline zone (SAV, MAI), was reduced by land-use intensification due to the higher microbial demand for fresh substrate (Figure 2.2-6).

Macronutrient contents in leaf litter of managed ecosystems were two to five times higher than in natural forests (Figure 2.1-4). Independent from elevation, HOM and COF at Mt. Kilimanjaro had higher N contents and therefore lower C:N ratios in leaf litter than natural forests. N-deprived plants usually have a high C:N ratio in litter (Chave et al., 2010). Fertilization in agroforestry systems leads to higher N contents in plants and consequently in leaf litter (O'Connell and Grove, 1993). Furthermore, the introduction of crops such as Musa ssp. and Coffea ssp. affects the nutrient content of vegetation and litter in general. As a result, the annual N deposition by litterfall in HOM and COF increased and N cycling in these ecosystems was enhanced. This is in line with Zech et al. (2011), who found evidence for accelerated N-cycling in the cultivated areas of Mt. Kilimanjaro. Fertilization with N and P also increases the content of other macronutrients in leaf litter (O'Connell and Grove, 1993). This corresponds to our findings that the content of most macronutrients in land-use ecosystems either increased or remained on the same level compared to natural forests. Decomposition is generally accelerated by a higher macronutrient content (Allison and Vitousek, 2004; Debusk and Reddy, 2005). The abundant macronutrients in the litter of the investigated agricultural ecosystems therefore imply an accelerated C and nutrient turnover in the respective ecosystems. Easily available substrate is decomposed faster, and soil respiration (i.e. soil CO_2 efflux) is generally higher in soils of intensively managed versus natural ecosystems at Mt. Kilimanjaro (Mganga and Kuzyakov, 2014). Together with tillage and crop removal, this explains the lower C and N stocks in the topsoil of agroforestry systems compared to natural forests at Mt. Kilimanjaro (Table 2.1-1). Consequently, the conversion of natural

forests to perennial plantations or homegardens probably represents a source of atmospheric CO_2 despite their structural resemblance to natural forests.

Land-use intensification from semi-natural savanna to maize monocultures and from traditional homegardens to large-scale coffee plantations decreased C stabilization and showed the tendency to increase decomposition rates (Figure 2.2-6). The total content of soil organic matter and microbial biomass commonly decrease with land use intensification (Don et al., 2011; Junior et al., 2016). This effect was also found at Mt. Kilimanjaro (Pabst et al., 2013). However, at the same time decomposition rates at Mt. Kilimanjaro tended to increase while C stabilization decreased. This is in contrast to previous findings that connected land-use intensification to decreasing decomposition rates (Attignon et al., 2004; Violita et al., 2016). Even under similar environmental conditions as compared to the lower slopes of Mt. Kilimanjaro (i.e. western Kenya, 1500 m), Kagezi et al. (2016) found a decrease of decomposition rates with agricultural land use. This decrease of SOM decomposition can be connected to the application of N fertilizers and reduced microbial biomass (Zang et al., 2016). Decomposition studies tend to exhibit strong site and method specific variation (Makkonen et al., 2012) and land-use intensification was likewise found to increase decomposition of litter and soil organic matter (Lisanework & Michelsen, 1994; Guillaume et al., 2015). Decreasing decomposition with higher land-use intensity is often related to changes in decomposer communities (Kagezi et al., 2016). Recent studies from Mt. Kilimanjaro found only minor effects of land-use change on overall arthropod abundance and composition (Röder et al., 2016) but indicated accelerated organic matter turnover on agricultural sites (Becker et al., 2015). In addition, glucose decomposition increases with land-use intensification from savanna to maize fields and homegardens to coffee plantations (Mganga & Kuzyakov, 2014). This is because soil microbes in these ecosystems are less efficient in SOM decomposition but at the same time more demanding for new C sources (Pabst et al., 2016), reducing S values on agricultural sites (Figure 2.2-6). This concept relates decomposition patterns primarily to the microbial decomposers nutritional status (Manzoni et al., 2008). Considering the features of the TBI method (i.e. standardized litter, exclosure of exogeic and >0.25 mm fauna) this points out the importance of pre-existing soil nutrient conditions on litter decomposition and C stabilization.

1.3.3 Effects of elevation on soil microbial communities and organic matter composition

1.3.3.1 Effects of elevation on microbial communities

Total PLFA content increased with elevation until *Ocotea* forest (2100 m), reaching a maximum of 2100 nmol g^{-1} soil, followed by a decrease in (sub-) alpine ecosystems (Figure 1.3-2). Gram-negative bacteria abundance, making up for 25-40% of total PLFAs, mainly determined this trend. Actinomycetes, fungi and arbuscular mycorrhizal fungi followed a U-shaped pattern and gram-positive

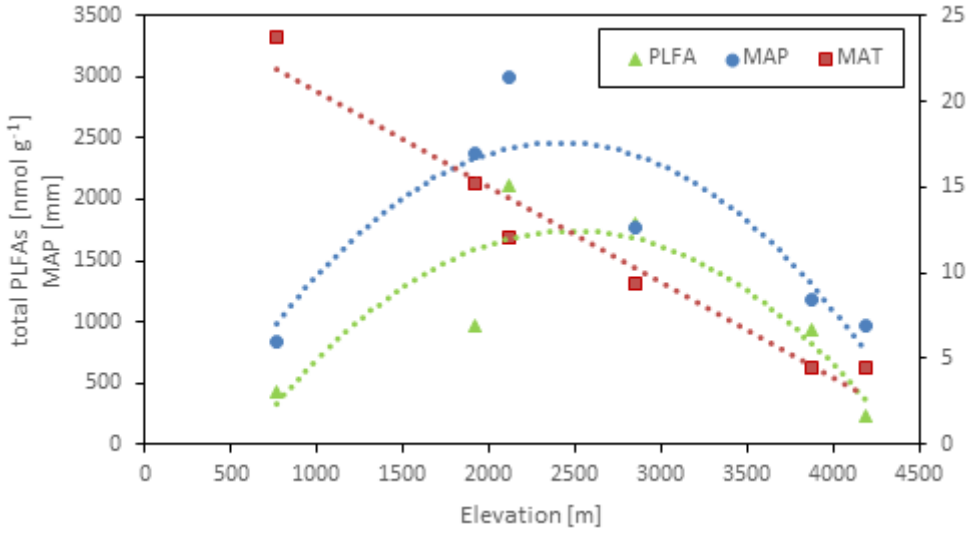

Figure 1.3-2: Mean Total PLFAs content, mean annual precipitation (MAP), and mean annual temperature (MAT) in six ecosystems along the elevation gradient of Mt. Kilimanjaro.

bacteria abundance decreased with elevation (Figure 2.4-2). Total PLFA content is a proxy for microbial biomass and therefore strongly correlated C contents as well as to previously reported MBC values (Pabst *et al.* 2013, Study 6). As proposed by Pabst (2015), elevation patterns of these variables are a combined result of the strong climate dependency of net primary productivity (NPP) and microbial activity. Annual moisture availability and moderate temperatures at mid-elevation (2100 m) increase NPP (i.e. leaf and root inputs) and turnover rates, simultaneously increasing C content and thus microbial biomass content. While NPP at low elevation (e.g. RAU) might be potentially high in rainy season, seasonal variations are large (Study 2) and strongly reduce productivity in dry season (Otieno *et al.* 2010). Low temperatures at high elevation decrease the activity of microorganisms (Study 2); however, they do not necessarily decrease the amount of soil microbial biomass (Blume *et al.* 2002). This indicates that low inputs at high elevation (Ensslin *et al.* 2015; Hemp 2006a) decrease of total PLFAs from 3800 m to 4100 m, mainly due to a low vegetation cover at Helichrysum (Gütlein *et al.* 2016).

Changes in the composition of microbial communities along the slope of Mt. Kilimanjaro are a result of this climatic optimum gradient and the consequent niche differentiation through certain groups. A partial redundancy analysis (RDA) was used to distinguish the effects of soil parameters and the underlying climatic conditions. The combined RDA model was highly significant (p-value < 0.001) and explained 65% of the variance in the PLFA dataset. Soil N content was the main factor contributing to RDA1 (r = -0.79), while soil C/N ratio was the strongest related to RDA2 (r = -0.89). Variation in the soil parameters (partial RDA) explained 19% of the total variance in PLFAs (Figure 2.4-6). Climatic variable (MAT, MAP) alone explained 6%. The interaction of soil parameters with climatic variable added 44% of the explained variance. Hence, both effects have to be considered as combined factors explaining

microbial community changes along the elevation gradient of Mt. Kilimanjaro. Gram-negative bacteria dominated the microbial communities throughout the elevation gradient. Their content peaked at 2900 m elevation, which agrees with increasing of bacterial richness at mid elevation (Singh et al. 2012). Gram-negative bacteria are usually more active at high elevation and more resistant to freeze–thaw cycles than gram positives. Gram-positive bacteria contributed mainly to microbial community composition below 2900 m. Their content decreased at higher elevation. This decrease is common for alpine soils and related to the weak tolerance of gram-positive bacteria to low temperatures and freeze-thaw cycles (Margesin *et al.* 2009). Direct climatic effects aside, The G+/G- ratio indicates substrate availability for microorganisms (Hammesfahr *et al.* 2008). Therefore, the relatively high abundance of gram-negative bacteria at mid elevation is explained by an increase of soil C content and the overall substrate availability. Fungal PLFAs were highest in colline RAU and alpine *Helichrysum* ecosystems (Figure 2.4-2). Above 2000 m, fungi increased linearly with elevation. Fungi are usually more resistant to cold and dry environments (Schinner & Gstraunthaler 1981; Ma *et al.* 2015). Accordingly, fungi/bacteria ratio reflects this pattern. In terms of soil conditions, fungal PLFAs increased with decreasing N content, as fungi are more adapted to low N supply, compared to gram-negative bacteria. In addition, the pH values increased at the highest elevation indicating an increasing role of fungi in the microbial community in alpine ecosystems (Zhang *et al.* 2013; Xu *et al.* 2014).

1.3.3.2 Effects of elevation on soil C chemistry

Pyrolysis fractions (>280°C) quantitatively dominated the soil organic matter composition (Figure 2.5-1). The contribution of volatile compounds in SOM increases with elevation (Table Supplementary 2.5-2), indicating an increase of easily available SOM components. While the thermally volatile fraction is nearly absent in lowland RAU forest soil, sub-montane *Erica* forest and alpine *Helichrysum* SOM already loose considerable amounts of volatile compounds below 280 °C.

Patterns of alkanes/-enes/-ols with elevation were similar for thermal desorption and pyrolysis steps and were highly correlated with total C content in soil. Both had their minimum at low elevation (RAU and FLM) and peaked in cloud forests (FOC and FPO) (Figure 2.5-4). These compounds were the major components of SOM in montane cloud forests (2100-2900 m), especially in the volatile fraction. They were also the main factor separating ecosystem characteristics along the elevation gradient (Figure 2.5-2). In soil, n-alkanes and n-alkenes occur in free form or bound in SOM by non-covalent binding (Lichtfouse *et al.* 1998). Decomposition leads to relative enrichment of aliphatic compounds in organic soil (Biester *et al.* 2014). Especially mid-chained alkanes and alkenes are considered relative recalcitrant products of vegetation litter degradation (Buurman *et al.* 2007; Vancampenhout *et al.* 2010). The increase of alkanes/-enes/-ols at around 2000 m can be a result of high leaf litter inputs (Becker *et al.* 2015) and incomplete decomposition. Further degradation and consequent increase of aromatic compounds was suppressed by the steady delivery of fresh litter inputs. C excess limits

degradation of less easily available compounds (Chen *et al.* 2014), explaining contrary elevation trends for more labile compounds.

Percentage of most easily degradable SOM compounds followed a decrease-increase pattern along the elevation gradient, reaching a minimum at around 2000 m a.s.l. (Figure 2.5-4). This included fatty acids and fatty acid esters, lignin monomers and phenolic compounds. These are seen as part of a labile C pool in soil and are readily decomposed in soil with high biological activities (Aerts 1997; Mueller *et al.* 2013). Phenols in SOM can be derived from various polymeric sources (Otto & Simpson 2006), but are mainly seen as decomposition products of lignin (Hedges & Mann 1979; Min *et al.* 2015). Soil lignin content peaks at low elevation (RAU) and in sub-montane Erica forest. Lignin mainly originates from leaf litter and woody debris and its content in soil is strongly depending on decomposition rates (Aerts 1997). Therefore, enriched soil lignin content reflects a skewed input-turnover balance. Decomposition rates below 1000 m are generally low due to the restricted productivity in dry season at Mt. Kilimanjaro (Study 2). In contrast, montane forest ecosystems (FLM, FOC, FPO) have high inputs but even higher decomposition rates compared to RAU and (sub-) alpine ecosystems (FER, HEL) (Becker *et al.* 2015; Study 2), which explains low contents in between 2000 and 3000 m. Above the tree line (i.e. HEL) low amounts of woody inputs decreases lignin content in soil. The different source and low decomposition at *Helichrysum* is reflected by a strong relative increase of volatile lignin components (Figure 2.5-3).
N containing compounds (amino N and N-heterocycles) in Mt. Kilimanjaro soils followed two contrasting trends with elevation. While amino N had their maximum at around 2000m, N-Heterocycles decreased at mid-elevation (Figure 2.5-4). The origin of N-containing components in SOM is not completely clear and can be either attributed to microbial or vegetal precursors (Vancampenhout *et al.* 2010). Still, amino acids, whether plant litter or microbial product, are easily degradable and part of a 'fast-cycle' turnover (Curry 1993). They mainly occur in fresh Litter and upper soil horizons (van Bergen *et al.* 1998). N-heterocycles (such as Pyridines, Pyrroles and Indole) are more stable and are products of the microbial decomposition of lignin or amino acids in further degraded SOM (Schulten & Schnitzer 1997; Chiavari & Galletti 1992). Strong N limitation and high perennial productivity in mid-elevation ecosystems might induce increased decomposition of N compounds. The more stable pool (N-heterocycles) is reduced (Sims 2006) and transferred into a fast cycling pool (amino N) and the aboveground biomass (Curry 1993).

1.3.4 Spatial interaction of above and belowground processes

Tropical alpine *Helichrysum* and savanna are open landscapes that are characterized by a patchy vegetation cover. The spatial distribution of these aboveground patterns strongly affected soil C and nutrient storage as well as CO_2 fluxes in both ecosystems. However, the underlying mechanisms were related to ecosystem specific properties. Both ecosystems showed a strong interaction of above and

belowground patterns and processes, controlling nutrient availability, and eventually greenhouse gas fluxes. High N retention in combination with low soil N_2O fluxes indicates N limitation in both ecosystems (Gerschlauer *et al.* 2016, Study 6). While precipitation had no effect on biogeochemical cycles in *Helichrysum* (Figure 2.6-4), soil microbial activity in savanna strongly increases at higher moisture availability (Pabst *et al.* 2016). Therefore, the study on Helichrysum is probably representative on a perennial scale whereas results from savanna are solely describing dry season patterns. This raises questions for future research on warming effects at Helichrysum and wet season processes in savanna.

Soil under shrub covered patches at *Helichrysum* had between 60% and 170% higher content of total and microbial C and N compared to low-vegetation patches (Table 2.6-2). The higher amounts of aboveground litter under shrubs facilitate microbial community growth and soil C stabilization (Sun *et al.* 2016). Consequently, higher substrate availability and soil microbial biomass lead to higher respiration rates with increased vegetation cover (Wang *et al.* 2003 Table 2.6-4). Higher autotrophic respiration from larger root density additionally contributed to elevated CO_2 fluxes from vegetated patches. Root and microbial respiration are positively related to temperature and solar radiation (Fitter *et al.* 1998; Luo *et al.* 2006). Solar radiation triggers root respiration via photosynthesis and subsequent stimulation of root exudation (Kuzyakov & Gavrichkova 2010), which in turn feeds back on microbial respiration (Kuzyakov & Domanski 2000). However, lower soil temperatures due to shading reduce emissions from shrub compared to herb patches (Figure 2.6-2). This indicates that changes in soil temperature strongly controlled soil N and C cycling in the tropical alpine *Helichrysum*. Positive correlation between CO_2 fluxes and N mineralization, without the effect of nitrification (Table 2.6-5, Table 2.6-6), indicate that heterotrophic microorganisms outcompete autotrophic nitrifiers. This suggests that increased N turnover rates at vegetated plots, caused by higher litter production and rhizodeposition (Hodge et al. 2000; Schimel and Bennett 2004; Phillips et al. 2011; Kuzyakov and Blagodatskaya 2015), do not enhance the risk of N loss, as long as the C:N ratio is not narrowing. In contrast, plants may even further compete with nitrification for soil NH_4^+. In this context, increasing microbial inorganic N immobilization (Table 2.6-7) and N retention capacity (Table 2.6-3) at shrub plots is pointing at intense plant-microbe competition for the limited N resources. Even though intense microbial competition may reduce short-term plant N availability, the process of internal N recycling along microbial loops also enables ecosystem nitrogen retention. This can even lead to sustainable nitrogen provision to plants, since plants on the long term may better compete versus microbes due to their longer and higher N storage capacity (Kuzyakov et al. 2013, Hodge et al. 2000). Paleoclimatic studies have shown movements in the vegetation belts of Mt. Kilimanjaro (Zech, 2006; Zech et al. 2014) and future climate change might increase vegetation cover at Helichrysum. Currently, about 60% of the *Helichrysum* ecosystem is covered with vegetation. Nitrogen turnover rates would increase in

parallel to vegetation cover (Table 2.6-7), without opening the N cycle. Therefore, *Helichrysum* ecosystem may be rather vulnerable to expected increase of atmospheric N deposition (Dentener et al. 2006; Vitousek et al. 1997) which may narrow the soil C:N ratio and thus could increase nitrification.

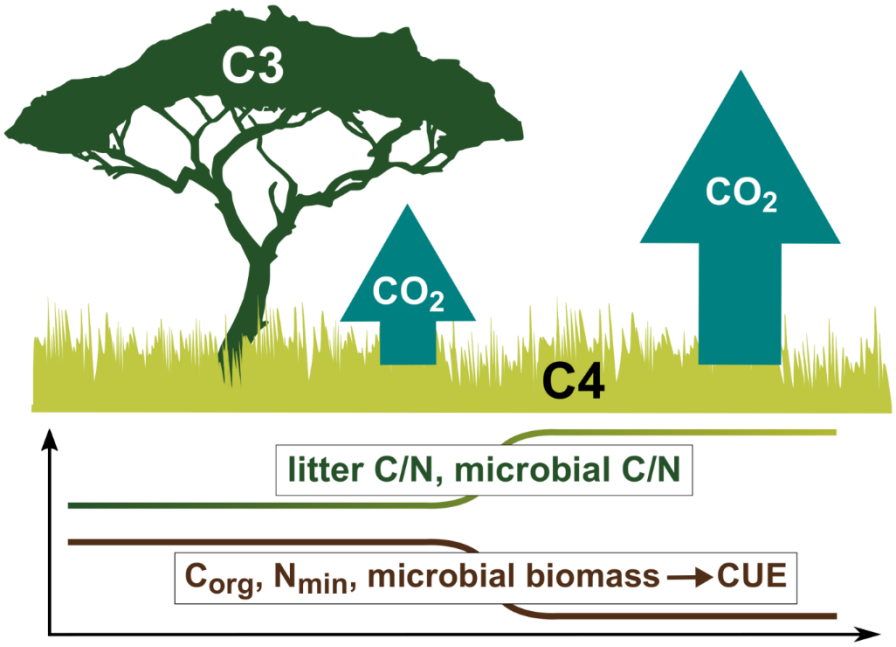

Figure 1.3-3: Effect of savanna trees on soil C and nutrient pools, carbon use efficiency (CUE) and related changes in soil respiration.

Soil C and N contents under savanna trees were about 40% higher compared to open area – similar redistribution occurred for microbial biomass, mineral N, available nutrient cations and soil pH (Study 7 – Appendix). These effects were strictly bounded to the crown area and the upper soil horizons (Figure 2.7-1, Appendix), indicating a spatially limited source of nutrient supply. N-fixation by the legume Acacia species is often regarded as a major source for the increase of fertility under savanna trees (Yelenik and others 2004). However, no effect on litter quality and soil properties was found, comparing a leguminous versus non-leguminous tree species (Table 2.7-2). Secondary effects of an altered species composition in the herb layer under the tree, as suggested by Bernhard-Reversat (1982) could be ruled out, since neither tree nor grass and herb roots showed noteworthy nodulation during dry season. Under dry conditions, symbiotic N-fixation is shifted to lower horizons (Vetaas 1992). While this may still play a direct role for plant and tree nutrition, the N availability in the microbial active topsoil horizons is independent from N-fixing effects and overall pool sizes are unaffected. Instead, the main responsible sources are the amount and quality of plant litter and throughfall water (Perakis and Kellogg 2007), which is in agreement with the theory that savanna trees act as vertical nutrient pumps (Ludwig and others 2004). Isotopic signatures of soil and plant material allowed partial quantification of this process: Shifts in soil $\delta^{13}C$ values under the crown towards the signal of tree leaf litter suggested that tree leaf litterfall contributes about 15% of SOM (Figure 2.7-2),

and is a major driver maintaining higher SOM levels under the crown. However, higher SOM and nutrient content under the crown did not result in higher C mineralization. Instead, higher CO_2 efflux was measured under open area (Figure 2.7-3). Under the conditions of this study, effects of soil moisture and autotrophic respiration were neglectable (Figure 2.7-1; Balogh *et al.* 2016; Kühnel 2015), hence increased CO_2 are most likely linked to lower leaf litter quality and microbial carbon use efficiency under C_4 grasses (Blagodatskaya *et al.* 2014a). C_4-grass litter had a wider C:N ratio than tree litter, which requires microorganisms to dispose of the C surplus via increased respiration to achieve their optimum C:N stoichiometry (Spohn 2015). This was further indicated by a wider microbial C:N ratio under open compared to crown area and a negative correlation of CO_2 fluxes and mineral N availability. Both are strong indicators of microbial N deficiency, amplifying microbial activity and N mining (Nicolardot *et al.* 2001; Sinsabaugh *et al.* 2013).

1.4 Conclusions

This research provides new insight into the effects of land-use and elevation on SOM characteristics and biogeochemical cycles on Mt. Kilimanjaro. These cycles and their underlying mechanisms were described and analyzed with regard to aboveground vegetation patterns under strongly contrasting climatic conditions.

On the southern slope of Mt. Kilimanjaro, leaf litter fall, decomposition seasonality and C stabilization were strongly dependent on climatic conditions along the elevation gradient. Annual leaf litter production decreased at higher elevations due to lower temperatures and reduced primary production. Decomposition rates are reduced by seasonal moisture limitation on the lower slopes (below 1900 m) and temperature limitation at high elevation (above 2850 m). Ecosystems at mid elevation (between 1900 and 2200 m) represent the zone of sufficient moisture and temperature conditions, with the highest plant biomass and productivity. High litter input and fast turnover regulate the C sequestration in these ecosystems and lead to increased amounts of volatile SOM compounds. However, at the same time stabile soil C pools increase through the excess of fresh C inputs. Climatic restraints control decomposition and C stabilization in lower and higher elevation zones. Due to their temperature sensitivity, (sub-) alpine Afromontane ecosystems must be considered future atmospheric CO_2 sources. Climatic variables are more important than litter quality and decomposer community complexity for controlling litter decomposition along the large climate gradient of Mt. Kilimanjaro.

The elevation trends are reflected by microbial abundance and changes in microbial community composition. Soil microbial biomarker contents at Mt. Kilimanjaro (between 800 and 4200 m), followed a bell-shaped curve with elevation, with a maximum at 2100 m. Gram-negative bacteria dominate the microbial community in Mt. Kilimanjaro soils, accounting for 25-40%, and, thus, determining the major trend of PLFAs distribution with elevation. With increasing elevation, gram-positive bacteria are replaced by fungi in response to the harsh environmental conditions in the alpine zone above 4000 m (low temperature, low soil C and N contents). These variations are indirectly dependent on climatic factors, and are explained by changes in vegetation composition and soil parameters. The optimal conditions for microbial biomass in mountain soils commonly occur at elevations around 2000 m, mainly because optimal properties combination of climate conditions for vegetation and soil development.

Land-use intensification decreases the stabilization of new C inputs in the transition from savanna to maize monocultures and from traditional homegardens to large-scale coffee plantations. Conversion of natural forests to sustainably or intensively used agroforestry systems leads to direct (change of

dominant species) and indirect (increased nutrient uptake after fertilization) enrichment of macronutrients in leaf litter. The change in litter quality reduces the C:N ratio, increases the C and nutrient turnover rates in soil, and so, accelerates the ecosystem C and nutrient cycles. This results in decreased C stocks in agroecosystems, with consequences for their fertility and ecosystem vulnerability. This calls for considering these effects when addressing land-use change and evaluating the sustainability of agroforestry and plantation management. Vegetation cover controls the spatial distribution of substrate availability in alpine *Helichrysum* and colline savanna ecosystems. This affects CO_2 fluxes in both ecosystems, however due to contrasting processes: Carbon mineralization rates at *Helichrysum* sites are mainly controlled by substrate availability from vegetation inputs. In contrast, dry season C fluxes in savanna are more related to litter substrate quality.

Litter inputs and root exudation from herbs and shrubs increase C and N availability in *Helichrysum* ecosystem. Hence, microbial biomass and activity increase, and, together with higher autotrophic respiration from larger root densities, elevate CO_2 fluxes from vegetated patches. N turnover at *Helichrysum* sites is primarily temperature controlled, and due to shallow, well-draining soils, is less affected by changes in soil moisture. The nitrogen cycle is tight and dominated by closely coupled ammonification-NH_4^+-immobilization, which is not prone to N losses. This cycling might accelerate if vegetation cover increases with progressive warming. An expected increase of atmospheric N deposition may be followed by higher nitrification due to narrowed soil C:N ratios. This transiently opens the N cycle, which means losses of N to the atmosphere and waters from the hitherto undisturbed *Helichrysum* ecosystem.

Savanna trees (C_3), whether leguminous or non-leguminous, increase soil fertility in the C_4 grassland through locally higher litter inputs and quality under the crown. This is the result of an active vertical transport by the trees (nutrient pumping) and a passive accumulation of C and N from litterfall over time. Thus, soil C pools and fluxes are directly related to the spatial abundance of trees and react more rapidly to increased tree cover than to vertical tree growth. In the open grassland and against the background of low N availability, the wide C:N ratios of C_4-grass litter reduce the carbon use efficiency of soil microbes. This increases microbial respiration and the CO_2 efflux from soil. Therefore, savanna trees affect soil C storage through two processes: first by actively increasing biomass inputs, and second by passively suppressing output mechanisms.

In conclusion, the combined effects of seasonal and long-term climatic conditions, land-use change as well as related variation in above and blowground ecosystem characteristics control biogeochemical cycles in East-African ecosystems. Thus, projecting effects of climate change and regionalizing C cycling patterns must consider the broad spectrum of these factors.

1.5 Contribution to studies

Study 1: Annual litterfall dynamics and nutrient deposition depending on elevation and land use at Mt. Kilimanjaro
Status: Published in *Biogeosciences* (2015), 12, 5635-5646, doi:10.5194/bg-12-5635-2015

Authors	*Contribution*
Joscha Becker	Laboratory work; Data analysis; Writing
Holger Pabst	Study design; Laboratory work; Commenting
James Mnyonga	Field work; Laboratory work; Commenting
Yakov Kuzyakov	Study design; Commenting

Study 2: Teatime on Mount Kilimanjaro: Seasonal variation in standardized litter decomposition and effects of elevation and land use
Status: Submitted, under review since 19.02.2017

Authors	*Contribution*
Joscha Nico Becker	Study design; Field work; Laboratory work; Data analysis; Writing
Yakov Kuzyakov	Study design; Commenting

Study 3: Climatic controls of leaf-litter decomposition and decomposer communities along an elevation gradient of Mt. Kilimanjaro
Status: Extended abstract

Authors	*Contribution*
Joscha Nico Becker	Study design; Field work; Laboratory work; Data analysis; Writing
Antonia Mayr	Laboratory work; Commenting
Yakov Kuzyakov	Study design; Commenting

Study 4: Soil microbial community structure in forest soils along the elevation gradient of Mount Kilimanjaro
Status: Manuscript in preparation

Authors	*Contribution*
Anna Gunina*	Study design; Laboratory work; Data analysis; Writing
Joscha Nico Becker*	Study design; Field work; Data analysis; Writing
Andreas Hemp	Data contribution; Commenting
Luo Yu	Supporting data analysis; Commenting
Davie Jones	Supporting data analysis; Commenting
Yakov Kuzyakov	Study design; Commenting

*both authors contributed equally to the study

Study 5: Ashes to Ashes: Thermal Characterization of Soil Organic Matter Composition in Mount Kilimanjaro Andosols
Status: Manuscript in preparation

Authors	*Contribution*
Joscha Nico Becker	Study design; Field work; Laboratory work; Data analysis; Writing
Michaela Dippold	Laboratory work; Data analysis; Commenting
Andreas Hemp	Data contribution; Commenting
Yakov Kuzyakov	Study design; Commenting

Study 6: Nitrogen turnover and greenhouse gas emissions in a tropical alpine ecosystem, Mt. Kilimanjaro, Tanzania
Status: Published in *Plant and Soil* (2017), 411, 243-259, doi:10.1007/s11104-016-3029-4

Authors	*Contribution*
Adrian Gütlein	Study design; Field work; Laboratory work; Data analysis; Writing
Markus Zistl-Schlingmann	Field work; Laboratory work; Data analysis; Writing
Joscha Nico Becker	Field work; Laboratory work; Data analysis; Commenting
Natalia Sierra Cornejo	Field work; Laboratory work; Data analysis; Commenting
Florian Detsch	Field work, Data analysis; Commenting
Tim Appelhans	Supporting data analysis; Commenting
Dietrich Hertel	Supporting data analysis; Commenting
Yakov Kuzyakov	Supporting data analysis; Commenting
Ralf Kiese	Study design; Writing

Study 7: Legume and non-legume trees increase soil carbon sequestration in Savanna
Status: Published in *ECOSYSTEMS* (2017), 20, 989-999, doi:10.1007/s10021-016-0087-7

Authors	*Contribution*
Joscha N. Becker	Study design; Field work; Laboratory work; Data analysis; Writing
Adrian Gütlein	Field work; Laboratory work; Data analysis; Commenting
Natalia Sierra Cornejo	Field work; Laboratory work; Commenting
Dietrich Hertel	Study design; Commenting
Ralf Kiese	Supporting data analysis; Commenting
Yakov Kuzyakov	Study design; Commenting

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2 Publications and Manuscripts

2.1 Study 1:
Annual litterfall dynamics and nutrient deposition depending on elevation and land use at Mt. Kilimanjaro

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Abstract

Litterfall is one of the major pathways connecting above- and belowground processes. The effects of climate and land-use change on carbon (C) and nutrient inputs by litterfall are poorly known. We quantified and analyzed annual patterns of C and nutrient deposition via litterfall in natural forests and agroforestry systems along the unique elevation gradient of Mt. Kilimanjaro.

Tree litter in three natural (lower montane, *Ocotea* and *Podocarpus* forests), two sustainably used (homegardens) and one intensively managed (shaded coffee plantation) ecosystems was collected on a biweekly basis from May 2012 to July 2013. Leaves, branches and remaining residues were separated and analyzed for C and nutrient contents.

The annual pattern of litterfall was closely related to rainfall seasonality, exhibiting a large peak towards the end of the dry season (August – October). This peak decreased at higher elevations with decreasing rainfall seasonality. Macronutrients (N, P, K) in leaf litter increased at mid elevation (2100 m a.s.l.) and with land-use intensity. Carbon content and micronutrients (Al, Fe, Mn, Na) however, were unaffected or decreased with land-use intensity.

While leaf litterfall decreased with elevation, total annual input was independent of climate. Compared to natural forests, the nutrient cycles in agroforestry ecosystems were accelerated by fertilization and the associated changes in dominant tree species.

2.1.1 Introduction

With their high biodiversity and importance for the global carbon (C) cycle, tropical forests are often highlighted as ecosystems of specific research interest (Brown, 1993; Sayer et al., 2011). Tropical forest ecosystems account for one third of the terrestrial net primary production (NPP) (Saugier et al., 2001) and contain more than half of the world's terrestrial species (Groombridge and Jenkins, 2002). Tropical forests also act as a net sink for CO_2 (FAO, 2010) and contain roughly 25% of the terrestrial biosphere C (Bonan, 2008).

Tree litterfall is one of the major pathways in C and nutrient cycles that connect above- and belowground processes (Vitousek and Sanford, 1986). As an important and regular source of nutrients and organic matter, litterfall has been well studied over the past decades (Vitousek, 1984; Meier et al., 2005; Carnol and Bazgir, 2013). Nonetheless, litterfall varies considerably between ecosystems, depending on climate, tree species composition, stand structure and soil fertility (Vitousek and Sanford, 1986). Elevation is strongly affecting these parameters in montane ecosystems (Ensslin et al., 2015; Pabst et al., 2013) and is of particular importance regarding potential ecosystem shifts through climate change (Beniston, 2003). Therefore, the effect of elevation on litterfall is an important indicator for estimating future changes in ecosystem cycles.

Land-use change affects numerous biological, chemical and physical factors as well as their interactions, leading to a high complexity and unpredictability of anthropogenic effects on ecosystem functions (Groffman et al., 2001). Especially the functioning of C and nutrient cycles under natural and disturbed conditions is important to assess the overall impact of anthropogenic land use on tropical forest ecosystems. As reviewed by Don et al. (2011), soil organic matter decreases up to 30% by converting tropical forests to agricultural systems. These effects might still be underrepresented in estimates of overall ecosystem C fluxes (de Blécourt et al., 2013).

This underrepresentation is particularly relevant because deforestation and conversion to intensive agriculture are common transformations in tropical regions and are projected to remain a major issue in the future (Lewis, 2006). Between 2000 and 2005, forest cover in Africa decreased by 11.5 million ha (Hansen et al., 2010) and this number is feared to further increase (UCS, 2011). The deforestation rate in Tanzania, for example, is already one of the largest in Africa (Fisher, 2010). In contrast to other tropical regions, it is mainly driven by small-scale farming for regional food production. Moreover, there was a considerable intensification of agricultural land use at Mt. Kilimanjaro within the last 50 years (Misana et al., 2012).

Most of the recent research on nutrient cycling in tropical forest ecosystems has been conducted in the Neotropics and Southeast Asia (Zhou et al., 2006; Chave et al., 2010; Celentano et al., 2011;

González-Rodríguez et al., 2011; Fontes et al., 2014; Vasconcelos et al., 2008), while African forests, especially montane rainforests in East Africa, have received much less attention (Schrumpf et al., 2006; Dawoe et al., 2010). Mt. Kilimanjaro offers the possibility to investigate nutrients cycles and litterfall along an elevation gradient were soils have a similar age and developed from the same parent material (Dawson, 1992). We are aware of only one study that published data on nutrient cycling with partial focus on litterfall in Mt. Kilimanjaro ecosystems (Schrumpf et al., 2006). Various studies in other ecosystems have shown that artificial nutrient addition accelerate nutrient cycles (Allison and Vitousek, 2004; Forrester et al., 2005; Homeier et al., 2012). It remains unclear how agricultural land use affects nutrient balances and its interrelation to litter quantity, quality and the above- and belowground element cycles in tropical (agro)ecosystems.

Our primary objective was to assess the effect of climate and of agricultural land use on litterfall and nutrient and carbon cycles in the dominant ecosystems of Mt. Kilimanjaro. Therefore, we (1) collected the annual litter deposition and examined the litterfall dynamics throughout the year, (2) measured the annual C and nutrient return and (3) compared differences between natural and managed ecosystems and address implications for the ecosystem nutrient cycle.

2.1.2 Methods

2.1.2.1 Study site

The study was conducted on the south-western slope of Mt. Kilimanjaro (3°4’33”S, 37°21’12”E), Tanzania, along an elevation gradient from 1 275 to 2 850 m a.s.l. Our study was part of the German Research Foundation Project: Kilimanjaro ecosystems under global change. This interdisciplinary project provided a number of long term research locations, plots, data and facilities along the south-western slope of Mt. Kilimanjaro. Six research sites were selected according to the joint study design. Each is representing either a typical tropical montane forest zone or a representative land-use class of the region (Table 2.1-1). Lower montane forest (FLM), *Ocotea* forest (FOC) and *Podocarpus* forest (FPO) are three natural sites located in Kilimanjaro National Park with minor anthropogenic impact. Nonetheless, illegal logging for firewood and building material may occur, especially in the lower FLM areas (Lambrechts et al., 2002; Rutten et al., 2015). The vegetation and zonation of these ecosystems was classified and described in detail by Hemp (2006a). Summarily, FLM is dominated by *Macaranga kilimandscharica, Agauria salicifolia* and partly *Ocotea usambarensis*, while at higher elevation *Ocotea usambarensis* prevails, accompanied by *Cyathea manniana* (FOC). The forest above 2 800 m a.s.l. is dominated by *Podocarpus latifolius* together with *Prunus africana* and *Hagenia abyssinica* (FPO). Two Chagga homegardens (HOMa, HOMb) represent a traditional form of sustainably managed agroforestry with sporadic organic fertilization with manure and household waste (Fernandes et al., 1986). Homegardens are multilayered agroforestry systems with *Musa* ssp. and *Coffea* ssp. as

dominant crops under remnant forest trees (e.g. *Albizia schimperiana, Cordia africana*) and cultivated fruit trees (e.g. *Persea Americana, Grevillea robusta*)(Hemp, 2006b). Shaded coffee plantation (COF) represented an intensively managed land-use type with regular application of mineral fertilizers and pesticides. A detailed description of land-use history of Mt. Kilimanjaro was given by Pabst (2015) and further information on aboveground biomass and vegetation structure is available from Ensslin et al. (2015).

The climate at Mt. Kilimanjaro is characterized by a bimodal rainfall regime with a short rainy season around November and a longer one from March to May (Hemp, 2006a). Mean annual precipitation (MAP) varies depending on elevation and exposition between 1 336 mm and about 3 000 mm per year (Table 2.1-1). Mean annual temperature (MAT) ranges from 9.8 °C to 20.9 °C and monthly means vary around ±3 °C.

The comparison of ecosystems and litterfall on Mt. Kilimanjaro is especially beneficial because the soils have a similar age and developed from similar parent material over the last 0.2 to 2.3 Mio years (Dawson, 1992). These parent materials are formed by volcanic rocks such as basalt, trachyte and olivine basalts. Soils are classified as Andosols with folic, histic or umbric topsoil horizons with accordingly high C contents in the upper horizons (Zech 2006), often underlain by C rich paleosol sequences (Zech et al., 2014). Water extractable and microbial biomass C increase with elevations and decrease with management intensity (Pabst et al., 2013).

Table 2.1-1: Land-use classification, topographic and climatic information and C and N stocks in 0-10 cm soil depth of research plots on the southern slope of Mt. Kilimanjaro

Ecosystem	Plot ID	Land-use class	Elevation (m a.s.l.)	MAP (mm yr^{-1})[a]	MAT (°C)[b]	Soil C (mg cm^{-3})[c]	Soil N (mg cm^{-3})[c]
Chagga homegarden	HOMa	Agricultural, traditional	1275	1336	20.9	24.7	2.1
Coffee plantation	COF	Agricultural, intensive	1305	1485	20.2	19.3	1.9
Chagga homegarden	HOMb	Agricultural, traditional	1647	2616	17.3	36.1	2.7
Lower montane forest	FLM	Natural, disturbed	1920	2378	15.3	45.8	3.1
Ocotea forest	FOC	Natural	2120	2998	11.2	55.8	3.2
Podocarpus forest	FPO	Natural	2850	1773	9.8	53.5	2.6

[a] mean annual precipitation (Appelhans and others 2014)
[b] mean annual temperature in 2012 (Appelhans, unpublished)
[c] stocks in 0-10 cm soil depth (calculated from Pabst and others (2013))

2.1.2.2 Sampling

Within each ecosystem, 10 litter traps (1m², 1mm mesh size) were installed as replicates along two 100m transects (5 per transect). Due to the areal structure of one of the homegardens (HOMb), the

number of litter traps had to be reduced and only five replicates could be installed. To exclude undergrowth, net heights were set between 20 and 100cm above ground. Between April 2012 and July 2013, litter was collected twice a month.

Litter samples were oven-dried for one week at 60 °C and then weighed. Within the two-week sampling interval the weight loss by decomposition was presumed negligible. Litter was manually sorted into leaves, branches (<2 cm in diameter) and a rest fraction containing blossoms and fruits as well as unidentified materials. Wooden material >2 mm is too persistent to be evaluated within the timescale of our study and was thus excluded from analysis. Leaf litter samples were coarsely ground and stored in paper bags for further analysis.

2.1.2.3 Analyses of carbon and nutrient contents

We expected leaves to contain most of the litter nutrients (Yang et al., 2004). Therefore, nutrient analyses were limited to the leaf fraction. Leaf litter samples were bulked randomly and divided into two subsamples from five nets per time step. Nutrient content of leaf litter was analyzed from six sampling dates equally distributed over one year. In line with Celentano et al. (2011) we refrained from seasonal subdivision because most nutrients show low seasonal variation. A total number of 12 samples per ecosystem were fine ground and analyzed for C and nutrient contents. C and N contents were determined with a dry combustion automated C:N analyzer (Vario EL, Elementar). After a preparative pressure digestion, inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro Analytical Instruments) was used to determine contents of major macro- (Ca, K, Mg, P, S) and micro- (Al, Fe, Mn, Na) nutrients. All chemical analyses were conducted in the laboratory of the Department of Soil Science of Temperate Ecosystems, University of Göttingen.

2.1.2.4 Calculations and statistical analyses

Annual litter deposition per ecosystem was calculated as the average from nets over one year (June 2012 to May 2013). Monthly deposition rates were calculated assuming a constant amount per day for each sampling interval. For missing values we assumed a linear behavior of litterfall between the previous and the following date. Nutrient deposition was calculated as the product of annual leaf deposition and mean nutrient content.

As our data do not meet the requirements for ANOVA and non-normal distribution must be assumed (Shapiro-Wilk test, $p < 0.05$), we applied non-parametric statistics. Significant differences were detected using the Kruskal-Wallis test with a Bonferroni correction at p-level = 0.05 (Katz, 2006). The presented data are means of 5 to 10 replications ± standard error (SE).

All statistical analyses were conducted in R 3.0.1 (R Core Team, 2013) using core and agricolae (Mendiburu, 2014) packages as well as the ggplot2 package for data visualization (Wickham, 2009).

2.1.3 Results

2.1.3.1 Annual amount of litterfall

The annual amount of total litterfall was independent of land use and elevation, whereas the amount of leaf litter in natural forests decreased with elevation (Figure 2.1-1). The total annual input varied from 4.6 Mg ha^{-1} in HOMa to 10.7 Mg ha^{-1} in HOMb. Accordingly, HOMb had a significantly higher total litterfall than HOMa as well as FOC and FPO.

Total litterfall was dominated by the portion of leaves, contributing between 61% (FPO) and 74% (HOMb). The annual value in FLM was significantly higher than in FPO (Figure 2.1-1). Deposition of branches and rest were on the same level for all sites: each constituted less than 30% of total litterfall.

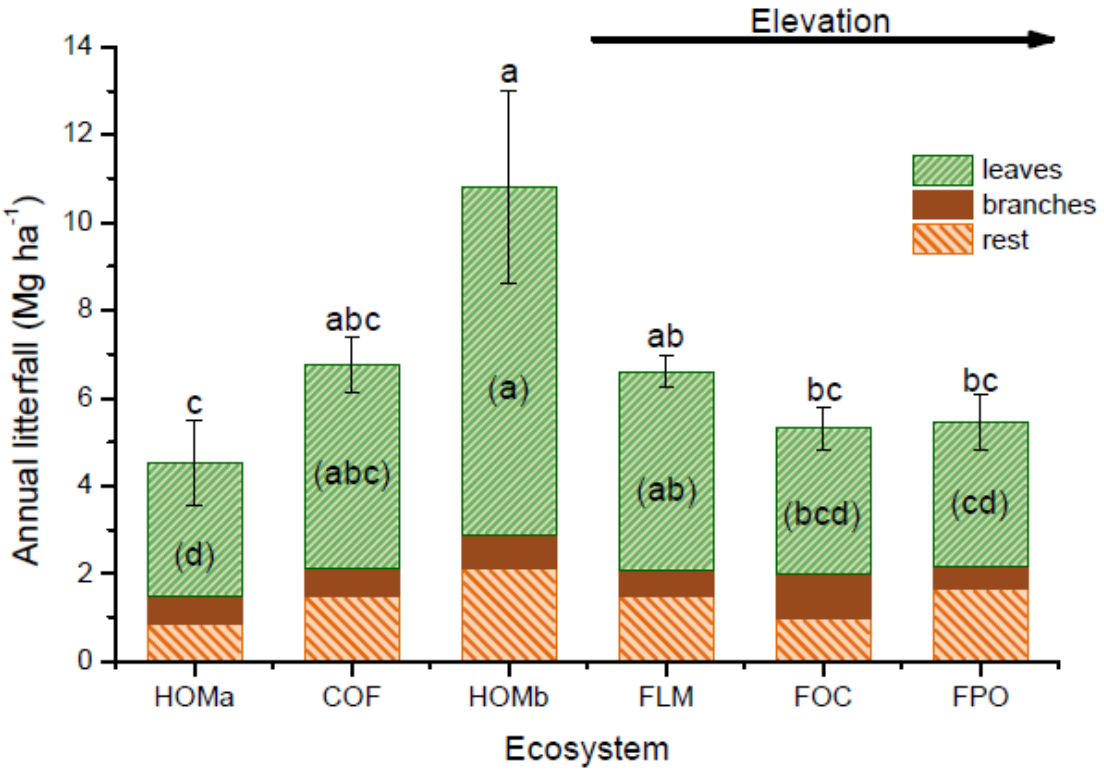

Figure 2.1-1: Annual litterfall and its components (2012 to 2013) in Chagga homegardens (HOMa & HOMb), shaded coffee plantation (COF), lower montane forest (FLM), *Ocotea* forest (FOC) and *Podocarpus* forest (FPO). Error bars indicate standard errors for total amount with significance levels shown as small letters a-c ($p \leq 0.05$). Letters in brackets (a-d) indicate significance levels for leaf fraction only.

2.1.3.2 Seasonal dynamics of litterfall

The seasonal patterns of litterfall were the same for natural and agroforestry systems if compared on the closest elevation level. In forests at higher elevation the seasonality was less pronounced and the peak values shifted from the end of the dry season towards the rainy season (Figure 2.1-2).

Similar to the annual litterfall, changes in monthly litterfall were determined by the portion of leaves. Maximum values in homegardens, COF and FLM were recorded between the mid- and late dry season (Figure 2.1-2). A second smaller peak appeared in the second rainy season around April. Within these

peaks, monthly litterfall increased three- (HOMa) to nine-fold (COF) in agroforestry systems. In natural forests, peaks increased about 350% in FLM, 300% in FOC and 450% in FPO. In FOC and FPO the first peak was delayed until November or December and was extended because litterfall rates remained high in the short dry season between January and March. Litterfall maxima within the year were positively related to elevation (Figure 2.1-3). Deposition patterns of branches were independent of seasons, and peaks occurred erratically (Figure 2.1-2). The deposition of the rest fraction did not follow pronounced dynamics but the peaks tended to increase during the rainy seasons.

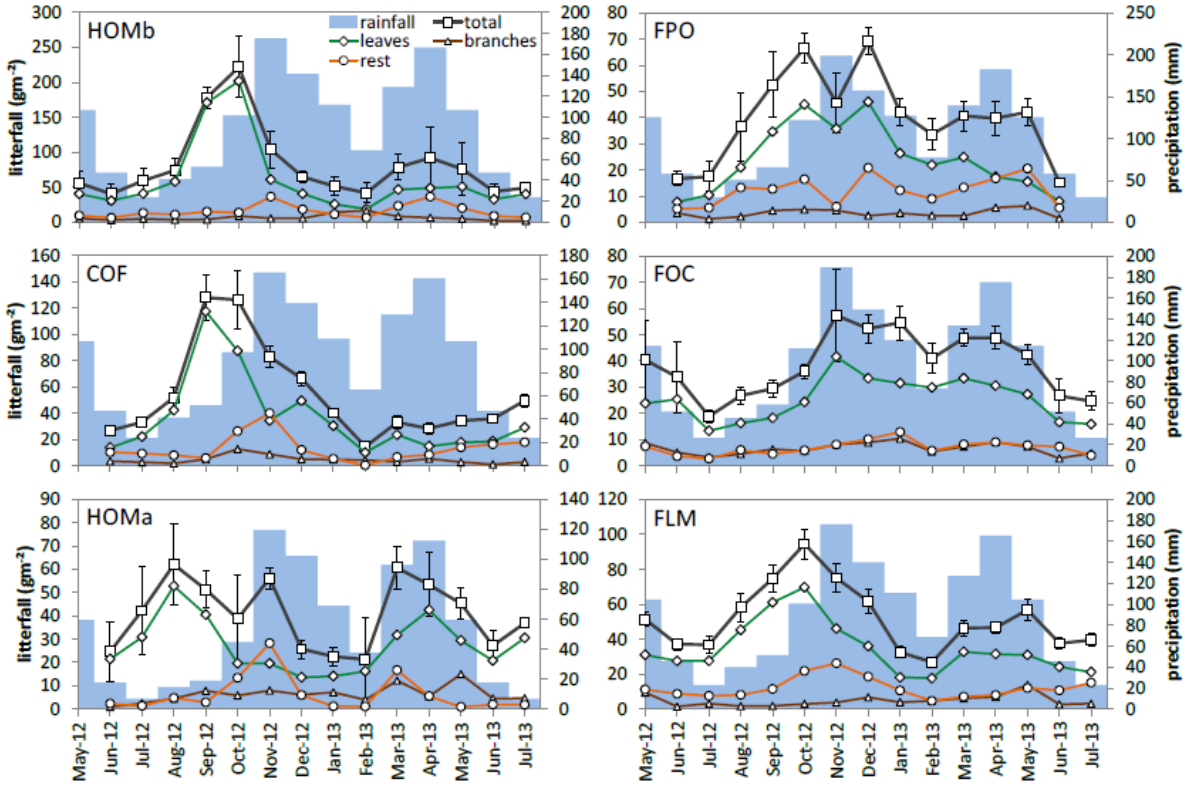

Figure 2.1-2. Monthly litterfall from May 2012 to July 2013 in Chagga homegardens (HOM), shaded coffee plantation (COF), lower montane forest (FLM), *Ocotea* forest (FOC) and *Podocarpus* forest (FPO). Total litterfall (squares) is divided into leaves (diamonds), branches (triangles) and rest (circles). 10-year-mean of monthly precipitation (2000 to 2010, TRMM, http://pmm.nasa.gov) is indicated as bars. Standard errors (SE) are displayed by error bars.

2.1.3.3 Nutrient contents and deposition

Agroforestry systems showed higher macronutrient content and deposition rates than natural forests (Table 2.1-2). With increasing elevation in the natural forests, nine of eleven analyzed nutrients followed a hump-shaped pattern with the highest content in FOC (2120 m a.s.l.) and lower contents in FLM (1920 m a.s.l.) and FPO (2850 m a.s.l.) (Appendix Table 2.1-3).

The N, P, and S contents in leaves under agricultural land use were significantly higher compared to those in natural forests (Figure 2.1-4; Appendix Table 2.1-3). Potassium was enriched in the leaf litter of managed ecosystems (7.4 to 15.8 mg g^{-1}) versus most natural forests (3.1 to 7.2 mg g^{-1}). The contents

of C, Al, Mg, Fe, and Ca were independent of land use. Due to the similar C and the increased N content, the C:N ratio was significantly lower in managed ecosystems. It ranged from 16.9 (± 0.6) to 20.4 (± 0.6) in agroforestry systems and from 32.1 (± 0.4) to 44.9 (± 0.5) in natural forests. Na and Mn contents were lower under agricultural land use (Table 2.1-2).

Table 2.1-2: Annual nutrient deposition via leaf litterfall (Mean ± SE, kg ha-1 yr-1) from six ecosystems at Mt. Kilimanjaro

	Homegarden-a	Coffee plantation	Homegarden-b	Forest lower montane	*Ocotea* forest	*Podocarpus* forest
(kg ha^{-1} yr^{-1})						
C	1454.1 ± 294.5^{c}	2230.8 ± 160.4^{ab}	3948.2 ± 606.8^{a}	2169.1 ± 71.1^{ab}	1635.7 ± 134.1^{bc}	1600.8 ± 176.2^{bc}
N	87.0 ± 17.6^{bc}	110.3 ± 7.9^{ab}	233.5 ± 35.9^{a}	48.7 ± 1.6^{cd}	51.9 ± 4.3^{cd}	38.2 ± 4.2^{d}
Al	2.9 ± 0.6^{b}	5.1 ± 0.4^{a}	6.1 ± 0.9^{a}	1.9 ± 0.1^{b}	4.5 ± 0.4^{a}	2.4 ± 0.3^{b}
Ca	54.6 ± 11.1^{ab}	63.5 ± 4.6^{a}	63.0 ± 9.7^{a}	30.0 ± 1.0^{c}	33.6 ± 2.8a^{b}	29.8 ± 3.3^{c}
Fe	3.4 ± 0.7^{abc}	3.8 ± 0.3^{ab}	5.2 ± 0.8^{a}	1.3 ± 0.0^{d}	2.6 ± 0.2^{bc}	2.4 ± 0.3^{c}
K	22.6 ± 4.6^{b}	59.9 ± 4.3^{a}	125.4 ± 19.3^{a}	14.0 ± 0.5^{c}	13.0 ± 1.1^{c}	23.6 ± 2.6^{b}
Mg	12.2 ± 2.5^{ab}	9.9 ± 0.7^{ab}	15.8 ± 2.4^{a}	8.4 ± 0.3^{bc}	9.0 ± 0.7^{b}	4.8 ± 0.5^{c}
Mn	0.4 ± 0.1^{c}	1.0 ± 0.1^{bc}	0.9 ± 0.1^{bc}	2.3 ± 0.1^{a}	2.2 ± 0.2^{a}	2.7 ± 0.3^{a}
Na	0.5 ± 0.1^{c}	1.0 ± 0.1^{b}	1.7 ± 0.3^{a}	1.9 ± 0.1^{a}	2.0 ± 0.2^{a}	0.7 ± 0.1^{bc}
P	5.2 ± 1.1^{ab}	5.3 ± 0.4^{bc}	10.9 ± 1.7^{a}	3.0 ± 0.1^{cd}	2.6 ± 0.2^{d}	2.4 ± 0.3^{d}
S	5.2 ± 1.0^{b}	7.4 ± 0.5^{a}	15.7 ± 2.4^{a}	4.8 ± 0.2^{b}	4.0 ± 0.3^{bc}	2.9 ± 0.3^{bc}

Superscript letters indicate significant differences between sites (Kruskal-Wallis Test; p-level ≤ 0.05)

The effect of land use on the annual nutrient deposition was buffered by the amount of litterfall, but remained present. HOMb had the highest C and nutrient deposition (except for Mn and Na) via litterfall compared to all other ecosystems (Table 2.1-2). The coffee plantation also had significantly higher N, P, K, Fe, and Ca deposition than all natural forests. Due to minimal litterfall in HOMa the annual nutrient deposition was low despite high concentrations in leaves. The deposition of most macronutrients in HOMa was still higher or on the same level as in natural forests. The Al and Na deposition was unaffected by land-use intensity. Annual Mn deposition was significantly higher in natural forests than in managed sites.

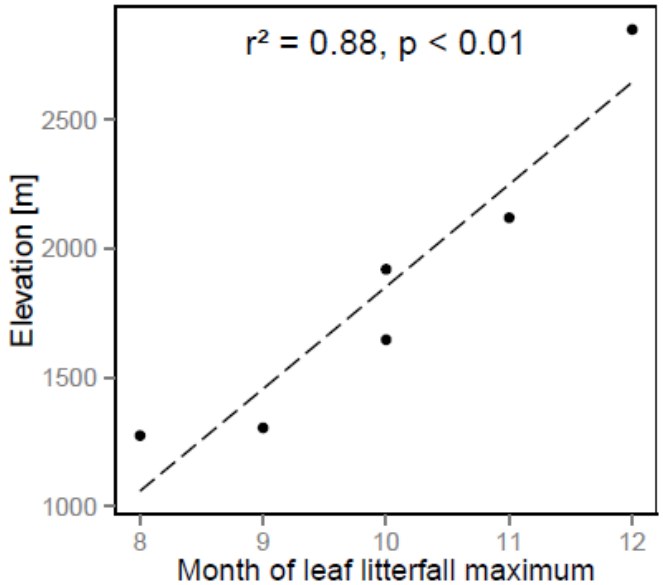

Figure 2.1-3: Linear regression between elevation and month of highest leaf litterfall in six ecosystems of Mt. Kilimanjaro.

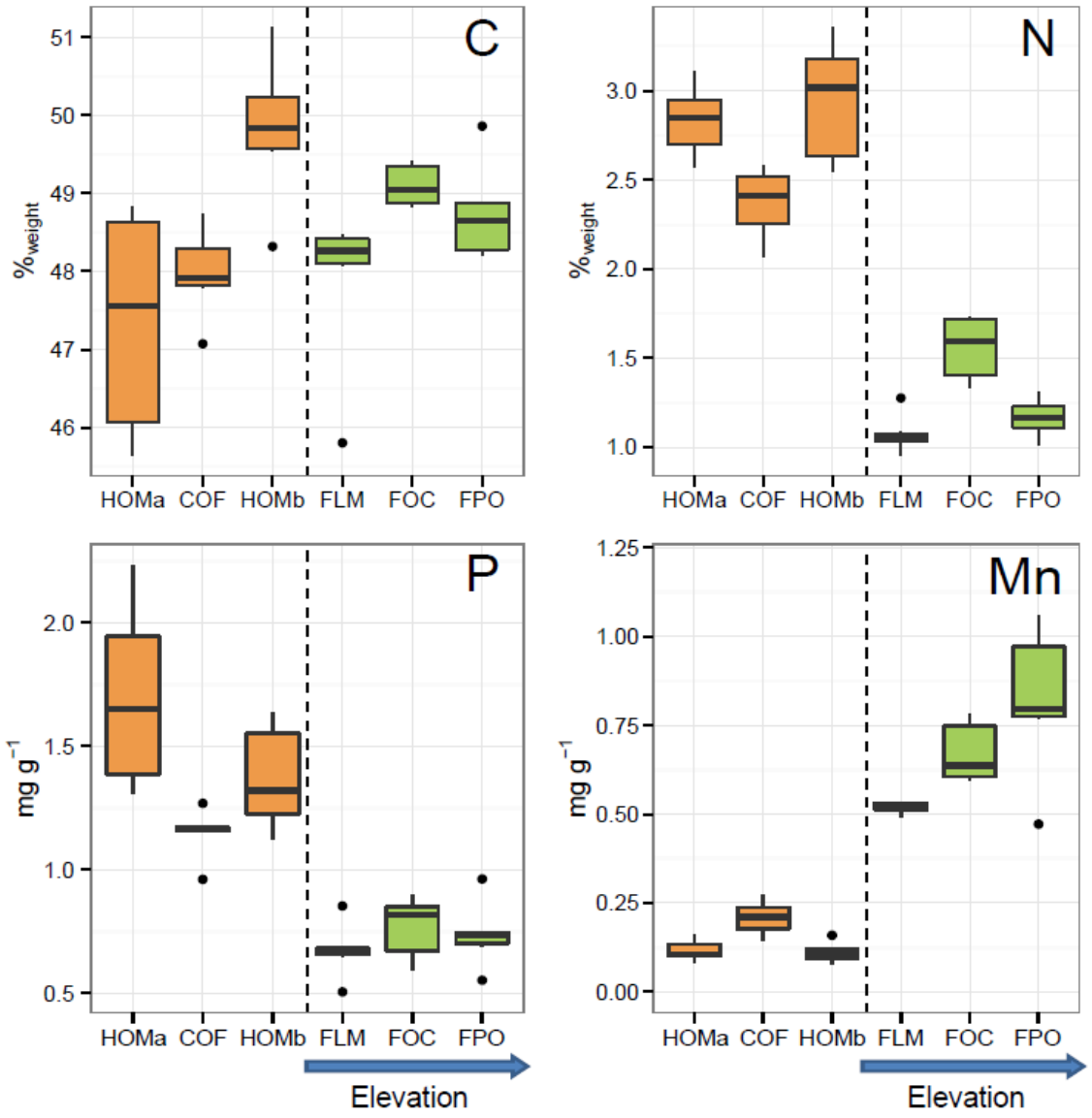

Figure 2.1-4: Contents of selected elements (C, N, P, Mn) in leaf litter from six ecosystems at Mt. Kilimanjaro. Medians, interquartile distances and extreme values are displayed as bold lines, boxes with whiskers and dots, respectively. Managed (left) and natural (right) ecosystems are separated by dashed line.

2.1.4 Discussion

2.1.4.1 Litterfall characteristics

The amounts of litterfall in Mt. Kilimanjaro ecosystems were within the common range for tropical mountain forests and followed a pronounced seasonality dependent on climatic variations. The annual leaf litterfall (4.6-10.7 Mg ha^{-1}) was also within the same range as at various other tropical sites (Chave et al., 2010; Zhang et al., 2014). A previous study at Mt. Kilimanjaro found similar amounts of fine litterfall (7.5 Mg ha^{-1}) at an elevation of 2250 to 2350 m. a.s.l. (Schrumpf et al., 2006). Lisanework and Michelsen (1994) reported annual fine litter production ranging from 5.0 Mg ha^{-1} to 6.5 Mg ha^{-1} in tree plantations and 10.9 Mg ha^{-1} in a natural forest in the Ethiopian highlands. Similar results were found

for cacao plantations in lowland humid Ghana where total litter ranged from 5.0 Mg ha^{-1} to 10.4 Mg ha^{-1} (Dawoe et al., 2010). The portion of leaf litter commonly varies between 60% and 90% (Lisanework and Michelsen, 1994; Schrumpf et al., 2006, Zhou et al., 2006; González-Rodríguez et al., 2011). Accordingly, leaf portions in Mt. Kilimanajro litterfall (60-75%) were at the lower end of tropical forest values.

The factors affecting litterfall amounts are succession stage, tree age and dominant plant or tree species (Barlow et al., 2007; Celentano et al., 2011). Varying management practices and crops in homegardens may alter these factors. The heterogeneity of the traditional agroforestry systems explains the low annual litterfall in HOMa. Compared to HOMb, there were more banana plants (Musa ssp.) in HOMa, which were manually cut as a management practice and thus were not accounted for by our litter traps.

Litterfall peaks during the dry season are well documented in tropical forests and plantation systems and mainly reflect drought stress (Okeke and Omaliko, 1994; Barlow et al., 2007; Selva et al., 2007). A recent meta-analysis by Zhang et al. (2014) has shown that this connection is a characteristic feature of tropical ecosystems. Leaf aging, caused by photoinhibition, stomatal closure and subsequent leaf overheating, might lead to leaf shedding at the end of the dry season (Röderstein et al., 2005). As a side effect, trees are preparing for the upcoming season of highest net primary production. By contrast, the peaks during the rainy season are the result of strong winds and thunderstorms (Dawoe et al., 2010; González-Rodríguez et al., 2011). This explains the observed increase in peaks of branch and rest deposition during wet months.

2.1.4.2 Effects of elevation

The Mt. Kilimanjaro forest ecosystems are characterized by the absence of a pronounced trend of total annual litterfall with elevation. When the leaf fraction was compared separately though, the annual deposition was significantly higher in FLM than in higher forests (FOC, FPO) (**Error! Reference source not found.**). Leaf litter production is considered to depend on temperature and thus decreases at higher elevations (Okeke and Omaliko, 1994; Zhou et al., 2006; Girardin et al., 2010). Nonetheless, a series of other studies from various ecosystems also show no decrease with elevation (Röderstein et al., 2005; Köhler et al., 2008). Within our elevation range of ~900 m in natural forests, the percentages of leaf litterfall were too small to determine a notable decrease of total litterfall with elevation. Sporadic sampling at higher elevations (data not shown) indicated that a litterfall decrease would become apparent in ecosystems above 3000 m a.s.l.

Seasonal variability of leaf litterfall in the natural forests on Mt. Kilimanjaro followed a U shaped pattern with increasing elevation (Figure 2.1-2). In tropical montane forests, the seasonality of litterfall

is generally low compared to tropical lowland forests (Chave et al. 2010). We observed the weakest seasonal variation in *Ocotea* forest in 2190 m a.s.l., featuring the highest annual precipitation and least varying soil moisture conditions (Table 2.1-1). At FPO (2850 m a.s.l.) seasonality increased again with lower MAP and an increasing temperature limitation. Litter production at higher elevation was distributed over the warmer period between October and May when canopy productivity is usually higher (Girardin et al., 2010). This pattern is based on the dependency of litterfall seasonality on rainfall intensities as well as temperatures (Zhou et al., 2006; Chave et al., 2010). Changes of seasonality patterns occurred within 200 m elevation difference (FLM to FOC). This suggests that elevation effects can easily overlay biome specific litterfall patterns and can contribute to the explanation of variabilities in large scale data (Zhang et al., 2014).

We found no consistent effect of elevation on litter nutrient content within the agroforestry systems (Appendix Table 2.1-3). This indicates a strong overlay of elevation effects by land-use practices. This enables discussing the changes in contents along an elevation gradient only by comparing natural forests with each other. Carbon and most nutrient contents in leaf litter followed a hump-shaped pattern with elevation. This pattern is typical for other ecosystem properties along montane elevation gradients (Kluge et al., 2006; Mölg et al., 2009). It is also present for MAP at Mt. Kilimanjaro (Table 2.1-1) as well as for aboveground biomass (Ensslin et al., 2015). Pabst et al. (2013) reported hump-shaped soil moisture curves and mirroring patterns for soil pH from the same Kilimanjaro ecosystems. Both parameters control soil nutrient availability and they are without a doubt also key factors for variations of nutrient uptake by plants and consequently for the litter nutrient contents.

2.1.4.3 Effects of land use

The contents of most macronutrients in leaf litter of managed ecosystems were two to five times higher than in natural forests. This suggests that the chemical composition of leaf litter at Mt. Kilimanjaro was significantly altered by land use and the associated change of dominant plant or tree species.

Especially for studying land-use effects it can be difficult to find adequate and comparable sites. At Mt. Kilimanjaro there is nearly no natural forest below and no land use above 1800 m a.s.l. Given this limitation to our study design we will only discuss land-use effects that are significant when compared on the closest elevation levels (FLM and HOMb). According to Hemp (2006) Mt. Kilimanjaro exhibits a strong ecological zonation. FLM and HOMb are both located in the same altitudinal zone (i.e. lower montane) and were selected to represent the respective zone of natural species composition (Ensslin et al., 2015). Therefore, we assume low elevation related variability. This assumption is also supported by the similar litter peak seasonality in both ecosystems (Figure 2.1-3) Several studies from the tropics focus on nutrient contents in leaf litter of agricultural plantations (Beer, 1988; Dawoe et al., 2010), tree

plantations (Sharma and Pande, 1989; Carnol and Bazgir, 2013) and natural forests (Dent et al., 2006; Lu and Liu, 2012). Some studies also compared tree plantations to natural forests (Lisanework and Michelsen, 1994; Celentano et al., 2011). However, the results vary considerably between study sites and are not directly comparable to each other. For example, the N content in litter is higher in Ethiopian natural forests than in tree plantations (Lisanework and Michelsen, 1994), while the opposite results were recorded from Costa Rican sites (Celentano et al., 2011). Independent from elevation, HOM and COF at Mt. Kilimanjaro had higher N contents and therefore lower C:N ratios in leaf litter than natural forests (Figure 2.1-4). Nitrogen is a limiting factor in tropical montane forests (Vitousek, 1984; Fisher et al., 2013), and N-deprived plants usually have a high C:N ratio in litter (Chave et al., 2010). We expect two processes to mitigate the natural N limitation. First, the introduction of crops such as Musa ssp. and Coffea ssp. affects the nutrient content of vegetation and litter in general. Second, fertilization leads to higher N contents in plants and consequently in leaf litter (O'Connell and Grove, 1993). As a result the annual N deposition by litterfall in HOM and COF increased and N cycling in these ecosystems was enhanced. This is well in line with Zech et al. (2011), who found evidence for accelerated N-cycling in the cultivated areas of Mt. Kilimanjaro. Fertilization with N and P also increases the content of other macronutrients in leaf litter (O'Connell and Grove, 1993). This corresponds to our findings because the content of most macronutrients in land-use ecosystems either increased or remained on the same level compared to the natural forests. Specific micronutrient fertilization can be ruled out in homegardens (Fernandes et al., 1986). Consequently, micronutrients were either unaffected (Al, Fe) or decreased under managed conditions (Mn, Na).

2.1.4.4 Implications for ecosystem cycles

The effects of land use and elevation on litterfall and nutrient contents also lead to two specific implications for C and nutrient cycles at the ecosystem level. The first implication can be drawn from the seasonal dynamics of litterfall. Litterfall peaks at the end of the dry season promote an accumulation of particulate organic matter on the surface soil. This accumulation entails increased microbial activity and mobilization of C and nutrients during the following wet season (Sayer et al., 2007; Blagodatskaya et al., 2009). Several studies reported a peak in freshly mobilized C and nutrients in the early wet season, increasing the possibility of leaching or translocation to deeper soil layers (Qiu et al., 2005; Pabst et al., 2013). As a consequence, an increased nutrient deposition via litterfall might not necessarily result in higher nutrient availability, but may actually increase nutrient losses. The investigated agricultural ecosystems at Mt. Kilimanjaro experience distinct climatic seasonality and accumulate large amounts of litter at the end of dry season. This implies that the nutrient cycles in these ecosystems are especially vulnerable to changes in vegetation structure and species composition.

The altered nutrient deposition rates lead to the second implication regarding turnover rates and C losses from soils. There is ambiguous information on the effects of single nutrient addition and fertilization on the decomposition rates of leaf litter (Khan et al., 2007; Grandy et al., 2013). While N or P addition alone might delay nutrient mobilization, decomposition is generally accelerated by a higher macronutrient content (Allison and Vitousek, 2004; Debusk and Reddy, 2005). In addition, Debusk and Reddy (2005) postulated that this acceleration is independent of soil nutrient content. The abundant macronutrients in the litter of the investigated agricultural ecosystems therefore imply an accelerated C and nutrient turnover in the respective ecosystems. Easily available substrate is decomposed faster, and soil respiration (i.e. soil CO_2 efflux) is generally higher in soils of intensively managed versus natural ecosystems at Mt. Kilimanjaro (Mganga and Kuzyakov, 2014). Together with tillage and crop removal, this explains the lower C and N stocks in the topsoil of agroforestry systems compared to natural forests at Mt. Kilimanjaro (Table 2.1-1). As a consequence, the conversion of natural forests to perennial plantations or homegardens probably represents a source of atmospheric CO_2 despite their structural resemblance to natural forests.

2.1.5 Conclusions

At the southern slope of Mt. Kilimanjaro, the annual pattern of litterfall depends on seasonal climatic conditions. Seasonality at lower elevations leads to a distinct peak of litter production in the late dry season (August – October) that is less pronounced at higher elevations. Annual leaf litter production decreased at higher elevations due to lower temperatures and reduced primary production. Nonetheless, other litter components (branches and rest) mask this effect and total annual litterfall was independent of climate and land-use.

Conversion of natural forests to sustainably or intensively used agroforestry systems leads to direct (change of dominant species) and indirect (increased nutrient uptake after fertilization) enrichment of macronutrients in leaf litter. The change in litter quality reduces the C:N ratio, increases the C and nutrient turnover rates in soil and so, accelerates the ecosystem C and nutrient cycles. This is followed by decreased C stocks in agroecosystems, with consequences to their fertility and ecosystem vulnerability. This calls for considering these effects when addressing land-use change and evaluating the sustainability of agroforestry and plantation management.

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2.1.8 Appendix A

Appendix Table 2.1-3: Nutrient content in leaf litter (± SE) from six ecosystems at Mt. Kilimanjaro, Tanzania

	Chagga homegarden 1(b)	Chagga homegarden 4(a)	Coffee plantation	Forest lower montane	*Ocotea* forest	*Podocarpus* forest
(‰$_{mass}$)						
C	49.82 ± 0.38^{a}	47.36 ± 0.43^{b}	47.97 ± 0.35^{b}	47.88 ± 0.28^{b}	49.09 ± 0.41^{a}	48.75 ± 0.62^{ab}
N	2.95 ± 0.14^{a}	2.83 ± 0.11^{a}	2.37 ± 0.10^{b}	1.08 ± 0.08^{d}	1.56 ± 0.07^{c}	1.16 ± 0.08^{d}
C:N	17.09 ± 0.77^{d}	16.85 ± 0.63^{d}	20.40 ± 0.61^{c}	44.93 ± 0.52^{a}	32.10 ± 0.40^{b}	42.30 ± 0.50^{a}
(mg g^{-1})						
Al	0.77 ± 0.12^{ab}	0.94 ± 0.17^{ab}	1.10 ± 0.18^{ab}	0.43 ± 0.18^{c}	1.36 ± 0.19^{a}	0.74 ± 0.19^{bc}
Ca	7.95 ± 0.26^{a}	17.77 ± 1.09^{cd}	13.65 ± 1.80^{a}	6.63 ± 2.00^{d}	10.09 ± 2.18^{b}	9.08 ± 1.88^{bc}
Fe	0.66 ± 0.11^{a}	1.10 ± 0.29^{a}	0.82 ± 0.29^{a}	0.29 ± 0.30^{b}	0.79 ± 0.30^{a}	0.72 ± 0.29^{b}
K	15.83 ± 1.51^{a}	7.36 ± 2.45^{b}	12.87 ± 2.78^{ab}	3.08 ± 3.12^{c}	3.89 ± 3.09^{c}	7.17 ± 2.29^{b}
Mg	1.99 ± 0.05^{bc}	3.99 ± 0.24^{a}	2.14 ± 0.34^{bc}	1.86 ± 0.33^{cd}	2.70 ± 0.41^{a}	1.47 ± 0.38^{d}
Mn	0.11 ± 0.01^{d}	0.12 ± 0.01^{d}	0.21 ± 0.01^{c}	0.52 ± 0.01^{b}	0.67 ± 0.01^{ab}	0.82 ± 0.01^{a}
Na	0.22 ± 0.04^{b}	0.17 ± 0.04^{b}	0.22 ± 0.03^{b}	0.41 ± 0.03^{a}	0.60 ± 0.03^{a}	0.21 ± 0.03^{b}
P	1.37 ± 0.09^{ab}	1.70 ± 0.07^{a}	1.15 ± 0.05^{b}	0.67 ± 0.05^{c}	0.77 ± 0.09^{c}	0.74 ± 0.15^{c}
S	1.98 ± 0.05^{a}	1.68 ± 0.08^{ab}	1.59 ± 0.09^{b}	1.06 ± 0.10^{cd}	1.19 ± 0.10^{c}	0.89 ± 0.12^{d}

Superscript letters indicate significant differences between the sites (derived from Kruskal-Wallis Test; p-level ≤ 0.05).

2.2 Study 2:
Teatime on Mount Kilimanjaro: Seasonal variation in standardized litter decomposition and effects of elevation and land use

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2.2.1 Abstract

Decomposition is one of the most important processes in ecosystem carbon (C) and nutrient cycles, and is a major factor controlling ecosystem functions. The functioning of Afromontane ecosystems and their ability to provide ecosystem services are particularly threatened by climate and land-use change. Our objectives were to assess the effects of climatic conditions (elevation and seasonality) and land-use intensity on litter decomposition and C stabilization in ten ecosystems along the unique 3000 m elevation gradient of Mt. Kilimanjaro.

Tea-Bag Index parameters (decomposition-rate-constant *k* and stabilization-factor *S*) were used to quantify decomposition of standardized litter substrate. Nine pairs of tea bags (green and rooibos tea) were exposed in each ecosystem during the short-wet, warm-dry, long-wet and cold-dry season.

Decomposition rate increased from *k*=0.007 in savanna (950 m elevation), up to a maximum of *k*=0.022 in montane cloud forest (2100 m). This was followed by a 50% decrease in (sub-)alpine ecosystems (>4000 m). Savanna experienced the strongest seasonal variation, with 23 times higher *S*-values in dry season compared to wet season. The conversion of savanna to maize monocultures (~1000 m), and traditional agroforestry to large-scale coffee plantations (~1300 m) increased mean *k*-values, and stabilization factors were about one third lower.

Forests between 1900 and 2100 m represent the zone of sufficient moisture and optimal temperature conditions. Seasonal moisture (lower slope) and temperature limitation (alpine zone) decreases litter decomposition. Mt. Kilimanjaro ecosystems are highly sensitive to land-use change, which accelerates ecosystem cycles and decreases C stabilization.

Keywords: East Africa, Tropical mountain forest, Land-use change, Carbon cycle, Tea Bag Index, Elevation gradient

2.2.2 Introduction

Decomposition of plant residues and organic matter in soil is a major flux in global carbon (C) cycling, and contributes about 58 Pg C $year^{-1}$ to emissions into the atmosphere (Houghton, 2007). At the global scale, litter decomposition and recycling is controlled by climatic factors and soil properties (Aerts, 1997). At the local scale, secondary regulators, such as litter quality, (plant species composition) and consumer organisms, play a greater role for decomposition in natural ecosystems (Makkonen *et al.*, 2012). However, the importance of these factors also changes throughout the decomposition process (Bonanomi *et al.,* 2013). These factors are also directly depending on climatic conditions and therefore can be attributed to the specific ecosystem characteristics along elevation gradients (Wilcke *et al.*, 2008; Röder *et al.*, 2016). It is important to understand general and specific ecosystem mechanisms, to estimate and predict consequences of future climate change scenarios for global C and nutrient fluxes (Stuart Chapin III *et al.*, 2009). A standardized approach is necessary to identify these mechanisms and to examine the role of environmental drivers of decomposition in highly diverse ecosystems (Didion *et al.*, 2016). Previous studies used cotton strips or standardized leaf litter mixtures (Harrison *et al.*, 1988; Wall *et al.*, 2008). However, these methods required multiple measurements in time and were labor intensive, thus could not achieve high resolution required for global modelling. Keuskamp *et al.* (2013) presented an easily applicable method that enables decomposition measurements with a single sampling time, the Tea Bag Index (TBI). Using this method allows to identify seasonal environmental drivers, even under logistically demanding conditions.

As one of the most important steps in organic matter and nutrient cycles, litter decomposition has been extensively studied over the past decades (Vitousek, 1984; Berg, 2000; Singh *et al.*, 2016). However, most studies were conducted in temperate and boreal ecosystems and data from tropical regions is still scarce, and have high uncertainties (Zhang *et al.*, 2008).

There are even fewer studies considering the effects of climatic conditions along tropical altitudinal gradients on decomposition. Most of these studies either looking at comparably short gradients (Ostertag *et al.,* 2003, Guo *et al.,* 2007, Illig et al., 2008), or excluded certain factors, such as seasonality (Coûteaux *et al.,* 2002). In general, research on C cycling in tropical ecosystems has focused on Southeast Asia and South and Central America (e.g. Powers *et al.*, 2009). In contrast, African ecosystems have received much less attention in global assessments (Zhang *et al.*, 2008). The knowledge gap is especially large when it comes to East African mountain forests and effects of anthropogenic disturbances. This underrepresentation is of particular relevance because montane East Africa is an ecological and biodiversity hotspot (Mittermeier, 2004) and deforestation and land-use intensification are rapidly ongoing (Lewis, 2006).

With its large deforestation rates, Tanzania is one of the areas most affected by land-cover change (Fisher, 2010). For example, Mt. Kilimanjaro region experienced considerable intensification of

agricultural land use within the last 50 years (Misana *et al.*, 2012). Despite the risks for ecosystem services, this offers valuable possibilities to study effects of these anthropogenic factors on ecosystem C cycling. Land-use change can alter numerous ecological factors, which in turn, affect ecosystem functions and lead to high complexity and unpredictable implications of these changes (Groffman *et al.*, 2001). To assess the anthropogenic impacts on C sequestration in tropical forest ecosystems, it is important to understand the functioning of C recycling through decomposition under natural and disturbed conditions. Current estimates might still underrepresent effects of converting tropical forests to agricultural land (Blecourt *et al.*, 2013). It is yet unclear how climate and agricultural land use affect C cycling in Afromontane ecosystems.

We used the unique elevation gradient of Mt. Kilimanjaro to investigate the effects of climate and land use on standardized litter decomposition. This allows drawing inferences about the dominating ecosystems of East Africa, covering a broad range of climate and land-use conditions. These are the first data on decomposition of plant materials from Mt. Kilimanjaro ecosystems and our contribution to the Tea Bag Index project (www.teatime4science.org).

Our first objective was to assess the effects of climatic conditions (changing with elevation) on decomposition and C stabilization in ecosystems with similar soil parent material. Secondly, we investigated the seasonal variations in decomposition and C stabilization along a climate and land-use gradient. We hypothesize, that (1) decomposition rates are increasing under seasonally stable climatic conditions (i.e. mid-elevation), that (2) seasonality is more important at low elevation (semi-arid climate) compared to higher elevation, and that (3) land-use intensification increases decomposition rates and reduces C sequestration potential.

2.2.3 Methods

2.2.3.1 Study site

The study sites are located at the southwestern slope of Mt. Kilimanjaro (3°4’33”S, 37°21’12”E) and cover an elevation gradient from 951 to 4190 m a.s.l. (Table 2.2-1). Ten plots (0.25 to 1.00 ha) were selected, representing typical natural and agricultural ecosystems of the region as characterized by Hemp (2006a). The colline area, below 1200 m, is naturally covered with savanna woodland (SAV) dominated by *Acacia* species (Becker *et al.*, 2016). This natural vegetation is increasingly transformed into arable land for intensive maize and sorghum production (MAI) (Lambrechts *et al.*, 2002). The densely populated area between 1200 m and 1800 m is mainly covered by *Chagga* homegardens (HOM) and Coffee plantations (COF). Homegardens are multilayered agroforestry systems with *Musa* ssp. and *Coffea* ssp. as dominant crops under fruit and remnant forest trees (e.g. *Albizia schimperiana, Grevillea robusta*) (Hemp, 2006b). They are traditionally managed with sporadic addition of organic fertilizers and household waste (Fernandes *et al.*, 1986). Shade-coffee plantations (COF) are an

intensively managed land-use type, with regular application of mineral fertilizers and pesticides. We categorized land-use intensity of these sites according to the indices proposed and calculated by Classen *et al.* (2015) and Schellenberger-Costa *et al.* (2017) (Supporting Table 2.2-2). These indices consider factors such as annual biomass removal, input of fertilizers and pesticides, vegetation structure as well as surrounding land-use types.

Table 2.2-1: Land-use classification, annual precipitation (MAP), mean annual temperature (MAT) and soil characteristics (in 0-10 cm) of the research sites on Mt. Kilimanjaro

Ecosystem	Plot ID	Land-use class	Elevation (m a.s.l.)	MAP* (mm)	MAT* (°C)	Soil C (mg g^{-1})	Soil N (mg g^{-1})	Soil pH
Savanna	SAV	Natural, disturbed	951	663	23.7	27.5	2.0	5.38
Maize field	MAI	Agricultural, intensive	1009	744	22.6	14.5	1.2	4.56
Chagga homegarden	HOM	Agricultural, traditional	1275	1267	20.8	38.4	3.5	5.42
Coffee plantation	COF	Agricultural, intensive	1305	1250	20.1	18.9	1.8	4.28
Lower montane forest	FLM	Natural, disturbed	1920	2257	15.3	134.8	9.2	4.34
Ocotea forest	FOC	Natural	2120	2500	12.1	214.6	12.4	3.49
Podocarpus forest	FPO	Natural	2850	2063	9.4	205.9	10.0	3.83
Erica forest	FER	Natural	3880	1389	4.5	137.5	7.6	4.5
Helichrysum	HEL1	Natural	3880	1417	5.3	131.3	8.8	5.0‡
Helichrysum	HEL2	Natural	4190	1308	4.5	29.8	2.4	5.2

* Appelhans et al. (2014)
‡ Estimated from Gütlein et al. (2016)

Five natural sites were located inside the Kilimanjaro National Park along the Machame and Umbwe ridges. The Lower montane forest (FLM) at 1920 m is dominated by *Macaranga kilimandscharica*, *Agauria salicifolia* and occasional *Ocotea usambarensis. Ocotea* forest (FOC) at 2120 m is defined by the lone dominance of *O. usambarensis* and tree fern, such as *Cyathea manniana.* The forest at 2850 m was classified as *Podocarpus* forest (FPO) and is dominated by *Podocarpus latifolius* together with *Prunus africana* and *Hagenia abyssinica.* In the subalpine zone around 4000 m (FER), *Erica trimera* is dominating and can reach up to 10 m growth height. Between 4000 and 4500 m (HEL), the alpine forest is displaced by *Helichrysum* cussion vegetation with tussock grasses (Ensslin *et al.*, 2015). An additional HEL plot (HEL1) was added to represent the zone of ongoing vegetation shift between *Erica* and *Helichrysum.*

Climate at Mt. Kilimanjaro follows a bimodal rainfall regime with a short rainy season between October and December and a longer rainy season from March to May (Hemp, 2006a). Interpolated, mean annual and monthly (2011-2014) meteorological data from the study sites are available from Appelhans et al. (2014). Mean annual precipitation (MAP) varies between 663 mm and about 2500 mm per year (Table 2.2-1). Mean annual temperature (MAT) ranges from 4.5 °C to 23.7 °C.

The comparison of ecosystems on Mt. Kilimanjaro is especially valuable because soils have a similar age and developed from similar parent material. In the colline zone, soils developed on erosion deposits from Mt. Kilimanjaro and were classified as Vertisols. Soils in the forest zone were classified as Andosols with folic, histic or umbric topsoil horizons and accordingly high C contents in the upper horizons, often underlain by C rich paleosol sequences (Zech 2014). In the alpine zone, dominating soil types are mainly Leptosols and Vitric Andosols. These soils developed from volcanic rocks, such as basalt, trachyte and olivine basalts (Dawson 1992).

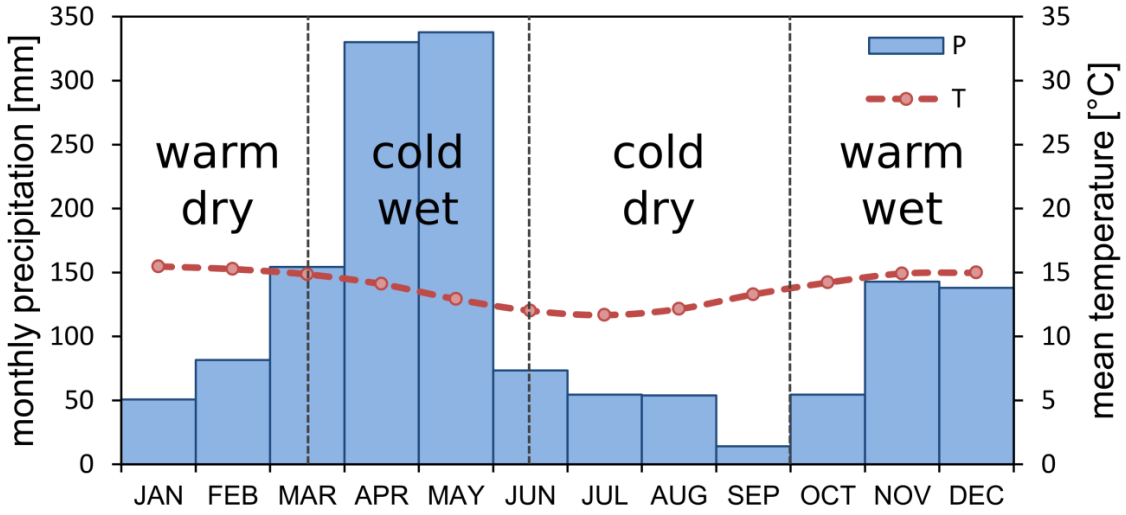

Figure 2.2-1. Annual variation in temperature (T, red dashed line) and monthly precipitation (P, blue bars) averaged over 10 ecosystems at Mt. Kilimanjaro slopes. Details for individual ecosystems are available from Appelhans et al. (2014)

2.2.3.2 Sampling and analyses

We used the Tea-Bag Index (TBI), as introduced by Keuskamp *et al.* (2013), to assess seasonal effects on decomposition of a standardized substrate. At each of the ten plots, nine pairs of litterbags (green tea & rooibos tea) were buried in 8 cm depth along a 100 m transect parallel to the line of the slope. The litterbags were exposed for ~90 days before collection. This was repeated during the short-wet (October-December 2014), warm-dry (December-March 2014), long-wet (March-July) and cold-dry season (July-September) (Figure 2.2-1). The recovered litterbags were dried at 60°C for 48 hours and weighed afterwards.

TBI is based on the decomposition rate constant (k) and stabilization factor (S). Both were calculated according to Keuskamp *et al.* (2013). In short: The k value is calculated from mass loss W after incubation time t, assuming a double-exponential decomposition due to faster decomposition of hydrolysable fractions (a) and relative increase of the more recalcitrant fraction (1 - a) over time (eq. 1).

$$W(t) = ae^{-kt} + (1 - a) \qquad \text{(eq. 1)}$$

Environmental conditions can alter the stability of less recalcitrant compounds, reducing the mass loss of the originally hydrolysable (i.e. chemically labile) fraction. This inhibiting effect is therefore referred to as *S* (eq. 2), with a_g being the decomposed fraction and Hg the hydrolysable fraction of green tea.

$$S = 1 - \frac{a_g}{H_g} \qquad \text{(eq. 2)}$$

2.2.3.3 Statistical analyses

The effect of elevation was assessed by linear regression at p-level ≤ 0.05. The polynomial degree of the model fit was determined using Akaike's Information Criterion (AIC) on linear, second-order and third-order models. We identified seasonal variations by comparing slopes and intercepts of the final regression models using analysis of covariance (ANCOVA) (p≤0.05). Effects of land use were compared separately for each elevation class (*colline* and *montane*). Significant effects were determined by using linear mixed effect model ANOVA for nested designs with season as random factor (p≤0.05). Seasonality of both TBI parameters (*k* and *S*) was related to seasonal amount of precipitation and mean temperature in each ecosystem using partial correlation to correct for T and P respectively (Supporting Table 2.2-2). Continuous measurements of climatic variables were available only from SAV, FLM, FPO and FER (Supporting Table 2.2-3), thus we limited our analysis to these sites. All statistical analyses were conducted in R 3.3.1 (R Core Team, 2016).

2.2.4 Results

2.2.4.1 Effect of elevation

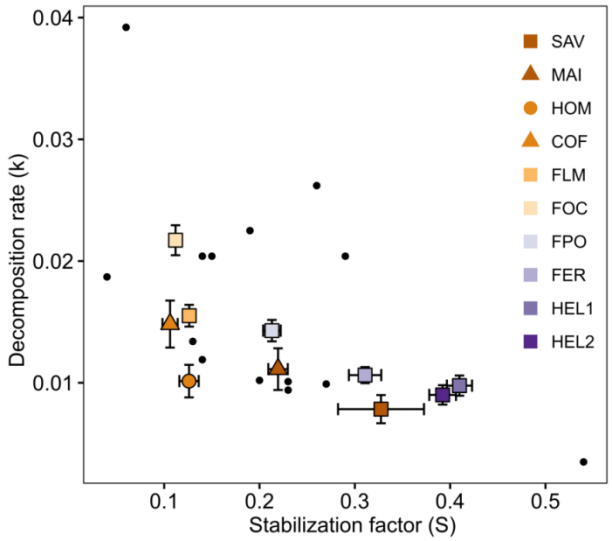

Figure 2.2-2. Annual means of Tea Bag Indicees: decompostion rates (k) vs. stabilization factor (S) in ten ecosystems along the elevation gradient of Mt. Kilimanjaro: Savanna (SAV), maize (MAI), homegarden (HOM), coffe plantation (COF), lower montane forest (FLM), *Ocotea* forest (FOC), *Podocarpus* forest (FPO), *Erica* forest (FER) and *Helichrysum* (HEL). Land-use classes are presented as: Natural and semi-natural ecosystems (squares), extensive agroforestry (circle), intensive land use (triangle). Black dots indicate global TBI references taken from Keuskamp et al. (2013).

Mean annual decomposition rate constant *k* decreased logarithmically with increasing stabilization factor S (Figure 2.2-2). Average *S* values were highest in alpine and sub-alpine ecosystems as well as in SAV. FOC exhibited the maximal *k* values.

Average *S* values were highest in alpine and sub-alpine ecosystems as well as in SAV. FOC exhibited the maximal *k* values. Annual means of *k* and *S* were strongly affected by elevation (Figure 2.1-3). These relationships were best explained by left skewed third-order (or higher) polynomial functions (Supporting Table 2.2-3), indicating stronger effects within the colline and lower-montane zones compared to the montane and alpine zones.

Mean decomposition rate increased from *k*=0.007 in SAV, up to a maximum of *k*=0.022 in FOC. The increase of *k* was followed by its decrease to around *k*=0.010 in the (sub-) alpine ecosystems. Stabilization factor decreased from SAV (*S*=0.33) to COF or FOC (*S*=0.11) and strongly increased again to a maximum of *S*=0.41 in the alpine *Helichrysum* ecosystem.

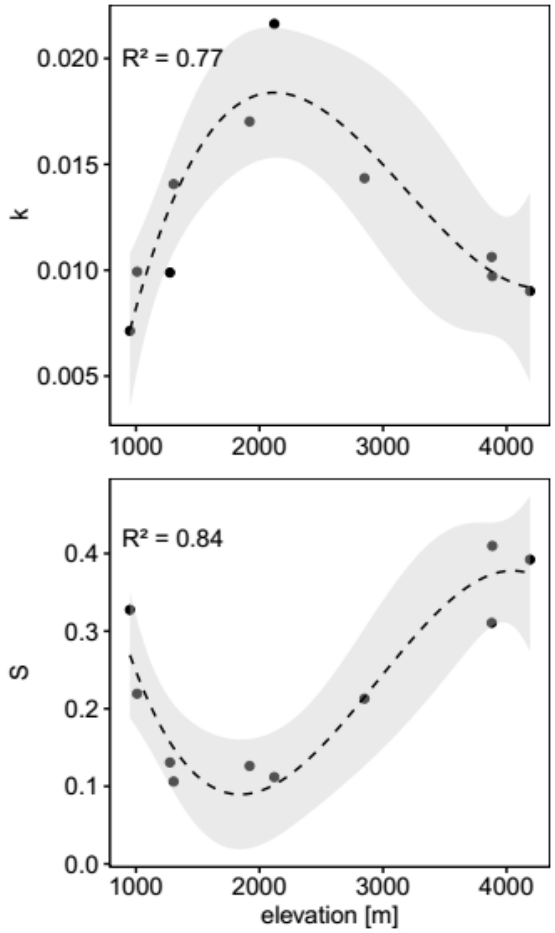

Figure 2.2-3. Annual means of Tea-Bag Index decompostion rate constant (k) and stabilization factor (S) in ten ecosystems along an elevation gradient at Mt. Kilimanjaro. Dashed lines and grey areas indicate best fit polynomial regression and respective areas of 95%-confidence.

2.2.4.2 Effect of seasonality

During all seasons, we found the highest decomposition rates in the mid-elevation forest belt (Figure 4). However, during both warm seasons the peak is shifted upslope.

Regression slopes between *k* and elevation differed significantly between seasons ($p \leq 0.05$). Maximum *k* values in cold-wet and cold-dry season were found at 2220 m in FOC. During the warm-dry season, *k* peaks at 2850 m (FPO). At most sites below 2220 m, seasonal maxima were found during the longer cold-wet season with the highest precipitation. While at higher elevation, maxima occurred solely during the warm-wet season.

Seasonal fluctuations strongly affected stabilization factor in SAV (Figure 4). In all ecosystems, the *S*-factor values were highest during the cold dry season. This seasonality was less influential at mid elevation. Both, highest and lowest *S* values were measured for wet and dry season in SAV, respectively. The mean *S* values in SAV during cold and dry season (*S*=0.54) were about 23 times higher compared to the warm-wet season (*S*=0.02). The lowest seasonal fluctuation was measured for FOC, where *S* varied between 0.13 during the cold dry season and 0.09 during the warm-wet season.

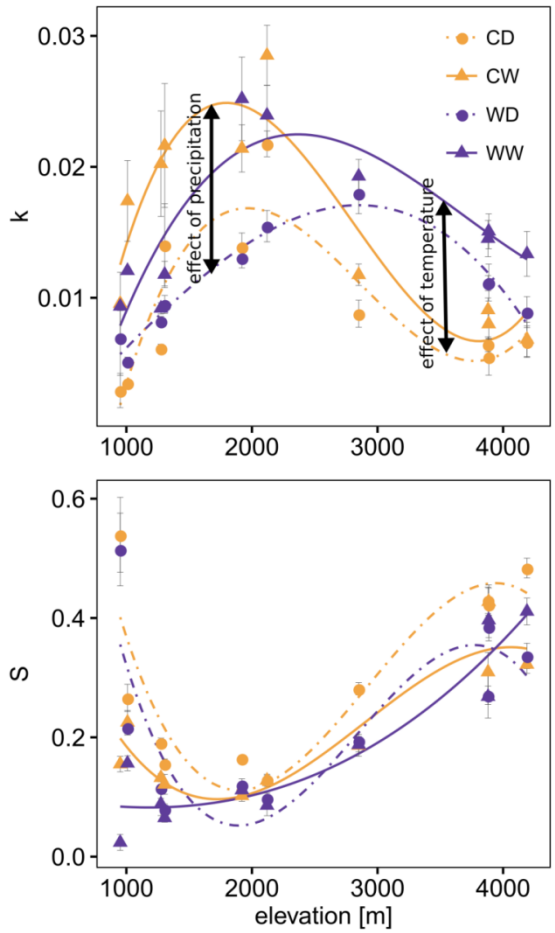

Figure 2.2-4. Seasonal variability of Tea-Bag Index decompostion rates (*k*) and stabilization factor (*S*) in ten ecosystems along an elevation gradient at Mt. Kilimanjaro. Linetypes indicate 3rd-order polynomial fits for cold-dry (CD), cold-wet (CW), warm-dry (WD) and warm-wet (WW) seasons. Arrows indicate the range of seasonal variation as maximal effects of temperature and precipitation.

Partial correlation between *k* in natural ecosystems and precipitation was significant (p≤0.05) except at mid elevation (FPO) (Figure 2.2-5). At mid and high elevation (FPO & FER) *k* was significantly affected by temperature. The correlation between stabilization factor and seasonal precipitation linearly decreased with elevation. Contrary, the stabilization factor was significantly affected by temperature, already at FLM and above.

2.2.4.3 Effects of land use

Land-use intensification slightly increased decomposition rates and significantly decreased S values (Figure 2.2-6). In both elevation zones, mean annual *k*-values increased by about 30% with land-use intensity, but these effects were not significant when considering seasonal variations (SAV-MAI: p = 0.14 & HOM-COF: p = 0.16). Mean annual stabilization factor in the colline zone decreased from 0.33 in SAV to 0.22 in MAI. Likewise, the stabilization factor in COF was around 20% lower compared to HOM.

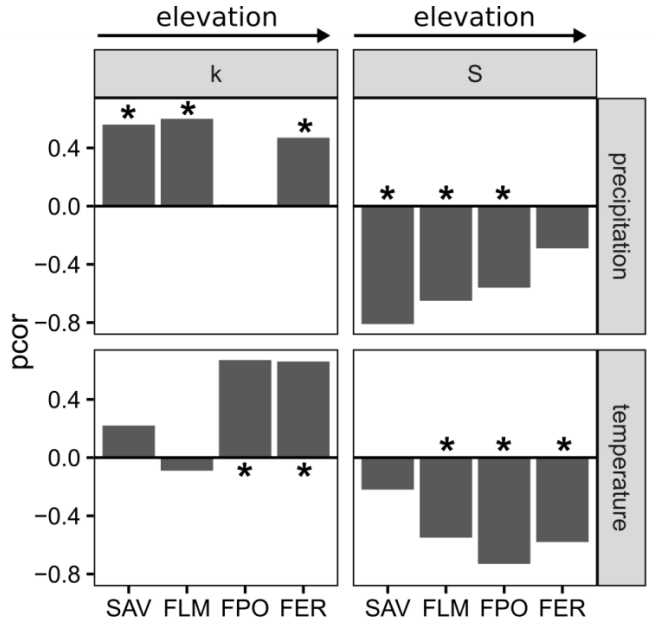

Figure 2.2-5. Partial correlation coefficients of both Tea-Bag Indices: k (left) and S (right) with seasonal precipitation (top) and seasonal mean temperature (bottom) in four natural ecosystems: Savanna (SAV, 950 m), lower montane forest (FLM, 1900 m), *Podocarpus* forest (FPO, 2850) and *Erica* forest (3880 m) on Mt. Kilimanjaro. Significant correlations (p ≤ 0.05) are highlighted (*).

2.2.5 Discussion

2.2.5.1 Evaluation of TBI indices

All measured values of k and S and their variances were in a similar range as global reference data derived from Keuskamp *et al.* (2013), but mean annual *k* values were mainly on the lower half (Figure 2). The mean *k* values in the Kilimanjaro forest belt (i.e. FOC, FLM, FPO) were comparable to temperate forest sites from Keuskamp *et al.* (2013), but were not as high as in tropical moist or lowland forests. Lower slopes of Kilimanjaro region are under stronger water limitation than lowland forests in Central and South American tropics (Legates & Willmott, 1990), while lower MAT at high elevation restricts decomposition. Annual *S* means covered the whole range of global reference values. In cold alpine and

semi-arid savanna ecosystems, *S* was higher compared to most reference sites (except desert). This supports the underlying assumption of the TBI, that *S* is strongly depending on environmental and climatic factors (Berg & Meentemeyer, 2002) and can reflect climatic limitation.

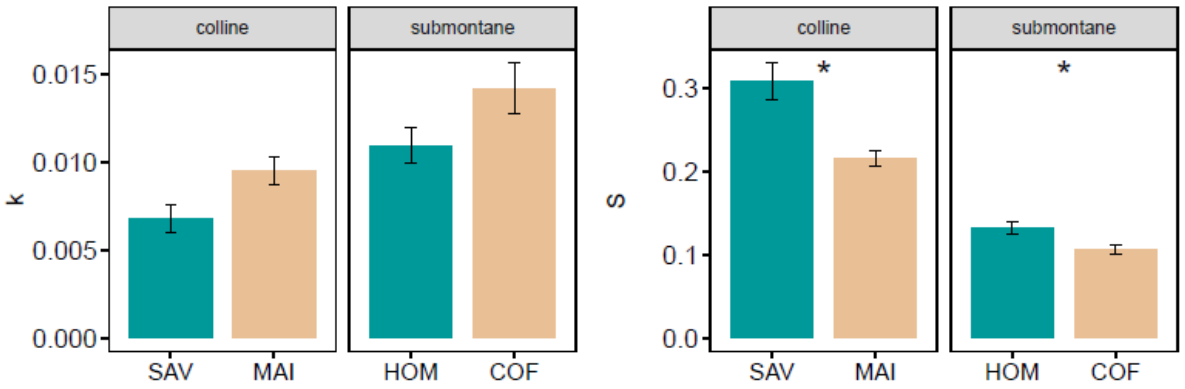

Figure 2.2-6: Effect of land-use change from semi-natural savanna (SAV) and traditional agroforestry (HOM) to maize field (MAI) and coffee plantation (COF), on mean annual TBI decomposition rates (k) and stabilization factor (S). Significant differences ($p < 0.05$) are indicated (*) according to linear mixed effect model ANOVA for nested design with seasons as random effect

The TBI appears to be a valid and reproducible method for estimating decomposition rates and C stabilization potential at Mt. Kilimanjaro and our results are consistent within this context. However, further improvements of the TBI method might be recommended (Didion *et al.*, 2016). Measurements are limited to 3 months of incubation but are highly sensitive to seasonal fluctuations. If the TBI data should contribute to a global annual modelling, this should be considered in method standardization.

2.2.5.2 Effects of elevation

Elevation (i.e. climatic conditions) had a strong effect on decomposition rate and stabilization factor (Figure 2.2-7). Both parameters have their critical values at mid elevation: the decomposition rate *k* – its maximum, and the stabilization factor *S* – its minimum (Figure 2.2-3).

Unimodal and U-shaped patterns are typical for various ecosystem properties along montane elevation gradients (Kluge *et al.*, 2006; Campos *et al.*, 2014). Peaks at mid elevation were recently found for photosynthesis (NDVI), soil C content, litter quality and species abundance at Mt. Kilimanjaro (Hemp, 2006a; Pabst *et al.*, 2013; Becker *et al.*, 2015; Röder *et al.*, 2016). Especially the distribution of aboveground biomass is distinctly hump shaped at Mt. Kilimanjaro (Ensslin *et al.*, 2015). The maximum occurs in FLM and FOC, between 2000 and 2500 m elevation. This mid-elevation peak of ecosystem productivity is highly correlated with precipitation, i.e. water availability, (Röder *et al.*, 2016) and it can be directly linked to decomposition patterns (Figure 2.2-3).

Seasonal temperature variations start to affect C stabilization at FLM (1920 m) and become increasingly important at higher elevation (Figure 2.2-5). Precipitation can be seasonally limiting below FPO (< 2850 m). However, FLM and FOC represent the interception zone between mostly sufficient

moisture availability and temperature. This indicates that C sequestration in these ecosystems is mainly driven by amounts of litter input and productivity. At lower and higher elevation, decomposition is restrained by climatic factors.

Ecosystems at lower elevation are highly subjected to seasonal moisture limitation (Appelhans *et al.*, 2016). Especially in semi-arid environments, low water availability negatively affects litter decay rates (Incerti *et al.*, 2011). During the rainy season, soil microbial activity in savanna strongly increases (Otieno *et al.*, 2010) and the turnover is less selective regarding organic matter quality (Davidson & Janssens, 2006). This effect is only present in semi-arid elevation zones (i.e. colline and submontane). At mid-elevation *S* values were low and unaffected by seasonality, thus the preference of easily available substrate was rather constant throughout the year.

In upper montane and alpine environments (≥2850 m), the decomposition was strongly limited by temperature (Figure 2.2-5) and increased during the warm seasons (Figure 2.2-4). This is commonly expected because temperature sensitivity of decomposition is generally higher at low temperatures (Davidson & Janssens, 2006) and at higher elevation (Schindlbacher *et al.*, 2010; Blagodatskaya *et al.*, 2016). Another factor that might reduce decomposition specifically in *Podocarpus* forest (2850 m) is the regular water logging of soil due to clouds inhibiting evaporation (Bruijnzeel & Veneklaas, 1998). However, neither negative nor positive effects of precipitation were found during the seasons (Figure 2.2-5). Strong seasonality in *Erica* and *Helichrysum* ecosystems implies strong dependency on climate variables and low potential to adapt to fast climate changes compared to lower elevation forests (Hemp & Beck, 2001). The projected increase of surface temperature (Bradley *et al.*, 2006) will reduce the stabilization of fresh C and accelerate organic matter decomposition. Therefore, future soil C losses into the atmosphere might be considerably large and fast in East African mountain ecosystems.

2.2.5.3 Effects of land use

Land-use intensification from semi-natural savanna to maize monocultures and from traditional homegardens to large-scale coffee plantations decreased C stabilization and showed the tendency to increase decomposition rates (Figure 2.2-6; Figure 2.2-7). The total content of soil organic matter (SOM) and microbial biomass commonly decrease with land use intensification (Don *et al.*, 2011; Junior *et al.*, 2016). This effect was also found at Mt. Kilimanjaro (Pabst *et al.*, 2013). However, at the same time decomposition rates at Mt. Kilimanjaro tended to increase while C stabilization decreased. This is in contrast to previous findings that connected land-use intensification to decreasing decomposition rates (Attignon *et al.*, 2004; Violita *et al.*, 2016). Under similar environmental conditions as compared to the lower slopes of Mt. Kilimanjaro (i.e. western Kenya, 1500 m), Kagezi *et al.* (2016) found decreased decomposition rates on agricultural compared to natural sites. This decrease of organic matter decomposition can be connected to the application of N fertilizers and reduced microbial

biomass (Zang *et al.*, 2016). Decomposition studies tend to exhibit strong site and method specific variation (Makkonen *et al.*, 2012) and land-use intensification was likewise found to increase decomposition of litter and SOM (Lisanework & Michelsen, 1994; Guillaume *et al.*, 2015). Decreasing decomposition with higher land-use intensity are often related to changes in decomposer communities (Kagezi *et al.*, 2016). Recent studies from Mt. Kilimanjaro found only minor effects of land-use change on overall arthropod abundance and composition (Röder *et al.*, 2016) but indicated accelerated organic matter turnover on agricultural sites (Becker *et al.*, 2015). Also, glucose decomposition increases with land-use intensification from savanna to maize fields and homegardens to coffee plantations (Mganga & Kuzyakov, 2014). This is because soil microbes in these ecosystems are less efficient in SOM decomposition but at the same time more demanding for new C sources (Pabst *et al.*, 2016), reducing *S* values on agricultural sites (Figure 2.2-6). This concept relates decomposition patterns primarily to the microbial decomposers nutritional status (Manzoni *et al.*, 2008). Considering the features of the TBI method (i.e. standardized litter, exclosure of exogeic and >0.25 mm fauna) this points out the importance of pre-existing soil nutrient conditions on litter decomposition and C stabilization.

2.2.6 Conclusions

This is the first study that gives insight into mechanisms of organic matter decomposition in Mt. Kilimanjaro ecosystems, representing a broad range of natural and agricultural areas in East-Africa. Soil organic matter turnover and stabilization at Mt. Kilimanjaro is strongly dependent on the climatic conditions along the elevation gradient. Ecosystems at mid elevation (between 1900 and 2200 m) represent the zone of sufficient moisture and optimal temperature conditions, with the highest plant biomass and productivity. High litter input and fast turnover regulate the C sequestration in these ecosystems, while climatic restrains control decomposition and C stabilization in lower and higher elevation zones. Decomposition in the colline savanna, Africa's most abundant biome, is strongly controlled by seasonal moisture limitation and highly sensitive to changing rainfall patterns. Small seasonal temperature variations had a strong effect on decomposition in *Erica* and *Helichrysum* sites (> 3000 m), implying a strong temperature sensitivity of these ecosystems. Therefore, with raising global temperatures, soils in (sub-) alpine Afromontane ecosystems must be considered potential future atmospheric CO_2 sources.

Land-use intensification at Mt. Kilimanjaro decreases soil C sequestration potential by increasing microbial demands for fresh C sources. The transformation of natural savannas to maize monocultures and from traditional subsistence farming to large-scale plantations may have strong negative impact on the C stocks of East-African soils. Especially considering the future increase in population and thus food-demand land-use intensification is likely to substantially act as a future CO_2-source in this area, too.

We conclude that decomposition rates in East-African ecosystems are controlled by the combined effects of long-term climatic conditions, seasonal variability and land-use change. Thus, projecting effects of climate change and regionalizing C cycling patterns must consider these factors. Especially for conducting short term decomposition experiments with standardized litter (e.g. TBI) in semi-arid or temperature limited regions, the consideration of seasonal variations, as a major controlling factor of decomposition, is required.

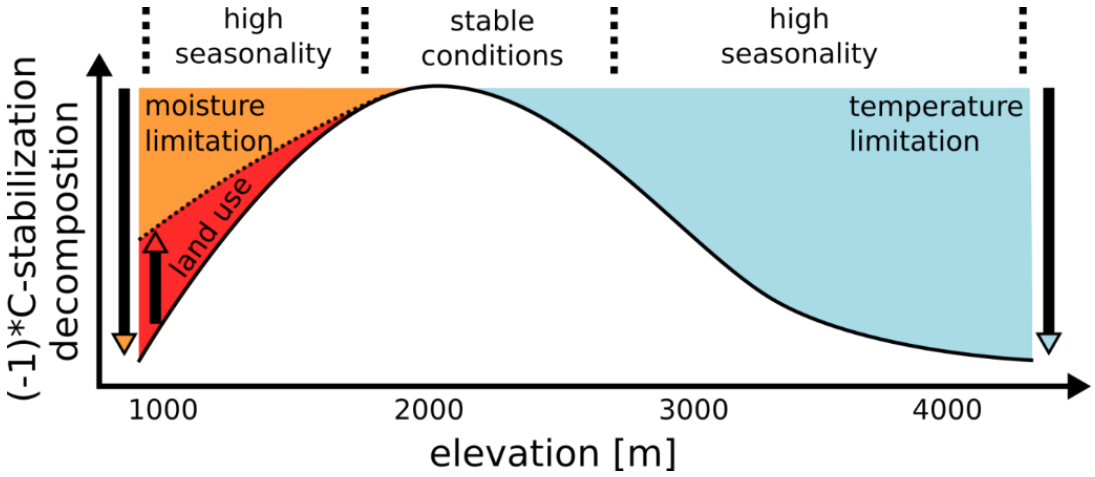

Figure 2.2-7: Conceptual outline of climatic and land-use effects on standardized litter decomposition at Mt. Kilimanjaro. Arrows indicate effect direction of increasing land-use intensity and decreasing temperature and precipitation.

2.2.7 Acknowledgements

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2.2.9 Supporting tables

Supporting Table 2.2-2: Land-use indices for savanna (SAV), maize field (MAI), homegarden (HOM) and coffee plantation (COF). Higher values (0 to 1) indicate stronger anthropogenic disturbance.

Ecosystem	Category	Land-use index[1]	Disturbance index[2]
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

[1] includes vegetation structure, data and method available from Classen *et al.* (2015)
[2] no vegetation structure included, data and method available from Schellenger-Costa *et al.* (2017)

Supporting Table 2.2-3: Comparison of polynomial regression fits for mean annual *k* and *S* with elevation.

	polynomial degree	R^2	p-value	AIC
k				
	1st order	-0.12	n.s.	-74.8
	2nd order	0.68	0.007	-86.8
	3rd order	0.78	0.007	-89.7
	4th order	0.74	0.026	-88.0
S				
	1st order	0.35	0.042	-14.9
	2nd order	0.73	0.004	-22.9
	3rd order	0.84	0.003	-27.7
	4th order	0.86	0.006	-29.0

Supporting Table 2.2-4: Seasonal climate variables from four sites at Mt. Kilimanjaro. Savanna (SAV), Lower montane forest (FLM), *Podocarpus* forest (FPO), *Erica* forest (FER).

PlotID	Season	Precipitation [mm]	Mean temperature [°C]
SAV	warm-wet	285	24.2
SAV	cold-dry	50	21.7
SAV	cold-wet	350	23.6
SAV	warm-dry	83	25.3
FLM	warm-wet	398	15.9
FLM	cold-dry	161	12.9
FLM	cold-wet	756	14.9
FLM	warm-dry	65	17.5
FPO	warm-wet	812	9.8
FPO	cold-dry	134	8.1
FPO	cold-wet	1307	9.2
FPO	warm-dry	520	10.6
FER	warm-wet	89	4.9
FER	cold-dry	39	3.4
FER	cold-wet	311	4.3
FER	warm-dry	260	5.5

2.3 Study 3: Climatic and decomposer community effects of leaf-litter decomposition along the elevation gradient of Mt. Kilimanjaro

Extended abstract

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2.3.1 Abstract

Decomposition of plant litter, as one of the major process in ecosystem cycling is depending on ecosystem specific characteristics, including temperature, precipitation, plant species composition, availability of substrate, and decomposer organisms. Therefore, it is important to identify ecosystem specific patterns to understand competition and demand of plants and decomposers for nutrient sources as well as their response to global changes. In this study, we relate previously collected data from Mt. Kilimanjaro with a new decomposition dataset to provide further insight on C and nutrient cycling in Mt. Kilimanjaro ecosystems.

Native leaf litter was exposed for one year in four forest ecosystems along an elevation gradient from 1900 to 3900 m of Mt. Kilimanjaro. Mesh sizes of 0.2, 2 and 5 mm were used to selectively exclude decomposer organisms. Initial and final content of litter nutrients (C, N, P, K, S, Ca, Al, Fe, Mg, Mn and Na) was used to calculate annual release rates.

The effect of elevation on litter decomposition was the same between native and standardized litter in previous studies. Annual litter-mass loss decreased for about 30% between 2100 and 2900 m and was mostly unaffected by accessibility for decomposer communities. However, under the most favorable condition (1900 – 2200) annual litter-mass loss decreased for about 15% from large to small mesh size. The annual release of nutrient cations was negatively correlated to initial C to nutrient ratios.

Climatic variables are more important than litter nutrients and decomposer community complexity for controlling litter decomposition along the large climate gradient of Mt. Kilimanjaro. Ecosystem specific nutrient demand is reflected in release rates from litter decomposition.

2.3.2 Introduction

Tropical mountain ecosystem are characterized by huge climatic gradients and a large percentage of endemic species, making them global hotspots of biodiversity (Gradstein et al. 2008). The specific biodiversity structure feeds back on ecosystem processes and changes the ecosystem resilience to environmental change (Stuart Chapin III et al. 2009). Thus, ecosystem functions and mechanisms are highly responsive to climatic variability. Montane elevation gradients, with their large climatic diversity, provide the ideal conditions to investigate the response of biogeochemical cycles to climatic changes (Wang et al. 2016).

Decomposition of plant litter is a major process in ecosystem carbon and nutrient cycles (Vitousek 1984). The rate and effectiveness of litter turnover depends on ecosystem specific characteristics, including temperature, precipitation, plant species composition, availability of substrate, and decomposer organisms (Gavazov 2010; Dale et al. 2015). Decomposition of litter substrate is directly linked to mineralization rates and nutrient cycling per unit soil organic matter, thus low decomposition rates could induce nutrient shortage at high elevation (Gütlein et al. 2016; Unger 2010). In contrast, fast litter turnover in tropical lowland forests leads to a strong dependency of plants and microbes on direct nutrient supply from decomposition.

Decomposer organisms are responsible for organic matter breakdown and nutrient release, which in turn promotes plant growth and fitness (Poveda et al. 2005). Concurrently, plant-microbial competition enhances the release of specific elements (Semmartin et al. 2004). Decomposition rates therefore are closely linked to diversity of plant and decomposer community structure (Liu et al. 2010). These conditions and processes are strongly varying between ecosystems and biomes (Wardle 2002). Therefore, it is important to identify ecosystem specific nutrient recycling to understand competition and demand of plants and decomposers for nutrient sources (Stuart Chapin III et al. 2009; Brovkin et al. 2012). Currently there is a lack of data from low latitude regions (Zhang et al. 2008), despite their importance for global assessments on ecosystem climate response. In this study, we aim to relate previously collected data from Mt. Kilimanjaro with a new decomposition dataset to provide further insight on C and nutrient cycling in Mt. Kilimanjaro ecosystems.

Our first objective was to assess the effects of climatic conditions (i.e. elevation) and decomposer communities on C and nutrient release from native leaf litter in ecosystems with similar geogenic nutrient supply (i.e. initial substrate/bedrock conditions). Secondly, to put these effects in context with previous studies on litter inputs (Becker et al. 2015), standardized litter decomposition (Study 2) and C storage and mineralization at Mt. Kilimanjaro (Pabst et al. 2016).

2.3.3 Methods

This was study was conducted at the Machame ridge of Mt. Kilimanjaro (3°10'26"S, 37°14'22"E). Four natural forests sites were selected, covering an elevation gradient from 1900 to 3900 m a.s.l. This included lower montane forest (FLM), *Ocotea* forest (FOC), *Podocarpus* forest (FPO) and *Erica trimera* forest that were previously studied and described by Becker et al. (2015), Hemp (2006) and Study 2. Comparing these ecosystems is particularly advantageous because soils are of similar age and developed from similar parent materials. These soils are Andosols with folic, histic or umbric topsoil horizons (WRB 2014) and accordingly high C contents in the upper horizons (Zech et al. 2011).

Five grams of previously collected leaf litter was dried (60°C, 36 h) and exposed for one year in field microcosms. These microcosms were covered with mesh of 0.2, 2 and 5 mm sizes to selectively exclude decomposer fauna (Makkonen et al. 2012). Each mesh size was replicated six times per site. Triplicates were placed along two transects parallel to slope and with a minimum distance of 25 m.

2.2. Laboratory and Data Analyses

After exposure, decomposer organisms were extracted from litter samples and identified under the stereo microscope. Litter C and N contents were determined by automated dry combustion (Vario EL, Elementar). Preparative pressure digestion, followed by inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro Analytical Instruments) was used to determine contents of major macro- (Ca, K, Mg, P, S) and micro- (Al, Fe, Mn, Na) nutrient cations. Annual decomposition rates of leaf litter were calculated from differences in dry weight before and after one-year exposure. Annual mass loss was used to calculate nutrient release (eq. 1):

$$\frac{(W_{t0} * c_{t0}) - (W_{t1} * c_{t1})}{W_{t0}} \qquad (1)$$

With W_{t0} being the litter exposed in each cosm, c_{t0} the average litter nutrient content before exposure and W_{t1} and c_{t1} being the litter weight and nutrient content after recovery, respectively. Effects of meshsize and sites were assessed by analysis of variance (ANOVA) with TukeyHSD post-hoc comparison at p-level 0.05. Requirements for ANOVA were tested using Shapiro-Wilk test (normality) and Bartlett's test (homogeneity of variances) at p-level 0.05. All statistical analyses were conducted in R 3.3.1 (R Core Team 2016).

2.3.4 Results and Discussion

Litter decomposition decreased at higher elevation and was unaffected by accessibility for decomposer communities in most ecosystems, except FOC (Figure 2.3-1). Annual litter-mass loss decreased from 0.41 g per g initial litter mass in FLM and FOC to 0.30 g in FPO and further to 0.27 g in FER. The decreasing pattern with elevation was in line with results from the same sites at Mt. Kilimanjaro using

standardized litter substrate (Study 2): Decomposition reaches a maximum at around 2000 m a.s.l. and decreases at higher elevation due to temperature limitation. These resembling trends indicate that in Mt. Kilimanjaro forests - along the elevation gradient of 1900 to 3900 m - climatic drivers are more important for controlling litter decomposition rates between ecosystems than changes in leaf litter quality. Generally, native and standardized litter react similarly to environmental changes (Didion et al. 2016) and decomposer communities show little specialization and high metabolic flexibility in processing plant litter of different origins (Makkonen et al. 2012). Furthermore, trends along the montane elevation zones can be regarded equivalent to longitudinal biome zonation (Stevens 1992) and decomposition patterns between biomes are usually controlled by climatic factors (Berg et al. 1993). Litter traits can have a strong effect on litter decomposition (Cornwell et al. 2008). However, litter quality is a less important regulator under unfavorable conditions (Coûteaux et al. 2002). Its effect is even more reduced when plant biodiversity is high because decomposer communities are diverse and less selective regarding species specific traits.

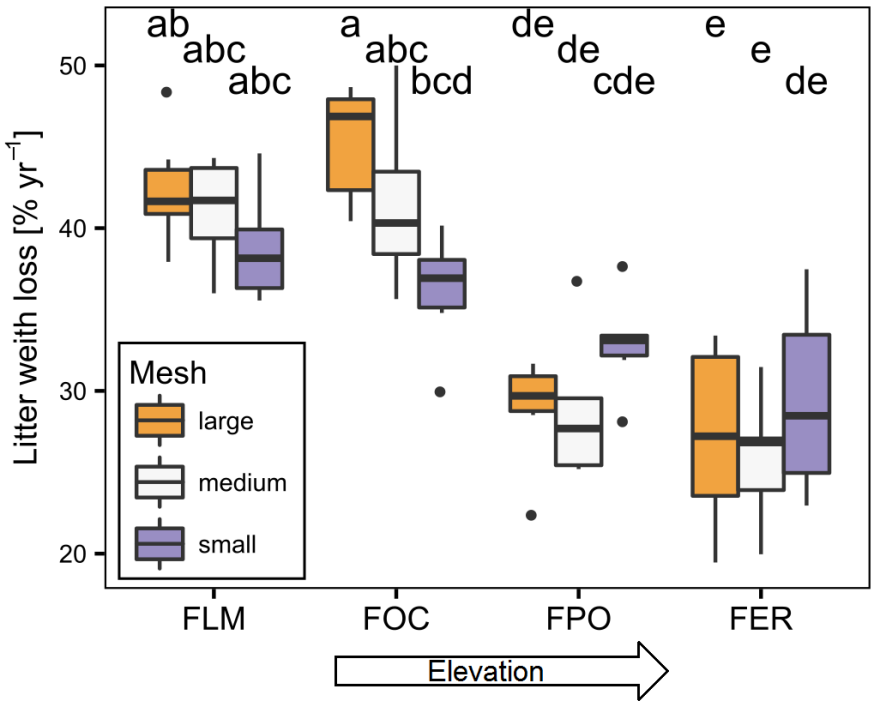

Figure 2.3-1: Annual litter-mass loss through decomposition in four forest ecosystems at Mt.Kilimanjaro. Accessibility for decomposers through mesh size 0.25, 2 and 5 mm is indicated by colour. Small letters (a-e) indicate significant differe differences according to ANOVA with TukeyHSD post-hoc comparison ($p \leq 0.05$).

There was no overall effect of mesh size on decomposition rates (Figure 2.3-1). However, at mid elevation (FOC) annual litter-mass loss decreased for about 15% from large to small mesh size ($p=0.019$). Here and tendentially in FLM, increased accessibility for soil fauna led to increased litter decomposition. This is in line with results from (Wall et al. 2008; Makkonen et al. 2012), who reported

that faunal effects on decomposition are mainly important in tropical forests or when climatic conditions are most favorable.

Table 2.3-1: Correlation between carbon (C) to nutrient ratio in litter and average annual nutrient release from decomposition per plot (n=4). Significance levels are indicated as ‘, * and ** for 0.1, 0.05 and 0.01 respectively

ratio	*r*	*p*-value
C:N	-0.94	0.064’
C:Al	-0.99	0.013*
C:Ca	-0.52	0.480
C:Fe	0.23	0.771
C:K	-0.98	0.023*
C:Mg	-0.88	0.119
C:Mn	-0.82	0.176
C:Na	-0.99	0.011*
C:P	-0.97	0.032*
C:S	-0.94	0.061*

FLM and FOC are ecosystems with relatively stable climatic conditions throughout the year (Appelhans et al. 2016) and consequently accelerated litter turnover (Study 2). Furthermore, the combination of high decomposer and floral diversity, through higher plant biomass, can increase decomposition rates (Ebeling et al. 2014). All these variables are higher in FLM and FOC compared to higher elevation ecosystems (Röder et al. 2016; Ensslin et al. 2015; Hemp 2002).

The annual release of nutrient cations was mainly negatively correlated to the respective initial C-nutrient ratios (Table 2.3-1). While this relationship was strongly expressed by macro nutrients (N, P, K, S) and Al, most micro and ballast element releases were not related to initial C-nutrient stoichiometry. Especially P and N limitation can lead to microbial mining for the respective nutrient from low quality sources (Sinsabaugh et al. 2013). Thus, litter breakdown increased when C-nutrient ratios were wide.

Table 2.3-2: Annual carbon and nutrient release per gram of exposed leaf litter

	FLM	FOC	FPO	FER
C [%]	23.29 ± 0.47^{c}	22.74 ± 0.76^{c}	17.23 ± 0.46^{b}	13.12 ± 0.8^{a}
N [%]	0.01 ± 0.03^{a}	1.19 ± 0.08^{c}	0.19 ± 0.03^{b}	0.74 ± 0.03^{d}
P [mg/g]	4.87 ± 0.21^{c}	0.17 ± 0.10^{a}	0.6 ± 0.11^{a}	1.85 ± 0.14^{b}
K [mg/g]	4.19 ± 0.13^{c}	2.34 ± 0.07^{b}	1.16 ± 0.03^{a}	2.68 ± 0.13^{b}
S [mg/g]	43.39 ± 3.41^{c}	$-2.11* \pm 0.61^{a*}$	13.32 ± 1.54^{b}	19.88 ± 3.78^{b}
Al [mg/g]	0.16 ± 0.00^{c}	$-0.21* \pm 0.03^{a}$	0.06 ± 0.00^{b}	0.16 ± 0.00^{c}
Fe [mg/g]	$-1.06* \pm 0.08^{a}$	$-0.83* \pm 0.08^{a}$	0.48 ± 0.23^{b}	5.62 ± 0.53^{c}
Ca [mg/g]	11.28 ± 0.49^{c}	10.27 ± 0.62^{c}	$-14.89* \pm 1.12^{a}$	$-3.43* \pm 0.96^{b}$
Mg [mg/g]	10.41 ± 0.49^{b}	1.49 ± 0.19^{a}	9.32 ± 0.88^{b}	0.84 ± 0.15^{a}
Mn [mg/g]	3.05 ± 0.13^{d}	1.7 ± 0.17^{c}	$-1.13* \pm 0.14^{b}$	$-2.31* \pm 0.24^{a}$
Na [mg/g]	2.1 ± 0.08^{c}	$-0.13* \pm 0.05^{a}$	1.33 ± 0.08^{b}	0.01 ± 0.14^{a}

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C loss decreased with elevation from 23% (FLM) to 13% of initial litter mass in (FER) (Table 2.3-2) and was the main factor for total weight loss (R^2=0.93, $p<0.001$). N release was highest in FOC and FER reaching 1.2% and 0.7% respectively. Indicating strong reliance of these ecosystems' productivity on N recycling through leaf litter (Parton et al. 2007). P, S, Al and Fe releases decreased from FLM to FOC and increased again at higher elevation. Release of P was particularly low and not different from zero in FOC and FPO (Table 2.3-2). Assuming low particulate P losses through the small mesh below the microcosms, P losses could only occur through leaching. If these fractions are retained by high microbial P demand losses would decrease (McGroddy et al. 2008), as microbial biomass was included in our final content measurements. Including measurements of gaseous N fluxes, N retention and soluble N and P fractions might help to explain these discrepancies. However, these interpretations are rather speculative and further discussion would go beyond the constraints of this thesis.

Negative values were calculated for some nutrients (Al, Fe, Ca, Mg) and may indicate contamination with soil particles. Other than that, we used average initial nutrient contents for calculation release rates, which might result in negative values if variability is high and losses are low.

2.3.5 Conclusions

This study provides additional understanding of the biogeochemical cycling of Mt. Kilimanjaro forest ecosystems. In context with previous studies, we showed that climatic variables are more important than litter nutrients and decomposer community complexity for controlling litter decomposition along the large climate gradient of Mt. Kilimanjaro. Initial litter nutrient content is an important variable for nutrient release. However, annual release rates vary considerably between ecosystems and indicate high demand for litter recycling.

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2.4 Study 4: Soil microbial community structure in forest soils along the elevation gradient of Mount Kilimanjaro

Manuscript in preparation

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2.4.1 Abstract

Climate is crucial for the development of mountain ecosystems, including vegetation and soils. Due to strong interactions between environmental variables, plant communities and edaphic properties, it is unclear, how each factor controls microbial community structure. We used the unique elevation gradient of Mt. Kilimanjaro along a transect of ~3500 m to prove the effects of a) mean annual temperature (MAT: from +4.7 to +23.7 ºC), b), mean annual precipitation (MAP, 845 to 3000 mm) and c) edaphic factors on the content of soil microbial biomass and particular microbial groups.

Topsoil samples (0-10 cm) were collected from six natural forest ecosystems from 740 to 4190 m a.s.l. Microbial community structure was assessed by phospholipid fatty acids (PLFAs). To generalize the effects of MAP and MAT on the total soil microbial biomass, a literature about the total PLFAs content in soils of the mountain forest ecosystems in humid continental, humid subtropical, temperate continental, monsoon, and semiarid climates was reviewed.

Total PLFAs content followed bell shape curve with its maximum at 2120 m a.s.l. (2 µmol g^{-1} soil), which is explained by optimal combination of annual mean temperature (>10 °C) and precipitation (3000 mm). The minimum PLFAs content (0.2 µmol g^{-1} soil) was found at the location with the lowest temperature and lowest productivity (4190 m a.s.l). The meta-analysis showed that PLFAs content peaked in mountain forest soils worldwide around 2000 m independently from the biogeographical region. Thus, bell shaped curve of PLFAs distribution with a peak of around 2000 m a.s.l. may be a general pattern in mountain forests.

Gram-negative PLFAs (25-40 %), which determined the distribution of total PLFAs along the elevation gradient, dominated microbial communities. Contents of gram-positives bacteria decreased, reacting on the decrease of MAP and MAT with elevation. In contrast, fungi and actinomycetes followed a U-shaped distribution, which reflect their adaptation to low precipitation, MAT and low nutritional status of the soils at the highest elevation. The principal component analysis of PLFAs distribution along the elevation revealed the preferences of distinct microbial communities for the low (below 3000 m) and high elevations (above 3000 m). Soil parameters (C, N, pH) explained 19% of the total variance (partial RDA) of PLFAs, whereas climatic variables (MAT, MAP) alone explained 2%. Consequently, the effect of climate on the formation of microbial community structure in mountain regions is indirect and is mediated through plant productivity and soil properties.

Keywords: Climate effects, Elevation gradient, Environmental variables, PLFAs, Microbial community structure, Ecological niche differentiation

2.4.2 Introduction

The structure of microbial communities governs the allocation of carbon (C) in soil and affects ecosystem C cycles (Schimel and Schaeffer 2012). In turn, chemical soil properties, plant community type, and climatic variables contribute to the development of soil microbial community structure. The major edaphic factors affecting the distribution of microbial communities are soil pH (Meng Xu, 2014) and C/N ratios of plant residues and subsequent soil organic matter (SOM). Therefore, acidic pH and wide C/N ratio of SOM, promote the development of fungal populations and are less favorable for bacteria (Zhang et al., 2013, Xu et al., 2014; Bossuyt et al., 2001). Bacterial populations are suppressed in coniferous forests (Saetre and Baath, 2000), whereas stimulated in grassland soils (Djukic et al., 2010). At the same time, contribution of fungal biomass to microbial communities is higher in forest soils compared to grassland soils (Joergensen and Wichern, 2008). However, at the large scales (continental and global) effects of plant communities on soil bacterial and fungal diversity weakens (Tedersoo et al., 2014; Fierer and Jackson, 2006), while climatic factors become more important (Tedersoo et al., 2014). Mean annual precipitation (MAP) has a strong positive effect on the richness of fungal communities, and the closer an ecosystem is located to the equator, the richer fungal soil community becomes (Tedersoo et al., 2014). Thus, it is still an open question, which factors control the soil microbial community composition, especially in places with strong climatic variability – such as mountain ecosystems.

The elevation gradient of a mountain provides an ideal situation to investigate the response of biogeochemical ecosystem characteristics to climatic variability (i.e. temperature and precipitation) (Wang et al. 2016). MAP and mean annual temperature (MAT) change gradually along the slope, which leads to an expressed ecosystem zonation along the elevation (Hemp 2006). Soil properties are also strongly affected by climate along mountain slope (Silver 1998, Antonio Vaquez and Givnish 1998, Seibert et al. 2007). Firstly, increasing precipitation accelerates nutrient losses from soil, which decreases pH and, secondly, the decreasing temperature suppresses decomposition of plant litter and increases the C/N ratio of SOM (Wang 2016, Yoh 2001). Thus, both factors (plant community change and a shift in soil chemical properties) can alter microbial community structure with elevation. However, it is still unclear, whether these factors have a direct impact or if their effect is mediated by the climatic variables.

Both, MAP and MAT affect the microbial community structure in soils of mountain climosequences. The negative effect of MAP was shown for the elevation gradient of 540-2360 m located in temperate monsoon climate, whereas positive effect of temperature was observed (Xu et al., 2014); bacterial diversity was strongly correlated with MAP at the 100-1950 m elevation gradient located in subtropical moist climate (Singh et al., 2014). In contrast, MAP had a rather weak effect in the humid continental

climate, whereas the effect of MAT was prevailing (Zhao, 2014). Thus, the climatic zone as well as the length of climosequence transect can affect the MAT or MAP impact on soil microbial community structure. To reveal the impact of both climatic variables, mountain ecosystems allocated in various elevations should be compared.

Development of natural forests on similar soil parent material and along the elevation gradient allows investigating the formation of microbial communities and reveals the dominant factors affecting their composition. The Kilimanjaro mountain climosequence was chosen for this study, because it has i) broad range of climatic variables due to long elevation gradient (from 767 to 4190 m), ii) identical parent material on all sites (volcanic materials), iii) similar time of soil formation and iv) natural vegetation (represented by forests and alpine heather) with dominance of broadleaf species.

Additional data on the total PLFA content in the forest mountain ecosystems were collected from the literature, to reveal the general effect of elevation, MAP, and MAT on PLFAs content. Based on the literature data we hypothesized that i) total PLFA content will be lower at the highest elevation (harsh weather conditions) compared to middle and low elevations, ii) the sites where MAP or MAT are shifted in both directions from optimal conditions will have different microbial community composition compared to plots with optimal conditions.

Based on these hypotheses and previous findings the objectives of the study were i) to evaluate the distribution of total microbial biomass (assessed by PLFA analysis) and particular microbial groups along the mountain climosequence, ii) to reveal the effect of climatic (MAT and MAP) and edaphic factors (C, N and pH) on the distribution of soil microbial communities and consequently iii) to find optimal climatic conditions for development of total soil microbial biomass and microbial groups.

2.4.3 Material and methods

2.4.3.1 Study site

The study sites are located on the southern slope of Mt. Kilimanjaro (3°4’33”S, 37°21’12”E), Tanzania. Soils at Mt. Kilimanjaro were classified as Andosols with folic, histic or umbric topsoil horizons (WRB) and were formed over a similar time span from volcanic rocks, including trachyte, olivine basalt and basalt. The climate is characterized by a bimodal rainfall regime with a long rainy season from March to May and a short rainy season between October and December (Appelhans et al. 2016). Mean annual precipitation (MAP) varies between about 750 and 3000 mm, dependent of elevation and exposition. Mean annual temperature (MAT) ranges from 5 °C to 25 °C and monthly means vary around ±3 °C.

Six research sites were selected representing natural forest and alpine ecosystems along the elevation gradient from 767 to 4190 m above sea level: Lowland dry broadleaf forest (RAU) dominated by *Milicia*

excelsa, Macaranga capensis, Oxystigmna msoo, Newtonia buchananii and *Albizia gummifera*, lower montane forest (FLM) dominated by *Macaranga kilimandscharica, Agauria salicifolia* and partly by *Ocotea usambarensis*, *Ocotea* forest (FOC) dominated by *Ocotea usambarensis* and *Cyathea manniana*, *Podocarpus* forest (FPO) dominated by *Podocarpus latifolius* with *Prunus africana* and *Hagenia abyssinica*, *Erica* bush (FER) dominated by *Erica trimera* and *Helichrysum* cushion (HEL) dominated by *Helichrysum newii, H. citrispinum* and *H. forskahlii* and tussock grasses (Ensslin et al. 2015). A detailed description of the ecosystems is available from Hemp (2006).

2.4.3.2 Soil sampling and analysis

Soil samples were taken in October 2014. At each site, four subplots (5x5 m) were selected. Five topsoil samples (0-10 cm depth) per subplot were taken randomly and pooled to reflect ecosystem heterogeneity. The samples were sieved (2 mm), and roots and plant materials were removed. Field samples were separated into two portions: one was dried at room temperature and the other was frozen (-20 ºC) until biomarker analysis. Soil carbon (C) and nitrogen (N) contents were measured using an elemental analyzer (Vario EL II, Germany). Soil pH was measured in water (soil to water ratio 1:5).

2.4.3.3 Extraction of PLFAs

Extraction of PLFAs from the soil samples was done according to Frostegard (1991). Briefly, lipids were extracted by one phase mixture of chloroform, methanol and citric acid (0.15 M, pH 4.0) (ratio 1:2:0.8 (v/v/v)). The 19:0 phospholipid (dinonadecanoylglycerol-phosphatidylcholine) was used as internal standard one and was added to the each soil sample prior to extraction (25 µL, 1 µg μL^{-1}) (Gunina et al., 2014).

The lipids were separated to neutral-, glyco- and phospholipids on the silica column, by eluting them from the column by chloroform (5 mL), acetone (20 mL) and methanol (20 mL), respectively. Phospholipid fraction was collected, saponified (0.3 M solution of BF_3 in methanol) and PLFAs were methylated (1 M solution of NaOH in methanol) and fatty acids methyl esters (FAMEs) were extracted to hexane. The FAMEs were dried under N_2 stream, and redissolved in toluene (185 µL) with addition of internal standard two (15 µL of 13:0 fatty acid methyl ester, 1 µg μL^{-1}).

The PLFAs were measured by GC-MS, with following parameters: a 15 m HP-1 methylpolysiloxane column connected to a 30 m HP-5 (5% Phenyl)-methylpolysiloxane column (i.d. 0.25 mm, film thickness of 0.25 µm), rate of the He flow was 2 ml min^{-1}, injection volume was 1 µL. The temperature program of GC-MS was set up to 80 ºC and then ramped to 164 ºC at 10 ºC min^{-1}, then to 230 ºC at 0.7 ºC min^{-1} and finally to 300 ºC at 10 ºC min^{-1}. Quantity of PLFAs was calculated based on the 29 external standards (Gunina et al., 2014), which were prepared at six concentrations (Apostel et al., 2014). Final content of PLFAs was presented as molar percentages (mol %). Classification of PLFAs was done

according to existing data on their presence in various microorganisms (Leckie, 2005; Lewandowski et al., 2015): for G- bacteria the 16:1ω7c, cy17:0, 18:1ω7c, 18:1ω9c, cy19:0 PLFAs were used, for G+ i15:0, a15:0, i16:0, i17:0 PLFAs were used, for actinomycetes (Ac) 10Me16:0 and 10Me18:0 were used, for fungi and arbuscular mycorrhiza fungi (AMF) 18:2ω6 and 16:1ω5c PLFAs were used, respectively.

2.4.3.4 Statistical analysis

The mol % of PLFAs were subjected to principal component analysis to reveal the major variation pattern. The scores of the first two components from the PCA were used to separate the soils formed at various elevations. Redundancy Analysis (RDA) was conducted to evaluate relation between PLFAs and environmental factors in all ecosystems. Explanatory (i.e. environmental) variables were preselected to prevent multicollinearity (variance inflation factor < 10). The RDA results were presented as correlation plot (type 2 scaling). The arrow projection on the 3rd and 4th axes equals the score of environmental variables on the respective RDA axis. Angles between arrows indicate strength of correlation. The coefficient of determination was corrected for the number of variables (adjusted R^2). Analyses were conducted in R v3.3.1 (R core team, 2008) using the ''vegan'' package for community data analysis (Okansen et al 2016). Variance partitioning by partial RDA (pRDA) was conducted to determine partial linear effects of each explanatory matrix in the RDA model (eq. 1) (environmental variables: MAT, MAP and soil parameters: N, C/N ratio, pH) on the response data (PLFAs).

$$PLFA = N + C/N + pH + MAP + MAT \qquad \text{(eq. 1)}$$

2.4.4 Results

2.4.4.1 Effect of elevation, temperature and precipitation on PLFAs.

The MAT decreased with elevation, whereas MAP peaked at 2100 m and decreased afterwards (Figure 2.4-1). The total PLFAs followed the trend of MAP distribution, with the maximum of 2100 nmol g^{-1} soil at mid elevation (2100 m) (Figure 2.4-1). The G- bacterial PLFAs followed a bell-shaped curve with elevation, whereas actinomycetes, fungi and AMF showed U-shaped curves. The content of G+ bacterial biomarkers decreased with elevation (Figure 2.4-2). Thus, microbial groups have a various behavior to elevation change, and, due to the domination of G- bacterial biomarkers in PLFAs composition (25-40%), this group determined the general PLFAs trend.

Total PLFAs content decreased with decreasing precipitation, whereas it had bell-shaped relationships with MAT (Figure Supplementary 2.4-9). Distinct microbial biomarkers were affected in three ways by MAP decrease: G- bacterial PLFAs decreased, fungal PLFAs increased and other groups had no significant trends (Figure 2.4-3). Most of the group specific PLFAs decreased with decreasing MAT, and only G- and fungal biomarker contents increased. Content of total PLFA decreased with decreasing precipitation, whereas it had bell-shaped relationships with MAT. Distinct microbial biomarkers were

affected in three ways by MAP decrease: G- bacterial PLFAs showed decrease, fungal PLFAs increased, and other groups showed no significant trends. Most of the biomarkers decreased with decreasing MAT, and only G- and fungal biomarker contents increased.

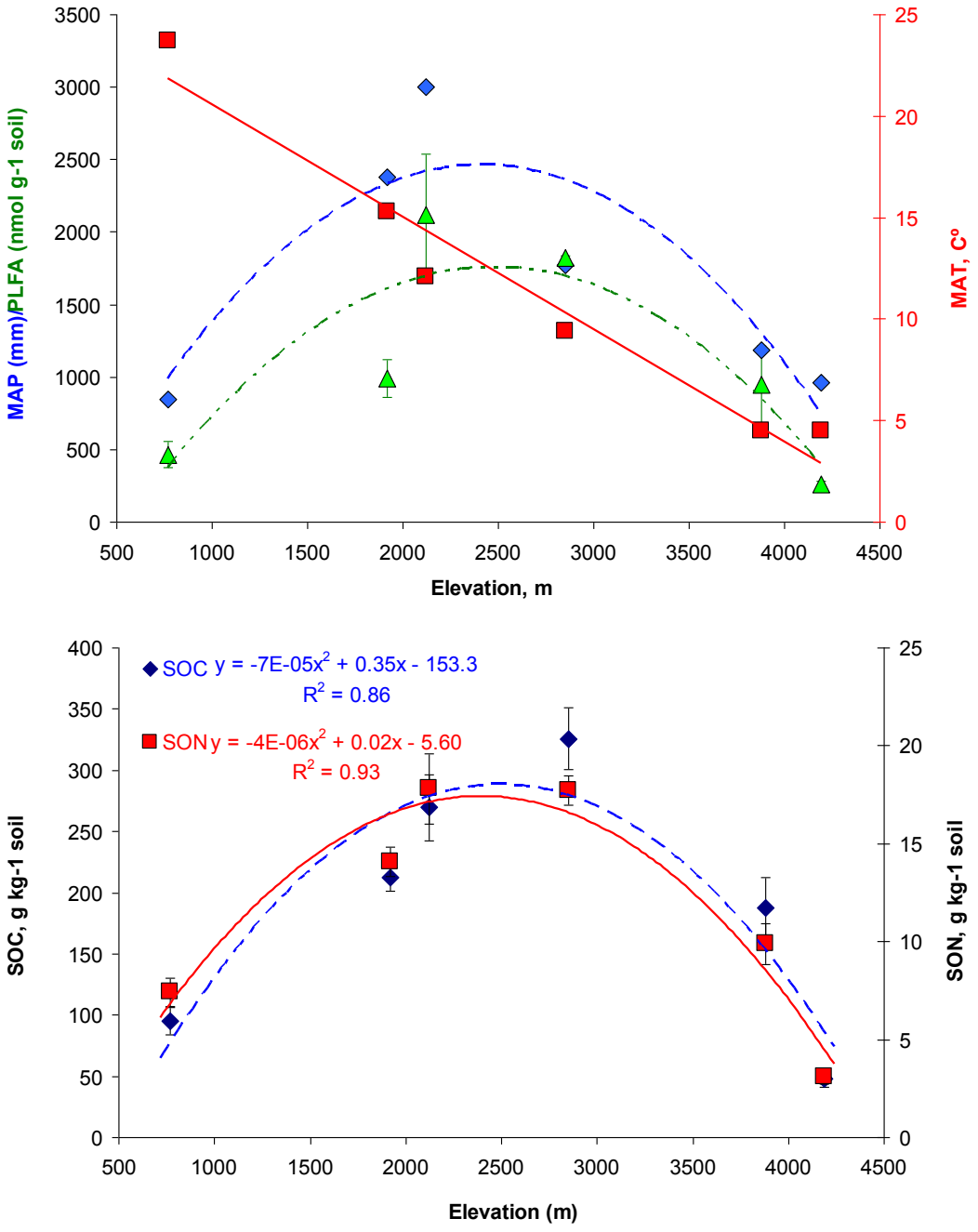

Figure 2.4-1: Mean annual temperature (MAT) and mean annual precipitation (MAP), soil organic C and N, and total PLFAs content along the 3500 m elevation gradient of Mt. Kilimanjaro.

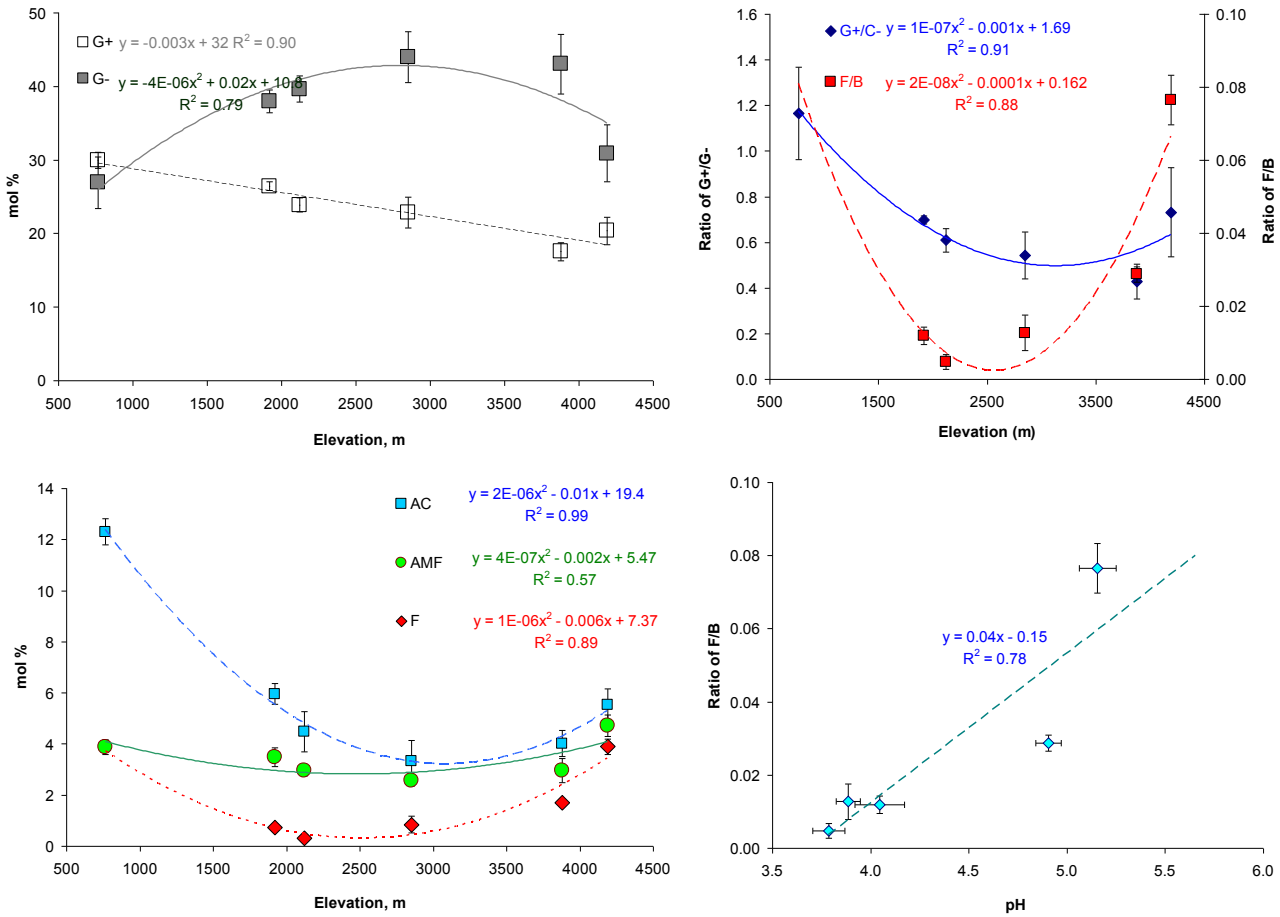

Figure 2.4-2: Specific microbial PLFA biomarker contents along a 3500 m elevation gradient at Mt. Kilimanjaro: Gram-positive (G+) and gram-negative (G-) bacteria, actinomycetes (Ac), putative arbuscular mycorrhizal fungi (AMC), and fungi (F). Data is presented as ecosystem means and standard error (n = 4).

2.4.4.2 Effect of soil properties and plant community on PLFA content and composition

Total C and N contents showed increase with elevation until 2800 m, and decreased at the highest elevations (Figure 2.4-1). Total PLFAs content increased with soil C and N contents (Figure Supplementary 2.4-11), showing quadratic (with C) and linear (with N) relationships. Increasing of soil C and N content increased G- biomarkers content, whereas other biomarkers decreased (VAM, F, Ac) or were unaffected (G+) (Figure 2.4-4). The increase of soil pH from 4.0 to 7.5 stimulated fungal and actinomycetes biomass, whereas G- bacterial PLFAs decreased, and G+ as well as VAM did not show consistent trends (Figure Supplementary 2.4-10).

The PCA explained 67 % of PLFAs variability. Investigated plots showed a distinct discrimination for microbial community composition (Figure 2.4-5): soils allocated below 3000 m were separated from those above, along the PC 1. The G- bacterial biomarkers (18:1ω7, 18:1ω9 and 16:1ω7) were responsible for separation of soils at low and high elevations. The PC2 separated ecosystems at the highest (HEL) and the lowest elevations (RAU, FLM) from the other sites. The RDA model was highly significant (p-value < 0.001) and explained 65% of the variance in the PLFA dataset. RDA axis one

(RDA1) and two (RDA2) explained the 79% and 16% of the within model variance, respectively. Soil N content was the main factor contributing to RDA1 (r = -0.78), while soil C/N ratio was the strongest related to RDA2 (r = -0.89). The C/N ratio was negatively correlated with MAT and soil pH. The MAP and soil N were positively correlated, but unrelated to C/N ratio and MAT. Variation in the soil parameters (pRDA) explained 19% of the total variance in PLFAs (Figure 2.4-6). Climatic variable (MAT, MAP) alone explained 2%. The interaction of soil parameters with climatic variable added another 44% of the explained variance.

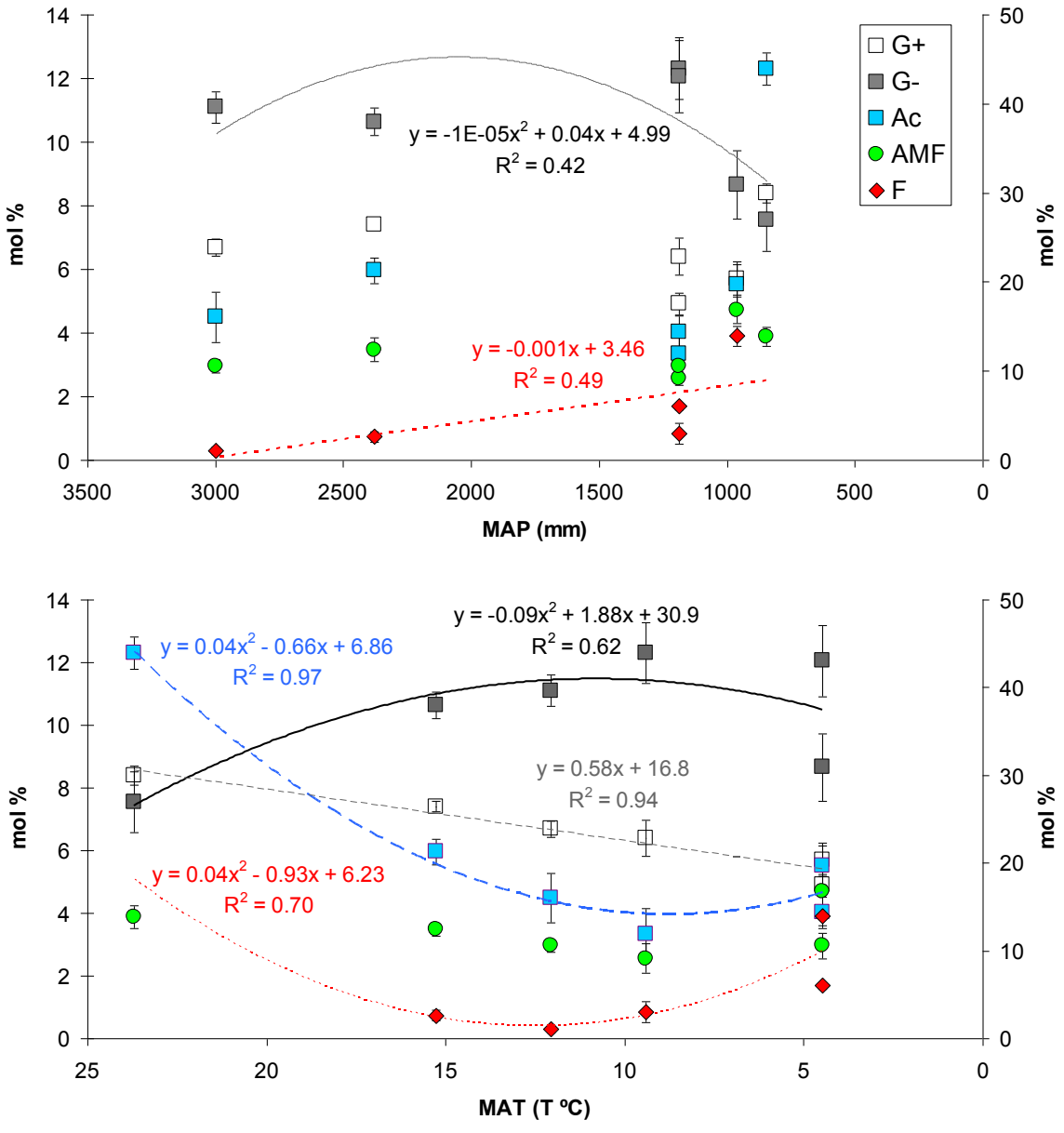

Figure 2.4-3: Microbial biomarkers contents with mean annual temperature (MAT) and mean annual precipitation (MAP) for the 3500 m elevation gradient for the Mt Kilimanjaro: Gram-positive (G+) and gram-negative (G-) bacteria, actinomycetes (Ac), putative arbuscular mycorrhizal fungi (AMC), and fungi (F).

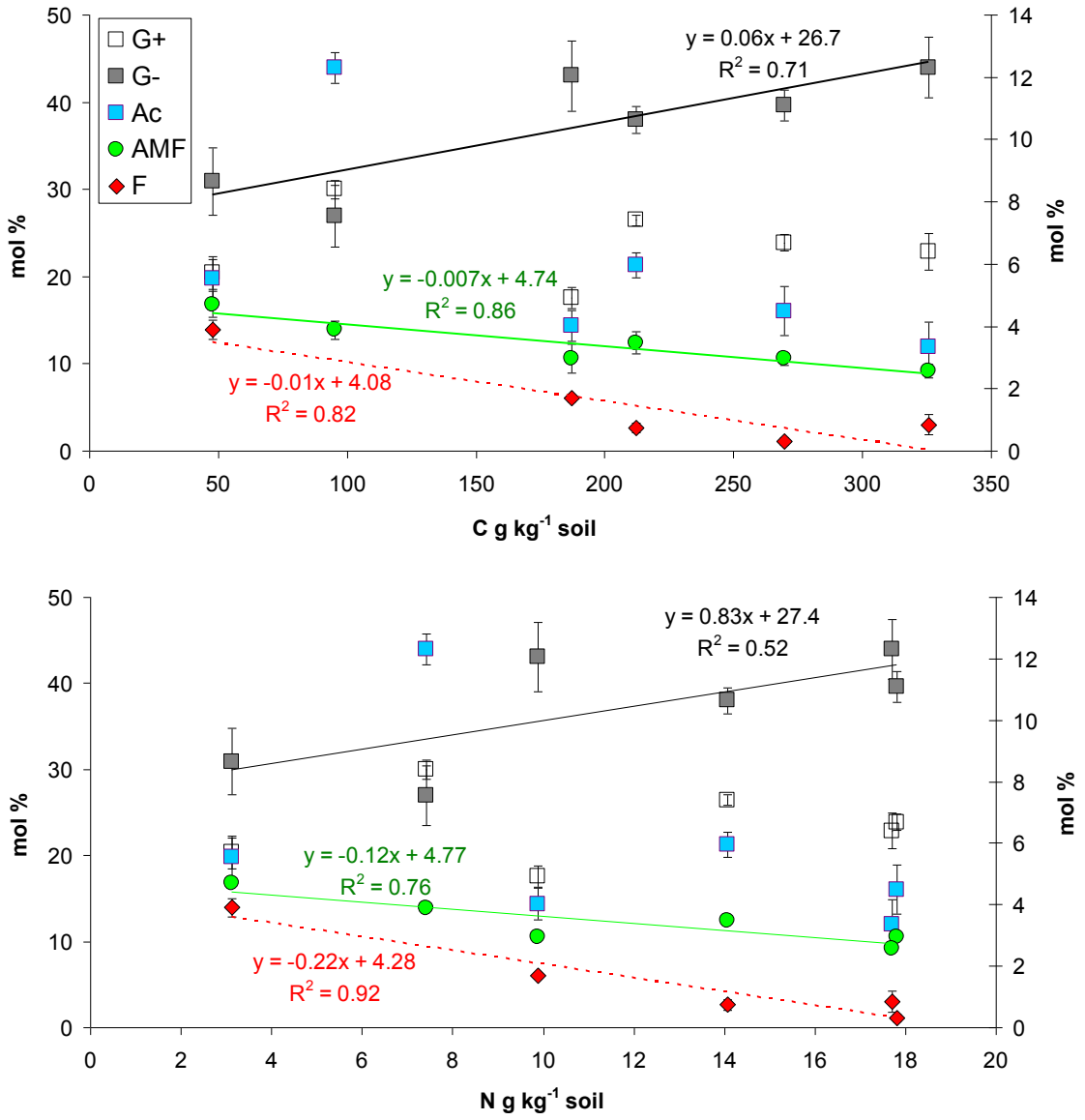

Figure 2.4-4: Microbial biomarker contents with soil C (top) and N (bottom) contents along a 3500 m elevation gradient of Mt Kilimanjaro. Gram-positive (G+) and gram-negative (G-) bacteria, actinomycetes (Ac), putative arbuscular mycorrhizal fungi (AMC), and fungi (F).

2.4.5 Discussion

2.4.5.1 Changes of soil properties with elevation

The distribution of soil chemical properties (C, N & pH) (Figure 2.4-1, Figure Supplementary 2.4-10) along the elevation gradient was well in line with previous findings from the experimental sites at Mt. Kilimanjaro (Ensslin et al., 2015; Becker et al., 2015; Pabst et al., 2013; Pabst et al., 2016). These changes reflect direct effects of climatic variables on mountain ecosystems. The bell-shaped distribution of soil C and N contents are the consequences of decreasing MAT with simultaneous increase of MAP. Both climatic variables affect net primary production, and consequently the amount of aboveground biomass and litter inputs (Ensslin et al., 2015, Becker et al., 2015), and thus, regulate the amount of C and N entering the soil (Becker et al. 2016). MAP strongly affected soil pH, which

followed the precipitation gradient and decreased with elevation due to the leaching of cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) from the soil profile by high rainfall (Hemp, 2006b).

2.4.5.2 Effect of elevation, temperature and precipitation on total PLFAs.

Total PLFAs content was ten times higher than reported earlier for mineral forest soils (Moore-Kucera and Dick, 2008; Myers et al., 2001; Murugan et al., 2014) and was within the range reported for organic soil horizons (Ushio et al., 2008; Baath et al., 1995). Recalculated data (PLFAs content per g of soil organic C) showed values between 3.8 - 7.5 µmol PLFAs g^{-1} C, which are higher than reported for other organic mountain soils (4 µmol PLFAs per g^{-1} C) (Djukic et al., 2010). Even at the highest elevations (3800-4200 m), the content of total PLFAs was higher (5 - 6 µmol PLFAs per g^{-1} C) than found for comparable sites (1.5 - 3.5 µmol PLFAs g^{-1} C) (Xu et al., 2014). These specific differences can be related to the low MAT in these studies (from -2.4 to +4 °C), than in our experimental sites (+9.4 to +4.5 °C).

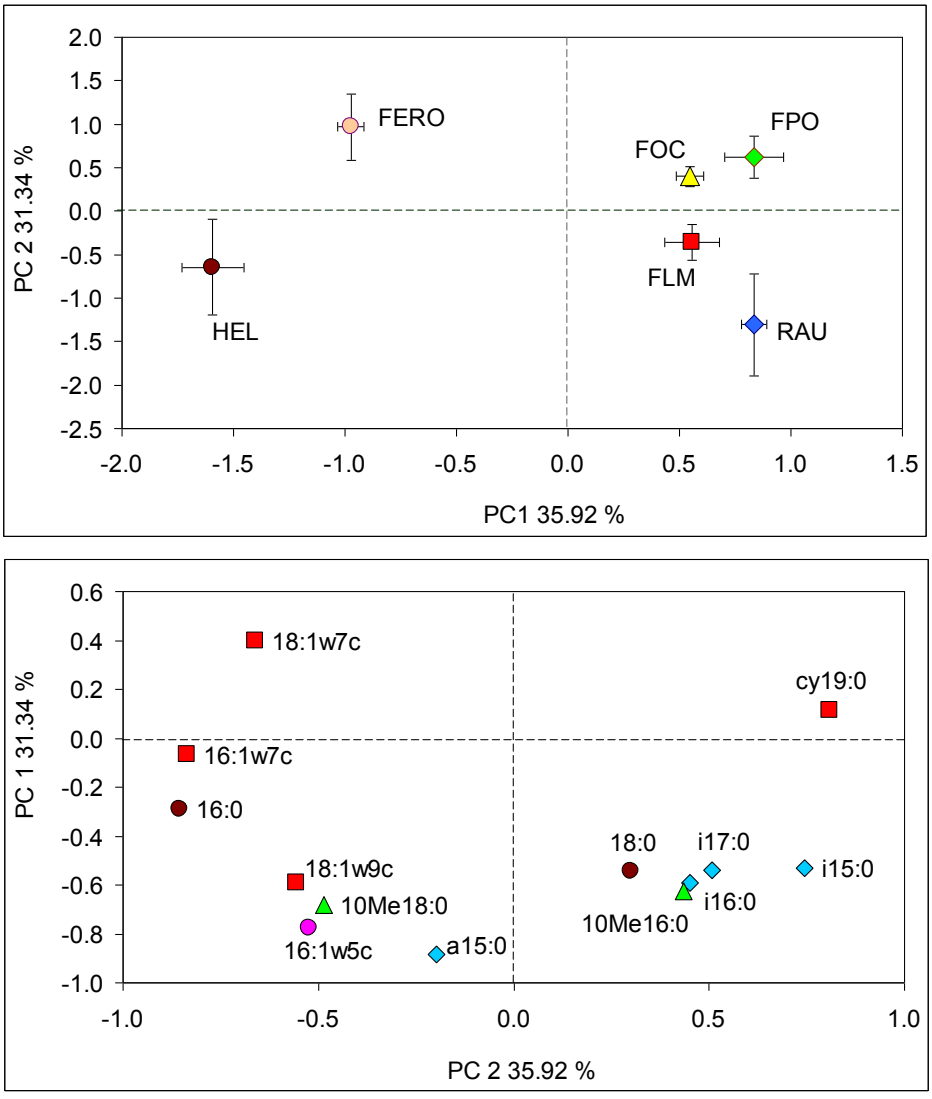

Figure 2.4-5: PCA score plot separating ecosystems on PC1 and PC2 (top) and loadings for the PLFAs (bottom). Lowland evergreen broadleaf forest (RAU), lower montane evergreen forest (FLM), montane evergreen *Ocotea* forest (FOC), upper montane evergreen *Podocarpus* forest (FPO), subalpine *Erica* forest (FER), alpine *Helichrysum* cushion vegetation (HEL).

A bell-shaped relationship between total PLFAs content and elevation was found with its maximum at 2100 m (Figure 2.4-1). Such pattern can a sequence of combination of optimal climatic conditions (MAP and MAT), as well as the highest plant productivity at this elevation. Similar results were found for a 540 - 2360 m elevation sequence in the northeast China mountain forests (Xu et al., 2014). This can be a result of developing the organisms with different ecological strategies, and thus, their similar contribution to the total biomass with elevation in various mountain ecosystems (Singh et al., 2012).

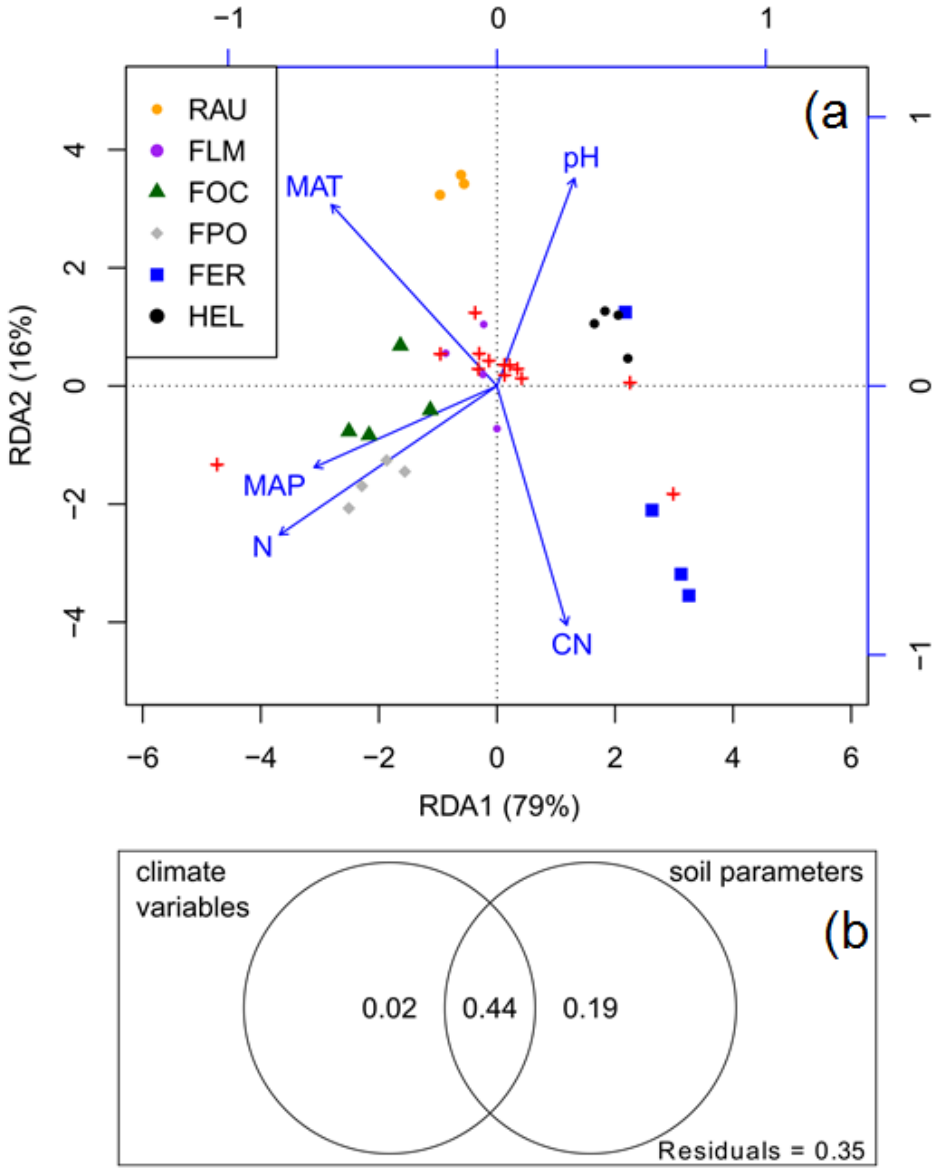

Figure 2.4-6: Type II scaled Redundancy Analysis (a) of the relation between PLFAs and environmental factors at six Mt. Kilimanjaro ecosystems. The arrow projection on the axis equals the score of environmental variables on the respective RDA axis. Angles between arrows indicate strength of correlation. Partial Redundancy Analysis (b) shows single and combined contribution of climate and environmental variables for explaining the model variance.

To prove the optimum for microbial biomass experimentally obtained in our study, we collected literature data on the effects of elevation on the total PLFA contents in mountain forests soils from various biogeographical regions (Figure 2.4-7). This meta-analysis showed that soils located at the same elevation, but in the different climatic zones, vary in total PLFA contents for 40 times (between 0.2 and 8 µmol PLFAs g^{-1} C): the maximum PLFA content was recorded for tropical savanna climate (present study) and minimum for the temperate monsoon and humid continental zones. However, the maximum PLFAs content was found at around 2000 m in all regions, which shows that a mid-elevation peak of PLFAs observed in present research can be taken as a general trend. Plotting the total PLFAs content against MAP revealed maximum PLFAs is common in soils located in the tropical savanna climate (Figure 2.4-7), and a minimum for temperate monsoon climate. Thus, not precipitation alone, but a combination of climatic variables drives the microbial biomass development in the mountain soils.

2.4.5.3 Effect of soil properties and plant communities on PLFAs composition.

PCA analysis distinctly separated high elevation ecosystems (3800 and 4200 m) from one located below 3000 m (Figure 2.4-4). Such separation can be explained by i) climatic factors, namely MAT, which was the lowest for the FER and HEL plots, ii) soil nutritional status - low C and N contents, and iii) low amount of above ground biomass (Ensslin et al 2015). The G- bacteria, arbuscular micorrhizal fungi (16:1w5) and actinomycetes (10Me18:0) contributed the most to separation of high altitude soils (3800-4200 m) from low altitude ecosystems. Contribution of G- bacteria increased from subalpine (1700 m) to alpine soils (2400 m), which is related to the tolerance of G- bacteria to freeze–thaw cycles (Margesin et al., 2008), common for FER and HEL plots, where freezing occurs on a daily basis (Hemp 2006, Gütlein et al 2016). The G- PLFAs peaked at the 3000 m elevation, which agrees with increase of bacterial richness at mid elevation reported by Singh et al. (2012) and shift in bacterial community composition from G+ to G- (Margesin et al., 2008). Decrease of G- bacteria at the highest elevation is a consequence of open vegetation cover within the *Helichrysum* ecosystem (4200 m), and, thus, the low amount of easily available root exudates (Gütlein et al., 2016), which are the preferred C source for this microbial group.

In contrast, G+ bacterial groups contributed the most to microbial communities at the low elevations (below 767 m), but their content decreased along the climosequence. A similar trend for G+ bacteria was reported for alpine soils (Margesin et al., 2008), and is related to low tolerance of this group to harsh weather conditions (i.e. low temperature and a daily freeze-thaw cycles common for HEL and partly for FER ecosystems) (Figure 2.4-3). The G+/G- ratio, characterizing starvation stress for microorganisms (Hammesfahr et al., 2008), decreased with elevation (Figure 2.4-2). The decrease of starvation stress is explained by an increase of SOC content, creating better condition for functioning

of G- bacteria compared to G+. In contrast, the highest stress found at RAU (760 m) ecosystem was due to low SOC content, which favors development of G+ bacteria.

Reported increase of fungi content with elevation (Figure 2.4-2) is connected with three reasons: 1) decrease of litter decomposability and increase of its C/N ratio, which facilitates fungal development, 2) general adaptation of fungi to low soil N supply, 3) and adaptation to harsh (dry and cold) environmental conditions, which include decrease MAT and MAP along the climosequence (Xu et al., 2014, Cheng et al., 2015, Zhang et al., 2013, Schinner and Gstraunthaler, 1981; Ma et al., 2015).

The RDA analysis (Figure 2.4-5) was consistent with the trends of distinct microbial biomarkers distribution and showed that climatic variables affected PLFAs composition in two opposite directions, with temperature being more important. Soil chemistry controls PLFAs composition in three directions: total N, C/N ratio and inversely to both soil acidity. The pRDA analysis showed that climatic factors indirectly affect the PLFAs through changes in vegetation and soil parameters.

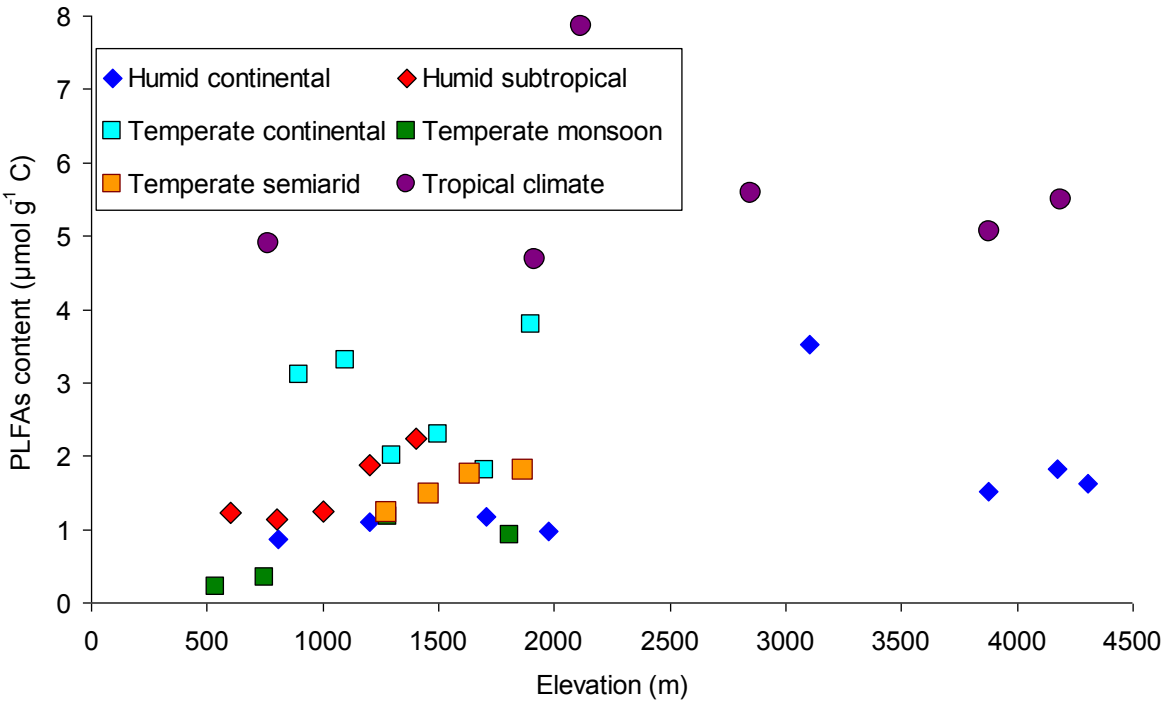

Figure 2.4-7: Literature derived total PLFA contents in forest soils along mountain elevation gradients in mountain ecosystems of various climatic zones.

2.4.6 Conclusions

Development of natural forests on similar parent material and along the elevation gradient allows investigating the effects of climatic variables on the formation of soil microbial communities. The study of soil microbial community structure in natural ecosystems of Mt. Kilimanjaro (from 770 until 4200 m), revealed a bell-shaped curve of total biomarkers (PLFAs) contents with elevation, with a maximum at 2100 m. Literature review has shown that both, MAP and MAT affect the PLFAs content not only in

the studied Mt. Kilimanjaro ecosystems, but in other mountain ecosystems as well, and total PLFAs content peaks at the mid-elevation (~2000 m) as a general trend in a broad range of ecosystems around the world.

Soil microbial communities at the highest elevation ecosystems (above 3000 m) were distinctly different from those at lower elevations (below 3000 m). Gram-negative bacteria dominated the microbial community in Mt. Kilimanjaro soils, accounting for 25-40%, and, thus, regulating the major trend of PLFAs distribution with elevation. With increasing elevation, gram-positive bacteria were replaced by fungi as a reaction to the harsh environmental conditions in the alpine zone above 4000 m (low MAT, and soil C and N contents). These variations were indirectly depending on climatic factors, and mainly explained by changes in vegetation composition and soil parameters. We conclude that the optimal conditions for microbial biomass in mountain soils are common at elevations between 1700 and 2700 m, mainly because optimal combination of climatic conditions for vegetation and soil development.

2.4.7 Acknowledgements

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2.4.8 References

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2.4.9 Supplementary figures

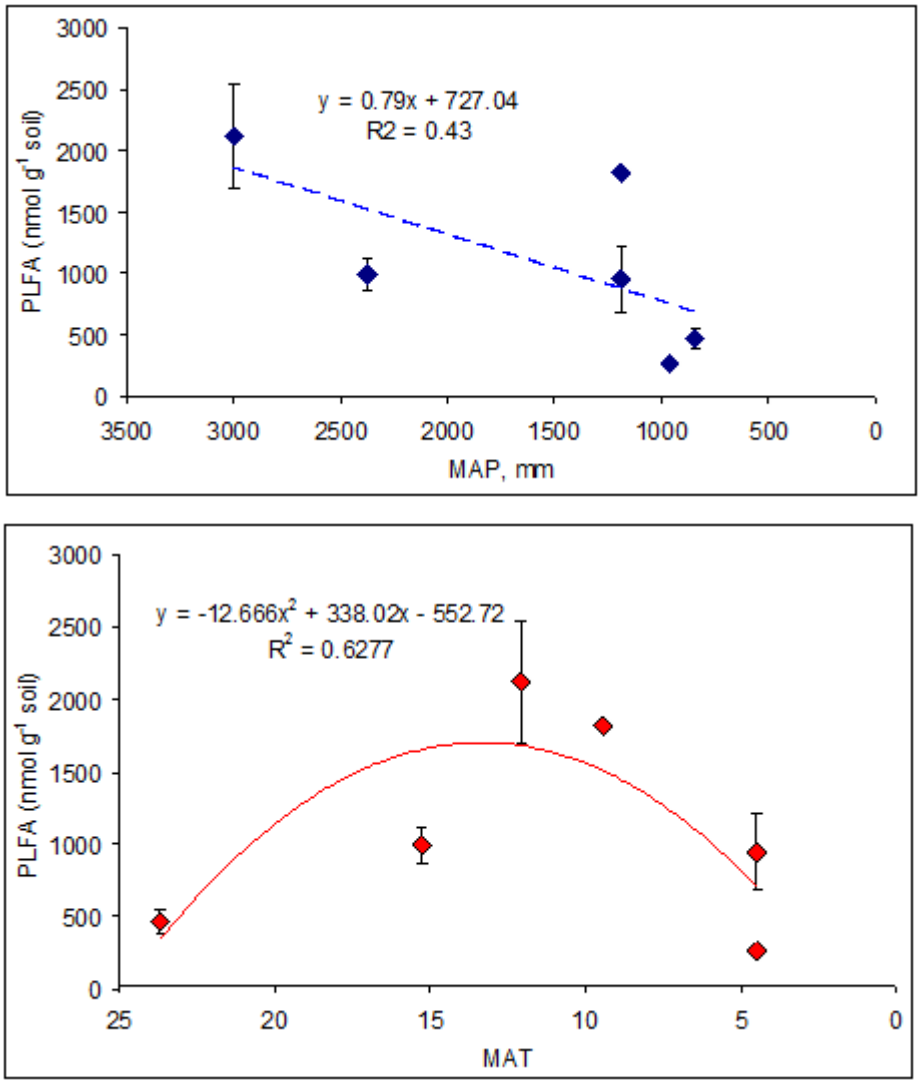

Figure Supplementary 2.4-8: Changes of microbial biomarkers content with mean annual precipitation (MAP) (top) and mean annual temperature (MAT) (bottom) for the 3500 m elevation gradient for the Mt Kilimanjaro.

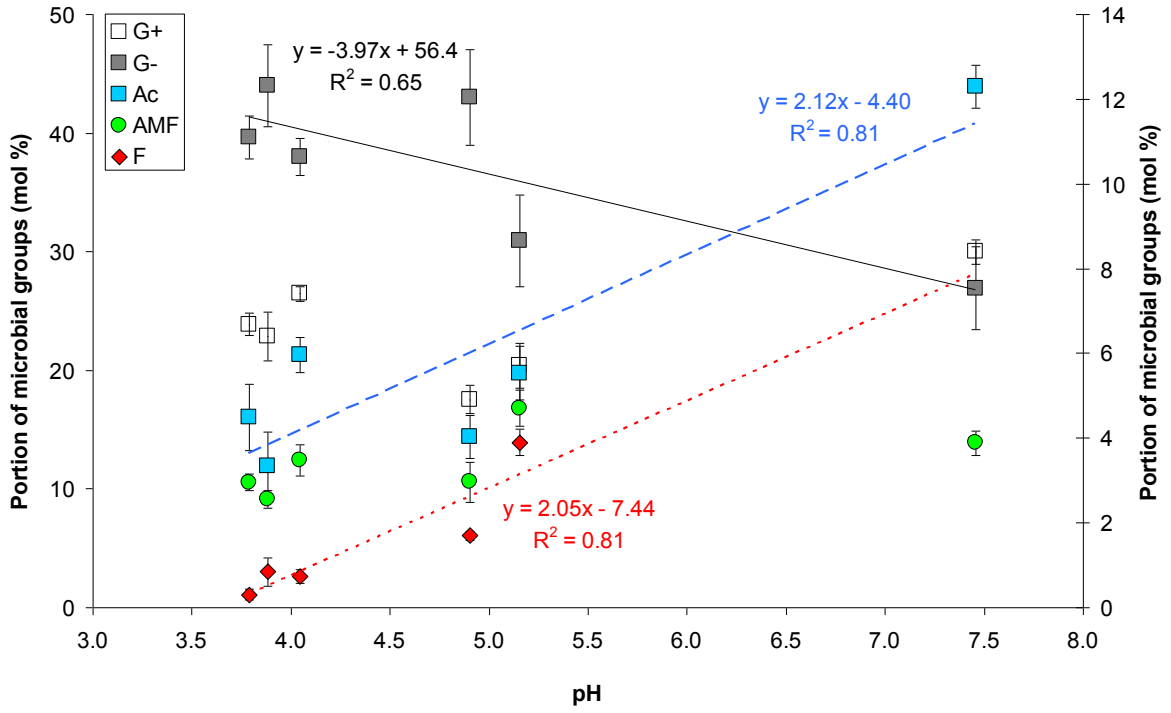

Figure Supplementary 2.4-9: Changes of microbial biomarker contents with soil pH along the 3500 m elevation gradient of Mt. Kilimanjaro.

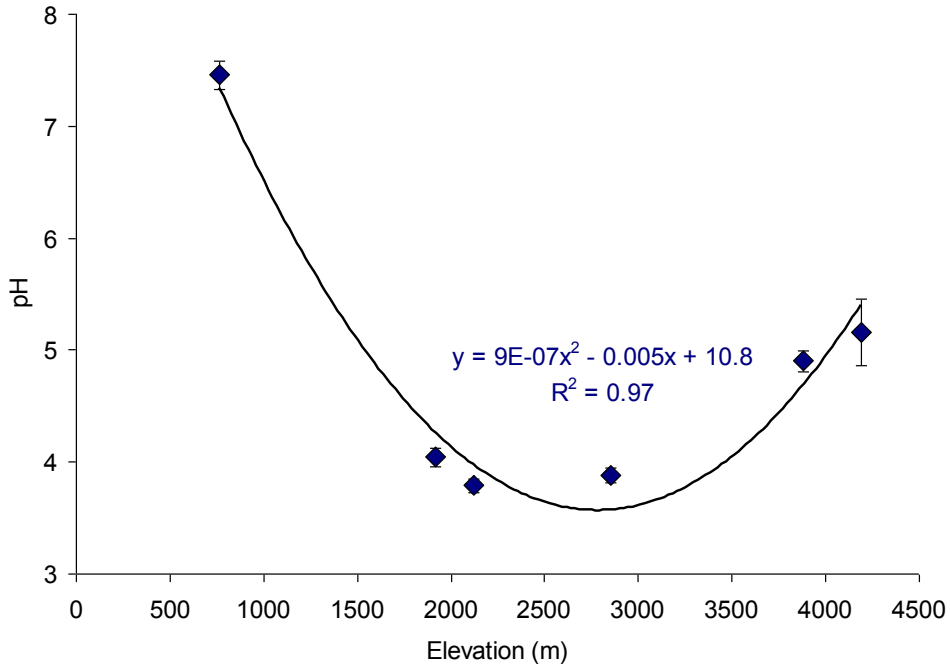

Figure Supplementary 2.4-10: Soil pH values along the 3500 m elevation gradient of Mt. Kilimanjaro

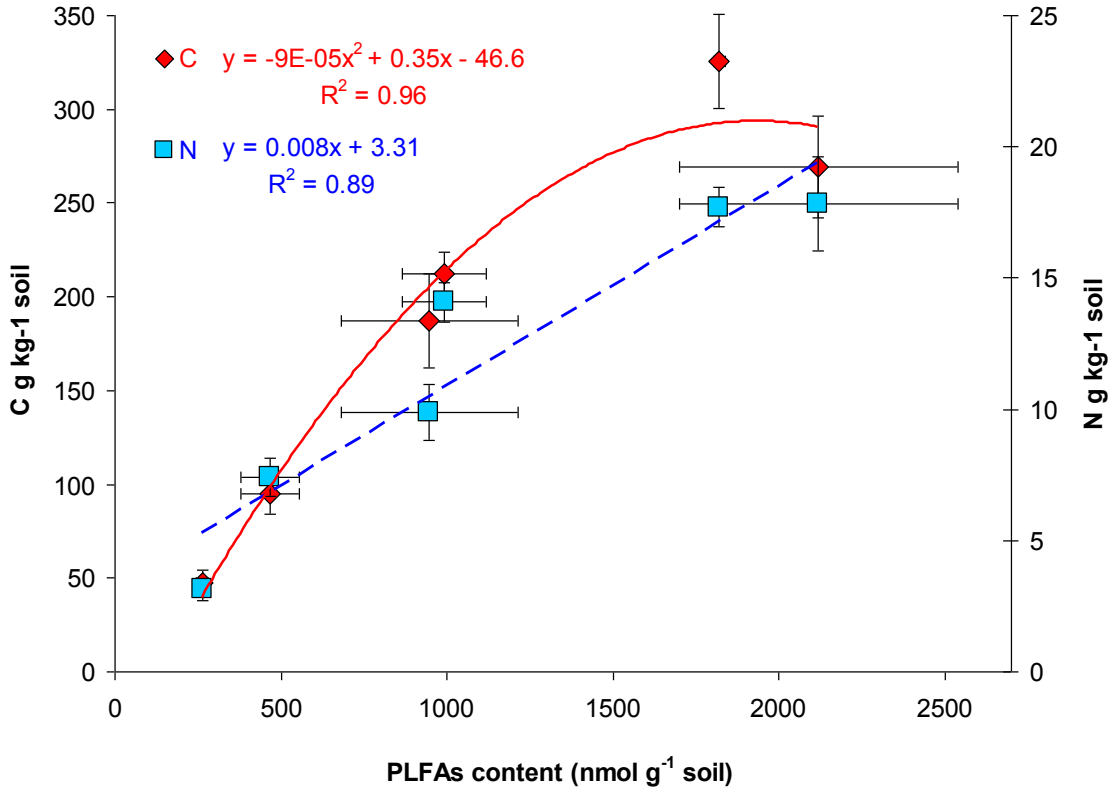

Figure Supplementary 2.4-11: Changes of microbial biomarkers content with soil C and N contents along the 3500 m elevation gradient of Mt Kilimanjaro

2.5 Study 5: Thermal and Structural Characterization of Soil Organic Matter Composition at Mount Kilimanjaro

Manuscript in preparation

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2.5.1 Abstract

Tropical mountain ecosystems cover a broad variety of climatic and vegetation zones and are a global hotspot of biodiversity. However, these ecosystems are severely threatened by climate and land-use change, which also strongly affect soil properties. Mt. Kilimanjaro with its associated large climate and land-use gradients provides a unique opportunity to observe and more fully interpret ecosystem responses to climate and land use change. Montane Andosols are of specific interest regarding carbon (C) sequestration and ecosystem services. They are characterized by thick litter layers and A-horizons that contain up to 20% organic C and are expected to promote soil carbon stabilization and storage.

Our objectives are to identify key soil organic matter compounds that are affected by the different climatic conditions along a ~3000 m elevation gradient and how changes in SOM composition is related to ecosystem specific characteristics. Furthermore, we aim to estimate quantitative changes in the specific C fractions and relate these changes to C turnover processes in various ecosystems. Therefore, topsoil samples were thermally decomposed using evolving gas analysis mass spectrometry (EGA-MS) and analytical double-shot pyrolysis gas chromatography mass spectrometry (Py/GC-MS). EGA curves were used to assess quantitatively the results of Py/GC-MS.

Thermal desorption chromatograms show a relative increase of volatile C compounds in higher mountain forests followed by a decrease in alpine ecosystems. More stable fractions were affected contrarily which is closely related to the overall ecosystem productivity. Cloud forest types possess a similar organic matter composition with higher percentage of stabile n-alkyl lipids and isoprenoid derivates. Polysaccharides and lignin derivates have their maxima at mid elevations due to decreasing inputs with elevation as well as slow decomposition at high elevations.

Soil organic matter composition in Mt. Kilimanjaro forests is strongly dependent on a precipitation and temperature equilibrium. Hence, high productivity at mid-elevation levels leads to increased amounts of volatile compounds but at the same time increases stabile carbon pools.

Keywords: Py GC-MS, EGA, tropical mountain forest, East Africa, Carbon Cycle

2.5.2 Introduction

Soils are the largest terrestrial carbon (C) reservoir and account for more than 2500 GT C of which more than 60% is part of soil organic matter (SOM) (Lal 2008). The amount of organic C that is stored in soil depends on the interaction of climate variables, soil mineralogy, input from vegetation and decomposer organisms (Vitousek, Sanford 1986; Doetterl et al. 2015; Blagodatskaya et al. 2014). Understanding the functioning of this pool is of major importance for understanding the global C cycle and its response to climate and land-use change (Lal 2004; Lehmann, Kleber 2015). Composition and quality of SOM are strongly related to the input, the stability and the turnover of C in soil (Allison, Vitousek 2004; Ng et al. 2014; Chen et al. 2014). While a lot is known about quantitative effects on soil C, the variation of SOM chemistry across ecosystem scales and its relation to climate, vegetation and abiotic factors remains poorly understood (Vancampenhout et al. 2010). Recent studies have shown that SOM chemistry is strongly varying on ecosystem scale (Vancampenhout et al. 2009; Plante et al. 2011; Yassir, Buurman 2012) and can easily change with vegetation and climatic boundary conditions (Andersen, White 2006; Stewart et al. 2011; Carr et al. 2013; Amelung et al. 1997). These efforts were a huge step in understanding ecosystem specific conditions and mechanisms of soil C sequestration and turnover dynamics. However, they also indicated that previous results cannot be easily applied to other regions and local fingerprints are necessary for global estimations of soil C dynamics (Schmidt et al. 2011).

Tropical mountain ecosystems are characterized by a large variety of climatic and biogeographic zones and are global hotspots of biodiversity (Myers et al. 2000). With their high belowground C sequestration potential (Wilcke et al. 2008), these ecosystems are of major importance concerning effects of global change on soil properties. Mt. Kilimanjaro with its associated large elevation (i.e. climatic) gradient provides a unique opportunity to observe and more fully interpret ecosystem responses to climate change, specifically regarding soil C balances. The major soil types in the forests of Kilimanjaro's southern slope are Andosols (Zech 2006). These soils are characterized by pronounced organic layers and thick A-horizons that contain up to 20% organic C. Andosols particularly promote soil C stabilization and storage through the formation of stabile organo-mineral complexes with aluminosilicates such as allophanes and imogolites (Aran et al. 2001). Soil minerals are selective regarding complexation with organic compounds (Adhikari, Yang 2015) and thus can change overall SOM composition in Andosols (Buurman et al. 2007; González-Pérez et al. 2007). It is yet unclear how these processes are affected by climatic conditions and how SOM composition in general changes along large climatic gradients.

Our objectives were to identify key SOM compounds that are affected by the different climatic conditions along a ~3500 m elevation gradient of Mt. Kilimanjaro. Further, to estimate quantitative

changes in the specific C fractions and relate these changes to ecosystem turnover processes and C cycle. Therefore, topsoil samples were thermally decomposed by analytical pyrolysis gas chromatography mass spectrometry (Py/GC-MS). This is a powerful tool to identify organic fractions and their relative contribution to SOM (Saiz-Jimenez, Leeuw 1986). By using evolving gas analysis mass spectrometry (EGA-MS) curves, this can be extended to quantitative assessment and inferences about SOM stability (Plante et al. 2009). EGA curves can also be used as indicators of humification status and stability of SOM (Katsumi et al. 2016).

We hypothesize that (1) stabile C pool increase at mid elevation, (2) which is related to an accumulation of aromatic compounds and (3) ecosystem specific characteristics in alpine environments alter SOM composition.

2.5.3 Methods

2.3.1 Study site

The study was conducted on the southern slope of Mt. Kilimanjaro (3°4’33”S, 37°21’12”E), Tanzania. Along an elevation gradient from 770 to 4200 m a.s.l., six research sites were selected, each representing a typical natural forest or alpine ecosystem of the region (Table 2.5-1). The lowland broadleaf forest (RAU) is part of the Rau Forest Reserve, near Moshi town (770 m). Important species in its upper tree layer are *Milicia excelsa, Khaya anthotheca, Oxystigmna msoo, Newtonia buchananii* and *Albizia gummifera*. *Trilepisium madagascariense, Tabernaemontana elegans, Blighia unijugata, Lecaniodiscus fraxinifolius* and *Trichilia emetica* build up a second tree layer. In the dense shrub layer *Allophylus pervillei, Blighia unijugata, Rothmannia urcelliformis, Turraea holstii, Vernonia amygdalina* and *Acalypha ornata* dominate. Lower montane forest (FLM), middle montane *Ocotea* forest (FOC), upper montane *Podocarpus* forest (FPO), subalpine *Erica* forests (FER) and alpine *Helichrysum* cushion vegetation (HEL) are located in Kilimanjaro National Park. According to fog-water input and structure (e.g. richness in epiphytes) the forests of the middle and upper montane and subalpine zone, in particular on the southern slope, can be defined as “cloud forests” (Hemp 2010). Hemp (2006; 2008) offers a detailed description and classification of these ecosystems. Summarily, FLM is dominated by *Macaranga kilimandscharica*, *Syzygium guineense, Agauria salicifolia* and partly *Ocotea usambarensis*. At higher elevation (FOC) *Ocotea usambarensis* prevails, accompanied by *Xymalos monospora, Ilex mitis* and *Cyathea manniana*. The forest above 2800 m a.s.l. is dominated by *Podocarpus latifolius together with Prunus africana* and *Hagenia abyssinica* (FPO). In the subalpine zone at around 4000 m (FER), *Erica trimera* is dominating and can reach up to 10 m growth height. Between 4000 and 4500 m (HEL), the alpine forest is displaced by *Helichrysum* cushion vegetation with a herb layer of about 30% dominated by *Helichrysum newii*, *H. citrispinum* and *H. forskahlii* and grasses. Logging for firewood and building material occurs, especially in RAU and the lower FLM areas (Lambrechts et al. 2002).

The climate at Mt. Kilimanjaro follows a bimodal rainfall regime with long rains from March to May and a shorter rainy season between October and December (Appelhans et al. 2016). Mean annual precipitation (MAP) varies between 845 mm and about 3000 mm, dependent of elevation and exposition. Mean annual temperature (MAT) ranges from 4.5 °C to 23.7 °C and monthly means vary around ±3 °C.

Soils in the southern forest zone were classified as Andosols with folic, histic or umbric topsoil horizons and accordingly high C contents in the upper horizons (Zech 2006). In the alpine zone, Leptosols and vitric Andosols are prevalent (WRB 2014). Soils have developed from volcanic rocks, such as basalt, trachyte and olivine basalts over the last 0.2 to 2.3 Mio years (Dawson 1992). The similar parent material throughout the elevation gradient makes the comparison of ecosystems on Mt. Kilimanjaro especially beneficial, because soil conditions are mainly a function of local ecosystem characteristics.

Table 2.5-1: Site specific topographic and climatic information as well as C and N contents in 0-10 cm soil depth for six ecosystems on the southern slope of Mt. Kilimanjaro

Ecosystem	ID	Elevation [m]	MAT [ºC]	MAP [mm]	C [%]	N [g kg^{-1}]	pH
Lowland forest	RAU	767	23.7	845	9.5±1.1	7.4±0.1	7.5±0.1
Lower montane forest	FLM	1920	15.3	2378	21.2±1.1	14.1±0.1	4.0±0.1
Ocotea forest	FOC	2120	12.1	2998	27.0±2.7	17.8±0.2	3.8±0.1
Podocarpus forest	FPO	2850	9.4	1773	32.6±2.5	18.0±0.1	3.9±0.1
Erica forest	FER	3880	4.5	1188	18.7±2.5	10.0±0.1	4.9±0.1
Helichrysum cushion	HEL	4190	4.5	962	4.8±0.7	3.1±0.0	5.2±0.0

2.3.2 Sampling and laboratory analyses

Soil samples were taken in March 2014. Four subplots (5x5 m) were selected at each corner of each plot. At each subplot, five topsoil samples (0-10 cm depth) were taken with a soil probe and pooled to reflect ecosystem heterogeneity. The samples were sieved (2 mm), and roots and plant materials were removed. Field samples were dried at 105 °C for 46 hours and ground for further analysis.

Soil samples were taken in March 2014. Four subplots (5x5 m) were selected at each corner of each plot. At each subplot, five topsoil samples (0-10 cm depth) were taken with a soil probe and pooled to reflect ecosystem heterogeneity. The samples were sieved (2 mm), and roots and plant materials were removed. Field samples were dried at 105 °C for 46 hours and ground for further analysis.

Total C and N content was measured using a dry combustion automated C:N analyzer (Vario EL cube, Elementar). Evolved gas analysis (EGA-MS) was conducted using a multi-Shot Pyrolyzer (EGA/PY-3030D, Frontier Lab, Koriyama, Fukushima, Japan) coupled to a GC (7890A, Agilent, Santa Clara, CA, USA) and MS detector (7000C Triple Quadrupole, Agilent, Santa Clara, CA, USA). The sample was heated constantly in a micro furnace from 100 to 600°C. The evolved gases flow to the detector without chromatographic separation (EGA tube, L = 2.5 m, I.D. = 0.15 mm, Frontier Lab). The same Instrumental setup as for EGA-MS was used for pyrolysis-gas chromatography mass spectrometry

(Py/GC-MS), exchanging the column for a high temperature Ultra ALLOY® Metal Capillary Separation Column (L=30 m, I.D. = 0.25mm, Frontier Lab). Double-Shot analysis was performed to increased resolution in MS spectra by separating the release of chemically sorbed compounds (thermal desorption: 100-280 °C) and cracking of covalent bounds (pyrolysis: 280-600 °C) (Derenne, Quénéa 2015). MassHunter Workstation Software (V. B.06.00, Agilent Inc, 2012) was used to identify peaks (> 0.5% of relative maximum peak height) with manual adjustment and identify compounds using software NIST08 library and pyrolysis-GC/MS literature. Compounds were subsumed in twelve classes according to chemical, genetical and analytical similarities: Alkanes/-enes/-ols, alkyle aromatics, fatty acids and fatty acid esters, lignin monomers, phenols, sterols, terpenes and isoprenoids, polyaromatics, polysaccharides, amino N, heterocyclic N.

2.3.3 Calculations and statistical analyses

EGA curves were normalized and compared between ecosystems using 95% confidence intervals. Previous research indicated that relationships between different ecological variables, e.g. total biomass and elevation at Mt. Kilimanjaro follow a unimodal trend (Becker et al. 2015, Pabst et al. 2016, Ensslin et al. 2015). Therefore, compound percentage along the elevation gradient was evaluated by second order polynomial regression. Multivariate statistics were used to evaluate relationships between chemical SOM composition and forest types: Principal components analysis (PCA) was visualized via type I scaling biplots.

All statistical analyses were conducted in R 3.3.2 (R Core Team 2016) using Bolstad2 and Agricolae packages (Mendiburu 2014; Curran 2013) as well as ggplot2 package for data visualization (Wickham 2009).

2.5.4 Results

2.5.4.1 EGA-MS analysis

All EGA curves show a distinct peak around 460°C and two less expressed peaks around 300°C and 370°C (Figure 2.5-1). Within plot variation is low and signals were very ecosystem specific (Table Supplementary 2.5-2). The lowland tropical forest (RAU) has a neglectable percentage of volatile SOM. The percentage of volatile compounds (i.e. thermally desorbed fraction) varied between 0.5 and 5.5%. It increases with elevation until crossing the tree line at HEL, where it decreases again. Ecosystems at higher elevation show an early peak in the low temperature zone and subalpine *Erica* forest (FER) starts to loose volatile compounds already below 280 °C.

2.5.4.2 Multivariate analysis of SOM chemistry

SOM composition in the pyrolyzed fraction separates well on the PC1 axis (61%) for ecosystems along the elevation gradient (lowland<montane>alpine) (Figure 2.5-2). This is strongly correlated to

Alkanes/-enes/-ols (positive) and heterocyclic N compound percentage (negatively). PC2 (16%) explains more within plot variation in the lower elevation and separates the sub-alpine from alpine ecosystem, mainly through lignin (positive), Polyaromatics and aromatics percentage (negatively). Around 75% variance of the thermal desorbed SOM fractions was explained by PC1 (48%) and PC2 (27%) (Figure 2.5-2). PC1 again, was positively related to Alkanes/-enes/-ols percentage. PC2 was strongly related to sterols (positive) and polysaccharides (negative).

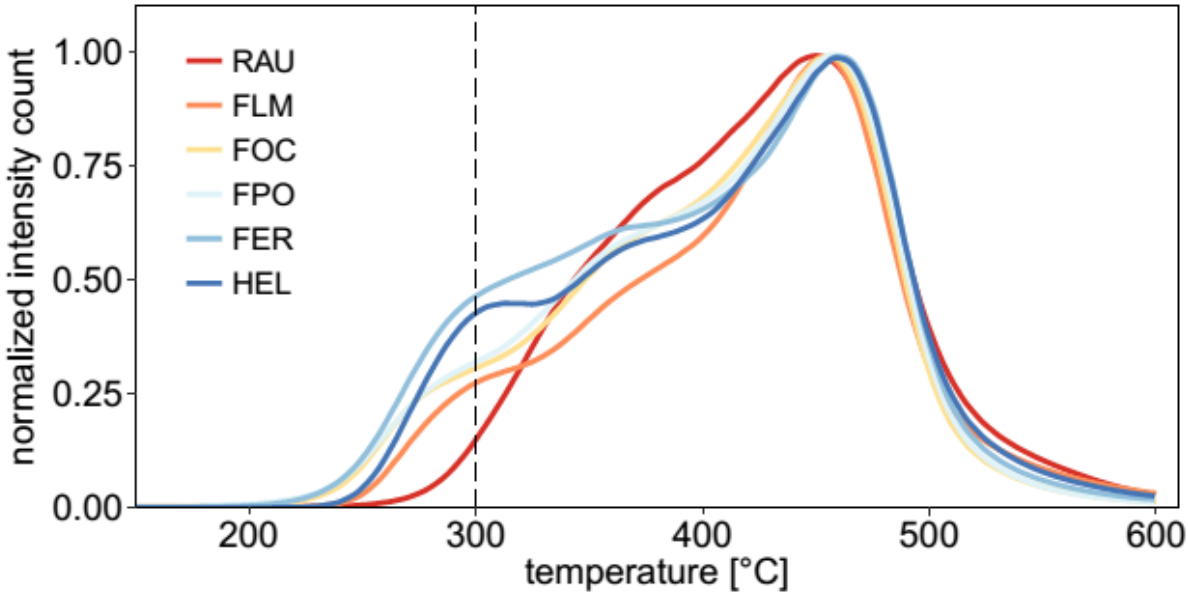

Figure 2.5-1: Average EGA curves for soil samples from six Kilimanjaro ecosystems (n=4). Dashed line sepparates temperature zones for thermal desorption (100-300 °C) and pyrolysis (300-600 °C).

2.5.4.3 Effect of elevation on thermally desorpable compounds

No compounds were detectable in the thermal desorption step at low elevation (RAU, 750m). Along the remaining elevation from 1920 to 4120 m, most compounds fractions followed an increase-decrease trend or reverse (Figure 2.5-3).

Alkanes/-enes/-ols strongly increased to a maximum of nearly 60% at FPO (2900 m) and decreased to around 15% in the (sub-) alpine ecosystems (FER and HEL). Aromatic compounds followed no clear trend with elevation, but reached a maximum at FLM (1920 m). Fatty acids and fatty acid esters expressed a small peak at FOC and, fatty acids in particular, a second peak at high elevation – with up to 25% contribution of FAs at HEL (4120 m). Lignin monomers contributed between 5 and 12% to volatile SOM composition. This percentage decreased with elevation until a sudden increase in the alpine *Helichrysum* area (4120 m). Phenols were not present at higher elevation and strongly increased only at FLM. Sterols decreased with elevation, except for a strong peak at the *Erica* forest (3880m) with nearly 10 times increased values. Terpenes and isoprenoids followed no clear trend with elevation, but were slightly enriched in FLM and FER. Polysaccharides varied between 3 and 8% in all ecosystems,

except for a strong peak in alpine *Helichrysum*. Both N compound fractions followed a similar trend with elevation. Maxima were reached at FLM (1920 m) followed by a decrease with higher elevation.

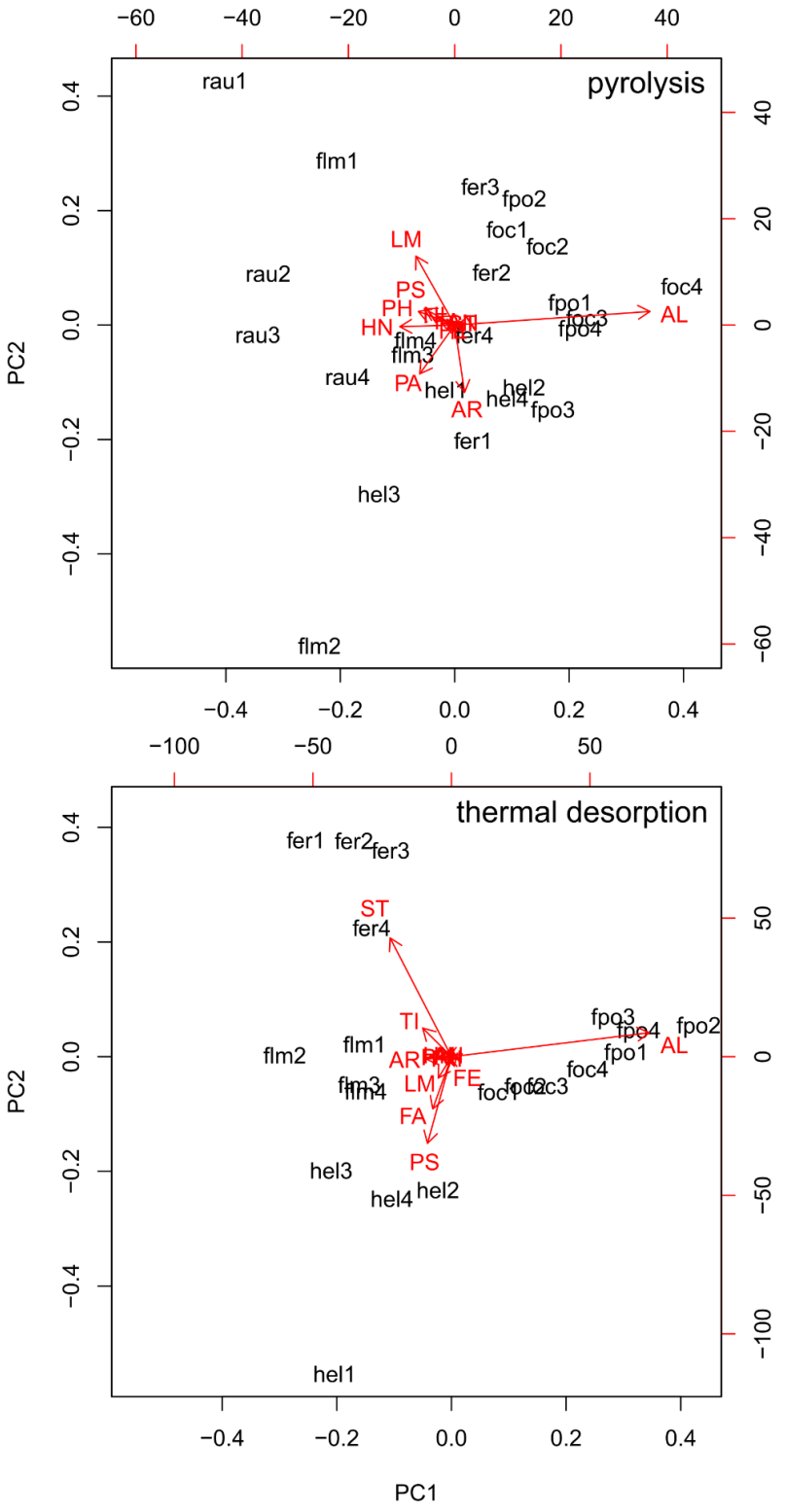

Figure 2.5-2: PCA biplot (Type I scaling) for pyrolyzed components and thermally decomposed components in six ecosystems (n = 4) of Mt. Kilimanjaro. Compounds were abbreviated as: AL – Alkanes/-enes/-ols, AR – Alkyle aromatics, FA – Fatty acids, FE – Fatty acid esters, LM – Lignin monomers, PH – Phenols, ST – Sterols, TI – Terpenes and Isoprenoids, PA – Poly aromatics, PS – Polysaccharides, AN – aminoacids, HN – heterocyclic N.

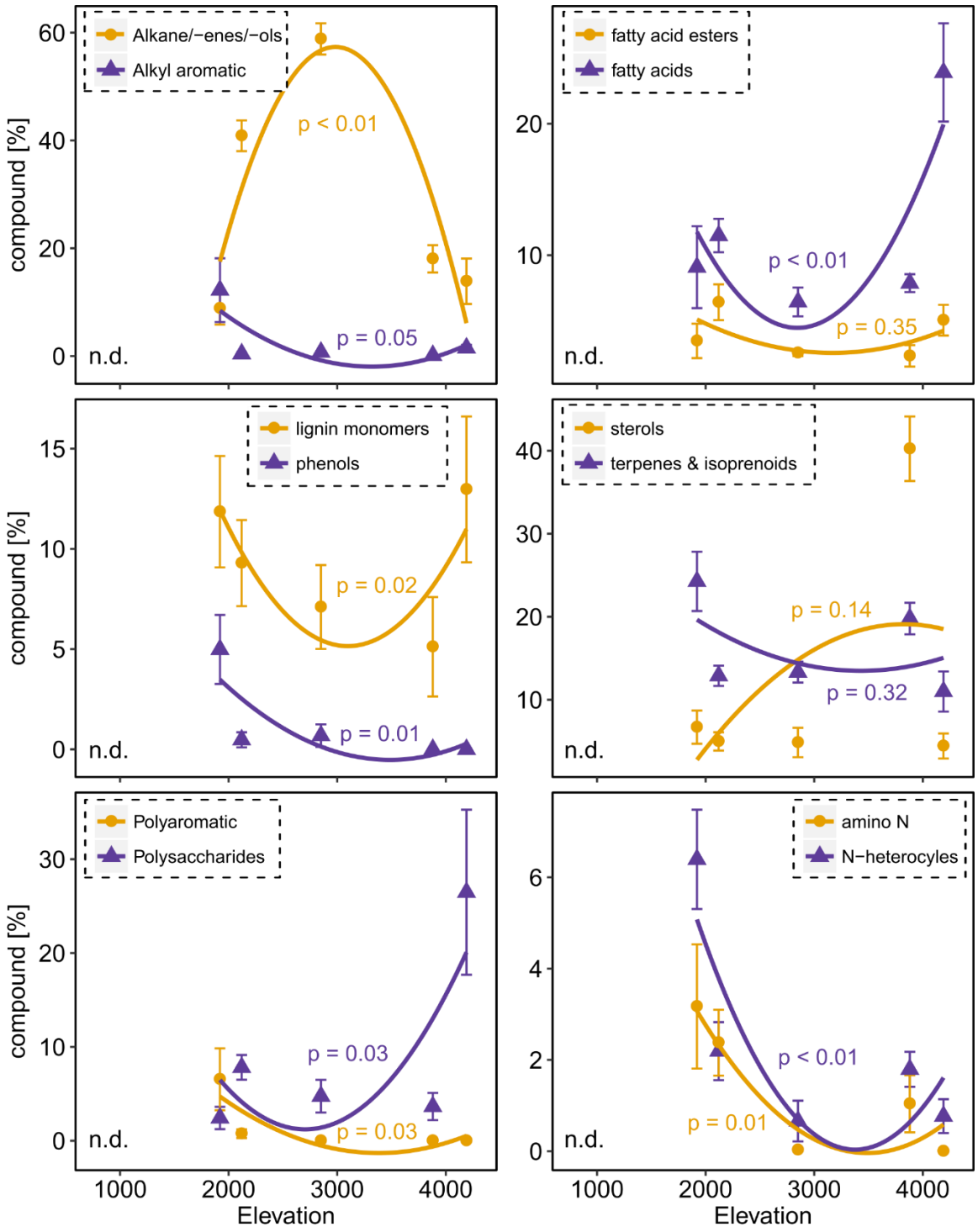

Figure 2.5-3: Percentage of thermally desorped compounds from soil organic matter in six ecosystems along the elevation gradient of Mt. Kilimanjaro. Small letters (a-c) indicate significant difference between ecosystems (*p < 0.05*) according to *Kruskal-Wallis* test with *Benjamini-Hochberg* correction for multiple comparisons

2.5.4.4 Effect of elevation and ecosystems

Similar to thermal desorption, most compounds in the pyrolysed fraction followed a parabolic trend with elevation and increased or decreased at mid elevation (Figure 2.5-4). Percentage of alkanes/-enes/-ols varied between 4% and 30%, with a maximum at mid elevation (2120 m). Alkyl aromatic compounds were slightly above 20% on all plots and showed no trend with elevation. Fatty acids and fatty acid esters contributed with less than 3% to SOM composition on all plots. Both decreased to a minimum at *Podocarpus* forest (2900 m), followed by an increase at higher elevation. Polycyclic aromatic compounds had no clear trend with elevation and contributed with around 15% to SOM composition. Maxima and minima were found at directly adjacent ecosystems: 18 % at FLM and 11% FOC. Lignin monomers generally had a U-shaped trend with elevation; however, a sudden decrease appeared above the tree line in the alpine zone (HEL). There were nearly no sterols found at Rau forest (750 m). However, same as isoprenoids, contents increased with elevation to their maxima at *Ocotea* Forest (2120 m) and afterwards decreased again. Polysaccharides linearly decreased with elevation, from around 10% in Rau forest to less than 4% at *Helichrysum*. Amino N compounds were highest at mid-elevation (FLM and FOC). Opposed to this, N-heterocycle percentage was highest at low elevation (RAU) and decreased in the cloud forests (FOC and FPO, followed by an increase in the (sub-) alpine zone (FER and HEL).

2.5.5 Discussion

2.5.5.1 SOM resistance to pyrolytic degradation

Pyrolysis fractions (>280°C) quantitatively dominated the SOM composition (Figure 2.5-1). The contribution of volatile compounds in SOM increases with elevation (Table Supplementary 2.5-2), indicating an increase of easily available SOM components. While the thermally volatile fraction is nearly absent in lowland RAU forest soil, sub-montane *Erica* forest and alpine *Helichrysum* SOM already loose considerable amounts of volatile compounds below 280 °C.

EGA intensities (counts mg^{-1} C) were within the range of previously reported values for Japanese Andisols (USDA) and showed a similar curve against temperature (Katsumi 2016, Figure 2.5-1). The release of volatile compounds with sample heating can be linked to either their chemical composition or their binding and complexation with mineral particles. Early and late peaks in EGA curves are connected to more labile OM components (e.g. lipids) and recalcitrant OM (e.g. lignin), respectively (Katsumi 2016). Low percentage of measurable volatile components in soils with andic characteristics is commonly explained by complexation with Al hydroxides or aluminosilicates (Shoji et al. 1994). However, neither mechanism would explain the elevation pattern at Mt. Kilimanjaro. The volatile signal more or less follows the amount of total organic C. Therefore, EGA results could not directly be

related to the chemical SOM composition. Most SOM compounds showed either decrease-increase, increase-decrease or site specific patterns with elevation.

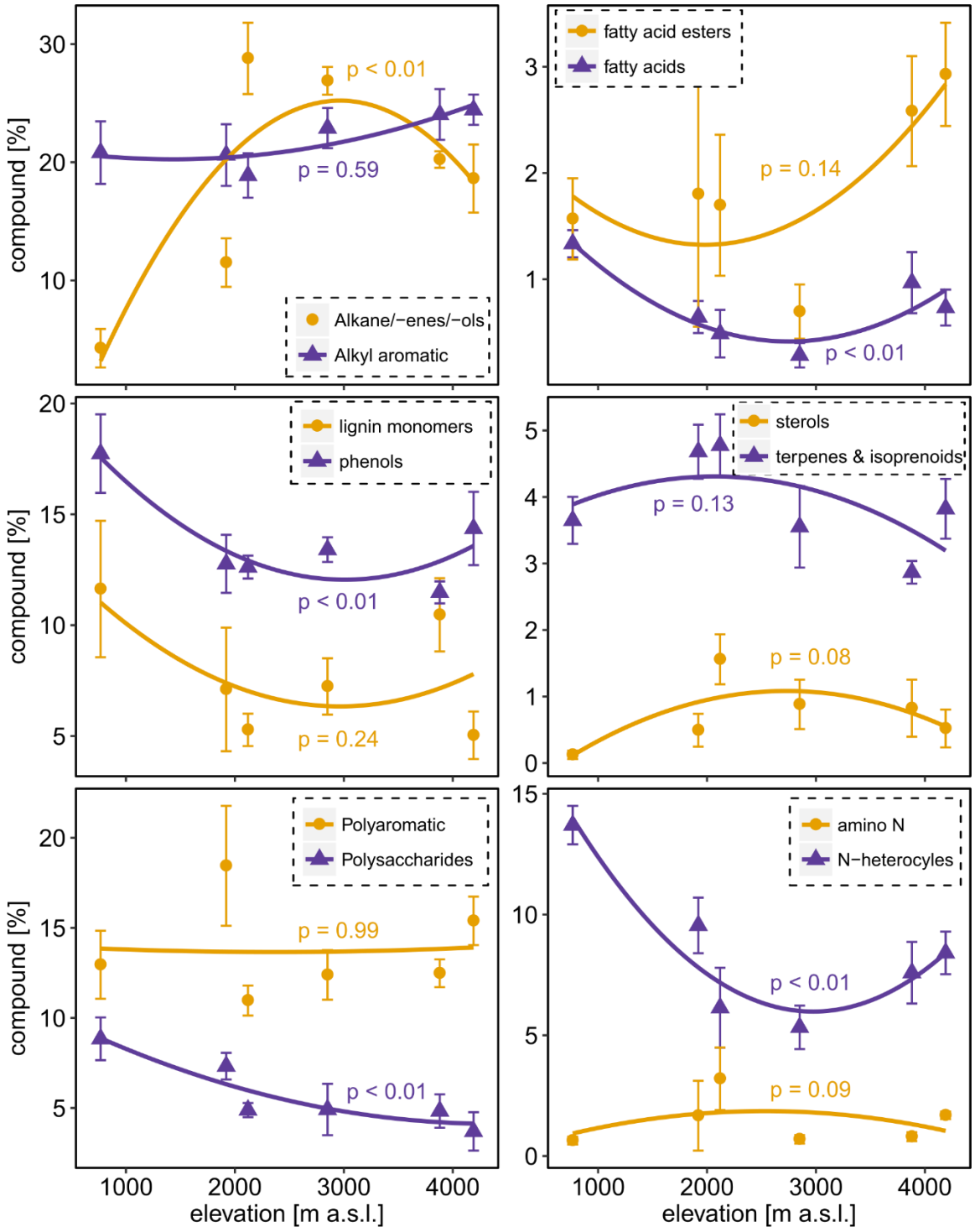

Figure 2.5-4: Percentage of pyrolysis compound classes from soil organic matter in six ecosystems along the elevation gradient of Mt. Kilimanjaro. Small letters (a-c) indicate significant difference between ecosystems ($p < 0.05$) according to *Kruskal-Wallis* test with *Benjamini-Hochberg* correction for multiple comparisons

2.5.5.2 Compounds with maximum at mid elevation

Patterns of alkanes/-enes/-ols with elevation were similar for thermal desorption and pyrolysis steps and were highly correlated with total C content in soil. Both had their minimum at low elevation (RAU and FLM) and peaked in cloud forests (FOC and FPO) (Figure 2.5-4, Table 1.2-1). These compounds were the major components of SOM in montane cloud forests (2100-2900 m), especially in the volatile fraction. They were also the main factor separating ecosystem characteristics along the elevation gradient (Figure 2). Depending on chain length, n-alkanes and n-alkenes originate from either fresh litter or microbial sources (Li et al. 2015). In soil, they occur in free form or bound in SOM by non-covalent binding (Lichtfouse et al. 1998). Decomposition leads to relative enrichment of aliphatic compounds in organic soil (Biester et al. 2014). Especially mid-chain alkanes and alkenes are considered relative recalcitrant products of vegetation litter degradation (Buurman et al. 2007; Vancampenhout et al. 2010). The increase of alkanes/-enes/-ols at around 2000 m can be a result of high leaf litter inputs (Becker et al. 2015) and incomplete decomposition. Further degradation and consequent increase of more stable compounds (e.g. aromatics) is reduced by the steady delivery of fresh litter inputs. C excess limits degradation of less easily available compounds (Chen et al. 2014; Guenet et al. 2010), explaining contrary elevation trends for more labile compounds.

Sterols, terpenes and isoprenoids occur in plant waxes, free or bound to n-alkanoic acids or carbohydrates (Otto, Simpson 2006). They are highly volatile compounds (Rowan 2011) and thus together contribute between 20% and 60% to the thermally desorbed fraction. These fractions are freely available and easily decomposed under aerobic conditions (Mehrabanian 2013). Elevation patterns of the volatile fractions were therefore strongly related to litter input and ecosystems specific conditions. Sterols and triterpenoids are specifically produced by *Erica* species (Fokina et al. 1988) and can be used as biomarkers to trace *Ericaceous* inputs in soil (Pancost et al. 2002). This explains the sudden increase of both fractions in the desorption step of *Erica* forest SOM. However, sterols and terpenes released in the pyrolysis step are from microbial origin and stabilized through ester bonds (Gobé et al. 2000). As part of the same bound lipids, they followed the elevation pattern of alkanes.

2.5.5.3 Compounds with minimum at mid-elevation

Percentage of most easily degradable SOM compounds (fatty acids, fatty acid esters and lignin) followed a decrease-increase pattern along the elevation gradient, reaching a minimum at around 2000 m a.s.l. (Figure 2.5-4). Fatty acids and fatty acid esters showed a very similar pattern. Both are usually seen as part of a labile C pool in soil and are readily decomposed in soil with high biological activities (Mueller et al. 2013). Accordingly, their decreasing content in SOM until 2900 m is negatively related to the increase of microbial biomass (Pabst et al. 2013). Increasing content at 3900 m and above is the consequence of decelerated microbial decomposition. We have to note that pyrolysis of

fatty acids leads to their decarboxylation and thus underestimation in favor of alkanes and alkenes and might affect the visible trends (Saiz-Jimenez 1994).

Soil lignin content peaks at low elevation (RAU) and in subalpine Erica forest. Lignin mainly originates from leaf litter and woody debris and its content in soil is strongly depending on decomposition rates (Aerts 1997). Therefore, enriched soil lignin content reflects a skewed input-turnover balance. Decomposition rates below 1000 m are generally low due to the restricted productivity in dry season at Mt. Kilimanjaro (Study 2). The necessity of drought resistance of vegetation RAU might additionally increase litter derived lignin inputs as plant avoid cell wall damage from water stress through lignification (Moura et al. 2010). In contrast, montane forest ecosystems (FLM, FOC, FPO) have high inputs but even higher decomposition rates compared to RAU and (sub-) alpine ecosystems (FER, HEL) (Becker et al 2015, Study 2), which explains low contents in between 2000 and 3000 m. While litter fall decreases with elevation (Becker et al. 2015), litter inputs at FER are mainly derived from woody materials and the very sclerophyllous needle leaves and thus increase the relative lignin content in soil. Low decomposition rates at FER additionally reduced lignin degradation (Study 2). Above the tree line (i.e. HEL), lignin content is input controlled and low amounts of woody inputs decreases lignin content. The different source (i.e. no trees and shrubs herb layer) and low decomposition at *Helichrysum* is reflected by a strong relative increase of volatile lignin components (Figure 2.5-4) reflecting the signal of untransformed lignin input.

Phenolic compounds made up for about 15% and followed a similar trend as lignin with elevation. In alpine *Helichrysum* however, they deviate from this trend exhibiting a strong relative increase in the pyrolyzed fraction. Phenols are a major component of SOM (Otto, Simpson 2006) and can be derived from various polymeric sources (Otto, Simpson 2006), but are mainly seen as decomposition products of lignin (Hedges, Mann 1979; Min et al. 2015). The high phenol, yet low lignin content in HEL soil indicates a shift to other sources above the tree line. It might be the result of an expectable increase of plant root to shoot ratios in alpine ecosystems (Wilson 2016). Suberin can contribute significantly to phenol origin in SOM (Otto, Simpson 2006) and is a distinct biomarker for root derived SOM inputs (Nierop 2001; Spielvogel et al. 2014) and very likely dominates the pattern observed in the alpine zone at Mt. Kilimanjaro.

2.5.5.4 N compounds

N containing compounds (amino N and N-heterocycles) in Kilimanjaro soils followed two contrasting trends with elevation. While amino N had their maximum at around 2000m, N-Heterocycles decreased at mid-elevation (Figure 2.5-4). The origin of N-containing components in SOM is not completely clear and can be either attributed to microbial or vegetal precursors (Vancampenhout et al. 2010). Still, amino acids, whether plant litter or microbial product, are easily degradable and part of a ‘fast-cycle’

turnover (Curry 1993). They mainly occur in fresh litter and upper soil horizons (van Bergen et al. 1998). N-heterocycles (such as pyridines, pyrroles and indoles) are more stable and are products of the microbial decomposition of lignin or amino acids in further degraded SOM (Schulten, Schnitzer 1997; Chiavari, Galletti 1992). Strong N limitation and high perennial productivity in mid-elevation ecosystems might induce increased decomposition of N compounds. The more stable pool (N-heterocycles) is reduced (Sims 2006) and transferred into a fast cycling pool (amino N) and thus into aboveground biomass N (Curry 1993).

Note, the slight increase of N–compounds in alpine soils might stem from cell wall chitin of fungi (Mehrabanian 2013), which are increasingly abundant in the alpine zone of Mt. Kilimanjaro (Gunina et al, Study4). We are aware, that separating N compounds using py GCMS tends to be error-prone as amino acids are transformed into indoles, imidazoles and, most commonly, nitriles during pyrolysis (Schulten, Schnitzer 1997). We pooled nitriles and amino N together to reduce the effect of misclassification. However, percentage of N compounds is not always in line with total C:N indicating a potential additional methodological bias.

2.5.5.5 Site-specific patterns

Aromatic pyrolysis products originate from proteins, tannins and other polyphenols including charcoal and usually are an important fraction in tropical forest (Vancampenhout et al. 2009). Alkyl-aromatic compounds were unaffected by elevation and were nearly absent in the volatile fraction (Figure 2.5-3, Figure 2.5-4). A sudden peak in FLM might be related to the high amount of other aromatic compounds (e.g. polycyclic aromatics and phenols).

Polycyclic aromatic compounds are mainly derived from the incomplete combustion of organic material from burning fuels or forest fires (Rumpel et al. 2007; Abdel-Shafy, Mansour 2016). High amount of polycyclic aromatic compounds in SOM from natural sites is considered an indicator of previous wild fires (Vergnoux et al. 2011). Consequently, the lowest amounts of PAs were found in ecosystems with no or low fire disturbance (e.g. FOC and FPO) (Figure 2.5-4). Beside the subalpine zone, burning is quite frequent in the densely populated areas directly below the national park border. These fires sometimes affect the adjacent forest areas (Hemp & Beck 2001). The high contribution of polyaromatics in the pyrolized and the desorpable SOM fraction of FLM could be a result of a fire more than 100 years ago. Furthermore, particularly labile PAs are strongly related to sooth and ashes than to onsite burning residues (Han et al. 2015). Sooth particles are transported uphill by the orographic lift and subsequently deposited during the perennial rains (Mladenov et al. 2012). Such atmospheric depositions may have added to the polyaromatics in the pyrolized and the desorpable SOM fraction of FLM. In this context, we would have expected higher percentage of PAs in RAU, which is located in the direct vicinity of Moshi town. However, no such signal was found. While the semi-arid climate explains

low wet deposition of such particles and compounds, it remains unclear why RAU did not even receive a detectable proportion of polyaromatics by dry dust deposition (Lohse et al. 2008).

Polysaccharide content in soil linearly decreased with elevation (Figure 2.5-4). Polysaccharides in soils originally derive from plant inputs, mainly cellulose and hemicellulose. These are easily biodegraded and replaced by microbial polysaccharides that accumulate on the forest floor and in mineral horizons (Kögel-Knabner 2000). At least in arable soil, polysaccharides are equally abundant in the labile and the stabile C pool (Kiem, Kögel-Knabner 2003). Therefore, high polysaccharide content in soil can be related either to large amounts of fresh litter inputs or strong microbial turnover and stabilization of these inputs. However, without separating plant and microbial derived compounds reasons for the declining trend with elevation are not easily explained. There are no litterfall data available for RAU forest. However, cover of litter on the Rau plot was 100% compared to 10% in the FER plot (Hemp unpublished data). Therefore, we assume that inputs are a lot higher compared to (sub-) alpine ecosystems. This may also explain low percentage of polysaccharides in the thermally desorbed fraction of RAU and FLM, because cellulose signals in pyrolysis usually appear only at more than 300 °C (Wang et al. 2013). However, litter input alone cannot explain the similar contribution of polysaccharides to SOM at forests and at high elevation ecosystems. Unexpectedly high content of thermally desorbable polysaccharides in the alpine zone might be derived from bacteria producing extracellular biofilms for adapting to the cold environment (Limoli et al. 2015). Here, further investigations by compound-specific analysis would be required to confirm such potential explanations.

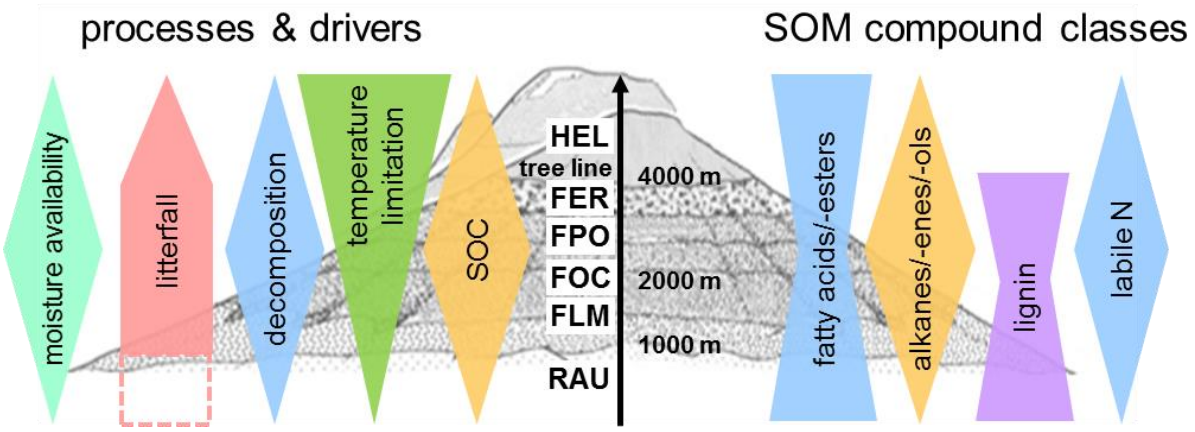

Figure 2.5-5: Schematic overview of processes and drivers affecting soil organic matter (SOM) composition in six along a 3000m elevation gradient of Mt. Kilimanjaro: Lowland evergreen broadleaf forest (RAU), lower montane evergreen forest (FLM), montane evergreen *Ocotea* forest (FOC), upper montane evergreen *Podocarpus* forest (FPO), subalpine *Erica* forest (FER), alpine *Helichrysum* cushion vegetation (HEL)

2.5.6 Conclusions

Results from EGA are very distinct and replicable for each ecosystem. However, EGA curves do not reflect the chemical composition derived from py-GC/MS and thus provide just very first insights into proportions of volatile compounds without allowing any conclusion on SOM quality and controlling dynamics. SOM chemistry varied considerably between ecosystems along the elevation gradient of Mt. Kilimanjaro. Fast decomposition rates around 2000 m a.s.l. lead to relative enrichment of litter degradation products, especially mid-chain alkanes and alkenes, in soil organic matter. C excess limits degradation of less easily available compounds, explaining a decrease-increase pattern along the elevation gradient for more labile compounds. Lignin derived compounds peak at low elevation (RAU) and in sub-montane Erica forest reflecting restricted decomposition rates below 1000 m in dry season, high inputs but even higher decomposition rates between 2000 and 3000 m and HEL low amounts of woody inputs decreases lignin content in soil. Nitrogen limitation and high perennial productivity in mid-elevation forests promotes decomposition of N compounds and shifts composition from a stable pool (N-heterocycles) into a fast cycling pool (amino N). Thus, we identified two main factors controlling SOM quality and composition: first, the rate and composition of OM inputs which is controlled by vegetation type and climatic characteristics, and second, the microbial decomposition rate controlled mainly by soil environmental parameters (e.g. temperature and soil moisture) and thus having its maximum at mid elevation.

2.5.7 Acknowledgements

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2.5.9 Appendix

Table Supplementary 2.5-2: Percent of the thermally desorbed fraction in EGA pyrograms (≤ 280°C). Mean values (mean), standard deviation (sd) and standard error of the mean (se) are presented for each ecosystem (n = 4). Small letters indicate significant difference according to ANOVA (p-level = 0.05).

	RAU	FLM	FOC	FPO	FER	HEL
mean	0.55[c]	2.76[b]	5.00[a]	5.30[a]	5.41[a]	3.36[b]
sd	0.12	0.67	0.38	1.01	0.30	0.95
se	0.06	0.34	0.12	0.51	0.15	0.48

2.6 Study 6:
Nitrogen turnover and greenhouse gas emissions in a tropical alpine ecosystem, Mt. Kilimanjaro, Tanzania

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2.6.1 Abstract

Background and Aims Tropical alpine ecosystems have been identified as the most vulnerable to global environmental change and despite their sensitivity they are among the least studied ecosystems in the world. Given the important role in constraining potential changes to the C balance, soil N turnover and plant availability in high latitude and high altitude ecosystems is still poorly understood.

Methods In this study, for the first time, a tropical alpine *Helichrysum* ecosystem at Mt. Kilimanjaro, Tanzania, at 3880 m altitude was characterized for its vegetation composition and investigated for major gross N turnover rates by the ^{15}N pool dilution method for three different vegetation covers. In addition greenhouse gas exchange (CO_2, N_2O and CH_4) was manually measured by use of static chambers.

Results Gross N turnover rates and soil CO_2 and N_2O emissions were generally lower than reported values for temperate ecosystems, but similar to Tundra ecosystems. Gross N mineralization, NH_4^+ immobilization rates and CO_2 emissions were significantly higher on densely vegetated plots than on low-vegetated plots. Relative soil N retention was high and increased with vegetation cover, which suggests a high competition of soil available N between microbes and plants. Due to high percolation rates, irrigation/rainfall had no impact on N turnover rates and greenhouse gas (GHG) emissions. Whereas soil N_2O fluxes were below the detection limit at all plots, soil respiration rates and CH_4 uptake rates were higher at more densely vegetated plots. Only soil respiration rates followed the pronounced diurnal course of air and soil temperature.

Conclusion Overall our data show a tight N cycle dominated by closely coupled ammonification-NH_4^+-immobilization which is little prone to N losses. Warming could enhance vegetation cover and thus, N turnover, but only more narrow C:N ratios due to atmospheric nitrogen deposition may open the N cycle of *Helichrysum* ecosystems.

Keywords Soil-N cycling, Gross-N turnover, ^{15}N-pool dilution, Greenhouse gas emission, Tropical alpine ecosystem

2.6.2 Introduction

Due to harsh environmental conditions pushing organisms close to their physiological limits, high latitude and high altitude ecosystems are among the most vulnerable ecosystems affected by global environmental changes. Furthermore, these ecosystems are exposed to extraordinarily strong warming well above the global average (Wookey et al. 2009). Typically, productivity of these ecosystems is strongly limited by availability of nitrogen (N) and phosphorus (P) (Shaver et al. 1992; Güsewell 2004; Weintraub & Schimel 2005). In a warming climate, the delicate balance of increased primary productivity - induced by higher nitrogen availability - and carbon (C) losses from promoted decomposition of SOM, may determine whether high latitude and high altitude ecosystems become a net sink or source for atmospheric carbon dioxide. Vice versa, the vegetation itself may exert feedback on soil C and N cycling through its litter quality, root exudation of labile organic compounds and via competition for organic and mineral nutrients (Rennenberg et al. 2009, Chapman et al. 2006). Despite the important role in constraining potential changes to the C balance, soil N turnover and plant availability in high latitude and high altitude ecosystems are still poorly understood (Weintraub and Schimel 2005). In particular this holds for tropical alpine ecosystems, which are considered to be one of the least well investigated ecosystems in the world (Buytaert et al. 2011). To our knowledge the study of Schmidt et al. (2009), is currently the only soil biogeochemical study providing gross N turnover rates for a tropical alpine ecosystem exposed to extreme diurnal temperature fluctuation. Studies on biogeochemical nutrient cycling are much more available for higher latitudinal and alpine ecosystems of the temperate zone (e.g. Jaeger III et al. 1999; Ernakovich et al. 2014; Clein and Schimel 1995; Alm et al. 1999; Gulledge and Schimel 2000; Kielland et al. 2006; Kielland et al. 2007; Kurganova et al. 2003). However, environmental conditions in tropical alpine ecosystems at >4000m are not directly comparable to those ecosystems due to generally lower atmospheric pressure, higher UV irradiance and different rainfall regimes. Even more, tropical alpine ecosystems are rather exposed to extreme diurnal temperature and radiation variations, whereas high latitude and alpine ecosystems are subject to strong seasonal variations of soil and air temperature as well as solar radiation resulting in highest activity of plant and biogeochemical soil processes in summer (Schmidt et al. 2009). Nevertheless, it was reported that even at periods with low soil temperatures (<5°C), and in particular at freeze-thaw events, microbes are still active and contribute to significant rates of gross soil N turnover (Schmidt et al. 2009; Mican et al. 2002; Wu et al. 2012, Wolf et al. 2010, Schütt et al. 2014) and associated N_2O emissions with significant or even dominating contribution to the annual budgets (Holst et al.; 2008; Luo et al. 2012). Various physical, chemical and biological processes and their interaction have been proposed to explain the occurrence of low temperature related N_2O emissions (De Bruijn et al., 2009; Matzner and Borken 2008). Due to pronounced diurnal changes in air and soil temperature freeze-thaw events could occur in tropical alpine ecosystems at unprecedented temporal

frequency likely to be disruptive to soil microbial communities with hitherto unresolved impacts on ecosystem availability of soil nitrogen (Larsen et al. 2002; Henry et al. 2007).

Therefore, for the first time we conducted a field study in an African *Helichrysum* ecosystem, with the aim of improving our understanding of soil nitrogen cycling and availability in a tropical high altitude site. The focus of this paper is on i) the quantification and characterization of key gross N turnover rates (i.e. mineralization, nitrification, microbial immobilization) and soil greenhouse gas (CO_2, N_2O, CH_4) exchange under different vegetation covers and ii) the influence of precipitation and freeze thaw cycles on biogeochemical processes.

Figure 2.6-1. Picture of the tropical alpine *Helichrysum* site (A) characterized by different vegetation classes (B: low-vegetation, C: herb and D: shrub).

2.6.3 Material and Methods

2.6.3.1 Site characteristics and sampling design

Mount Kilimanjaro is located in Tanzania, next to the border of Kenya (2°45’ to 3°25’ S and 37°00’ to 37°43 E) and is the highest peak on the African continent (5895 m. a.s.l.). Geologically it is a stratovolcano with a large spread of about 80 x 48 km (Downie et al. 1956). The study area (2500 m²) representing a tropical alpine ecosystem (3°053637’ S; 37°276770’ E, 3880 m a.s.l.) was selected in a slightly sloping area with no anthropogenic influence. The site is characterized by diurnal climate

Table 2.6-1: Classification (moss, herb, shrubs) and coverage of different plant species at non-vegetated, herb and shrub plots

Plot	Species	Mean cover class	Mean area cover	Vegetation type	Mean cover class	Mean area cover
Low veg	Mosses	+	<5%	Mosses	+	<5%
	Agrostis kilimandscharica	2	5-25%	Herbs	1	5-25%
	Haplosciadium abyssinium	+	<5%			
	Luzula abyssinica	2	5-25%			
	Pentaschistis borussica	+	<5%			
	Pentaschistis minor	1	5-25%			
	Alchemilla argylophylla	+	<5%	Shrubs	0	5-25%
	Alchemilla johnstonii	0	<5%			
	Euryops dacrydiodes	+	<5%			
	Helichrysum citrispinum	+	<5%			
	Helichrysum forskhalii	r	<5%			
	Helichrysum newii	1	5-25%			
	Helichrysum splendidum	1	<5%			
				Total	2	25-50%
Herb	Mosses	+	<5%	Mosses	+	<5%
	Agrostis kilimandscharica	1	5-25%	Herbs	2	25-50%
	Haplosciadium abyssinium	+	<5%			
	Luzula abyssinica	1	5-25%			
	Pentaschistis minor	+	5-25%			
	Alchemilla argyrophylla	1	5-25%	Shrubs	3	50-75%
	Alchemilla johnstonii	+	<5%			
	Alchemilla microbetula	+	<5%			
	Erica trimera	r	<5%			
	Euryops dacrydiodes	1	5-25%			
	Helichrysum citrispinum	1	5-25%			
	Helichrysum forskhalii	2	5-25%			
	Helichrysum newii	1	5-25%			
	Helichrysum splendidum	r	<5%			
				Total	4	50-75%
Shrub	Mosses	1	5-25%	Mosses	1	5-25%
	Agrostis kilimandscharica	+	5-25%	Herbs	+	<5%
	Haplosciadium abyssinium	+	<5%			
	Luzula abyssinica	+	<5%			
	Alchemilla argyrophylla	r	<5%	Shrubs	4	>75%
	Alchemilla johnstonii	+	<5%			
	Erica trimera	4	50-75%			
	Helichrysum citrispinum	+	<5%			
	Helichrysum newii	1	5-25%			
				Total	4	>75%
1) r	< 5%	single individual of the species with less than 5% coverage				
2) +	< 5%	2-20 individuals of a species and collectively cover less than 5%				
3) 1	< 5%	numerous individuals of a species collectively cover less than 5%				
4) 2	5% - 25%	species cover 5% and 25%				

5) 3	25% - 50%	species cover 25% and 50%
6) 4	50% - 75%	species cover 50% and 75%
7) 5	75% - 100%	species cover 75% and 100%

Coverage is expressed as percental contribution (area coverage) and classified (cover class) in the Braun-Blanquet scale, adapted by Mueller-Dombois and Ellenberg (1974)

with considerably high daily fluctuations in air temperature. The mean annual temperature is 5.3 °C and the mean annual precipitation is about 1417 mm (Appelhans et al. 2015a). The dominant vegetation species is alpine *Helichrysum* and a variety of mosses, herbs and also subalpine *Erica* shrubs (Hemp 2006) (Table 2.6-1). Thus, we defined three vegetation cover classes: low-vegetation (low-veg), herbal vegetation (herb) and shrub vegetation (shrub) (Figure 2.6-1). Regarding these categories, areal coverages were calculated from google maps satellite images by unsupervised k-means clustering, resulting in 40.5 % low-vegetation (10 cm height), 51.9 % herbs (30 cm height) and 7.6 % shrubs (260 cm height) (Table 2.6-2) at a total site area of 50x50m (Appelhans et al. 2015b). Within this area, three replicated plots per vegetation cover (app. 15 x 15m; N=3 * 3=9) were selected, each being represented by three randomly selected sampling locations (app. 1.5 x 1.5m; N=3 * 9=27). At any of the 9 plots replicated sampling locations were used to collect pooled samples for measurements of gross N turnover rates, GHG fluxes, microbial biomass, root abundance and other physicochemical soil properties (see section soil properties). At any of the 27 sampling locations relative abundance of each plant species was recorded based on a visual estimation of the space a species covered in the 1.5 – 1.5m area and expressed in the Braun-Blanquet scale, adapted by Mueller-Dombois and Ellenberg (1974). Information on the level of single plant species was aggregated and summarized as relative abundance of shrubs, herbs and mosses as well as the total vegetation coverage for any of the three vegetation classes (Table 2.6-1, Table 2.6-2).

The soil is a Vitric Andosol (WRB, 2014) characterized by partly shallow soil depths ranging from 5 to about 40 cm. Overall, an A-horizon of up to 10 cm depth was followed by either a B-horizon or bedrock, especially on surfaces without vegetation. An O-horizon was formed for the litter of the shrub vegetation.

Measurements of gross N turnover rates and GHG emissions were conducted between 25th – 30th November 2014. As an additive treatment to the vegetation cover classes each of the 27 sampling locations was irrigated (2.5 mm m^{-2}) at the end of 27th November, in order to simulate impacts of rainfall on N turnover processes and GHG emissions. Due to continuous heavy rainfall events soon after this irrigation event with even higher intensities during consecutive days, further irrigation was not necessary.

Table 2.6-2: Top soil (0-10cm) characteristics

Parameters		Low-veg			Herb			Shrub		
NH_4^+-N	[µg N / g BTG]	1.25	a	± 0.25	2.72	b	± 0.35	1.19	a	± 0.11
NO_3^--N	[µg N / g BTG]	0.84	a	± 0.18	0.47	ab	± 0.18	0.20	b	± 0.13
DON-N	[µg N / g BTG]	23.46	a	± 1.14	26.66	a	± 2.24	30.79	a	± 5.63
total extractable nitrogen	[µg N / g BTG]	25.55	a	± 1.37	29.85	a	± 2.57	32.03	a	± 5.53
total extractable carbon	[µg C / g BTG]	429.03	a	± 63.2	390.31	a	± 79.12	314.79	a	± 35.84
SOC (0-10 cm)	[%]	6.16	a	± 0.94	10.87	ab	± 1.09	12.32	b	± 2.09
N (0-10 cm)	[%]	0.46	a	± 0.06	0.71	a	± 0.07	0.74	a	± 0.1
C:N ratio (0-10 cm)		12.86	a	± 0.44	15.00	b	± 0.23	16.13	b	± 0.61
MBN	[mg/kg]	25.76	a	± 4.43	61.26	b	± 6.25	69.77	b	± 14.29
MBC	[mg/kg]	367.79	a	± 32.79	606.43	ab	± 51.64	834.43	b	± 144.8
MBC:MBN ratio		16.86	a	± 2.09	10.13	b	± 0.32	12.98	ab	± 0.83
bulk density	[g/cm3]	0.79	a	± 0.07	0.60	b	± 0.09	0.61	b	± 0.09
stone content	[%]	11.17	a	± 2.4	1.47	b	± 0.81	2.33	b	± 1.09
pH		5.30	a	± 0.1	4.80	b	± 0.1	4.80	b	± 0.1
live roots	[g l^{-1}]	0.75	a	± 0.14	0.51	a	± 0.1	0.92	a	± 0.19
dead roots	[g l^{-1}]	0.07	a	± 0.02	0.36	b	± 0.04	0.25	a	± 0.11
soil temperature (-2 cm)	[°C]	6,40	a	± 0,05	5,90	b	± 0,05	5,91	b	± 0,04
soil temperature (-10 cm)	[°C]	6.21	a	± 0.02	7.08	b	± 0.02	5.83	c	± 0.01
VWC	[Vol. %]	30.17	a	± 2.56	27.56	a	±2.60	26.37	a	0.93
area coverage	[%]	40.50	a		51.90	b		7.60	c	

DON dissolved organic nitrogen, *DOC* dissolved organic carbon, *TN* total extractable nitrogen, *TC* total extractable carbon; *SOC* soil organic carbon; *N* total soil nitrogen, *MBN* microbial nitrogen, *MBC* microbial carbon, *VWC* volumetric water content and area coverage of different vegetation classes of a tropical alpine *Helichrysum* site. Different superscript letters show significant differences between vegetation classes *($p \leq 0.05$)*

2.6.3.2 Gross nitrogen rates, dissolved inorganic N and organic C and N concentrations

For determination of gross N-turnover rates, soil sampling and ^{15}N labeling of the soil was carried on the 25th (no rain) and the 28th (irrigation/rain) of November 2014. Gross N turnover rates were quantified using the ^{15}N pool dilution technique described by Rosenkranz et al. (2005) and (Davidson et al. 1992) with slight modifications. At any of the 9 plots 300g (composite of the 3 sampling locations) from the upper mineral soil (0-10 cm) were sampled. Bulk soil was sieved (5 mm mesh width, Dannenmann et al. 2006) and a subsample of 150g was labeled either with 4.5 ml solution containing $(^{15}NH_4)_2SO_4$ or $K^{15}NO_3$ (50 atom% ^{15}N, N addition rate 3 mg N kg^{-1} dry soil) for investigation of gross N mineralization and nitrification rates, respectively. Isotope labeling of sieved soil was conducted by spraying the labeled solution on the soil as described by Dannenmann et al. (2009). One third of the ^{15}N labeled soil was extracted 15 min after labeling (t_1) and the second third incubated *in-situ*, covered with top soil layer material, for subsequent extraction 24 hours (t_2) later (for details see Dannenmann et al. 2009). The remaining 50 g were used for the determination of volumetric soil water content

(VWC) of the labeled soil. Additional 60 g of sieved unlabeled soil were used for measurements of VWC, dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) concentrations (Dannenmann et al. 2009). Further processing and analysis of soil extracts such as ^{15}N diffusion on acid traps, and analysis of isotopic signatures with EA-IRMS (Flash EA 1112 Series coupled to Finnigan Delta Plus XP, Thermo Fisher, USA); DIN (Epoch, BioTek Instruments Inc., USA) TN, DOC (Multi N/C 3100, Analytik Jena, Germany) were carried out at laboratory facilities of KIT IMK-IFU (Garmisch-Partenkirchen, Germany) and followed the protocols described by Dannenmann et al. (2009). Gross N mineralization and nitrification rates and NH_4^+ and NO_3^- consumption were calculated using the equations given by Kirkham and Bartholomew (1954). Microbial immobilization of NH_4^+ was calculated as $^{15}NH_4^+$ consumption minus gross nitrification, assuming that gaseous losses and heterotrophic nitrification of organic N were negligible (Davidson et al. 1991a). Microbial immobilization of NO_3^- was assumed to equal NO_3^- consumption. Based on the gained gross rates of inorganic N production and consumption, specific indicators of N cycling were calculated. The ratio of gross NH_4^+ immobilization plus gross NO_3^- consumption to gross N mineralization plus gross nitrification is referred to as relative N retention and the ratio of gross NH_4^+ immobilization to gross N mineralization is referred to as relative NH_4^+ immobilization.

2.6.3.3 Greenhouse gas measurements

For GHG exchange measurements (CO_2, N_2O and CH_4) one static chamber (25.2x15.2x14.7cm) was installed at each of the 27 sampling locations. A rubber sealing and clamps maintained gas tightness of the chamber at collars driven 3-5cm into the soil. The opaque polypropylene chambers were equipped with a rubber septum and a 30 cm long and 1/8 inch Teflon tubing to allow pressure equilibrations during sampling. Gas sampling was performed with a 60ml gas tight syringe (Omnifix®, B. Braun, Melsungen, Germany) equipped with a one way LuerLock stop cock (VWR International, Darmstadt, Germany). Over the whole measuring campaign four times a day (6:00, 9:00, 14:00 and 18:00), headspace gas was sampled at t1=0, t2=15, t3=30, t4=45 and t5=60 minutes after chamber closure in order to cover potential diurnal patterns. Sampling followed the gas pooling protocol of Arias-Navarro et al. (2013) by subsequently taking and mixing 15 ml gas samples from three replicated plot chambers at any sampling time t1 – t5 with one syringe. Thus, this approach integrates gas flux measurements at replicated sampling locations but still maintains plot replication. The total of 45 ml pooled sample was used to flush and finally over-pressurize (5ml) 10 ml glass vials (SRI Instruments, Bad Honnef, Germany). The samples were shipped to IMK-IFU (Garmisch-Partenkirchen, Germany) for further analysis using a headspace auto sampler (HT200H, HTA s.r.l, Brescia, Italy) coupled to a gas chromatograph (8610 C, SRI Instruments, Torrence, USA) equipped with an electron capture detector (ECD N_2O) and a flame ionization detector/ methanizer (FID: CH_4 and CO_2). Samples

were continuously calibrated with standard gas samples (N_2O: 406 ppb; CH_4: 4110 ppb; CO_2: 407.9 ppm, Air Liquide, Düsseldorf, Germany). Flux rates were calculated with R version 3.2.0 including HMR package 0.3.1 for calculation of GHG flux rates by linear increase or decrease in gas concentration over time (n = 5). Quality checks were applied and flux measurements were discarded at $r^2<0.6$. Mean detection limits (MDL) calculated according to Baker et al. (2003) were 0.17 mg CO_2-C, 5.3 µg, CH_4-C or 0.6 µg N_2O -N m^{-2} h^{-1}, respectively

2.6.3.4 Microbial biomass and fine root biomass

Soil samples were taken from 27 sampling locations (9 per vegetation class) with a steel corer (5 cm diameter) to a depth of 10 cm and separated into two depths: 0-5 cm and 5-10 cm. In three low-veg plots we only could take samples until 5 cm and 2.5 cm depth, because of underlying bedrock material. Samples were transferred into plastic bags and transported to the laboratory in Nkweseko station, Tanzania, and stored at 5°C. Processing of the samples was done within 60 days. All the macroscopically visible roots longer than 10 mm were extracted by hand with tweezers. The method described by Van Praag et al. (1988) and modified by Hertel and Leuschner (2002) was inapplicable under field conditions. Thus, roots were separated belonging to shrubs and the ones from grasses, herbs and mosses under the stereomicroscope. Also, we distinguished between live roots (biomass) and dead roots (necromass) by root elasticity and degree of cohesion of cortex, periderm and stele. An indicator of root death is a non-turgid cortex and stele, or the only presence of the periderm (Leuschner et al. 2001). Fine root biomass and necromass samples were dried at 70 °C (48 h) and weighed. After separation of roots, soil samples were stored in 60 ml PE-Tubes (VWR, Germany) at 4°C and shipped to Göttingen (Germany) for further analysis. Microbial biomass C (MBC) and microbial biomass N (MBN) were quantified by fumigation-extraction method following the protocol introduced by Vance et al. (1987).

2.6.3.5 Measurements of soil properties

All physicochemical soil properties were measured from pooled samples (N=3) at any of the 3 replicated vegetation plots (N=9). Soil pH was measured from air dried soil samples dissolved in 0.01 molar $CaCl_2$ solution with a SenTix 61 electronic pH-meter (WTW GmbH, Weilheim, Germany). Bulk density (BD) was calculated from oven dried (72 h at 105°C) undisturbed soil cores (100 cm^3) taken at 0-5cm soil depth. From the same samples stone fraction was measured as water displacement of stones >2mm. Carbon (C) and nitrogen (N) contents were determined using an automated C:N analyzer (Vario EL cube, Elementar, Germany). About 40 mg of dry soil were fine ground and combusted at 950°C. The evolving CO_2 and NO_x were then measured by a thermal conductivity detector.

Soil temperature was continuously (1 minute intervals) measured in 2 and 10cm soil depth over the whole measuring campaign at 27 sampling locations (EBI 20-TH1; ebro Eletcronic, Ingolstadt,

Germany). Means were calculated per vegetation class and soil depth. In addition to the determination of VWC from soil samples used for quantification of N turnover rates, VWC was also measured after GHG measurements in any chamber by a portable frequency domain sensor (GS3, Decagon Devices©, Pullman, USA).

2.6.3.6 Statistics

Kolmogorov–Smirnov statistics was applied to test normal distribution of data for any measured parameter. Since neither N gross turnover rates nor GHG emissions were normally distributed, we applied log transformation on N gross turnover rates and square root transformation on greenhouse gas data. Differences between the no-rain and irrigation/rainfall treatments for all sites were assessed using independent-samples t-test. For greenhouse gas data a two way ANOVA (Tukey's HSD) was conducted to test differences in time and between vegetation classes. Additionally, a one way ANOVA (Tukey's HSD) was executed for N-turnover rates and all other soil parameters to test for differences between vegetation classes. Correlation analyses between GHG, N turnover and soil parameters were conducted across all 9 plots using Pearson product-moment correlation coefficient. For identification of main controls of N gross rates and GHG emissions multiple stepwise regression analysis was applied. Level of significance was chosen at $p < 0.05$. All statistical analyses were calculated with IBM® SPSS® statistics 21 (IBM Corporation, New York, USA).

2.6.4 Results

2.6.4.1 Soil properties

The temperatures at 2 cm soil depth showed a strong diurnal cycle with a maximum of up to 22°C around noon and minimum 0°C in the early morning hours. Even though soil surface was covered with frost, minimum temperatures in 2cm soil depth were slightly higher than 0°C. Overall in 2 cm soil depth the mean diurnal temperature variation of 15°C was much higher compared to the temperature differences between the vegetation classes which were mostly <1°C. The temperature in 10cm soil depth showed a dampened diurnal variation with temporarily delayed maximum (12°C) and minimum temperatures (3°C) and a more pronounced difference (2°C) across the three vegetation classes (Figure 2.6-2). Over the whole measuring campaign mean soil temperatures at 2 and 10cm soil depth ranged between 5.9 – 7.1 °C with significantly highest values found in 2cm at the low-veg and in 10cm at the herb plots (Table 2.6-2).

In contrast to soil temperature, temporal variation of volumetric water content at all three vegetation classes was minor, even though soils were exposed to one irrigation and consecutive rainfall events since 28th November 2014 (Figure 2.6-2). For the low-veg and herb plots mean daily VWC ranged between 22 and 40 vol% with a tendency of decreasing VWC at beginning of the measuring campaign.

VWC at the shrub plots did not vary significantly over time and ranged between 26-28 vol%. Only the low-veg treatment showed an increase of VWC after irrigation. Mean VWC of the low-veg, herb and shrub treatments, measured daily at the GHG chamber positions, were not significantly different (Table 2.6-2) and in the same range than VWC measurements calculated from soil samples used for quantification of gross N turnover rates (Figure 2.6-3).

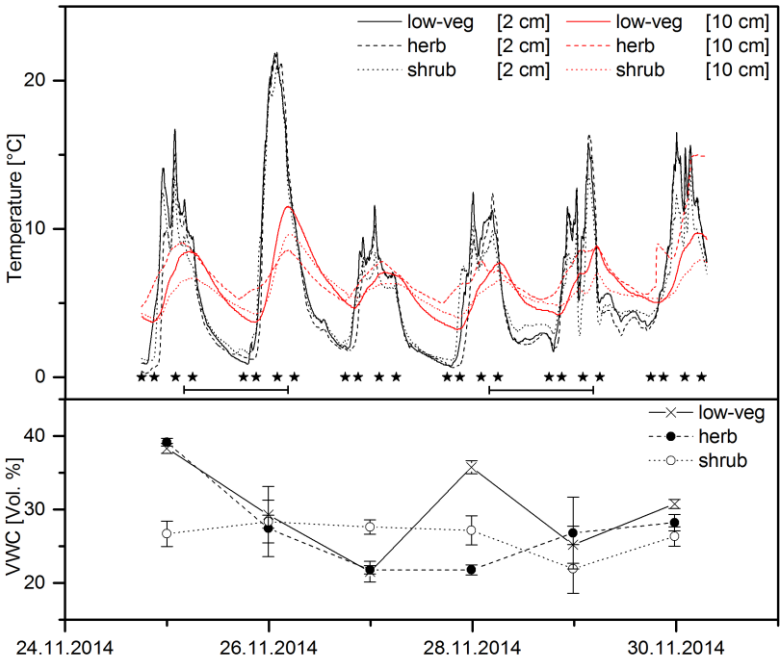

Figure 2.6-2: Course of soil temperature (2 and 10 cm) and volumetric soil water content (0-5 cm) at three vegetation classes of a tropical alpine *Helichrysum* site. Stars represent gas sampling times and lines below the stars the incubation time for the ^{15}N labeled soil.

Measurement of pH revealed more acidic conditions for the herb and shrub than for low-vegetated plots. Bulk density (BD) was higher for the low-veg plots (0.8 g cm^{-3}) compared to the herb and shrub plots (0.6 g cm^{-3}), whereas the C and N content as well as C/N ratio increased with vegetation cover (Table 2.6-2).

2.6.4.2 Gross N turnover rates and extractable soil C and N concentrations

At the first sampling time under no rain conditions gross N mineralization significantly increased with vegetation cover (Figure 2.6-3A). Rates on the herb plots were four times and on shrub plots more than 5 times higher than on the low-veg plots. Gross nitrification rates showed the same, though not significant trend as N mineralization rates but were four times lower than gross N mineralization rates on the low-veg and about ten times lower than on the vegetated plots. NH_4^+ immobilization rates significantly increased with growing vegetation cover. Gross NO_3^- consumption rates showed the same trend but were found to be much lower than NH_4^+ immobilization rates (Figure 2.6-3B).

For the sampling after the irrigation/rain event, magnitude and trends of gross N mineralization and nitrification rates across the three treatments were comparable to the no-rain situation. However, plant effects were less pronounced which resulted in diminished statistical significance of the differences across the vegetation cover treatments (Figure 2.6-3E). The same was true for NH_4^+

immobilization rates which were slightly lower in the vegetated plots compared to the no-rain situation. NO_3^--consumption rates declined after irrigation/rainfall and were detectable only in the shrub treatment.

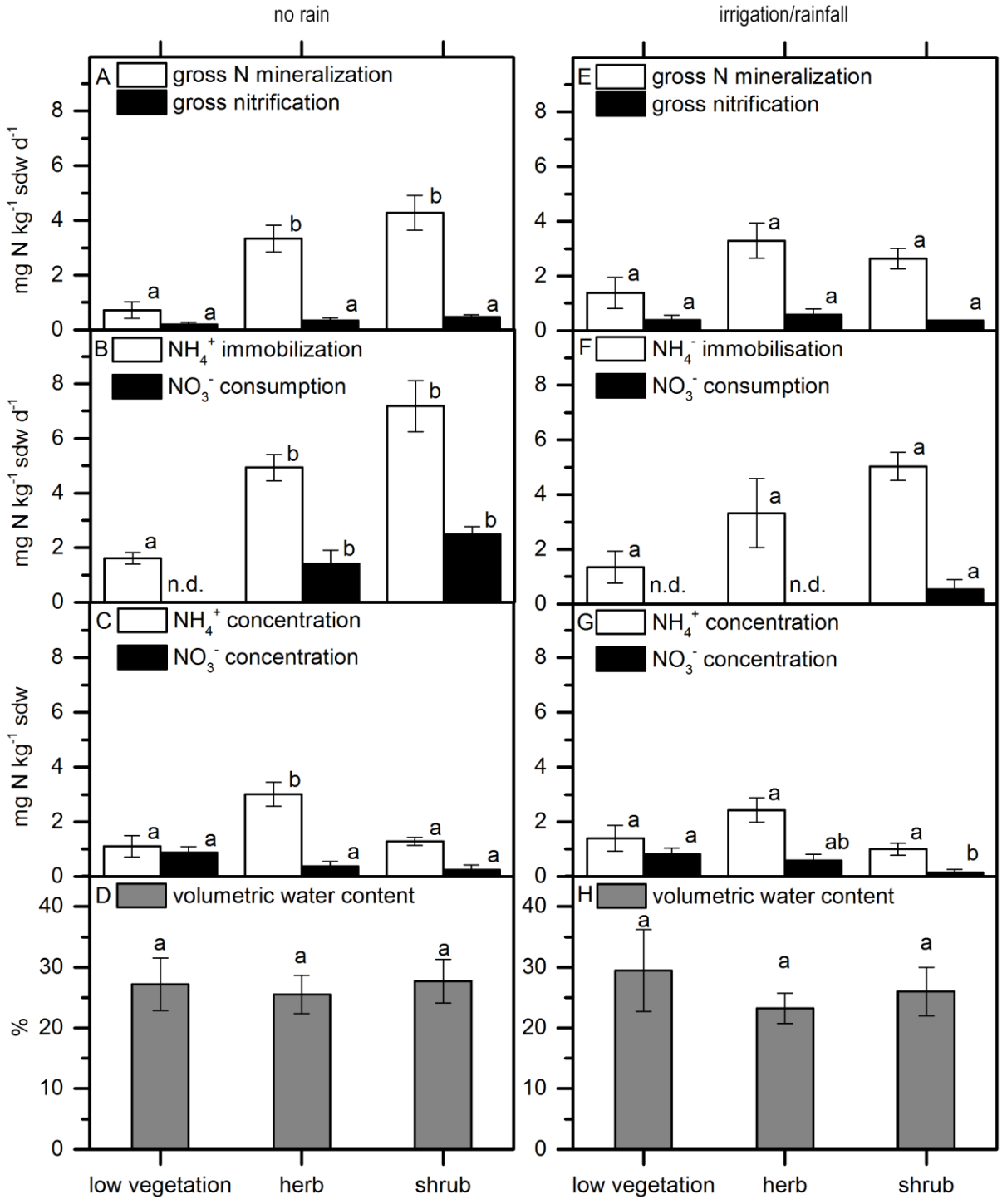

Figure 2.6-3: Gross N-turnover rates, soil N concentration and water content at three vegetation classes of a tropical alpine *Helichrysum* site. A-D represent measurements for no-rain, E-H represent measurements after irrigation (rain). Stars indicate times of GHG chamber measurements, lines indicate incubation time of gross N turnover measurements. A-Error bars are standard errors of the mean. Lower case letters represent significant difference ($p<0.05$) between the vegetation classes.

Before irrigation NH_4^+ and NO_3^- concentrations (Figure 2.6-3C) showed a different pattern across the three treatments than gross N turnover rates. NH_4^+concentrations were highest at the herb plots, while NO_3^- concentrations even showed a decreasing trend with increasing vegetation cover. After irrigation/rainfall mineral N concentrations were slightly lower but showed the same trends compared to the no-rain sampling. (Figure 2.6-3G). Across all vegetation classes NO_3^- concentrations were persistently lower than NH_4^+ concentrations, irrespective of irrigation/rainfall (Figure 2.6-3C and G). Overall the *Helichrysum* site was characterized by more than 10 times higher DON than DIN concentrations. DON concentrations did not differ significantly between treatments, nevertheless showed an increasing trend with increasing vegetation cover (Table 2.6-2).

Both relative N retention as well as relative NH_4^+ immobilization significantly increased in the presence of shrub as compared to the low-veg plots in the irrigation/rain treatment, but were not significantly affected by vegetation in the no-rain treatment (Table 2.6-3).

Table 2.6-3: **N turnover indicators for the three vegetation classes for no-rain, irrigation/rain and combined conditions. $Nret_{rel}$: relative N retention; $ImmNH_4^+rel$: relative NH_4^+ immobilization**

	Vegetation class	$Nret_{rel}$			$ImmNH_4^+{}_{rel}$		
no rain	low-veg	2.59	^{aA}	± 0.85	3.45	^{aA}	± 1.12
	herb	1.74	^{aA}	± 0.15	1.53	^{aA}	± 0.16
	shrub	2.07	^{aA}	± 0.08	1.69	^{aA}	± 0.06
Irrigation/ rain	low-veg	0.55	^{aB}	± 0.41	0.96	^{aA}	± 0.22
	herb	0.70	^{abB}	± 0.22	0.92	^{aB}	± 0.18
	shrub	1.89	^{bA}	± 0.2	1.93	^{bA}	± 0.09
combined	low-veg	1.26	^{a}	± 0.75	2.21	^{a}	± 0.55
	herb	1.22	^{a}	± 0.17	1.23	^{a}	± 0.07
	shrub	1.74	^{a}	± 0.09	1.82	^{a}	± 0.08

Superscript in small letters represent significant differences ($p<0.05$) between vegetation classes.
Superscript in capital letters represent significant differences ($p<0.05$) of no-rain and irrigation/rain within one vegetation class.
$Nret_{rel}$: relative N retention; $ImmNH_4^+rel$: relative NH_4^+ immobilization

2.6.4.3 Soil GHG emission CO_2, CH_4 and N_2O emissions

Since soil GHG emissions did not show any significant changes to the irrigation/rainfall event, data were aggregated over the whole measuring campaign (Table 2.6-4), and for evaluation of diurnal patterns divided into four classes representing different hours of the day (Figure 2.6-4).

Soil CO_2 emissions were low and ranged between 3.3 and 28.3 mg C m^{-2} h^{-1}. Emission were significantly higher on the herb and shrub plots compared to the low-veg plots (Table 2.6-4). At all plots, the highest CO_2 fluxes were measured at 2 pm and the lowest fluxes occurred at 6 am. This diurnal pattern was most obvious for the herb plots, which also showed highest daily maximum fluxes (Figure 2.6-4A). The difference between minimum and maximum fluxes at the shrub plots was lower but still higher than

at the low-veg plots which showed only a minor diurnal pattern. For all three vegetation classes chamber measurements revealed a net uptake of CH_4 into the soil, with rates ranging between -4.9 and -45.7 µg CH_4-C m^{-2} h^{-1} (Table 2.6-4). At the herb and shrub plots, uptake rates were significantly higher (app. 50%) than on the low-veg plots (Table 2.6-4). At medium and high vegetated plots diurnal patterns of fluxes were less pronounced than for CO_2 emissions and not existent at low vegetated plots (Figure 2.6-4B). For all vegetation classes N_2O emissions were below the detection limit (0.6 µg N_2O - N m^{-2} h^{-1}) and showed no diurnal pattern (Figure 2.6-4C, Table 2.6-4).

Table 2.6-4: **Compilation of minimum, mean, maximum and area weighted mean fluxes of CO_2 (mg C m^{-2} h^{-1}), CH_4 (µg C m^{-2} h^{-1}) and N_2O (µg N m^{-2} h^{-1}) for different vegetation classes and the whole *Helichrysum* ecosystem**

GHG emission	Vegetation class	min	max	mean		
CO_2						
[mg C m^{-2} h^{-1}]	low-veg	3.38	14.60	7.20	a	± 0.55
	herb	3.85	28.32	11.54	b	± 0.71
	shrub	4.96	17.42	10.86	b	± 0.56
	area weighted total			9.73		± 0.63
CH_4	low-veg	-3.64	-33.14	-15.37	a	± 2.24
[µg C m^{-2} h^{-1}]	herb	-4.91	-45.71	-22.44	ab	± 1.70
	shrub	-9.04	-33.90	-23.75	b	± 1.78
	area weighted total			-19.68		± 1.92
N_2O	low-veg	-2.69	3.48	0.25	a	± 0.23
[µg N m^{-2} h^{-1}]	Herb	-1.48	1.65	0.20	a	± 0.13
	shrub	-0.83	4.01	0.11	a	± 0.16
	area weighted total			0.21		± 0.17

Superscript letters show significant differences between vegetation classes ($p \leq 0.05$)

2.6.4.4 Microbial biomass (N and C) and fine root biomass

Microbial biomass N was significantly lower at low-veg plots compared to herb and shrub plots (Table 2.6-2). Microbial biomass C showed a comparable pattern across vegetation treatments, however with only significant differences between the low-veg and shrub plots. Overall at all vegetation classes, biomass of live roots was much higher than biomass of dead roots. Dead root abundance was significantly higher at the herb plots than at the low-vegetated and shrub plots. In contrast, abundance of live roots did not differ across vegetation treatments with herb plots tending to have lowest values (Table 2.6-2).

2.6.4.5 Correlation and controls of gross N turnover rates and GHG emissions

Both N mineralization and nitrification were positively correlated with soil CO_2 emission, but surprisingly no correlation was found between them. In addition N mineralization was also positively correlated with NH_4^+ immobilization and NO_3^- consumption. Also for the latter two a high positive correlation was found (Table 2.6-5). Stepwise linear regression revealed total extractable N, soil NO_3^-/

NH_4^+ concentration and MBN as main parameters controlling gross N turnover rates. Highest r^2 (> 0.9) of the regression was found for N mineralization and NH_4^+ immobilization by combination of three of the before mentioned parameters (Table 2.6-6). NO_3^- consumption as well as indicators of N cycling could be best explained either by soil NO_3^- or NH_4^+ concentration, however with much lower predictive power (r2<0.5). Note that nitrification, N_2O and CH_4 emissions could not be explained by any of the parameters.

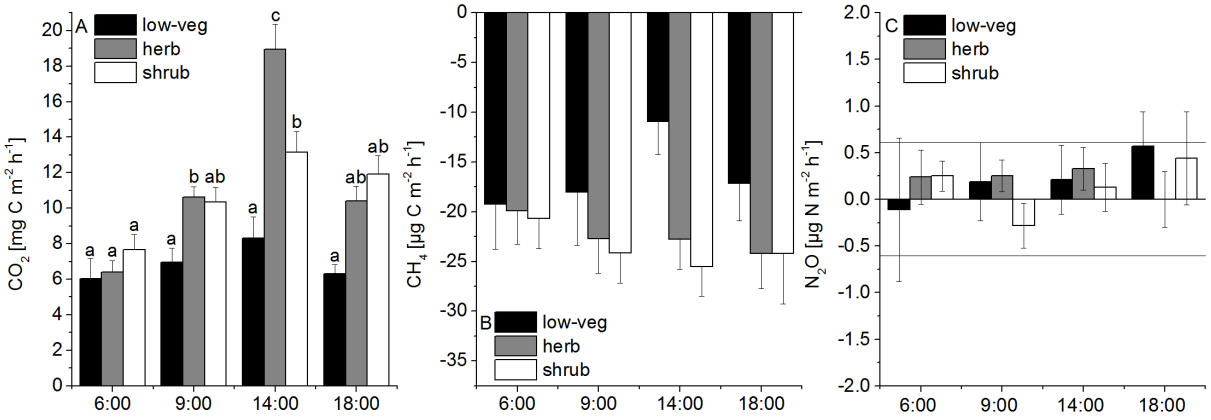

Figure 2.6-4 Diurnal patterns of soil GHG exchange (A: CO_2, B: N_2O, C: CH_4) at three vegetation classes of a tropical alpine *Helichrysum* site. Error bars represent standard error of the mean. Letters indicate significant ($p < 0.05$) temporal differences of fluxes within a vegetation class. Note no letters are presented for CH_4 and N_2O since no significant differences were detected. Lines at 0.6 and -0.6 in (Figure 2.6-4C), represent the MDL for N_2O measurements. Correlation coefficients of soil CO_2 emissions and temperature were 0.53 ($p<0.01$), 0.88 ($p<0.001$), 0.67 ($p<0.001$) for low-veg, herb and shrub plots.

2.6.5 Discussion

In the tropical alpine *Helichrysum* ecosystem variations in air and soil temperature are rather driven by diurnal (diff. 20°C) than seasonal patterns (diff 2°C of warmest and coldest month). Even though rainfall has a more pronounced seasonal pattern than air temperature, changes in soil moisture were not significant as proved by the results from the no-rain and irrigation/rain treatment (Table 2.6-7). That is related to a high vertical water percolation caused by high porosity and cleaved bedrock material. Regarding this specific soil conditions, we are convinced that the short term character of our study is not a significant limitation. In contrast to soil temperature and moisture, vegetation cover exerted pronounced effects on gross N turnover rates and GHG emissions. Therefore, gross N turnover rates and GHG emission sink or source strength presented, should be representative for the *Helichrysum* ecosystem investigated also for longer time scales. Accordingly, the following discussion focuses mainly on effects of vegetation cover.

2.6.5.1 Gross N turnover rates

Our approach of quantifying gross rates of N turnover together with extractable organic and mineral C and N substrates allowed a hitherto unavailable functional insight into N cycling of the *Helichrysum* ecosystems at Mt. Kilimanjaro. Overall, the N cycle was characterized by more than an order of

magnitude larger DON than mineral N availability, by high NH_4^+ immobilization rates and small nitrification rates with minimal soil NO_3^- concentrations, accompanied by an overall high microbial inorganic N retention capacity. This characterizes a rather undisturbed, N-limited and thus closed N cycle, which is confirmed also by extremely low N_2O emissions. Nevertheless, the high DON versus low mineral N availability is challenging the current paradigm of the N cycle, that depolymerization of organic macromolecules is the dominant "bottleneck" of overall N cycling (Schimel and Bennett 2004). At least for the tropical alpine *Helichrysum* ecosystem under investigation, nitrification seems to be the limiting step of overall N cycling.

Table 2.6-5: **Pearson's correlation coefficients (R) between N gross turnover rates and CO_2 emissions: NH_4^+ immob. = immobilization and NO_3^- cons. = consumption, *p <0.05, **p <0.01.**

	N mineralization	Nitrification	NH_4^+ immob.	NO_3^- cons.
CO_2	0.76*	0.74*	0.59	0.42
N mineralization		0.25	0.94**	0.75**
Nitrification			0.16	0.29
NH_4^+ immob.				0.88**

NH_4^+ immob. immobilization and *NO_3^- cons.* consumption
*p <0.05, **p <0.01.

Gross N mineralization rates (Table 2.6-7) were considerably higher on the vegetated plots and agree well with compiled data by Booth et al. (2005) for arctic/montane grassland ecosystems and Cookson et al. (2002) for winter conditions of soils in temperate regions. However, the area weighted gross nitrification rate for the *Helichrysum* site (Table 2.6-7), including all vegetation classes, is much lower, but in the same range as rates reported for an N-limited beech forest soil in southern Germany (Dannenmann et al. 2006). However, the latter as well as other studies, which report about boreal and alpine ecosystem nitrogen turnover processes (Clein and Schimel 1995; Jaeger III et al. 1999; Kielland et al. 2006; Schütt et al. 2014), are hardly comparable to the *Helichrysum* ecosystem. This is mainly due to different climatic (e.g. temperature, precipitation, and radiation regimes) and vegetation characteristics, i.e. larger vegetation cover, higher litter input and decomposition rates as compared to the *Helichrysum* site. Similarly, vegetation dependent variation of soil properties can also be observed at the site scale in our study, i. e., between the vegetation cover types at our *Helichrysum* site. Since larger vegetation cover leads to an increase of litter production and dead roots in the soil, SOM contents were found to increase with vegetation cover (Table 2.6-2), a finding in line with other studies (e.g. Prescott, 2010). Such plant-soil interactions provide the explanation for the observation of increased microbial biomass and gross N turnover rates with higher SOC contents (e.g., Geßler et al. 2005; Pabst et al. 2013), as also observed at our *Helichrysum* ecosystem (Table 2.6-7). Results of the regression analysis support this finding. From the total set of soil environmental parameters, except

nitrogen substrate, only MBN and SOC were selected as main controls for the dominating N processes of N mineralization and NH_4^+ immobilization.

Table 2.6-6: **Multiple regression analysis for identification of main environmental controls on gross N processes and greenhouse gas emissions. Selected variables**

	Parameter	Coefficient	Change in R^2	p value	Multiple R^2	Adjusted R^2	p value
gross N mineralization	Intercept	-17.858			0.947	0.928	<0.001
	TN	13.694	0.605	<0.001			
	NO_3^-	-0.697	0.896	0.018			
	MBN	0.045	0.947	0.004			
gross nitrification	none						
NH_4^+ immobilization	Intercept	-16.431			0.951	0.93	<0.001
	NO_3^-	-2.824	0.544	<0.001			
	TN	11.849	0.872	0.001			
	SOC	0.119	0.951	0.12			
NO_3^- consumption	Intercept	-0.418			0.804	0.782	<0.001
	NO_3^-	-1.498	0.804	<0.001			
rel. N retention	Intercept	0.028			0.402	0.335	0.036
	NO_3^-	-0.177	0.036	0.036			
Rel. NH_4^+ immob.	Intercept	2.616			0.479	0.422	0.018
	NH_4^+	0.512	0.479	0.018			
CO_2 flux	Intercept	5.901			0.46	0.382	0.045
	MBN	0.055	0.682	0.045			
N_2O flux	none						
CH_4 flux	none						

Discarded parameters (p>0.05): NH_4^+, NO_3^-, DON, total extractable N, total extractable C, SOC, N, MBC, live roots, dead roots

TN total extractable nitrogen, *NO_3^-* soil NO_3^- concentration, *NH_4^+* soil NH_4^+ concentration, *SOC* soil organic carbon, *MBN* microbial biomass N

The very low relative importance of nitrification versus NH_4^+ immobilization facilitated the overall closed N cycle of the *Helichrysum* ecosystem. Though it has been reported that nitrification might be more sensitive to low temperatures than ammonification (Cookson et al. (2002), the low nitrification rates of this study may also be related to the high DOC availability, which favors heterotrophic microbial NH_4^+ immobilization over gross autotrophic nitrification (Butterbach-Bahl & Dannenmann 2012). The trend of declining DOC with growing vegetation cover might also be explained by heterotrophic microbial NH_4^+ immobilization, which is, in contrast to the mainly autotrophic nitrification, a carbon consuming process (Rennenberg et al. 2001; Dannenmann, 2007; Sutton et al. 2011). The positive correlation between CO_2 fluxes and N mineralization and no correlation between nitrification and N mineralization (Table 2.6-5, Table 2.6-6) contrasts the general finding of other

studies (summarized by Booth et al. 2005). However, it supports the assumption of dominant heterotrophic microorganisms versus autotrophic nitrifiers. Heterotrophic microorganisms use NH_4^+ solely for growth, whereas autotrophic nitrifiers need NH_4^+ also for energy production, impairing their competition for NH_4^+ against microbial NH_4^+ immobilization at high DOC over N availability (Verhagen and Laanbroek 1991; Booth et al. 2005; Dannenmann, 2007). This suggests that increased N turnover rates at vegetated plots, caused by higher litter production and rhizodeposition (Hodge et al. 2000; Schimel and Bennett 2004; Phillips et al. 2011; Kuzyakov and Blagodatskaya 2015), do not enhance the risk of N loss, as long as the C:N ratio is not narrowing. In contrast, plants may even further compete with nitrification for soil NH_4^+. In this context, increasing microbial inorganic N immobilization (Table 2.6-7) and N retention capacity (Table 2.6-3) at shrub plots is pointing at intense plant-microbe competition for the limited N resources. This is further confirmed by e.g., declining NO_3^- concentrations and residence time of NH_4^+ (i.e., the ratio of NH_4^+ concentration to ammonification) with increasing vegetation cover (Figure 2.6-3). Even though intense microbial competition may reduce short term plant N availability, the process of internal N recycling along microbial loops also enables ecosystem nitrogen retention. This can even lead to sustainable nitrogen provision to plants, since plants on the long term may better compete versus microbes due to their longer and higher N storage capacity (Kuzyakov et al. 2013, Hodge et al. 2000).

Table 2.6-7: **Mean (no-rain and irrigation/rain treatment) gross N-turnover rates for three vegetation classes and for the whole (area weighted mean) *Helichrysum* ecosystem**

	low-veg		herb		shrub		area weighted mean	
	[µg N g^{-1} SDW d^{-1}]							
gross N mineralization	1.05 ^{a}	± 0.3	3.31 ^{b}	± 0.35	3.58 ^{b}	± 0.46	2.42	± 0.8
gross nitrification	0.29 ^{a}	± 0.09	0.46 ^{a}	± 0.11	0.42 ^{a}	± 0.04	0.39	± 0.05
NH_4^+ immobilization	1.48 ^{a}	± 0.27	4.13 ^{b}	± 0.65	6.26 ^{c}	± 0.64	3.22	± 1.38
NO_3^- consumption	n.d.	n.d.	0.49 ^{ab}	± 0.44	1.65 ^{b}	± 0.41	0.38	± 0.58
	[kg N ha^{-1} d^{-1}]							
gross N mineralization	0.83 ^{a}	± 0.29	1.97 ^{b}	± 0.7	2.17 ^{b}	± 0.82	1.52	± 0.42
gross nitrification	0.23 ^{a}	± 0.08	0.27 ^{a}	± 0.1	0.26 ^{a}	± 0.09	0.25	± 0.01
NH_4^+ immobilization	1.17 ^{a}	± 0.41	2.46 ^{b}	± 0.87	3.80 ^{c}	± 1.44	2.04	± 0.76
NO_3^- consumption	n.d.	n.d.	0.29 ^{ab}	± 0.1	1.00 ^{b}	± 0.38	0.23	± 0.37

Superscript in small letters represent significant difference ($p<0.05$) between vegetation classes

Currently, about 60% of the *Helichrysum* system is covered with vegetation. Palaeosols reflecting movements of vegetation belts caused by palaeoclimatic fluctuations (Zech, 2006; Zech et al. 2014) show that climate change may induce an increase in vegetation cover in the *Helichrysum* ecosystem. Since N turnover rates are highest at vegetated plots (Table 2.6-7), this may increase gross N turnover rates, but based on our findings this does not necessarily open the N cycle. Therefore, the *Helichrysum*

ecosystem may be rather vulnerable to expected increase of atmospheric N deposition in tropical regions of Africa (Dentener et al. 2006; Vitousek et al. 1997) which may narrow the soil C:N ratio and thus could increase nitrification, transiently opening the N cycle of the hitherto undisturbed ecosystem.

2.6.5.2 Greenhouse gas emissions

The area weighted mean CO_2 flux measured for the *Helichrysum* ecosystem was 86.4 g CO_2-C m^{-2} yr^{-1} which is only slightly higher than soil respiration rates reported for Tundra ecosystems (60 g CO_2-C m^{-2} yr^{-1}; Raich and Schlesinger 1992). Because decreasing temperatures inhibit soil respiration, we assume that similarly to Tundra ecosystems, soil respiration of the *Helichrysum* ecosystem at Mt. Kilimanjaro is mainly temperature limited. The total CO_2 production in intact soils is the sum of respiration from soil organisms, roots and mycorrhizae. Litter production, dead root decomposition and root exudates increase the organic matter inputs and thus soil respiration rates (Raich and Schlesinger 1992). Significant differences in organic matter inputs reflected by higher SOC contents at herb and shrub plots and highest live root abundance at shrub plots explain the increase of soil CO_2 emissions with increasing vegetation cover. Root respiration is positively correlated to temperature (Luo and Xuhui 2006) and solar radiation, the latter triggering root respiration via photosynthesis and subsequent stimulation of root exudation (Kuzyakov & Gavrichkova 2010). This is supported by our findings with more pronounced diurnal patterns of soil CO_2 emissions at the vegetated plots (Figure 2.6-4A). The slightly lower emissions on the shrub plots might be caused by lower soil temperatures during daytime due to higher shading compared to herbs (Figure 2.6-1; Figure 2.6-2). The minor influence of root respiration and lower SOM contents leads to the lowest temperature sensitivity of CO_2 emissions on the low-veg plots, which is also represented in the lower correlation coefficient with soil temperature (Figure 2.6-4A). Except soil temperature, also soil moisture has been found to correlate positively with soil respiration (e.g. Davidson et al. 1998; Raich and Tufekcioglu, 2000). Due to the high percolation rates, changes in soil moisture caused by irrigation/rainfall events were dampened, and had neither impact on N turnover rates nor GHG emissions. From this one can conclude that soil N and C cycling in the tropical alpine *Helichrysum* ecosystem is mainly controlled by changes in soil temperature.

During the whole measuring campaign the *Helichrysum* ecosystem was a net sink for atmospheric CH_4 for all vegetation classes. The area weighted mean uptake rate of 1.72 kg C ha^{-1} yr^{-1} is higher than the mean uptake rate of 1.12 kg C ha^{-1} yr^{-1} reported for Tundra ecosystems (Dutaur and Verchot, 2007), indicating a high adaptation of microorganism to the specific climatic and soil conditions. CH_4 uptake in soils is driven by oxidation via methanotrophic microorganisms (Conrad 1996, Butterbach-Bahl 2002) which is primarily influenced by diffusive properties regulating the availability of atmospheric CH_4 and oxygen in the soil (Ball et al. 1997; Boeckx et al. 1997) and therefore occurs predominantly in

the top soil (Bender and Conrad 1994; Steinkamp et al. 2001). The significantly lower CH_4 uptake rates on the low-vegetated plots may result from generally lower soil aeration caused by significantly higher soil BD (Table 2.6-2). In addition, during the observation period, soil moisture was highest at the low-veg plots (Figure 2.6-2) which further reduced gas exchange with the atmosphere and thus, lowered O_2 and CH_4 supply for methanotrophic microorganisms. Due to favoring physical soil conditions observed CH_4 uptake rates are highest in forest ecosystems (Dutaur and Verchot, 2007; Adamsen and King, 1993; Castro et al. 1995), which is also supported by Matzner and Borken (2008) who pointed out that vegetation generally enhances soil diffusivity. Various studies also showed a positive correlation of temperature and CH_4 uptake rates in particular for forest ecosystems (Butterbach-Bahl 2002; Kiese et al. 2008). Likewise, CH_4 fluxes at the vegetated plots show a weak diurnal trend with general lowest uptake rates at 6am (Figure 2.6-4B). Contradictory to our hypothesis, there was no impact of irrigation/rainfall on CH_4 uptake in any of the three vegetation classes which again can be attributed to the shallow soils and the high water drainage capacity.

The majority of N_2O fluxes of the *Helichrysum* ecosystem are below the mean detection limit, showing that N_2O emissions are negligible in the *Helichysum* ecosystem. N_2O production end emissions in soils predominantly occur indirectly via nitrification and directly via denitrification (Conrad 1996; Butterbach-Bahl et al. 2011). Since in our study nitrification rates are very low and denitrification proceeds mainly under anaerobic soil conditions at WFPS >70% (Butterbach-Bahl et al. 2013; Silver et al. 2001), none of the two relevant processes could produce significant amounts of N_2O. Contrary to our hypothesis, neither the vegetation nor irrigation/rainfall affected the magnitude of N_2O emissions. N_2O emissions were assumed to be higher on the vegetated plots since former studies revealed higher microbial biomass and activity as well as increased N-turnover to be positively correlated with N_2O emissions (e.g. Butterbach-Bahl et al. 2011). Due to the high rates of microbial NH_4^+ immobilization and high relative N retention, indicating low nitrogen availability in particular at vegetated plots (Table 2.6-3, Table 2.6-7), the increase of N_2O emissions with vegetation cover was likely hampered at the investigated *Helichrysum* ecosystem.

Contrary to our assumption, daily freeze-thawing was existent only at the soil surface and, thus in combination with low N availability did not affect the magnitude of N_2O emissions as reported for other ecosystems under similar climatic conditions (e.g. Holst et al. 2008). Since N_2O fluxes did not increase with vegetation cover, progressed warming and potentially associated expansion of vegetation will have only minor impacts on the overall N_2O budget of the *Helichrysum* ecosystem.

2.6.6 Conclusions

Our study is the first presenting N turnover processes and greenhouse gas exchange in an afro-alpine tropical ecosystem. N turnover at the investigated *Helichrysum* ecosystem is primarily temperature

controlled and due to shallow, well-draining soils, less affected by changes in soil moisture. SOM input from the vegetation and root exudates increase C and N substrate availability, and thus, increase microbial biomass and activity in vegetated patches. Overall this leads to higher N mineralization rates favoring subsequent microbial NH_4^+ immobilization. The high N retention and the low DIN concentrations reveal strong microbial competition for N, and thus, potential N limitation for plant growth. This indicates a rather closed N cycle, which is confirmed by the extremely low N_2O emissions. Most striking is the low nitrification, which seems to limit overall N cycling in the Helichrysum ecosystem. Nitrogen cycling will be accelerated if vegetation cover expands with progressed warming. Since this does not necessarily open the N cycle, the *Helichrysum* ecosystem may be rather vulnerable to expected increase of atmospheric N deposition. The latter could lead to narrowing of the soil C:N ratio, and thus, may increase nitrification and transiently opening the N cycle, which means losses of N to the atmosphere and waters of the hitherto undisturbed *Helichrysum* ecosystem.

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2.6.8 References

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2.7 Study 7: Legume and non-legume trees increase soil carbon sequestration in Savanna

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2.7.1 Abstract

Savanna ecosystems are increasingly pressured by climate and land-use changes, especially around populous areas such as the Mt. Kilimanjaro region. Savanna vegetation consists of grassland with isolated trees or tree groups and is therefore characterized by high spatial variation and patchiness of canopy cover and aboveground biomass. Both are major regulators for soil ecological properties and soil-atmospheric trace gas exchange (CO_2, N_2O, CH_4), especially in water-limited environments. Our objectives were to determine spatial trends in soil properties and trace-gas fluxes during the dry season and to relate above- and belowground processes and attributes.

We selected a Savanna plain with vertic soil properties, south east of Mt. Kilimanjaro. Three trees were chosen from each of the two most dominant species: the legume Acacia nilotica and the non-legume Balanites aegyptiaca. For each tree, we selected one transect with nine sampling points, up to a distance of 4 times the crown radius from the stem. At each sampling point we measured carbon (C) and nitrogen (N) content, $\delta^{13}C$ of soil (0-10, 10-30 cm depth) and in plant biomass, soil C and N pools, water content, available nutrients, cation exchange capacity (CEC), temperature, pH, as well as root biomass and greenhouse-gas exchange.

Tree species had no effect on soil parameters and gas fluxes under the crown. CEC, C and N pools decreased up to 50% outside the crown-covered area. Tree leaf litter had a far lower C:N ratio than litter of the C_4 grasses. $\delta^{13}C$ in soil under the crown shifted about 15% in the direction of tree leaf litter $\delta^{13}C$ compared to soil in open area reflecting the tree litter contribution to soil organic matter. The microbial C:N ratio and CO_2 efflux were about 30% higher in the open area and strongly dependent on mineral N availability. This indicates N limitation and low microbial C use efficiency in the soil of open grassland areas.

We conclude that the spatial structure of aboveground biomass in savanna ecosystems leads to a spatial redistribution of nutrients and thus in C mineralization and sequestration. Therefore, the capability of savanna ecosystems to act as C sinks is both directly and indirectly dependent on the abundance of trees, regardless of their N-fixing.

Keywords: Carbon-use efficiency; *Balanites aegyptiaca*; *Acacia nilotica*; Soil respiration; Spatial variability; C:N stoichiometry

2.7.2 Introduction

The savanna biome covers nearly 20% of the earth's terrestrial surface (Scholes and Walker 1993). It is a hotspot for biodiversity and wildlife conservation in temperate and tropical regions of America, Asia, Australia and Africa. Savannas are under strong pressure from climate and land-use changes. They are particularly threatened by desertification, shrub encroachment and conversion into arable land (Meyer and others 2007; Lambin and others 2003; Goldewijk 2001).

One of the main attributes that defines the savanna biome is the co-dominance of trees and grasses (Scholes and Archer 1997). Ecological interactions due to this contrasting vegetation cover have been a major research topic (Huntley and Walker 1982). Most research, however, has focused on species interactions or the impact of disturbances such as fire, grazing or droughts (Otieno and others 2005; Meyer and others 2009; Schleicher and others 2011). Other approaches estimated carbon (C) and nutrient stocks or fluxes in the ecosystem as a whole (Varella and others 2004; Veenendaal and others 2004; Grace and others 2006; Werner and others 2014; Chen and others 2016).

Several studies combined these approaches and analyzed the spatial effects of the highly heterogeneous vegetation cover on soil ecological properties (Bernhard-Reversat 1982; Belsky and others 1993; Hibbard and others 2001; Ludwig and others 2004; Perakis and Kellogg 2007; Rascher and others 2012; Otieno and others 2015). These studies have shown positive effects of trees on soil fertility, nitrogen (N) availability, understory growth and C pools compared to open grassland areas. This results in patchy areas of distinctly altered biogeochemical conditions: 'islands of fertility' (Garcia-Moya and McKell 1970). These changes in physical (e.g. water budget and temperature), chemical (pH, CEC, N content) and biological (microbial biomass and composition) soil properties result from a multitude of processes, including altered water balance, shading, and accumulation of biomass in the form of litter. It is often assumed that plant N-fixation, whether by the tree itself or by undergrowth species, is a main factor for the increased soil fertility of tree patches (Vitousek and Walker 1989; Sitters and others 2015). Legume trees such as Acacia species can resolve N limitation in African savanna grasslands (Ludwig and others 2001). However, the extent of this effect strongly depends on other limiting factors, such as nutrients and water (Vetaas 1992), and some studies did not show stronger effects of N-fixing tree species on soil parameters compared to other tree species (Bernhard-Reversat 1982; Belsky and others 1989).

Few studies have measured the broad spectrum of above- and belowground parameters and their interactions to determine the mechanistic effects of tree islands on soil C sequestration. The potential of an ecosystem to sequester C in soil is largely controlled by soil microbial activity and carbon use efficiency (CUE) (Bradford and Crowther 2013). If tree islands alter substrate quality and nutrient supply, this may also change microbial CUE. To date, little is known about how the affected properties

interact to control the C and N cycles, especially under water-limited conditions. While savannas are generally considered to be active or potential C sinks (Grace and others 2006), they act as a net source of CO_2 during the dry season (Miranda and others 1997). It remains unclear which factors regulate these C losses and how they are affected by the vegetation. Especially the spatial distribution of these variables and the connection between above- and belowground processes are important for understanding and predicting ecosystem changes. This is crucial in estimating vulnerability to climate and land-use change.

Our objective was to determine the interrelations and patterns of soil properties and soil greenhouse gas fluxes, depending on the spatial variability and characteristics of the vegetation (i.e. legume or non-legume tree). We hypothesize that (1) soil C and nutrient contents increase with the presence of trees through increased litter inputs (independent of tree species), (2) lower litter quality outside the crown area will result in reduced N availability and (3) C mineralization will increase due to higher microbial N mining outside the crown area.

2.7.3 Methods

2.7.3.1 Study site

The study was conducted in a semi-arid savanna plain of the Lake Chala Game Reserve, close to the Kenyan-Tanzanian border (3°18'39"S, 37°41'8"E). The research area covers about two hectares. It is located at the bottom of the southeastern slope of Mt. Kilimanjaro at an elevation of 950 m a.s.l. Soils of this area were classified as Vertisols and developed on erosion deposits from Mt. Kilimanjaro main peaks and from various parasitic volcanoes along the eastern slope (Kühnel 2015). These soils have high clay (66-79 %) and low sand (2 %) content in the upper 40 cm. Bulk density varies from 0.8 to 1.0 g cm^{-3} at 0-10 cm, and from 0.9 to 1.1 g cm^{-3} at 10-30 cm soil depth.

Mean annual temperature and precipitation are 21 °C and 536 mm respectively (Appelhans and others 2014). Rainfall mainly occurs over a short rainy season around November and a longer rainy season from April to June.

Table 2.7-1: **Tree characteristics and transect orientation**

ID	Species	tree height [m]	DBH [cm]	crown radius [m]	transect orientation [°N]
AN1	*Acacia nilotica*	4.9	73.6	3.0	142
AN2	*Acacia nilotica*	4.5	41.8	2.4	304
AN3	*Acacia nilotica*	2.8	32.2	1.9	84
BA1	*Balanites aegyptiaca*	3.0	46.0	1.8	338
BA2	*Balanites aegyptiaca*	2.6	35.0	1.5	302
BA3	*Balanites aegyptiaca*	4.0	50.4	2.2	316

The dominant woody plant species are various acacias (Acacia nilotica, Acacia senegal and Acacia tortilis) and Balanites aegyptiaca. The most abundant grass species are Heteropogon contortus, Eragrostis superba and Botriochloa insculpta, which all fix carbon by the C_4 pathway.

2.7.3.2 Field sampling

Field work was conducted during the dry season in September 2014. We identified the two dominant tree species in our research area: the leguminous A. nilotica, and the non-leguminous B. aegyptiaca. For both species we selected three solitary individuals that covered the common range of tree sizes in the region (Table 2.7-1). At each tree, one transect was placed in random orientation. Along each transect we selected nine sampling locations in relation to the respective crown radius r. Locations 50 cm, 0.50 and 0.66 times r distance from the stem represented the area under the canopy. The border zone was defined as 1 x crown radius. The open area outside the crown was sampled at distances of 1.5 and 2.0, 2.5, 3.0 and 4.0 x r.

At each sampling location, collars for greenhouse gas (GHG) chamber measurements were installed (383 cm^2). Before GHG measurements, we measured soil temperature and above-ground grass and herb biomass was collected from inside the collar area. Because of the dry conditions, these samples were assumed to represent dead plant material (i.e. undergrowth litter). GHG exchange was measured twice at each transect (9:00 and 12:00 o'clock, two transects per day). Opaque polypropylene chambers (25.2 x 15.2 x 14.7 cm) were fixed gas-tight to the collars and fluxes of CO_2, N_2O and CH_4 were calculated from concentration changes in the chamber headspace air (n=5 in 60 min). Soil cores were taken from the collar area with a closed soil-core sampler (30 cm x 5 cm Ø) and separated into 0-10 and 10-30 cm depths. Fine roots (<2 mm Ø) with length ≥10 mm were collected from each soil sample and stored at 4 °C until analysis. In each soil sample, total carbon (C) and nitrogen (N), microbial carbon (MBC) and nitrogen (MBN), water extractable carbon, density of living and dead roots, gravimetric water content, extractable nutrients (NO_3^-, NH_4^+ and cations of Al, Mg, K, Mn, Ca, Mg, Fe), cation exchange capacity (CEC), base saturation (BS), soil pH (in H2O and KCl), and bulk density were measured. Litter traps (70 x 70 cm) were placed under each tree and tree leaf litter was collected for one month.

2.7.3.3 Laboratory analyses

Soil chemical analyses were conducted in the laboratory of the Department of Soil Science of Temperate Ecosystems, University of Göttingen. Carbon and N contents were determined using a dry combustion automated C:N analyzer (Vario EL, Elementar). We considered total C as equal to organic C because the inorganic C content was negligible at our site (Kühnel and Becker, Unpublished Data). Microbial biomass C (MBC) and microbial biomass N (MBN) were estimated by fumigation-extraction (Vance and others 1987) with correction factors of 0.45 for MBC (Joergensen 1996) and 0.54 for MBN

(Joergensen and Mueller 1996). K_2SO_4-extractable C was taken as extractable organic C (Beck and others 1997). NH_4^+ and NO_3^- concentrations in the extracts were measured by continuous flow injection colorimetry (SEAL Analytical AA3, SEAL Analytical GmbH, Norderstedt, Germany). Samples were prepared by salicylate and dichloro-isocyanuric acid reaction for NH_4^+ and by cadmium reduction with NH_4Cl buffer for NO_3^-. Availability of major nutrient cations (Al_3^+, Ca_2^+, Fe_2^+, H^+, K^+, Na^+, Mg_2^+, Mn_2^+) was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro Analytical Instruments) following a preparative extraction in unbuffered salt solution (1.0 mol l^{-1} NH_4Cl). Total cation exchange capacity (CEC) and base saturation were calculated as described by Chesworth (2008). Soil pH was measured in H_2O as well as in KCl solution.

Dried and ground bulk soil, tree leaf litter and grass biomass samples were analyzed for ^{13}C natural abundance by isotope ratio mass spectrometry (Delta V Advantage with Conflo III interface, Thermo Electron, Bremen Germany) and a Flash 2000 elemental analyzer (Thermo Fisher Scientific, Cambirdge UK). Delta values ($\delta^{13}C$) are given as the divergence from the standard reference for ^{13}C to ^{12}C ratio (Vienna-PDB).

Fine root samples were analyzed according to Hertel and Leuschner (2002) with slight modification. Tree roots were separated from herb and grass roots under a stereomicroscope and separated into living and dead roots based on morphological criteria. All root samples were dried for 48 hours at 70 °C and weighed.

CO_2, CH_4 and N_2O concentrations from 10 ml vials of chamber headspace were determined at the IMK-IFU (Garmisch-Partenkirchen, Germany), using a gas chromatograph (8610 C, SRI Instruments, Torrence, USA) equipped with an electron capture detector and a flame ionization detector. Calculated flux rates were corrected for pressure and air temperature measured in the field. All flux rates lower than the minimum detection limit (CO_2-C: 0.09 mg m^{-2} h^{-1}; CH_4-C: 5.76 µg m^{-2} h^{-1}; N_2O-N: 0.83 µg m^{-2} h^{-1}), were set to zero.

2.7.3.4 Data analysis

Dixon's Q test was used to identify and remove outliers from each factor. We used all data points and applied linear mixed effect model (LME) analysis of variances for nested designs (each tree as random factor) at significance level $p<0.05$ to identify differences between the tree species (as fixed factor). The same method was used to compare areas below and outside the crown, with the addition of soil depth (if available) as a second fixed factor and using Tukey's HSD post-hoc adjustment for multiple comparisons. Satterthwaite approximation of degrees of freedom was used to correct for unbalanced replicate number when appropriate.

Variable interactions (GHG fluxes vs. soil parameters & tree characteristics vs. soil parameters) were analyzed by Pearson product-moment correlation at p-level < 0.05. Statistical analysis was conducted in R 3.3.0 (R Core Team 2013).

2.7.4 Results

2.7.4.1 Effect of tree species and characteristics

The total N content as well as extractable N fractions in soil under the crown were the same for both tree species and were also unaffected by structural variables such as tree size. For A. nilotica and B. aegyptiaca, mean N contents at 0-30 cm soil depth were 0.14 and 0.16%, respectively (Table 2.7-2). C content was also unaffected, and therefore the soil C:N ratio was the same under both tree species. The concentration of plant-available NO_3^--N varied from below the detection limit (0.15 mg l-1) to 1.05 mg l^{-1} under A. nilotica N and to 0.84 mg l^{-1} under B. aegyptiaca. Available NH_4^+-N was mainly below the detection limit and reached 0.85 and 0.75 mg l^{-1} for A. nilotica and B. aegyptiaca, respectively. Microbial C and N were the same in soil under both tree species. Tree height and crown radius positively affected water content at 0-10 cm ($p < 0.01$) but did not affect any other measured property at either depth under the crown ($p > 0.05$). As most of the soil attributes and GHG fluxes were unaffected by tree characteristics (Table 2.7-2), we pooled data from all trees of both species for further comparisons.

2.7.4.2 Soil properties and understory vegetation

C and N content, MBC and CEC at 0-10 cm depth decreased with distance from the tree (Appendix). Most of the decline occurred over the transition from crown cover to open area, and there were no further changes with greater distance from the tree. In soil below 10 cm, the decrease was less pronounced or completely absent. We therefore used crown and open area as distance classes for LME analysis. Values directly at the interface (1 radius) could be attributed to either of the distance classes and were not considered in further analysis.

In the upper 10 cm of soil, most variables were lower in the open area than under the crown (Figure 2.7-1). Carbon and N content as well as MBC decreased by about 25%. The extractable N fraction and MBN decreased by about 41%. The stronger decline of MBN versus MBC resulted in a wider microbial C:N-ratio in the open area. Gravimetric soil-water content was the same under both cover classes.

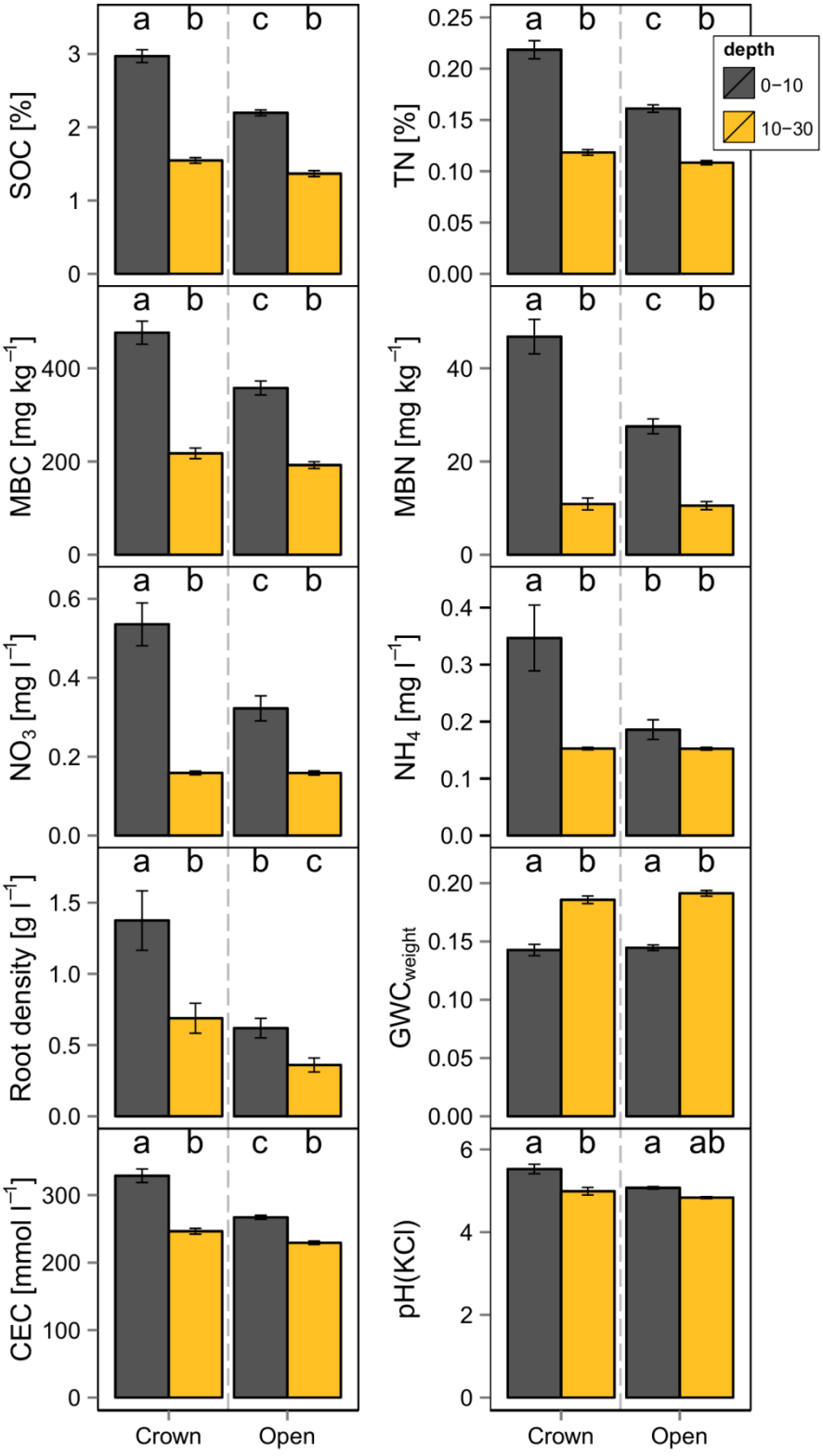

Figure 2.7-1 Soil properties at 0-10 and 10-30 cm depth, under the crown (n=18) and open area (n=30). Standard error of the mean is shown as error bars with significance levels (a-c) derived from mixed effect model ANOVA for nested designs ($p \leq 0.05$).

Table 2.7-2: **Effects of tree species on soil conditions at 0-30 cm depth, understory biomass (BM) and trace-gas fluxes under the crown (arithmetic mean ± standard error). P-values are derived from mixed-effect model for nested ANOVA.**

	Acacia nilotica	*Balanites aegyptiaca*	*p-value*
C [%]	2.00 ± 0.05	2.09 ± 0.08	0.2985
N [%]	0.14 ± 0.00	0.16 ± 0.01	0.3004
Soil C:N	13.5 ± 0.3	13.1 ± 0.1	0.5200
$\delta^{13}C$ [‰]	-14.6 ± 0.22	-14.6 ± 0.3	0.9334
$\delta^{15}N$ [‰]	5.79 ± 0.09	5.63 ± 0.07	0.2379
MBC [mg kg^{-1}]	287 ± 14	320 ± 22	0.4375
MBN [mg kg^{-1}]	18.9 ± 1.97	26.41 ± 2.52	0.2300
WOC [mg l^{-1}]	5.59 ± 0.30	6.08 ± 0.36	0.5066
NO_3^- [mg l^{-1}]	0.31 ± 0.03	0.26 ± 0.03	0.4654
NH_4^+ [mg l^{-1}]	0.20 ± 0.03	0.19 ± 0.02	0.4815
CEC [mmol kg^{-1}]	252 ± 4	255 ± 5	0.7990
BM [kg m^{-2}]	1.16 ± 0.09	1.36 ± 0.08	0.2403
BM C:N	59.0 ± 2.7	67.5 ± 5.1	0.3229
CO_2 [mg m^{-2} h^{-1}]	19.3 ± 1.2	20.3 ± 1.1	0.7365
N_2O [mg m^{-2} h^{-1}]	-0.50 ± 0.24	0.17 ± 0.15	0.5999
CH_4 [mg m^{-2} h^{-1}]	-20.0 ± 1.3	-19.8 ± 0.7	0.8504

Compared to the upper 10 cm of soil, the values of most parameters were lower at 10-30 cm soil depth (Figure 2.7-1). CEC decreased with soil depth and was about 7% lower outside the crown area. This effect was related to K^+ availability, which declined by 50%. The other dominant cations (Ca_2^+, Mg_2^+) decreased with soil depth but were unaffected by vegetation cover.

Above- and belowground grass and herb biomass was lower in the open area than under the crown (Table Appendix 2.7-4 Appendix Table A1). Living and dead roots in the topsoil (0-30cm) mainly originated from grass and herb species. The average N content in the grass biomass was 50% lower than in the tree leaf litter, and the C:N ratio was much wider in grass (40.6 ± 2.1, LME p-value = 0.0048).

2.7.4.3 Isotopic composition

The abundance of ^{13}C in soil under the crown was shifted towards the values of tree litter (Figure 2.7-2). The $\delta^{13}C$ composition of leaf litter from A. nilotica and B. aegyptiaca varied between -29.4‰ and -31.7‰. Delta values of grass biomass did not differ between the crown and open area, averaging -15.9‰ and -15.5‰, respectively. Due to the incorporation of grass biomass into soil organic matter (SOM), $\delta^{13}C$ values increased by about 1.0‰ on average. Mean $\delta^{13}C$ values in the top 10 cm were more negative under the crown than in open area (-16.8‰ and -14.5‰, respectively). $\delta^{13}C$ values increased evenly with soil depth under both cover types. Assuming similar ^{13}C fractionation during the incorporation of tree leaf and grass litter into SOM, we estimated the percentage of biomass input by trees: the isotopic composition in soil under the crown was shifted by 2.3‰ in the direction of tree leaf litter $\delta^{13}C$, which is equivalent to a 15% mass contribution of tree leaf litter to SOM (Figure 2.7-2).

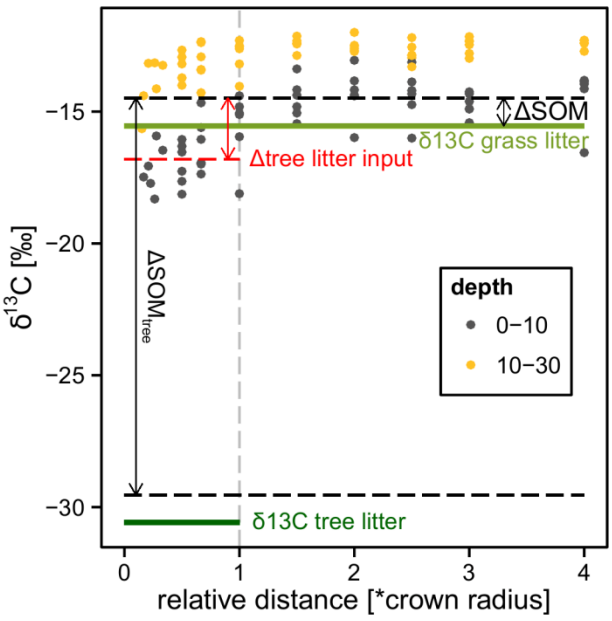

Figure 2.7-2 $\delta^{13}C$ at 0-10 and 10-30 cm soil depth with increasing distance from the stem. Solid lines indicate average $\delta^{13}C$ composition of tree and grass litter. Dashed lines show mean $\delta^{13}C$ values in 0-10 cm soil under the crown (black) and outside the crown (red) and potential value at 100% tree litter contribution. Black arrows indicate ^{13}C fractionation through grass litter incorporation in soil organic matter (ΔSOM) and the difference to tree litter incorporation (ΔSOMtree). Contribution of tree litter to SOM (Δtree litter input) is calculated as percentage of ΔSOMtree.

2.7.4.4 Soil greenhouse gas exchange

Measurements of greenhouse gas exchange revealed generally low soil CO_2 emissions, with higher rates in open areas than under the crown (Figure 2.7-3). At the same time the savanna ecosystem was a sink for CH_4, with average flux rates of -20 µg C m^{-2} h^{-1}. N_2O fluxes varied between -4.0 and 2.7 µg N m^{-2} h^{-1} but were not different from zero (t-test, $p>0.05$). Both N_2O and CH_4 fluxes were unaffected by vegetation cover.

GHG fluxes under the crown area were uncorrelated to soil properties at 0-10 cm depth (Table 2.7-3). Outside the crown, under low-nutrient conditions, there was a positive effect of soil water content on CO_2 efflux. Furthermore, CO_2 production was negatively correlated to NO_3^- availability, indicating a higher substrate turnover under nutrient-limited conditions. Flux rates of CH_4 were not related to any of the measured variables. N_2O fluxes in the open area were positively correlated to the C content in the soil ($r=0.55$, $p<0.01$).

Table 2.7-3: Pearson correlations coefficients between gas fluxes and selected soil properties at 0-10 cm under the crown (n = 18) and under open area (n = 30). Significance levels of p<0.05 and p<0.01 are indicated as * and ** respectively.

	C	N	MBC	MBN	NO_3^- (a)	NH_4^+ (a)	Living roots	Water content	T_{soil}	CEC	pH (H2O)
Crown area											
CO_2	-0.20	-0.13	-0.22	-0.12	-0.14	-0.40	0.15	0.16	-0.29	-0.52*	-0.11
N_2O	-0.05	-0.04	0.09	-0.01	-0.06	-0.04	-0.16	0.29	0.27	0.22	0.01
CH_4	-0.01	-0.07	-0.16	0.01	-0.37	0.06	0.29	-0.25	-0.25	-0.13	-0.17
Open area											
CO_2	-0.25	-0.08	0.23	-0.02	-0.55*	-0.31	0.25	0.37*	-0.15	-0.27	-0.20
N_2O	0.55**	0.30	0.10	0.29	0.26	-0.11	0.12	0.07	0.29	0.27	-0.04
CH_4	0.18	0.05	-0.10	0.07	0.28	0.01	-0.09	0.01	0.22	0.26	-0.05

(a) Values below detection limit were excluded

2.7.5 Discussion

2.7.5.1 Effects of savanna trees on soil C and nutrient contents

The presence of trees increased most soil fertility attributes as well as above- and below-ground grass biomass through higher litter inputs and quality. Tree species (and therefore N-fixing capability) had no effect on soil C, N or soil greenhouse gas fluxes under the crown.

The most apparent effect of trees was an increase in C and N content, microbial biomass (C and N), understory biomass and soil nutrient content. This is a common phenomenon for savanna ecosystems (Scholes and Archer 1997). It becomes more distinct with tree age and can remain for several years after tree dieback (Ludwig and others 2004). Nonetheless, the underlying mechanisms of this effect are under debate.

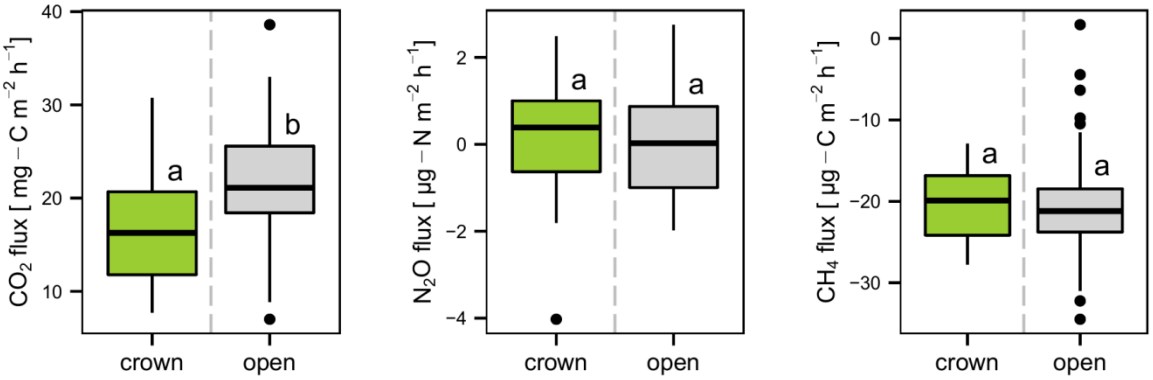

Figure 2.7-3 Soil greenhouse gas emissions under tree crowns and in open savanna area. Medians, interquartile range (IQR) and extreme values (>1.5 x IQR deviation) are displayed as bold lines, boxes with whiskers and dots, respectively. Significance levels derived from mixed effect model ANOVA for nested designs are shown as letters a-b ($p \leq 0.05$).

The N-fixing capability of Acacia species is often seen as one of the main mechanisms for subsequent C and nutrient accumulation under the trees (Yelenik and others 2004). In contrast to this interpretation, we found no effect on a large set of soil properties of a leguminous versus non-leguminous tree species (Table 2.7-2). Particularly, N content and availability as well as N_2O fluxes were the same under the crown of either species. Bernhard-Reversat (1982) attributed a similar finding (comparing B. aegyptiaca and A. senegal) to N fixation by an altered species composition in the herb layer under the tree, rather than by the tree itself. In our case, tree root densities in 0-30 cm soil depth were low and had nearly no visible nodules. Grass and herb roots showed no nodulation under or outside the crown. Even though nodulation potential increases with soil depth and maxima can occur more than 4 m below ground (Virginia and others 1986), we would expect at least a sporadic occurrence in the topsoil. Rhizobial nodulation depends on environmental conditions and decreases in dry soil. At the end of the dry season, topsoil horizons are dry and symbiotic N-fixation is shifted to

lower horizons (Vetaas 1992). While this may still play a direct role for plant and tree nutrition, the N turnover rates and N availability in the most microbially active soil horizons are independent of N-fixing effects.

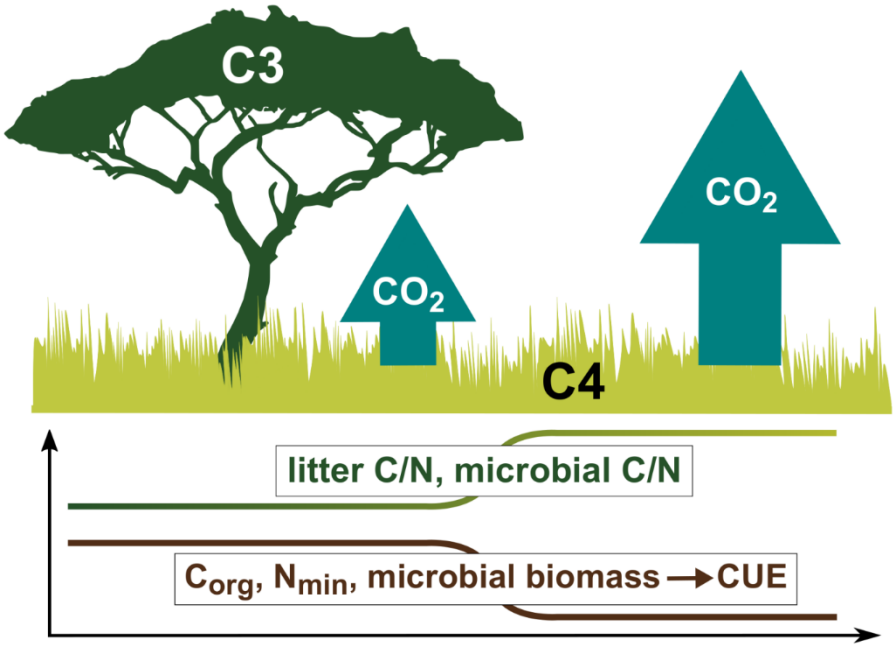

Figure 2.7-4 Effect of savanna trees on soil C and nutrient pools and related changes in soil respiration under dry conditions. The wide C:N ratio of C_4 plant litter reduces N availability (Nmin) and microbial biomass (MBC). Soil microbial C:N ratios and respiration increase due to low carbon use efficiency (CUE).

The higher soil C and N content is limited to the area under the crown and to the upper 10 cm of soil (Figure 2.7-1). This indicates a spatially limited source, such as the amount and quality of plant litter or throughfall water, as the main reason for increased C and N under the trees (Perakis and Kellogg 2007). Overall inputs from grass litter under and outside the crown did not differ in $\delta^{13}C$. A few grass biomass samples under the crown area, however, showed a lower $\delta^{13}C$ value, which implies the co-occurrence of C_3 herbs or grasses with the dominant C_4 grass species (Cerling and others 1997). This agrees with previous findings that the species composition in savanna herb-grass layers changes with varying tree cover (Belsky 1994; Ludwig and others 2004). Grass biomass, however, can only partially explain elevated soil C and nutrient contents. Soil $\delta^{13}C$ values under the crown were shifted towards the signal of tree leaf litter, suggesting that tree leaf litterfall contributes about 15% of SOM (Figure 2.7-2) and is a major driver maintaining higher SOM levels under the crown. This interpretation is supported by the fact that CEC (i.e. available nutrient cations: K+, Na+, Ca2+) showed a redistribution as well (Figure 2.7-1) and supports the theory that savanna trees act as nutrient pumps (Scholes 1990). Nutrients from the weathering zone are transported through the tree and return to the soil as litterfall, leachate, root litter or exudate. This explains the increase in cation availability in topsoil, followed by a decrease with soil depth. Similar to Ludwig and others (2004), there was no obvious lateral pump effect and the

absence of tree roots in the topsoil layers indicates a preferential vertical nutrient and water flow from deeper soil layers. Additionally, the accumulation of decomposition-resistant woody debris from trees and roots adds to higher C contents under crowns.

2.7.5.2 Interactions between variables

We expected the strongest effects of trees on soil microbial activity during the dry season because canopy shading and hydraulic lift are especially important under water-limited conditions (Horton and Hart 1998; Raz-Yaseef and others 2010). However, we found no differences in water contents under and outside the tree crowns at the end of the dry season (Figure 2.7-1). Water content was below or just around the permanent wilting point (Kühnel 2015), and all activities (i.e. biomass production, GHG fluxes) were reduced due to water limitation. This allowed us to look at other parameter effects on GHG exchange without the overriding effect of water content. While soil water content was constant under and outside the crown, CO_2 efflux was higher in the open area (Figure 2.7-3). This efflux trend was negatively related to fine root density. Because these variables are usually positively correlated under dry conditions (Ceccon and others 2011), we rule out a large contribution of rhizomicrobial and root respiration or an effect of water content. Instead, the higher CO_2 efflux outside the crown can be attributed to increased microbial respiration by decomposition of SOM and litter. CO_2 production under low-N conditions (i.e. outside the crown) is inversely related to NO_3^- availability (Table 2.7-3). Since NO_3^- addition is known to reduce microbial C mineralization (Burton and others 2004), this relationship might indicate N limitation. We found a stronger decrease in MBN than in MBC outside the tree crown, widening the microbial C:N ratio. These wide microbial C:N ratios are directly related to the C:N ratio of available substrate (Nicolardot and others 2001) and reflect a low carbon use efficiency (Sinsabaugh and others 2013; Blagodatskaya and others 2014). New available substrate for microbial turnover (i.e. litterfall from trees and grasses) differs in C:N ratio: leaf litter from C_4 grasses has a wider C:N ratio than litter from trees and C_3 grasses. This requires microorganisms to dispose of the C surplus via increased respiration to achieve their optimum C:N stoichiometry (Chen and others 2007; Spohn 2015).

2.7.5.3 Implications and relevance

We chose two widely different species and individual trees that cover the whole range of tree sizes in the study area in order to increase representativeness. All measured properties were in a typical range for soil characteristics, but the values were highly variable between trees and for each tree. Nonetheless, despite water limitation and the overall reduced biological activity (represented by low soil respiration rates), tree-cover effects on soil respiration were evident. Apparently, there is strong competition for nutrients within the microbial community in savanna soils, even under strongly water-limited conditions.

Tree effects on soil properties were independent of tree height, DBH and crown radius – all characteristics directly linked with tree age (Diallo and others 2013). Therefore, trees can affect the surrounding soil independent of their age. This indicates that soil C pools and fluxes react more rapidly to increased tree cover than to vertical tree growth. Tree cover is expected to change in natural savanna ecosystems of Africa due to improved wildlife management and climate change: On the one hand, the already decreased abundance of mega-herbivores and prevention of wildfires will increase tree-cover percentage (Staver and others 2011). On the other hand, the predicted irregularity of precipitation and increased air temperatures (IPCC 2013) might lower tree cover. This would in turn decrease soil fertility and directly increase CO_2 losses during the dry season because of lower carbon use efficiency (Figure 2.7-4). The potential of savanna ecosystems to act as a C sink, as proposed by Grace and others (2006), is very variable and directly depends on how the vegetation structure affects N availability.

2.7.6 Conclusions

The occurrence of trees (C_3) in a C_4 grassland increased soil fertility through higher litter inputs and quality in the local area under the crown. In soil deeper than 10 cm, the increase was less pronounced or disappeared completely. This effect is the result of active vertical transport by the trees (nutrient pumping) and a passive accumulation of C and N from litterfall over time. Tree species, whether leguminous or non-leguminous, had the same effects on soil properties. We conclude that soil C pools and fluxes are directly related to the spatial abundance of trees and react more rapidly to increased tree cover than to vertical tree growth.

In the open area and against the background of low N availability, the wider C:N ratio of C_4-grassesr compared to C3-tree litter inputs reduced the carbon use efficiency of soil microbes (Figure 2.7-4). This increased microbial respiration and the CO_2-efflux from soil. Therefore, savanna trees affect soil C storage through two processes, (1) actively by increasing biomass inputs and (2) passively by hampering output mechanisms.

2.7.7 Acknowledgements

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2.7.8 References

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2.7.9 Appendix

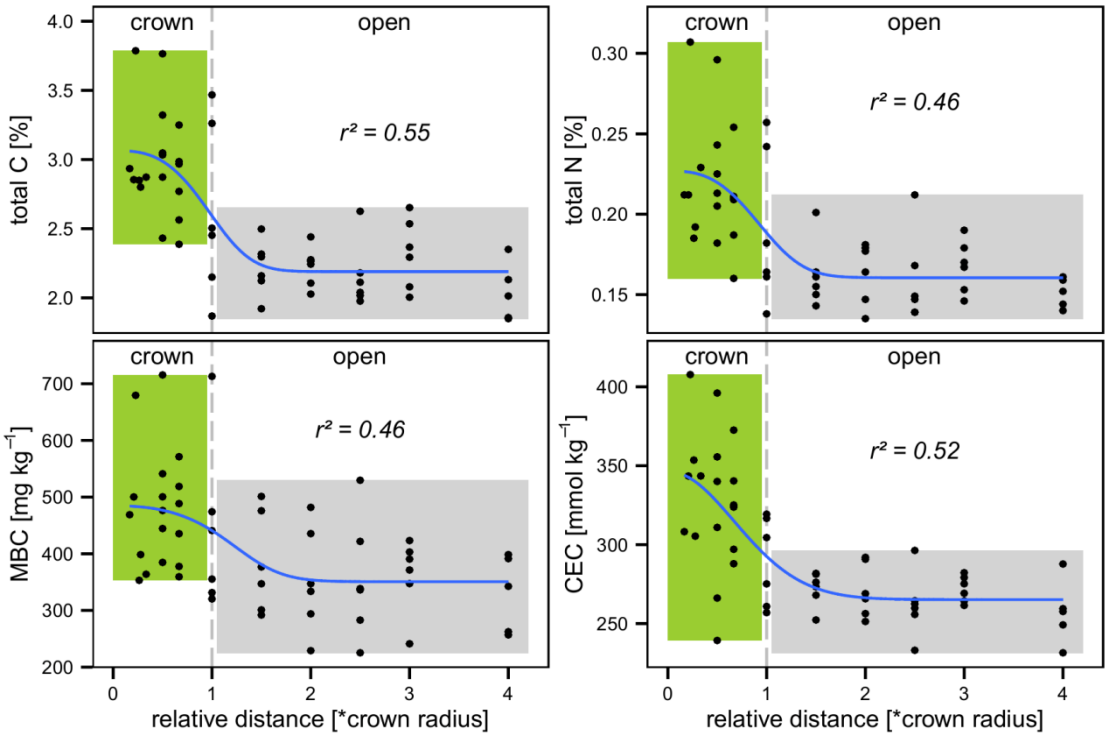

Figure Appendix 2.7-5: Total carbon (C) and nitrogen (N) content, microbial biomass (MBC) and cation exchange capacity (CEC) in soil of 0-10 cm depth in relative distance to the stem of two dominant savanna tree species.

Table Appendix 2.7-4: Means and standard errors of soil (in 0-10 and 10-30 cm depth) and understory vegetation properties under (Crown) and outside (Open) the tree crown. Small letters (a-d) indicate significant differences according to mixed-effect model for nested ANOVA with Tukey's HSD post-hoc comparison.

Position	Crown (n=18)		Open (n=29)	
Depth	0-10 cm	10-30 cm	0-10 cm	10-30 cm
C [%]	2.97±0.09^{a}	1.55±0.04^{c}	2.20±0.04^{b}	1.37±0.04^{c}
N [%]	0.22±0.01^{a}	0.12±0.00^{c}	0.16±0.00^{b}	0.11±0.00^{c}
C:N	13.71±0.21^{a}	13.08±0.16^{b}	13.71±0.19^{a}	12.59±0.22^{c}
WOC [mg l^{-1}]	6.46±0.47^{a}	5.53±0.21^{ab}	4.56±0.18^{bc}	4.38±0.18^{c}
MBC [mg kg^{-1}]	476.4±24.5^{a}	217.6±11.4^{c}	357.8±15.2^{b}	192.5±7.2^{c}
MBN [mg kg^{-1}]	46.8±3.7^{a}	10.9±1.3^{c}	27.5±1.6^{b}	10.5±0.8^{c}
MB C:N	10.64±0.45^{a}	18.98±1.46^{a}	13.52±0.53^{b}	19.82±1.37^{c}
NO_3 [mg l^{-1}]	0.54±0.05^{a}	0.16±0.01^{c}	0.32±0.03^{b}	0.16±0.01^{c}
NH_4 [mg l^{-1}]	0.35±0.06^{a}	0.15±0.00^{b}	0.19±0.02^{b}	0.15±0.00^{b}
Soil water [g g_{soil}^{-1}]	0.14±0.01^{a}	0.19±0.00^{b}	0.14±0.00^{a}	0.19±0.00^{b}
T_{soil} [°C]	36.3±0.9^{a}	NA	36.8±0.8^{a}	NA
pH(H_2O)	6.67±0.09^{c}	6.17±0.07^{b}	6.24±0.03^{a}	6.07±0.03^{ab}
pH(KCl)	5.52±0.12^{c}	4.99±0.09^{b}	5.07±0.03^{a}	4.83±0.02^{ab}
Grass biomass [kg m^{-2}]	1.5±0.13^{a}	NA	1.10±0.10^{b}	NA
Grass biomass C:N	51.18±2.81^{a}	NA	69.82±3.60^{b}	NA
$GrassRoot_{alive}$ [g l^{-1}]	1.31±0.21^{a}	0.61±0.10^{b}	0.62±0.07^{b}	0.36±0.05^{b}
$GrassRoot_{dead}$ [g l^{-1}]	0.32±0.06^{c}	0.22±0.04^{bc}	0.13±0.02^{ab}	0.10±0.01^{a}
$TreeRoot_{alive}$ [g l^{-1}]	0.06±0.03^{a}	0.07±0.02^{a}	0.00±.0.00^{b}	0.00±.0.00^{b}
$TreeRoot_{dead}$ [g l^{-1}]	0.01±0.00^{a}	0.01±.0.00^{a}	0.00±0.00^{a}	0.00±.0.00^{a}
CEC [mmol kg^{-1}]	328.7±10.0^{a}	246.4±4.3^{c}	267.1±3.0^{b}	229.4±2.7^{c}
$Al3^{+}$ [mmol kg^{-1}]	0.04±0.01^{a}	0.08±0.01^{bc}	0.05±0.01^{ab}	0.10±0.01^{c}
Ca^{2+} [mmol kg^{-1}]	182.7±6.5^{a}	130.0±3.0^{c}	147.2±1.9^{b}	122.7±2.3^{c}
Fe^{2+} [mmol kg^{-1}]	0.04±0.01^{a}	0.05±0.01^{a}	0.05±0.01^{a}	0.04±0.01^{a}
K^{+} [mmol kg^{-1}]	47.39±3.16^{a}	26.43±2.17^{b}	24.97±0.65^{b}	17.47±0.82^{c}
Mg^{2+} [mmol kg^{-1}]	97.20±2.03^{a}	88.25±1.50^{b}	93.59±1.08^{a}	87.78±0.69^{b}
Mn^{2+} [mmol kg^{-1}]	0.69±0.08^{a}	0.79±0.06^{a}	0.82±0.03^{a}	0.68±0.02^{a}
Na^{+} [mmol kg^{-1}]	0.62±0.04^{b}	0.79±0.05^{c}	0.39±0.02^{a}	0.59±0.52^{b}

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Legal Statement

I hereby declare that this Ph.D. dissertation has not been submitted to any other examining body either in its present or in a similar form.
Furthermore, I confirm that I have not applied for a Ph.D. at any other higher school of education. This dissertation was written independently and without any unauthorized aid.

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