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The role of the fine root system in carbon fluxes and carbon allocation patterns of tropical ecosystems along a climate and land-use gradient at Mount Kilimanjaro

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A mis padres y mi hermana,
por tanto amor

Summary

The combined effects of global warming and land-use conversion to human-modified systems are threatening biodiversity and ecosystem processes maintained by tropical mountains. To assess and predict the impact of global change on these ecosystems, it is crucial to understand the drivers and mechanisms of biodiversity and ecosystem processes. For instance, the study of carbon (C) and nitrogen (N) cycles is of key importance, as they encompass fundamental ecosystem processes such as carbon sequestration and storage, fluxes of C and N among ecosystem components as well as soil nitrogen turnover, which influence the performance of plant species and activity of soil microorganisms. Fine roots play a major role for most of the abovementioned ecosystem processes, as they represent the plant-soil interface. They are also essential plant organs for water and nutrient uptake. Thus, the study of fine roots' carbon economy contributes to the understanding of plant strategies in resource limiting environments and their role in ecosystem processes. Mount Kilimanjaro, in northern Tanzania, with its many different ecosystems (both natural and human-modified) across the large elevational gradient, represents a good opportunity to study the effects of climate and land use on the fine root system, as well as on below- and aboveground NPP relations and carbon allocation patterns. Along the present investigation, fine root bio- and necromass, fine root dynamics and fine root morphological traits across elevation and in different land-use systems were estimated. In addition, above and belowground NPP of woody plants along the elevation was quantified and carbon allocation patterns were assessed. Finally, the spatial vegetation heterogeneity of two contrasting natural ecosystems provided the opportunity to assess the strong link between plants and soil microorganisms by studying the effects of vegetation on belowground processes.

In the first study, fine root biomass and dynamics along the entire elevational gradient were investigated to assess plant carbon investment strategies to adapt to different environmental conditions. In addition, focusing on the tropical montane forest, the effects of elevation and associated biotic and abiotic factors on the fine root system were determined and the existence of a root economic spectrum (RES) was assessed. Ecosystems with pronounced resource limitation (savanna: water limitation, alpine heathland: N limitation) showed much higher root: shoot ratios (fine root biomass and production related to aboveground biomass) compared to tropical montane forest ecosystems. Moreover, the root: shoot ratio in the tropical montane forest increased exponentially with elevation but decreased with precipitation and soil nitrogen availability. The variation in root traits across the elevation gradient fits well within the concept of a multi-dimensional RES. In addition, the species identity of the dominant species had a strong effect on the properties of the fine root system. In conclusion, a general belowground shift in carbohydrate partitioning is evident

across the elevation in the tropical montane forest, suggesting that plant growth is increasingly limited by nutrient (probably N) shortages towards higher elevations.

In the second study, we aim to broaden our understanding of the effects of elevation on the carbon economy of plants from fine roots to the aboveground components. Focusing on the tropical montane forest, NPP above- and belowground was quantified, carbon allocation patterns were assessed and C and N return to the soil via leaf and fine root litter across elevation was determined. Total NPP-C declined and carbon allocation from above- to belowground tree organs showed a marked shift with increasing elevation. The C and N fluxes to the soil via leaf and fine root litter also diminished along the slope. These findings suggest that the decrease of total NPP across elevation is caused by decreasing carbon gain due to a lower leaf area index towards the subalpine *Erica* forest. This fact is consequence of increasing N limiting conditions at high elevations. The shift of carbon allocation from above- to belowground tree organs might contribute to acquire the limiting nutrients in these harsh environmental conditions.

In the third study, the effects of land-use change and ecosystem disturbance on fine root bio- and necromass, dynamics, morphological and chemical traits, as well as on the C and N fluxes to the soil via fine root litter were addressed. We found a consistent decrease of nearly all investigated variables with land-use change and disturbance. However, the traditional agroforestry systems (“Chagga homegardens”) maintained similar values as the natural montane forest for some of the fine root properties (e.g. stand fine root production, fine root litter quality) and outstanded for being a high dynamic ecosystem. *Podocarpus* forest disturbed by fire showed a markedly strong decline of C and N return to the soil via fine root mortality. These results indicate a modification of the fine root C stocks and the C and N supply to the soil from root litter decay with land-use change, which strongly affects the ecosystems' C and N cycle.

In the fourth and fifth studies, the vegetation effects on belowground processes (gross N turnover rates, soil C sequestration, greenhouse gas (GHG) fluxes) in ecosystems with strong harsh environmental conditions and patchy vegetation were determined. In the alpine *Helichrysum* heathland, gross N mineralization, NH_4^+ immobilization rates and CO_2 emissions were significantly higher on high-covered vegetation plots than on low-covered plots. Gross N turnover increased with vegetation cover, and thus, with supply of plant litter for the microbial community. The high relative soil N retention indicates high competition for N availability in the soil between microbes and plants and a tight N cycle dominated by tightly coupled ammonification-NH_4^+- immobilization in *Helichrysum* heathlands. In savanna woodlands, spatial trends (from the tree crown into the open grassland) in soil properties and GHG fluxes and related above- and belowground processes and attributes were determined.

Higher soil fertility, soil C and N content, microbial biomass and fine root density were found under the crown, whereas soil respiration rates, microbial and plant litter C:N were higher in open grasslands. Tree leaf litter held lower C:N than C_4 grass litter and contributed 15% of SOM. These patterns suggest that in the open grassland, high microbial competition, together with low substrate C:N from C_4 grasses lead to a low carbon use efficiency of soil microbial communities and a higher soil respiration. Hence, the spatial structure of the vegetation in savanna ecosystems results in a spatial redistribution of nutrients and thus in C mineralization and sequestration.

The present investigation contributes to a better comprehension of the effects of climate on woody plants carbon economy, with special attention on the role of the fine roots. In addition, it highlights the impacts of land-use change and disturbance on the fine root system and related carbon and nitrogen fluxes to the soil. Further, the acting mechanisms of the vegetation as a driver of belowground processes were determined. Finally, we highlight the importance of including fine root data in carbon studies in order to develop more accurate terrestrial ecosystem models, to better understand ecosystem functioning and to be able to predict ecosystems responses to disturbances.

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CHAPTER

1

General Introduction

1.1 Global change, tropical forests and the African continent

Global change affects many different aspects of the earth´s ecosystems, such as their properties, processes and interactions supporting life. Consequently, it is a threat to the natural balance of the entire system, its resilience and integrity (Vitousek, 1994; Steffen et al., 2004). Intensification of anthropogenic activities has led to the current acceleration of global change with consequences for the earth and for human populations. Biodiversity, biogeochemical cycles, water catchments, species distribution and community assemblies are only some of the earth´s affected properties (Sala et al., 2000; Steffan-Dewenter et al., 2007; Carpenter et al., 2009; Park et al., 2010; Clough et al., 2011). Global change is thus one of the biggest challenges for mankind, as it affects a large range of our life aspects: from health, agricultural productivity and traditional practices, resource availability and energy access, through economic and social politics, to human migrations and gender-equality opportunities (Nelson and Stathers, 2009; Hil et al., 2009; Rayner, 2010; Black et al., 2011; Arora-Jonsson, 2011; Hauck et al., 2019). Understanding ecosystem functioning is crucial to assess the impacts of a changing world. Ecologists can contribute to this aim by providing baseline knowledge on how ecosystem components, ecological interactions and feedbacks work under current environmental conditions. This information can be further used in predictive models and in national, regional and local management plans to respond to, as well as to rethink how to reduce and mitigate the effects of a rapidly changing world.

The main drivers of global change are climate and land-use change. Climatic models predict an increase of future temperature, whereas the trend is not clear for precipitation patterns. Current predictions determine a high regional variability and an increasing contrast between wet and dry regions and wet and dry seasons (IPCC, 2013). Currently, half of the released atmospheric carbon is sequestered by components of terrestrial (e.g. forests and soil microbial communities) and marine ecosystems (e.g. phytoplankton, redistribution of CO_2 to deeper waters). However, there is a high uncertainty about the future effects of climate change on the carbon uptake capacity of terrestrial ecosystems, as predictive models show contradictory results as towhether they will remain carbon sinks or whether they will rather become carbon sources (IPCC, 2013). Unclear physiological responses of ecosystem components to climate change lead to further uncertainties in future emission scenarios (Huntingford et al., 2013). The increase in atmospheric CO_2, together with the higher air temperatures, altered precipitation patterns and N depositions. These are among the major factors which may potentially affect ecosystem structure and functions, ecological interactions as well as processes such as carbon sequestration, mineralization rates, soil respiration, fine root dynamics, and nutrient uptake (Norby et al., 2004; Galloway et al., 2008; Malhi et al., 2014; Crowther et al., 2016). At the same time, these factors play a feedback on climate change (Bassirirad, 2000; Cox et al., 2000; Gill and Jackson, 2000; Malhi et al., 2014).

Land-use change is the second major driver of climate change. The 25% of the global greenhouse gas emissions originate from land-use change, mainly due to deforestation, rice crops, ruminant livestock and the use of fertilizers (IPCC, 2019). In the last centuries, the rates, scale and impacts of land-use changes have reached unprecedented levels (Lambin et al., 2001), currently affecting three quarters of global land surface (IPCC, 2019). Specifically, tropical forests have experienced increasing human use, with 25-50 % of their surface being converted for different activities, including agriculture, logging and attendant fire (Lewis, 2006; Lewis et al., 2015). The underlying causes of land-use change are site-specific processes related to demographic (e.g. growth and migration of human populations), economic (e.g. product prices, consumption patterns, access to credit, available technology), political (e.g. strength of the governance and democratic institutions) and ecological (e.g. suitable conditions for agriculture) dynamics (Lambin et al., 2001; Geist and Lambin, 2002; Millenium Ecosystem Assessment, 2005; Roebeling and Hendrix, 2010). The balance between natural resource conservation and the socio-economic factors, both influenced by climate change, determines the magnitude of anthropogenic land-use conversion.

Deforestation is one of the dominant drivers of land-use change. Although forest cover declined 3-6% since 1990, there is currently a reduction tendency of the deforestation rates (FAO, 2015; Keenan et al., 2015). The elimination of the forest and consequent conversion of land to anthropogenic activities activates a chain of effects on ecosystem properties and ecological interactions, with regional and even global consequences, triggering feedbacks on the system (Canadell, 2007). The lack of vegetation entails, among other consequences, the release of CO_2, decrease of C sequestration, soil erosion, water runoff and nutrient leaching; the absence of roots affects soil stability and porosity and the lack of litterfall cover leaves the soil unprotected (Cramer et al., 2004; Canadell, 2007). Besides, the decrease and/or change in plant litter quantity and quality leads to shifts in the microbial community composition and activity, which in turn affects the N turnover rates and, therefore, soil fertility (De Deyn et al., 2008). Thus, management practices of human activities have large influence on ecosystem processes. For instance, agroforestry systems might buffer some effects of land-use conversion, as properties from the natural forest are retained in the system (Tscharntke et al., 2011). Knowledge about how land-use conversion and management practices affect ecosystem components and ecological interactions is crucial for the decision-making process of land-use managers and politicians.

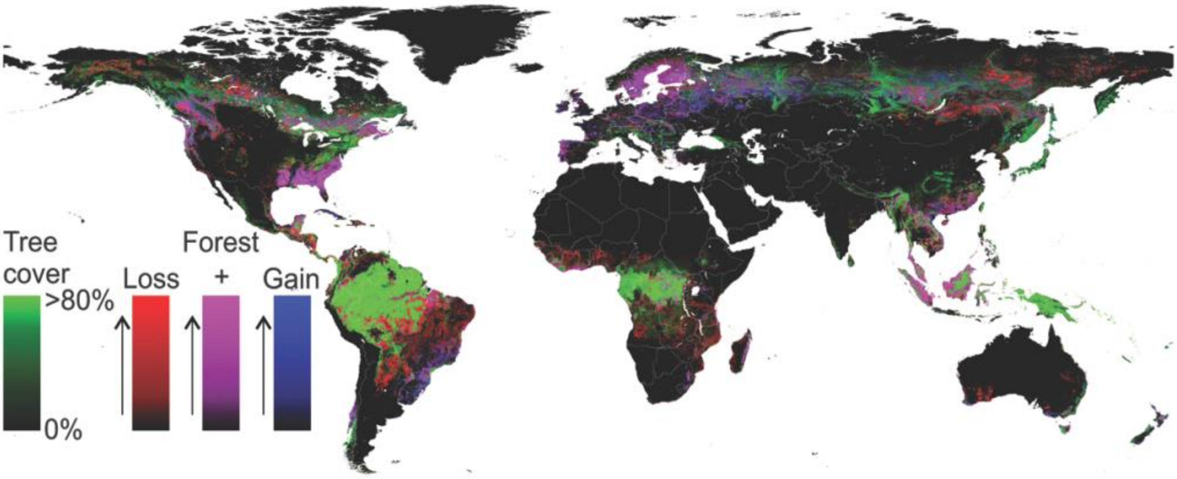

Figure 1.1: Global tree cover extent, gain and loss for the period 2000-2012. (Source: Hansen et al., 2013).

Tropical forests have a great influence on the climate and on biogeochemical cycles as they exchange large amounts of energy, water, carbon and nutrients with the atmosphere (Melillo 1993; Field et al., 1998; Lewis, 2006; Malhi, 2012). They process six times the carbon released by anthropogenic activities via photosynthesis and respiration (Lewis, 2006). Gross primary productivity (GPP) (carbon fixed in organic matter through photosynthesis) in this biome represents a 34% of global GPP, fivefold higher values than temperate and boreal forests. These high values are a result of the lack of a dormant period in tropical zones thanks to a higher irradiance, temperatures and water availability (Kricher, 2011; Malhi, 2012). They cover 13% of terrestrial area, and store 40-50 % of the C in terrestrial ecosystems (Houghton, 2005; Del Grosso, 2008; Lewis et al., 2009). Besides, these ecosystems host an extremely high biodiversity and have a strong influence on the climate through evapotranspiration, soil moisture and cloud cover (Betts, 2004, Gardner et al., 2009). Consequently, there is a strong feedback between tropical forests and climate change. Around 8% of the tropical forest area is considered montane (> 1000 m a.s.l.) (Spracklen and Righelato, 2014). Tropical montane forests maintain a cloud belt which is crucial for the maintenance of hydrological cycle (Bruijnzeel et al., 2014). Although they hold a lower GPP than tropical lowland forests (~50%), they still show a high carbon sequestration capacity (Leuschner et al., 2013; Malhi et al., 2017). In addition, they are crucial for nutrient cycles, the provision of food, medicine and wood products as well as for hosting a high biodiversity, especially of endemic species (Costanza et al., 1997; Toledo-Aceves et al., 2011). However, they are among the most threatened ecosystems in the world (Toledo-Aceves et al., 2011), mostly because of land-use and climate change (Bubb et al., 2004). Moreover, the elevational gradient inherent to mountain ecosystems entail a change of climatic conditions, vegetation structure and soil properties in a short space scale, offering the great opportunity to study ecosystem properties and processes under changing environmental conditions (Becker et al., 2007; Malhi et al., 2010).

African tropical forests play a key role for the global climate as they represent the second largest tropical forest region in the world, hosting 34% of the global tropical forest area. Through the allocation of 0.44 PgC of carbon per year in their trees (26 % of the tree C sink of tropical forests worldwide) they have a similar carbon sequestration potential than the Amazonian rain forest (Lewis et al., 2009). However, we have limited knowledge about their ecosystems' functioning, for instance, data on the processes involved in the carbon cycle on this continent (Lewis et al., 2009; Malhi, 2012). The predicted temperature increase in the region is 3 to 4°C until 2100 compared to 1999 (Christensen et al., 2007), while different trends on precipitation are reported among zones: an aggravated water stress is predicted for North Africa but an increment for the eastern part of the continent is expected (Christensen et al., 2007). An area of 11.5 Mio ha of forest cover was lost in the African continent between 2000 and 2005 (Hansen et al. 2010), the rates of forest loss in sub-Saharan Africa being among the fastest in the world (Fisher, 2010). The commodity crops (any crop that is traded) are expanding in sub-Saharan Africa, thereby increasing the pressure on tropical forests (Ordway et al., 2017). Focusing on Tanzania, the expansion rate of these crops was among the highest in Africa from 2000 to 2013 (Ordway et al., 2017). On the other hand, deforestation rates decreased in the country in the same period (Keenan et al., 2015). Deforestation of African forests may lead to a great impact on ecosystem functions and services.

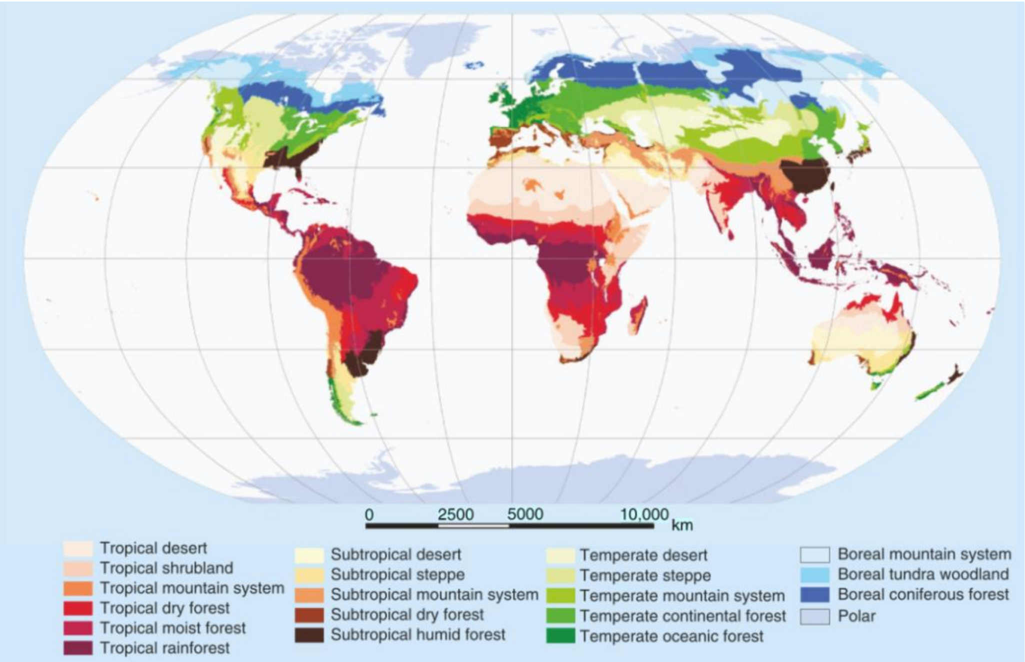

Figure 1.2: Ecological zones determined by FAO (Source: FAO, 2012).

1.2 The role of fine roots in ecosystem processes

Fine roots (i.e. ≤ 2 mm in diameter) are the essential organs for plant water and nutrient uptake. Representing the plant-soil interface, they are involved in multiple ecosystem functions and processes playing a key role in biogeochemical cycles (Vogt et al., 1996; Gill and Jackson, 2000). Even if they represent a small fraction of the total tree biomass, they are estimated to account for one third of the global annual net primary productivity (Jackson et al., 1997). Generally, they present high turnover rates and, therefore, short lifespans, from weeks to a couple of years (Eissenstat, 2000). This fact entails a rapid flux of C and N through the plant-soil interface. Besides fine root mortality, the release of exudates (e.g. sugars, aminoacids, organic acids) to the rhizosphere and the transfer of compounds to mycorrhizal fungi/N fixing bacteria, confers fine roots an important role as a source of C and N to the soil (Jones et al., 2005; Bardgett et al., 2014). Fine root litter quality has been found to be the driver of fine root decomposition (See et al., 2019). Through this property, fine roots have a main control on the soil microbial community composition and activity (De Deyn et al., 2008, Bardgett et al., 2014). For instance, plant litter with low quality (rich in lignin, high C:N ratio and N content) is more difficult to decompose. It also stimulates the growth of fungi communities over bacteria, as the former have enzymes that facilitate the decomposition of low quality material (De Deyn et al., 2008). Moreover, fine roots are the preferential substrate over leaf litter for different soil fauna organisms (Pollierer et al., 2007; Endlweber et al., 2009). In addition, they also modify their surrounding physical and chemical environment: fine root density shapes the soil affecting soil stability, porosity and bulk density (Bardgett et al., 2014). Through root penetration, they break the soil influencing moisture as well as water and nutrient leaching. By the release of ions during nutrient uptake and through rhizodeposition fine roots also alter soil chemical conditions (e.g. pH) (Jones et al., 2004; Marschner, 2012). All these functions and processes confer fine roots a strong impact on the C and N cycles.

Under different environmental conditions, plants follow distinct strategies in terms of their carbon investment into the fine root system in order to obtain the required resources. Therefore, fine root bio-necromass and dynamics (production and turnover) are affected by abiotic (e.g. mean annual temperature, mean annual precipitation, soil pH, soil fertility)as well as by biotic factors (e.g. aboveground biomass, stand basal area) (Cairns et al., 1997; Hertel and Leuschner, 2010; Moser et al., 2011). The first ones are related to soil fertility conditions and the second ones determine carbohydrate supply. Eissenstat et al. (2000) proposed a cost-benefit approach to optimize the investment of carbon and energy to the fine root system based on an acquisitive or a conservative strategy. These strategies are connected with fine root functions, which are, at the same time, intimately related to fine root morphological and chemical traits. Among the most commonly studied fine root traits are: specific root length (SRL, root length per weight), related to soil exploration; specific root area (SRA, root area per weight) connected to nutrient uptake capacity and mycorrhiza colonization; root diameter, determining the storage capacity; root tissue density (RTD, root weight per volume), linked to the carbon and nutrient storage, as well as to the defense against herbivory and drought; and root N content determining the metabolic activity such as nutrient uptake and respiration rates (Weemstra et al., 2016; Freschet and Roumet, 2017). In addition to their functional role, fine root morphological and chemical traits are assumed to affect fine root lifespan (Eissenstat et al., 2000; McCormack and Guo, 2014; Weemstra et al., 2016). However, not only carbon economics, but also biotic interactions such as mycorrhizal symbioses and herbivory drive fine root lifespan (Eissenstat, 2000).

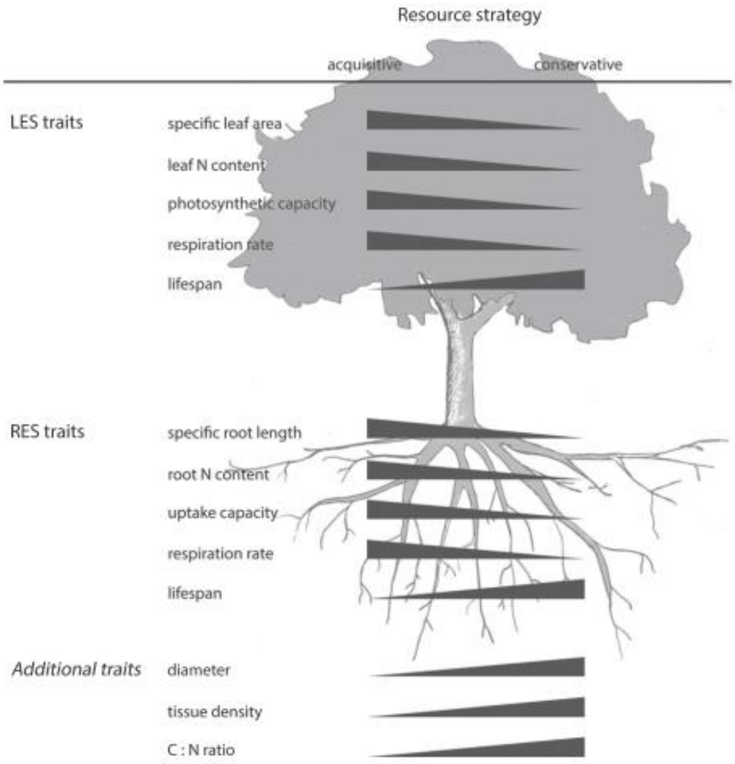

Figure 1.3: Resource economic strategies of leaves and fine roots in a leaf economics spectrum (LES) and root economics spectrum (RES) context. (Source: Weemstra et al., 2016).

Fine root traits, being genotypically determined, are also highly plastic and present a large intra and interspecific variability, being able to respond to changes in soil temperature, soil nutrient availability, as well as to species competition within a wide range of strategies of carbon investment into the fine root system (Pregitzer et al., 1993; Hodge, 2004; Chapman et al., 2012; Valverde-Barrantes et al., 2013; Bardgett et al., 2014; Kubisch et al., 2017). Studying patterns of fine root biomass, dynamics and morphological and chemical traits along different gradients contributes to a better understanding of their role in the C and N cycle, plant strategies and ecosystem functioning.

Our comprehension of the variation of the roots carbon economy among species and biomes is far from the level of knowledge we have about leaf functioning (Chen et al., 2013; Wright et al., 2014; Roumet et al., 2016). The concept of a root economic spectrum (RES), consisting on a trade-off among acquisition and conservation of resources with coordination among fine root traits is currently under research. The contradictory results between studies respond to the very different functions fine roots develope, the maintenance of mychorhiza symbiosis and the many different stimuli received from their environment (Kramer-Walter et al., 2016; Weemstra et al., 2016). Few studies have tested the existence of a RES at the community level. Further research in this direction contributes to advance in the comprehension of plant and ecosystem strategies in changing environmental conditions from a carbon economy perspective (Holdaway et al., 2011; Prieto et al., 2015; Kramer-Walter et al., 2016; Li et al., 2019).

1.3 Carbon and nitrogen cycle: the role of plants and soil microorganisms

Net primary productivity (NPP) (amount of organic carbon fixed in plant biomass as a balance of photosynthetic carbon gains and losses by autotrophic respiration) is a fundamental component of the C cycle. It depends on irradiance, climate, stand structure characteristics and functional traits, soil properties but also on nutrient availability (Schuur, 2003; Del Grosso, 2008; Moser et al., 2011; Chapin et al., 2011; Leuschner et al., 2013; Fyllas et al., 2017). The most limiting element for plant growth in most of the Earth´s ecosystems is nitrogen (N) (Elser et al., 2007). Tropical montane forest, for instance, is limited by this element (Tanner et al., 1998). Nitrogen is part of proteins, enzymes and nucleic acids among other cellular components, and is fundamental for life functioning. Carbon and nitrogen, as well as the other essential nutrients for life, are intimately related by ecological processes. Thus, it is important to understand the functioning, magnitudes and feedbacks of these cycles in tropical ecosystems as they process large amounts of these elements and are sensitive to shifts in a changing world.

Carbon allocation and storage, fine root turnover, fluxes of C and N from plant litter to the soil are key processes of the C and N cycle. Carbon allocation (how NPP is distributed in the plant) generally responds to a trade-off between plant organs in order to acquire the most limiting resource (e.g. light or water and/or nutrients) (Aragão et al., 2009; Moser et al., 2011; Malhi, 2012). At the same time, carbon allocation determines the fluxes of organic matter to the soil, as some components are more dynamic (e.g. leaves, fine roots) than others (e.g. coarse wood). Among tree components, fine roots are, by far, much less studied than the aboveground parts. Little is known about the relation of fine root traits and nutrient cycling, nutrient use efficiency or their fluxes to the soil via fine root death. In addition, to assess the carbon retention time in a pool is essential, as it controls plant carbon stocks (Friend et al., 2014). Alteration of the balance between C and N pools and its fluxes can lead to losses of these elements from the system. Fluxes of plant litter constitute a large input of organic matter to the soil. Their magnitude is determined by plant litter turnover rates and nutrient content, which depend on climatic conditions and soil fertility (Chapman et al., 2006; Becker et al., 2015). At the same time, these fluxes create a feedback on soil fertility through the return of nutrients to the soil (Chapman et al., 2006).

The tight relation among plants and soil microorganisms links the C with the N cycle (Paul, 2015). Plants provide substrate to soil microbiota through plant litter and root exudates, whereas the soil microorganisms provide them with plant N available forms. Plant litter is used as substrate by soil fauna and microorganisms for their growth. Part of this matter will stay as biomass in the soil fauna and microbial community, while another fraction will be released to the atmosphere by respiration. At the same time, this organic matter from plant litter can become part of the soil organic matter (SOM), defined as the ensemble of plant, microbial and faunal-derived debris in different stages of decomposition, microbial by-products, exudates and the microbial biomass itself (Gougoulias et al., 2014). SOM is an important stable carbon stock (60% of total soil C stocks) that can persist for thousands of years (Lal, 2008). Climate and land-use change can destabilize the steady state of SOM resulting in either C accumulation or losses (Davidson and Janssens 2006). Fine root litter contributes to a high proportion of the carbon stored in SOM, due to its high content of recalcitrant compounds that are difficult to decompose, but especially due to the formation of organominerals which confers physio-chemical protection to the organic matter against further mineralization (Rasse et al., 2005). Despite the important role of fine root fluxes to the soil and the use of root litter as substrate by soil fauna and the microbial community, there is not much information about these fluxes.

Soil microbial activity depends on temperature, soil moisture, soil properties (pH, texture, mineralogy), soil C and N content and plant litter quality (Booth et al., 2005; Davidson and Janssen, 2006; Gerschlauer et al., 2016). Among these factors, litter quality is the major driver of decomposition rates at a regional scale (Aerts, 1997). Together with the amount of plant litter, it affects microbial activity: high plant litter quality (high amount of C substrate and/or low C:N plant litter ratio) induce mineralization, while the contrary leads to immobilization and retention of N in the microbial community (Booth et al., 2005; Robertson and Groffman, 2007). Plant litter quality not only affects microbial activity but also its composition (De Deyn et al., 2008). Plant litter and the microbial community Together, these two components affect microbial efficiency and thus, the amount of CO_2 released to the atmosphere through respiration as well as the soil N available to plants.

There is a strong competition among fine roots and soil microorganisms for the same resources in the short term. However, in the long term, soil microorganisms are a source of N for fine roots. Rhizodeposition stimulates microbial activity and growth, triggering the breakdown of soil organic matter (a process known as ‘priming’) (Kuzyakov, 2000). After the growth of microbial biomass, it is consumed by protozoa and nematodes, with the consequent release of ammonia to the soil (Kuzyakov and Xu, 2013). Therefore, microbes retain for a time N, avoiding its losses, but, aftertheir consumption by other soil organisms, they provide this N to plants, this mechanism being called the ‘microbial-loop’ (Clarholm, 1985; Bonkowski, 2004).

Land-use change results on strong impacts on carbon and nitrogen cycles. In addition to changes related to the shifts on the plant community structure, species composition and management practices, land-use change has increased enormously the amounts of nitrogen available for plants due to the higher use of legume sp., fossil fuel combustion and N fertilizer (Bottomley and Myrold, 2007). The increase of grasslands and agriculture replacing forests has led to the liberation of protected N in soil organic matter. Nitrogen inputs entail positive (e.g. stimulation of plant, microbe growth, N immobilization), as well as negative (soil and aquatic acidification, nitrate leaching) consequences (Bottomley and Myrold, 2007).

The control of biogeochemical cycles in tropical ecosystems is extraordinarily complex. There is a need of studies on C and N cycling in tropical forests to understand how plant communities respond to different environmental conditions and consequently predict future changes (Clark et al., 2001). Specifically, it is necessary to include the response of ecosystem components to the N cycle in predictive models, as this essential element for life has a great influence on the C cycle and global climate. Studies on direct interactions of nutrients with carbon in tropical ecosystems are scarce (Townsend et al., 2011).

1.4 Climate effects on ecosystem properties and processes

Mountains present steep climate gradients across a small spatial scale. Thus, they are powerful natural laboratories to study the influence of environmental factors on ecological processes (Becker et al., 2007). The current properties and processes along elevational gradients have been shaped by the interactions among biotic and abiotic factors during long periods of time, leading to acclimation and adaptation of the species (Malhi et al., 2010). The rapid changes of the environmental conditions in a small spatial scale lead to high species turnover rates, mountains being biodiversity hotspots (Myers et al., 2000). In addition, mountain ecosystems are very sensitive to climate change. Species living in these systems typically have very constrained niches, especially in the tropics. Thus, small alterations of environmental conditions threaten the existence of these species (Janzen, 1967; Körner, 2003; Huber et al., 2005). Tropical mountain ecosystems are additionally more vulnerable to climate change as the magnitude of the predictive change in temperature is higher than for temperate regions (Schüler, 2012). Studies on environmental effects on ecosystem components, functions and processes across tropical elevational gradients are therefore crucially important. The insight gained from such studies may help to develop reliable predictions in the context of global change (Blois et al., 2013).

Across the elevation gradient, there are multiple interactions among changing climatic factors, soil properties and soil microbial and plant communities. The decrease of temperature along the slope, entails lower metabolic activity leading to a decrease of mineralization rates and to N limiting conditions for plants (Becker and Kuzyakov, 2018). Changes in precipitation affect soil moisture, which in turn has effects on microbial activity (Gerschlauer et al., 2016). At high elevations, there is an accumulation of soil organic matter resulting from low decomposition rates (Moser et al., 2010). Stand structure characteristics also change along the elevation. Tree height and aboveground biomass decrease, whereas stem density increases (Kitayama and Aiba, 2002; Moser et al., 2011). The interactions of these factors have consequences on the plant carbon investment strategies and on plant ecological competitiveness (Marschner, 2007; Moser et al., 2011). Not only N limitation, but strong winds, high UV radiation, fog and steep orography characterize high elevation ecosystems and determine the adaptations of plant species' physiognomy to these extreme conditions (Smith and Young, 1987; Holtmeier, 2009).

1.5 Land-use change and ecosystem disturbance at Mt. Kilimanjaro

At Mt. Kilimanjaro foothills, 40% of the savanna woodlands, dry forest and bushlands have been converted to crop fields in the period from 1976 to 2000. The predictions of a strong increase in African population growth until the year 2050 (United Nations, 2013) together with ecological, political and economic dynamics (Lambin and Meyfroidt, 2010), might probably increase the conversion of savannas to agricultural fields. Savanna ecosystems maintain important functions as they are hotspots for biodiversity, keep high amounts of C stocks in its soil and, at Mt. Kilimanjaro, are corridors for species migration between mountains (Hemp and Hemp, 2018; Peters et al., 2019).

In the lower montane zone, "chagga homegardens" cover 64 % of the eastern slopes of Mt. Kilimanjaro (Soini, 2005). In the last decades, homegarden size has decreased, with fewer trees than before and farm owners have intensified their production due to the lack of available land. Besides, the strong heritage traditions in Tanzania difficult the joining of homegardens from different owners (Maghimbi, 2007). Due to low coffee prices, farm owners have started to diversify their crops to groundnuts, sunflowers, tomatoes or green peppers (Soini, 2005) and even started to uproot the coffee plants, to grow vegetables or make space for cattle (Mhando, 2005). Others have focused on maize and rice growth in other areas of the Kilimanjaro region (Maghimbi, 2007). This agroforestry system, and the ecological functions it shares with the lower tropical montane forest, is therefore in danger. Similarly, coffee plantations have decreased their production in the last years due to higher temperatures, lower precipitation and because of the management of the plantations (Kumburu, 2012; Craparo et al., 2015). The coffee cooperatives are incentivizing the use of shade trees to lower the temperature and improve the harvest (Kumburu, 2012).

The Kilimanjaro National Park provides protection against tropical montane forest logging since 1974 in the middle montane *Ocotea* forest and since 2005 in the lower montane forest. Only the collection of fallen branches for firewood is allowed in the lower zone. However, at high elevations, lighting-caused fires and human-induced fires are intensified with drier conditions due to climate change and have led to the loss of 150 km^2 of upper montane and ericaceous forest since 1976 (Hemp, 2009). Preservation of these forests is critical, as they have a high potential for collecting cloud water (Bruijinzeel, 2001; Hemp, 2005) and providing this resource to the population settlements downhill.

1.6 Effects of spatial vegetation heterogeneity on ecosystem processes of extreme environments at Mt. Kilimanjaro

Vegetation has a strong influence on belowground processes through litter quality and root exudation, as well as through competition for nutrients.At the same time, soil microbial activity executes a feedback on the performance of the plants (Rennenberg et al., 2009; Chapman et al. 2006; Rodríguez et al., 2016). Thus, the linkage among plants and decomposers is crucial for ecosystem functioning (Wardle et al., 2004). In stressful ecosystems, there are plant species that, not only create microhabitats facilitating the establishment of other species (Jones et al., 1997; Callaway, 2007), but are co-drivers of essential ecosystem functions (Navarro et al., 2008). The patchy vegetation of these ecosystems allows the study of the tight relationship among above and belowground processes.

Spatial heterogeneity of vegetation is especially frequent in tropical ecosystems (Houghton et al., 2009), such as savanna woodlands and *Helichrysum* heathlands. These ecosystems are present at the lower and upper zones, respectively, of the elevation gradient at Mt. Kilimanjaro. The harsh conditions of these two ecosystems, to which plant species have developed adaptations, together with their spatial heterogeneity make them attractive sites for ecological research. In addition, both ecosystems are threatened: savannas by land-use conversion, fires and desertification and tropical alpine ecosystems by climate change (Goldewijk, 2001; Lambin et al., 2003; Meyer et al., 2007; Buytaert et al., 2011).

Savannas cover 20% of the land surface and 50 % of the African continent, they constitute a hotspot for biodiversity and wildlife conservation and account for 26% of terrestrial NPP (Scholes and Walker, 1993; Campbell, 1996; Malhi, 2012), having a great influence on the global C cycle. They are characterized by strong seasonal water limitation leading to strong eco-physiological adaptations of plants (Chen et al., 2004). The matrix of trees and grasslands results in a heterogeneous vegetation, which influences abiotic and biotic characteristics as well as ecosystem processes (Ludwig et al., 2004; Otieno et al., 2015). However, studies on the interactions among a broad number of ecosystem components and processes in order to understand the mechanistic controls of trees on ecosystem C and N cycling are still scarce. The water limiting factor together with the presence of N-fixing and non-fixing trees in savanna ecosystems makes the study of interactions of above and belowground processes in this ecosystem even more interesting.

Tropical alpine ecosystems are among the least studied ecosystems on earth (Buytaert et al., 2011). Plants in these ecosystems are adapted to extreme daily variation in solar radiation, air temperature and relative humidity (Nagy and Grabherr, 2009), as well as high UV-B radiation, low air water vapor pressure, winds, fog and, in some cases, seasonal drought (Smith and Young, 1987). Productivity is limited by soil N and phosphorous (P) availability (Güsewell, 2004). They also present heterogeneous vegetation, with plant patches among bare soil. Daily soil temperature variations, as well as contribution of plant litter to the soil, affect microbial activity, both being important controls of C and N cycling. These factors are influenced by the spatial distribution of vegetation. Despite the important role of soil N turnover and plant availability in constraining potential changes to the C balance, these processes are still poorly understood in high latitude and high altitude ecosystems (Weintraub and Schimel, 2005).

1.7 Project framework

The combined effects of global warming and land-use conversion to human-modified systems as well as land-use intensification are threatening biodiversity and ecosystem processes maintained by tropical ecosystems (see section 1.1). However, the impact of global change on biodiversity, biotic interactions (e.g. herbivory, parasitism, pollination, seed dispersal, decomposition, pathogen infections) and biogeochemical processes (e.g. water and nutrient fluxes, ecosystem carbon and nitrogen budgets) is still poorly understood

(Hegland et al., 2009, Forrest and Thompson, 2011, Schleuning et al., 2012). The steep changes of environmental conditions along mountain ecosystems provide a good opportunity to study ecological processes (and eventually the resulting ecosystem services). Specifically, Mount Kilimanjaro, in northern Tanzania, covers a huge elevational gradient from semi-arid lowlands through tropical montane forest to alpine vegetation, together with human-modified systems, being suitable for the study of ecosystem processes under global change.

The present study is part of the joint research project "Kilimanjaro under global change: Linking biodiversity, biotic interactions and biogeochemical ecosystem processes" (KiLi project), which is funded by the German Science Foundation (DFG research unit FOR1246). The main aim of this research unit is to assess the interactions among biodiversity and ecosystem processes along elevational gradients in both natural and human-disturbed ecosystems at Mt. Kilimanjaro (Tanzania, Africa). Within this context the major goals of the project are (KiLi proposal 2013):

1. Inferring the influence of climate and anthropogenic disturbance on both biogeochemical processes and biodiversity.

2. Quantifying biodiversity-ecosystem functioning relationships along elevational gradients.

3. Estimating resilience and adaptive potential of natural and modified ecosystems to global change.

4. Examining negative feedbacks of disturbance on local climate and ecosystem processes.

5. Quantifying temporal shifts in species distributions due to climate and land-use change.

The KiLi project is formed by seven closely linked subprojects and two central projects for coordination, data management and synthesis. The subprojects developed field data sampling, laboratory work; experimental studies; meta-analyses and modeling. They addressed diversity at different levels, from microbes to larger organisms, as well as biotic interactions, ecosystem properties and dynamics along the climate and land-use gradients.
Within this project framework, the present PhD research was embedded in the subproject 2, addressing above and belowground processes related to the plant system, with a special emphasis on fine roots (biomass, dynamics, morphological and chemical traits and fluxes with fine root mortality) representing the plant-soil interface. The investigation was developed in the framework of ecosystem functioning along elevation and land-use gradients and involved field data collection and laboratory work.

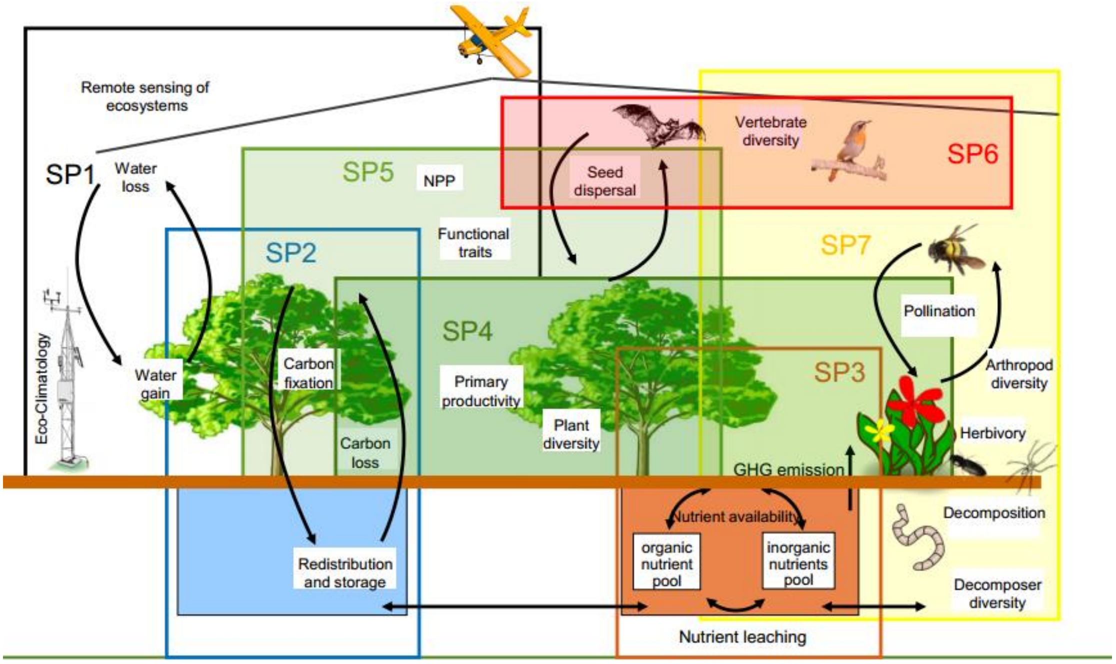

Figure 1.4: Interdisciplinary approach of the KiLi project, subprojects and addressed ecosystem properties and processes. (Source: Steffan-Dewenter, 2013).

1.8 Study area

The study sites are located on the southern and south-eastern slopes of Mt. Kilimanjaro in northern Tanzania (3°4´33´´S, 37°21´12´´E). The study covered an elevational gradient of 3680 m (870 to 4550 m a.s.l.), reaching from colline savanna to alpine *Helichrysum* heathlands. Vegetation at Mt. Kilimanjaro exhibits a vertical zonation (Hemp, 2006). Within each elevation zone, except for the alpine belt, natural and semi-natural ecosystems, together with areas of human-induced disturbance are present. The study design consists of 5 replicates of each natural and disturbed ecosystem type along the elevation. For the present research, 55 plots of 0.25 ha size were sampled. Detailed information about species composition in the different vegetation zones is described in Hemp (2006). Briefly, the foothills of the mountain (800 - 1100 m a.s.l.) are covered by savanna woodlands with *Acacia-Commiphora* trees and C_4 grasses dominating the landscape. This ecosystem is disturbed by its conversion into maize fields. In the densely populated area upslope, we selected two agroforestry systems to represent human-induced disturbance of the forest at this vegetation zone (1100-1800 m a.s.l.). The first one, the traditional multicrop system known as “Chagga homegarden”, consists of a mixed system of banana and coffee as dominant crops, together with cultivated fruit trees (e.g. *Persea americana*) and shade-tolerant crops (e.g. taro, yams and beans) under remnant forest trees (e.g. *Albizia schimperiana*, *Grevillea robusta*). The second one, coffee plantations, consists of coffee trees together with a low number of shade trees (e.g. *Gravillea robusta*, *Albizia sp*.). The lowland montane forest (1600 and 2000 m a.s.l.) is characterized by *Macaranga kilimandscharica, Agauria salicifolia* and, to a lesser degree, *Ocotea usambarensis*. The middle montane forest (*Ocotea* forest) (2100 and 2800 m a.s.l.) is dominated by *Ocotea usambarensis*, *Ilex mitis*, *Xymalos monospora* and the tree fern *Cyathea manniana*, and contains a dense understory layer. Due to its high commercial value, *Ocotea sp*. has been a target for selective logging until the year 1984 (Agrawala, 2013), resulting in some areas of disturbed forest. The upper montane *Podocarpus* forest (2700 - 3100 m a.s.l.) hosts *Podocarpus latifolius* as the dominant tree species, together with *Hagenia abyssinica* and *Prunus africana*. Human-induced fires changed species composition and forest structure, natural vegetation being replaced by *Erica excelsa* trees, which re-sprout from stumps after fire, leading to monodominant *Erica excelsa* patches (Hemp, 2005). In the highest forest zone, which reaches up to 3900 m a.s.l, *Erica* bushlands with some remnants of *Erica trimera* forests are dominant. We refer to the forest ecosystems described above as Mt. Kilimanjaro tropical montane forest.

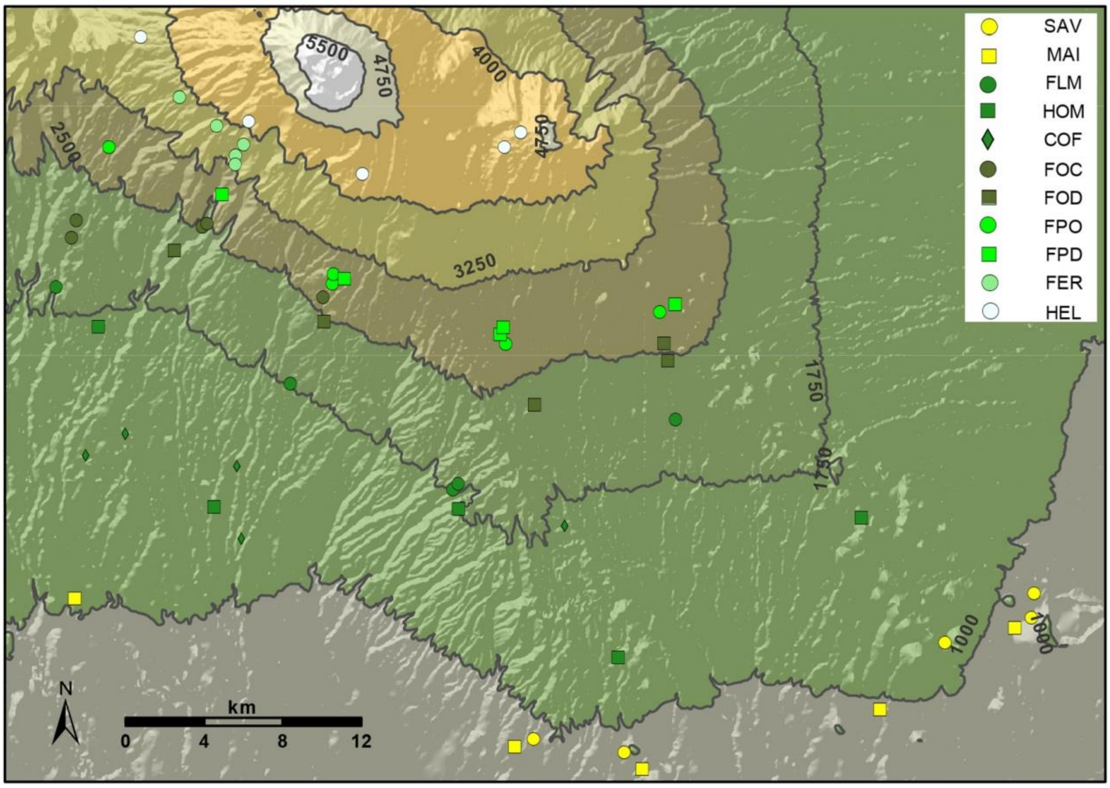

Figure 1.5: Study design consisting in 55 plots representing natural and human-induced ecosystems characteristic of the vegetation belts at Mt. Kilimanjaro. Plots are distributed in five transects along the elevation. SAV= savanna woodlands, MAI= maize fields, FLM= lower montane forest, HOM= homegardesn, COF= coffee plantations, FOC= Ocotea forest, FOD= Ocotea forest disturbed, FPO= Podocarpus forest, FPD= Podocarpus forest disturbed, FER= Erica forest, HEL= Helichrysum heathland. (Modified from Peters et al., 2019).

Higher up, in the tropical alpine zone (4550 m a.s.l.), heathlands with dwarf shrubs of *Helichrysum sp*. together with grasses dominate the landscape. The tropical montane forest and alpine plots are located inside Kilimanjaro National Park (except two lowland montane forests), while the savanna plots, maize fields, chagga homegardens and coffee plantations are outside the protected area.

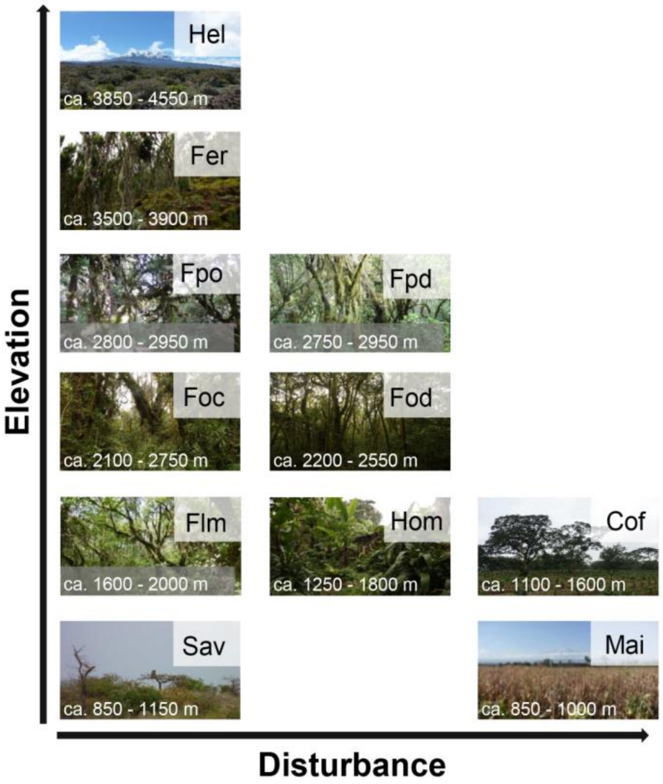

Figure 1.6: Study sites along the elevation and disturbance gradient at Mt. Kilimanjaro. SAV= savanna woodlands, MAI= maize fields, FLM= lower montane forest, HOM= homegardesn, COF= coffee plantations, FOC= Ocotea forest, FOD= Ocotea forest disturbed, FPO= Podocarpus forest, FPD= Podocarpus forest disturbed, FER= Erica forest, HEL= Helichrysum heathland. (Modified from Hemp, 2013).

Mean annual temperature ranges from 25°C at the foothills to 3°C in the afroalpine zone (Appelhans et al., 2015). Precipitation is characterized by a long rainy season from March to May and a shorter one around November (Hemp, 2006). Along the slope, mean annual precipitation exhibits a unimodal pattern with minimum values around 800 mm and maximum values around 2100 mm (Hemp, unpublished data).

The soils on the Kilimanjaro massif all have a roughly similar age and developed from the same volcanic deposits (Dawson, 1992). In the savanna, vertisols have developed, while in the forest zone andosols are predominant (Zech et al., 2014). In the alpine zone, soils are characterized as Leptosols and Vitric Andosols (WRB 2014).

1.9 Thesis structure and research hypothesis

This thesis dissertation consists of 5 studies and aims the following main objectives:

- To assess patterns of fine root biomass, dynamics and morphological traits as well as to clarify the existence of a RES across changing environmental condition along the elevation.
- To quantify above- and belowground NPP and carbon allocation patterns along the elevation and assess their relationship to abiotic and biotic factors.
- To determine the impacts of land-use change and ecosystem disturbance on the fine root system (biomass, dynamics and morphological and chemical traits) at different types of human-modified ecosystems.
- To disentangle the effects of vegetation on above-and belowground processes in ecosystems with strong resource limitation and high spatial vegetation hetereogeneity.

1. The first study focused on the fine root dynamics and fine root morphology in the six major natural ecosystem types across the elevation (Chapter 2). The study was divided int two sections: the first part covered the entire elevational gradient, from savanna woodland to *Helichrysum* heathlands. This approach enabled the comparison of the fine root system of ecosystems with different limiting resources and plant life forms. The second part focused only on the tropical montane forest, allowing the assessment of elevation effects and associated abiotic and biotic factors on the fine root related variables. Furthermore, it allowed the exploration of evidence in support of a RES.

   The following hypotheses were tested:

   (i) In ecosystems with harsher environmental conditions and lower productivity (in particular savanna and alpine scrub), FRB is lower and fine root turnover and root: shoot: ratios are higher than in the moist montane forest belt.

   (ii) The FRB:AGB and FRP:AGB ratios increase with elevation due to increasing nutrient (N) limitation.

   (iii) The independent and partly opposing trends in temperature, moisture and nutrient availability with elevation together with a turnover of species and functional types lead to great variation in root traits, which do not fit to a one-dimensional RES.

   In order to test these hypotheses, a fine root biomass inventory and the ingrowth core approach to estimate fine root production were carried out down to 40 cm soil depth. Mean fine root lifespan was estimated from the fine root biomass and production. Fine root morphological traits were determined by scanning the samples obtained in the fine root biomass inventory and N content was analyzed. Differences among fine root related variables of the natural ecosystems along the entire elevation were tested. Along the tropical montane forest, the relationships among fine root related variables and abiotic and biotic factors were addressed. In addition, we assessed the existence of a RES by determining the relationships among the fine root morphological and chemical traits.

2. The second study was limited to the natural ecosystems of the tropical montane forest. We aimed to quantify above- and belowground NPP and assess patterns of C allocation and aboveground wood residence time along the elevation. Additionally, the return of C and N to the soil via leaf and fine root mortality in the different ecosystems was estimated (Chapter 3).

   The following hypotheses were tested:

   (i) Total NPP declines and C allocation shifts to the fine root system as a consequence of increasing N limiting conditions with elevation.

   (ii) Nutrient use efficiency of productivity is higher at high elevations as a respond to the low soil N availability.

   (iii) C and N fluxes from fine root mortality to the soil are more important than from leaf litter at high elevations as a result of plant species adaptation to the harsh conditions.

   The annual NPP of aboveground wood (monthly stem increment measurements), aboveground litterfall (monthly collection of litterfall traps), fine root production (sequential coring approach down to 40 cm soil depth) and coarse roots (estimation from aboveground NPP) were estimated in three transects along the elevational gradient. By combining values of biomass and production, we obtained the carbon residence time for aboveground wood, coarse and fine roots. Chemical composition of the aboveground litter and fine roots was analyzed and the carbon and nitrogen fluxes to the soil via leaf and fine root mortality were estimated. In addition, nitrogen use efficiency (NUE) values from aboveground literfall and fine roots were calculated. Differences among the NPP components, total NPP, C and N fluxes via fine root and leaf litter, as well as canopy and fine root NUE were tested among the forest ecosystems along the slope. Further, the relationships of NPP components with changing environmental conditions along the slope were assessed.

3. In the third study, the impacts of land-use change and disturbance on ecosystem properties and processes involving fine roots were assessed (Chapter 4). To this end, we quantified fine root biomass and dynamics, C and N fluxes to the soil via fine root mortality and fine root morphological and chemical traits at four elevational zones (from the colline to the upper montane forest zone) covering different natural and disturbed ecosystems.

   This study aims the following objectives:

   (i) To determine the differences on fine root biomass, dynamics and traits among natural/semi-natural and disturbed ecosystems in each elevation zone.

   (ii) To assess the effects of land-use change on the C and N fluxes to the soil via fine root litter.

   To develop this study, a fine root biomass inventory and the estimation of fine root production with the ingrowth core method down to 40 cm soil depth was carried out. Turnover rates were estimated from fine root biomass and production. Fine root morphological traits were determined by scanning the samples from the fine root biomass inventory and N content was analyzed. Differences of the fine root related variables among natural and disturbed ecosystems in the different elevation zones were tested. Relationships among fine root related variables and abiotic and biotic factors were addressed as well.

4. In the fourth study, we investigated the effects of different vegetation cover categories on gross N turnover rates, greenhouse gases, soil properties and fine root density in the tropical alpine *Helichrysum* heathlands (Chapter 5).

   The objectives of this study were:

   (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.

   (ii) To analyse the influence of precipitation and freeze thaw cycles on biogeochemical processes.

   Vegetation composition in the three different vegetation cover categories (low, medium, high) was characterized. Major gross N turnover rates were investigated during a 6 day period with the 15N pool dilution method. In addition greenhouse gas exchange (CO_2, N_2O and CH_4) was manually measured with static chambers. Microbial biomass carbon and nitrogen were analyzed with the fumigation-extraction method and fine root density was estimated by differentiating among living and dead roots down to 10 cm depth. Furthermore, soil properties were assessed. Differences among the estimated factors based on vegetation-cover categories were tested. Relationships among gross GHG, N turnover and soil parameters were conducted across all nine plots and drivers of N gross rates and GHG emissions were determined.

5. The fifth study was settled on savanna woodland. Spatial trends in soil properties and trace-gas fluxes during the dry season were determined and above- and belowground processes and attributes were related (Chapter 6).

   The following hypotheses were tested:

   (i) Soil C and nutrient contents increase with the presence of trees through increased litter inputs (independent of tree species).

   (ii) Lower litter quality outside the crown area result in reduced N availability.

   (iii) C mineralization increase due to higher microbial N mining outside the crown area.

Three trees from each of the two most dominant species were chosen: the leguminous *Acacia nilotica* and the non-leguminous *Balanites aegyptiaca*. At each sampling point of the six transects (ranging from the selected trees to the open grassland) we measured: C and N content, $\delta^{13}C$ of soil (0-10, 10-30 cm depth) and of 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. Differences of these factors under the crown and in open grasslands were tested and relationships among variables were examined.

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CHAPTER

2

Biomass, morphology and dynamics of the fine root system across a 3000 m-elevation gradient on Mt. Kilimanjaro

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2.1 Abstract

Fine roots (≤ 2 mm) consume a large proportion of photosynthates and thus play a key role in the global carbon cycle, but our knowledge about fine root biomass, production and turnover across environmental gradients is insufficient, especially in tropical ecosystems. Root system studies along elevation transects can produce valuable insights into root trait-environment relationships and may help to explore the evidence for a root economics spectrum (RES) that should represent a trait syndrome with a trade-off between resource acquisitive and conservative root traits. We studied fine root biomass, necromass, production and mean fine root lifespan (the inverse of fine root turnover) of woody plants in six natural tropical ecosystems (savanna, four tropical mountain forest types, tropical alpine heathland) on the southern slope of Mt. Kilimanjaro (Tanzania) between 900 and 4500 m a.s.l.. Fine root biomass and necromass showed a unimodal pattern along the slope with a peak in the moist upper montane forest (~2800 m), while fine root production varied little between savanna and upper montane forest to decrease towards the alpine zone. Root:shoot ratio (fine root biomass and production related to aboveground biomass) in the tropical montane forest increased exponentially with elevation, while it decreased with precipitation and soil nitrogen availability (decreasing soil C:N ratio). Mean fine root lifespan was lowest in the ecosystems with pronounced resource limitation (savanna at low elevation, alpine heathland at high elevation) and higher in the moist and cool forest belt (~1800-3700 m). The variation in root traits across the elevation gradient fits better with the concept of a multi-dimensional RES, as root tissue density and specific root length showed variable relations to each other, which does not agree with a simple trade-off between acquisitive and conservative root traits. In conclusion, despite large variation in fine root biomass, production and morphology among the different plant species and ecosystems, a general belowground shift in carbohydrate partitioning is evident from 900 to 4500 m a.s.l., suggesting that plant growth is increasingly limited by nutrient (probably N) shortage toward higher elevations.

Keywords

Afroalpine heathland, fine root biomass, fine root production, root economics spectrum, root traits, root:shoot ratio, savanna, tropical montane forest

2.2 Introduction

Fine roots (i.e. roots ≤ 2 mm in diameter) are a small but functionally important component of plant biomass, which controls the uptake of water and nutrients and influences biogeochemical cycles through rapid biomass turnover (Vogt et al., 1996; Gill and Jackson, 2000). The lifespan of fine roots, in particular of the smallest 1st and 2nd order rootlets, is short, in woody plants typically not more than a few months (Eissenstat et al., 2000). Despite representing only a few percent of plant biomass, fine roots have been estimated to consume about a third of annual global net primary production, and they represent a major source of soil carbon through root death and rhizodeposition (Jackson et al., 1997; Jones et al., 2004).

Under contrasting environmental conditions, trees adopt different strategies of resource allocation to the fine root system, which all aim at minimizing the resource investment needed to secure nutrient and water uptake. These strategies vary with the species and functional groups, and in dependence on climatic and edaphic factors such as temperature, precipitation, nutrient availability, soil acidity and soil bulk density (Cairns et al., 1997; Hertel and Leuschner, 2002; Finér et al., 2011). In correspondence, large variation in fine root system size, mean fine root diameter, branching patterns, and fine root turnover has been found in different forest types, even when the general climate is similar (Leuschner and Hertel 2003; Finér et al., 2011).

Of the fine root properties, lifespan (i.e. the inverse of biomass turnover in a fine root population) has a particularly large influence on the carbon (C) and nitrogen (N) cycles in the rhizosphere and bulk soil. In the last decades, considerable research effort has been directed to those factors that are assumed to control fine root lifespan in different plant life forms and ecosystem types (Eissenstat et al., 2000; Yanai and Eissenstat, 2002; McCormack and Guo, 2014). Among the factors influencing root lifespan are seasonality, edaphic and climatic factors, and biotic conditions, notably species identity, assimilate availability, root infection by mycorrhiza or pathogens, and competition intensity (Eissenstat et al., 2000; Norby and Jackson, 2000; Eissenstat et al., 2013). Root morphological and chemical traits such as specific root length (SRL) (root length per root biomass), root tissue density (RTD) (root biomass per root volume), root N content, and root diameter may also have an important influence on fine root lifespan (Weemstra et al., 2016). Compared to the major advances in our knowledge about leaf functioning (Reich et al., 1997; Wright et al., 2014), little success has been made until recently in the understanding of variation in the carbon economy of roots from different species, functional types and biomes (Chen et al., 2013; Roumet et al., 2016).

Advancement in our understanding may happen with the introduction of the concept of a root economics spectrum (RES), which mirrors the leaf economics spectrum (LES). The RES concept postulates that a trade-off between acquisition and conservation of resources exists in fine roots, with covariation of fine root traits at the species and ecosystem level (Weemstra et al., 2016; Kramer-Walter et al., 2016; Li et al., 2019). Plants which follow an acquisitive belowground strategy should build roots with high nutrient and water uptake capacity, which typically is linked to high root N content, small root diameter and high specific root length (SRL) and surface area (SRA), and relatively short lifespan. In contrast, the roots of plants with a conservative belowground strategy should maintain roots with relatively low resource uptake rates, low N content, large root diameters but small surface development (low SRL and SRA), and long lifespan. While several trait correlations have been reported (e.g. a negative relation between root N content and lifespan, and a positive between root diameter and lifespan; McCormack et al., 2012; Reich, 2014), the existing empirical evidence for a one-dimensional RES along the acquisitive-conservative axis is not consistent (Weemstra et al., 2016). While a multi-dimensional RES may be a more appropriate concept (Kramer-Walter et al., 2016), it remains unclear whether a RES, if it exists at the species level, can be extrapolated to the community level, as very few studies have tested this hypothesis in natural ecosystems (Holdaway et al., 2011; Prieto et al., 2015; Kramer-Walter et al., 2016; Li et al., 2019). Further studies across different plant functional types and ecosystems along climatic and edaphic gradients using standardized methods are needed before the more general validity of a RES can be accepted.

Mount Kilimanjaro in tropical eastern Africa represents a unique place to study the change in root traits and root system properties along steep gradients in temperature, precipitation and nutrient (nitrogen) availability at a regional scale. The mountain hosts a great variety of tropical ecosystems including various mountain forest types, savanna woodland, and alpine scrub vegetation, which are dominated by different plant functional types. The plants in these communities represent contrasting life strategies and are constraint by different environmental factors including drought, nutrient deficiency and cold as well as disturbance agents such as fire and intensive herbivory. As a consequence, plant-internal allocation strategies and root:shoot ratios differ largely, which should lead to broad variation in rooting patterns and root dynamics across the Mt. Kilimanjaro ecosystem matrix. As far as we know, elevation transect studies on fine root biomass and its dynamics have been conducted in tropical mountains of South America (Ecuador: Röderstein et al., 2005; Graefe et al., 2008; Moser et al., 2011, Peru: Girardin et al., 2013, Bolivia: Hertel and Wesche, 2008) and South-east Asia (Malaysia: Kitayama and Aiba, 2002), but not in the tropical mountains of Africa. So far, no clear over-regional pattern has emerged from these studies, highlighting the need for further research. Studies along elevation transects may also allow predictions about future warming effects on the root system if the studied environmental matrix allows separating the driving factors.

In the framework of a comprehensive investigation of ecosystem structure and functioning along elevation and land-use gradients on Mt. Kilimanjaro (the 'KiLi Project' of the German Science Foundation DFG), we studied the fine root dynamics and fine root morphology in six major natural ecosystem types on the southern slope of Mt. Kilimanjaro. Main study aim was to identify patterns of fine root biomass, dynamics and traits along gradients in elevation and associated environmental factors, and to explore the evidence in support of the existence of a RES. Fine root biomass (FRB) and productivity were related to aboveground biomass (AGB) to obtain a measure of belowground carbon partitioning in the different ecosystems. From existing literature overviews of fine root biomass patterns (Leuschner and Hertel 2003, Hertel and Leuschner 2010; Finér et al., 2011), we hypothesized that (i) in the ecosystems with harsher environmental conditions and lower productivity (in particular savanna and afroalpine scrub), FRB is lower and fine root turnover and root:shoot ratios are higher than in the moist montane forest belt, (ii) the FRB:AGB and FRP:AGB ratios increase with elevation due to increasing nutrient (N) limitation; and (iii) the independent and partly opposing trends in temperature, moisture and nutrient availability with elevation together with the turnover of species and functional types lead to great variation in root traits, which do not fit to a one-dimensional RES.

2.3 Methods

2.3.1 Study area and design

The study was carried out within the framework of the KiLi project, a larger interdisciplinary research group (DFG-FOR1246) focused on "Kilimanjaro ecosystems under global change: linking biodiversity, biotic interactions and biogeochemical ecosystem processes". It is based on the premise that Mt. Kilimanjaro exhibits a vertical zonation of vegetation belts (Hemp, 2006a), which are addressed by studying representative ecosystem types. Our study sites correspond to the plots established in the joint design of the KiLi project, which were selected in terms of representability for a given ecosystem type. Plots were located in core zones of the vegetation belts in order to avoid ecotones (Peters et al., 2019). The plots are located in northern Tanzania (3°4´33´´S, 37°21´12´´E) on the southern and south-eastern slopes of the mountain. Due to their exposure to humid air masses advected from the Indian Ocean, the southern slopes of Mt. Kilimanjaro are characterized by higher humidity than the northern ones. We chose the moister side of the mountain because ecosystem diversity is greater here and the vertical climate gradient is more pronounced. The study covered an elevation distance greater than 3500 m (871 to 4550 m a.s.l.), reaching from the colline to the alpine belt. Mean annual temperature ranges from 25°C in the savanna at the foothills to 3°C in the afroalpine zone (Appelhans et al., 2015). Rainfall distribution along the slope is determined by the air masses of the intertropical convergence zone (ITCZ) and the south-easterly trade winds, resulting in a bimodal rainfall distribution with a long rainy season from March to May and a shorter one around November (Hemp, 2006a). Along the slope, mean annual precipitation exhibits a unimodal pattern with minimum values around 620 mm yr^{-1} at the foothills and maximum values around 2600 mm at 2200 m a.s.l in the middle montane forest, followed by a decrease to 1208 mm in the afroalpine heathlands (Hemp, 2006a; Appelhans et al., 2015).

The soils on the Kilimanjaro massif all have a roughly similar age and developed from the same volcanic deposits (Dawson, 1992). In the savanna, vertisols have developed, while at higher elevations, andosols are predominant (Zech et al., 2014). Soil depths in savanna, *Erica* forest and afroalpine belt generally do not exceed 30 cm, while they reach several m in the tropical montane forest belt.

Our investigation focuses on the six main natural ecosystem types present along the elevation gradient (Table 2.1), which were studied with five replicates each. In total we sampled 30 plots of 0.25 ha size each. Detailed information about the vertical vegetation zonation and the main plant species is given in Hemp (2006a), adopting the elevation zone terminology of Körner (2012). Briefly, the foothill zone between 800 and 1100 m a.s.l. is covered by savanna woodlands, with *Acacia-Commiphora* trees dominating the remaining natural vegetation. The lower montane forest zone between 1600 and 2000 m a.s.l. is characterized by *Macaranga kilimandscharica*, *Agauria salicifolia* and, to a lesser degree, *Ocotea usambarensis*. The middle montane forest (*Ocotea* forest) between 2100 and 2800 m a.s.l. is dominated by *Ocotea usambarensis, Ilex mitis, Xymalos monospora* and the tree fern *Cyathea manniana,* and contains a dense understory layer. The upper montane forest (*Podocarpus* forest) from 2700 to 3100 m a.s.l. hosts *Podocarpus latifolius* as the dominant tree species, together with *Hagenia abyssinica* and *Prunus africana*. In the highest forest zone, which reaches up to 3900 m a.s.l, *Erica* bushlands with some remnants of *Erica trimera* forest are dominant. We refer to these four forest ecosystems as the Mt. Kilimanjaro tropical montane forest. Higher up in the afroalpine zone, which extends to 4550 m a.s.l., heathlands with dwarf shrubs of *Helichrysum* species together with grasses dominate the landscape. The tropical montane forest and afroalpine plots are located inside Kilimanjaro National Park, while the savanna plots and two lower montane forest plots are outside the protected area.

Table 2.1: Site characteristics of the six community types along the slope on Mt. Kilimanjaro.

	Savanna	Lower montane forest	*Ocotea* forest	*Podocarpus* forest	*Erica* forest	*Helichrysum* heathland
Elevation (m a.s.l.)	(871-1130)	(1620-2040)	(2120-2750)	(2720-2970)	(3500-3910)	(3880-4550)
MAT (°C)	23.8 (0.5)	15.2 (0.4)	11.6 (0.4)	9.4 (0.2)	6.2 (0.7)	4.0 (0.4)
MAP (mm)	732 (50)	2164 (34)	2409 (80)	2055 (29)	1523 (64)	1295 (34.5)
Soil C:N ratio (g g^{-1})	14.4 (0.9)	14.5 (0.9)	18.80 (0.7)	18.80 (1.0)	19.62 (0.4)	10.49 (1.5)
pH (KCl)	5.38 - 7.27	4.23 - 5.30	3.49 - 4.25	3.83 - 5.35	4.45 - 4.54	5.00 - 5.30
WFPS (%) *	24.7 (11.7)	21.6 (8.3)	41.1 (13.2)	36.1 (11.5)	…	…
AGB (Mg ha^{-1})	7.90 (1.9)	360.1 (88.8)	280.5 (48.8)	366.7 (3.5)	57.6 (6.1)	6.3 (2.0)
Stem density (n ha^{-1})	45 (12)	388 (22)	309 (20)	516 (76)	2086 (724)	…
Basal area (m^2 ha^{-1})	0.9 (0.2)	49.5 (6.3)	46.9 (5.3)	58.7 (3.6)	15.8 (2.0)	…
Mean tree height (m)	4.6 (0.2)	17.7 (1.6)	12.0 (0.9)	16.1 (1.3)	5.8 (0.2)	…

Given are means wit SE (in brackets). *Soil moisture data was only available for one savanna, and two lower montane, *Ocotea* and *Podocarpus* forest. Note that AGB refers to tree and shrubs biomass only. MAT: mean annual temperature, MAP: mean annual precipitation, WPFS: water-filled pore space, AGB: aboveground biomass. Climatic data from Appelhans et al. (2015), topographic and stand structure data from Hemp (unpublished data), aboveground biomass data from Ensslin et al. (2015) (DBH ≥ 10 cm; allometric equations from Chavez et al., 2005), data for *Erica* forest stand structure from David Schellenberger-Costa (unpublished data) (DBH ≥ 5 cm; allometric equations from Dislich et al., 2009), soil data for mineral topsoil (0-10 cm) from Becker (unpublished data) and Gütlein et al. (2018).

2.3.2 Fine root biomass inventory

In each plot, at least 10 soil samples were taken at random positions down to 40 cm depth with a soil corer of 3.5 cm diameter. Twelve of the 30 plots were sampled more intensively; here, 15 samples were taken instead. Samples were stored in plastic bags at 5°C until processing. In the laboratory, samples were washed under running water over a sieve of 200 µm mesh size. All root fragments greater than 1 cm in length and ≤ 2 mm in diameter were selected and subsequently separated under the stereoscope into biomass (living) and necromass (dead) fractions. As indications of root death, we used the degree of root elasticity, the cohesion of cortex, periderm and stele, and the non-turgidity of the cortex (Leuschner et al., 2001). We further separated herb, grass and fern roots from tree and shrub roots using the lack of visible suberinization and specific root morphological characteristics as criteria. This was done by comparing the samples with root material taken from the study sites. In the subsequent analysis, we only considered tree and shrub roots, as grass, herb and fern roots have a very different life cycle. Root fractions were dried at 70°C for 48 hours and weighed, and the fine root biomass and necromass expressed in Mg d.m. ha^{-1} to 40 cm depth. In order to estimate the root necromass of fragments less than 1 cm in length, we followed the method introduced by Van Praag et al. (1988) and modified by Hertel and Leuschner (2002). Six samples per plot were selected, and after extracting larger root fragments (> 1 cm length) as described above; they were spread homogenously on a filter paper (730 cm^2) subdivided into 36 squares. From six randomly selected squares, root fragments were extracted under the microscope. We then extrapolated the mass of the collected small root fragments to the fine root necromass of the remaining samples that were not included in this more detailed analysis, using linear regression equations between the masses of the small root fragments and the larger dead fine root fraction. In cases where a regression equation could not be applied, a mean ratio of small to large root fractions was used.

2.3.3 Fine root morphological traits

Morphological traits of living fine roots were investigated prior to drying. Each root sample was scanned using an EPSON perfection V700 scanner (EPSON America Inc.). Specific root length (m g^{-1}), specific root area (cm^2 g^{-1}), mean root diameter (mm) and root tissue density (g cm^{-3}) were calculated from the scans and fine root biomass data using WinRhizo software (Régent Instruments Inc., Québec, Canada). We determined the C and N concentrations of the living fine root fraction with a CN elemental analyser (Vario EL III, Hanau, Germany). Three samples per plot were analyzed, with each sample consisting of two collected samples in the field that were mixed.

2.3.4 Fine root production and turnover

We estimated annual fine root production with the ingrowth core technique (Majdi, 1996), which has been used in studies worldwide (e.g. Chen et al., 2004; Hendricks et al., 2006; Handa et al., 2008; Adamek et al., 2011; Kubisch et al., 2017). Measurements in temperate forests have shown that this method tends to provide rather conservative values of fine root production compared with other approaches such as sequential coring and minirhizotrons (e.g. Hertel and Leuschner, 2002; Hendricks et al., 2006; Finér et al., 2011). Ingrowth cores have proven to be useful for studying differences in root production between sites, when root growth is fast as in tropical forests (Vogt et al., 1998), and when a large number of plots is investigated synchronously, as is the case in the KiLi project. With more labor-intensive methods such as sequential coring or mini-rhizotrons, it would have been possible to investigate only a small fraction of the plots, thereby limiting the potential for a comparison of the ecosystems. In September 2014 and February 2015 (dry season) we installed 10 ingrowth cores per plot at random locations in the topsoil down to a depth of 40 cm. After extraction of the soil with a corer of 3.5 cm in diameter, we removed all visible roots by hand and refilled the holes with the original root-free soil. We used a soil core of small diameter to minimize the disturbance produced by the method (Hertel and Leuschner, 2002). We restored the original soil horizon sequence and soil bulk density as good as possible. No mesh was used to avoid barriers for root growth and to retain natural conditions (Hertel et al., 2013; Kubisch et al., 2017) without impeding the access of the soil dwelling fauna. The locations were precisely marked with three plastic sticks to enable the correct insertion angle of the core during resampling. In addition, a PVC tube with exactly the same diameter as the soil corer (3.5 cm) was placed on the top of the soil. Resampling was done after one year. We could not collect data on 5 plots due to logistic problems. The soil samples were processed in the laboratory as described in the previous section (except for the high-resolution analysis of necromass). Fine root production was calculated as ingrown fine root biomass divided by the length of the time interval between the start of recolonization and harvest (Vogt et al., 1998).

To determine the start of recolonization in the different studied ecosystems, we carried out a side study. We placed four additional ingrowth cores in every plot and resampled each one of the cores during the next four months. Accordingly, recolonization started in the savanna and lower montane forest plots roughly two months after core installation and in the montane *Ocotea* forest, *Podocarpus* forest, *Erica* forest and the alpine dwarf shrub heathlands after three months. Fine root production values were extrapolated to one year to obtain an estimate of annual fine root production in Mg ha^{-1} yr^{-1}. Fine root turnover was calculated at the plot level by dividing annual fine root production by mean standing fine root biomass (Gill and Jackson, 2000). Lifespan was then calculated as the inverse of turnover. We assume a steady state between fine root mortality and productivity (Graefe et al., 2008).

2.3.5 Statistical analysis

We applied linear mixed effects models (LME) to determine differences in fine root biomass, necromass, productivity and morphological traits among the studied ecosystems. We used all data points and designated “plot” as a random effect and “ecosystem” as a fixed effect. The Satterthwaite approximation of degrees of freedom was applied to correct for unbalanced sample numbers. For mean root lifespan and nitrogen content, we used mean values per plot and applied ANOVA. Subsequently, Tukey’s HSD post-hoc adjustment for multiple comparisons was used to detect differences between ecosystems types. Linear and nonlinear regression analyses were applied to study in the mountain forest plots the relation a) between fine root biomass, necromass, productivity, lifespan and root morphological traits with the topographic, climatic, soil and stand structural characteristics; b) among fine root morphological and chemical traits and root lifespan; and c) between the FRB:AGB and FRP:AGB ratios and elevation, mean annual precipitation (MAP) and soil C:N ratio. We conducted the regression analyses with the plot means. A significance level of $p < 0.05$ was used throughout the analyses. Normality and homoscedasticity of model residuals were tested and in the case of lifespan, FRB:AGB and FRP:AGB ratio, data were log-transformed to meet these criteria. In the linear mixed effects models, we added the value of 1 to all fine root biomass and necromass data before log transformation due to the existence of several zero values. Fine root production was log-transformed. These statistical analyses were conducted with R software (R Core Team, 2013). The mixed effects models were calculated with the lmer function from the 'lmerTest' package (Kuznetsova et al., 2017). Finally, we carried out a principal components analysis (PCA) to assess the interrelation of the fine root-related variables, stand structure and soil properties among the different ecosystem types along the slope using CANOCO software, version 5.02 (Biometris, Wageningen, the Netherlands).

2.4 Results

2.4.1 Plant community differences in fine root biomass, necromass and root productivity along the elevational gradient

The fine root biomass and necromass, fine root productivity and lifespan of woody plants exhibited contrasting elevational patterns along the slope of Mt. Kilimanjaro. Fine root biomass and necromass revealed a unimodal curve with a peak in the *Podocarpus* forest zone (2720 – 2970 m a.s.l.) (Figure 2.1). Fine root production was unaffected by elevation below 3000 m a.s.l. but decreased higher up the slope toward the *Erica* forest and *Helichrysum* heathland. Furthermore, fine root lifespan linearly increased with elevation toward the tree line, which is formed by *Erica* forest at about 4000 m a.s.l., followed by a strong decrease toward the alpine *Helichrysum* heathland belt.

Mean fine root biomass and necromass increased threefold from the savanna to the *Podocarpus* forest, where maxima of 3.7 and 6.5 Mg ha^{-1}, respectively, were reached (Table S 2.1). The subsequent decrease toward the *Helichrysum* heathland led to biomass and necromass minima of 0.8 and 2.3 Mg ha^{-1}, respectively. In general, fine root necromass exceeded biomass 1.5- to 3-fold along the slope. Values of carbon content in the aboveground biomass, fine root biomass and in the soil (down to 40 cm depth) are shown in Table S 2.2.

Fine root production reached a maximum rate of 1.3 Mg ha^{-1} yr^{-1} in the upper montane *Podocarpus* forest without significant differences to the ecosystems downslope (Table S 2.1). The productivity decline in upslope direction toward the *Erica* forest (0.5 Mg ha^{-1} yr^{-1}) was significant.

The mean fine root lifespan of woody plants was lowest in the ecosystems downslope and upslope of the tropical montane forest belt, i.e. the savanna woodland and *Helichrysum* heathland (1.0 yr and 1.2 yr, respectively) (Table S 2.1), which are characterized by relatively harsh environmental conditions. Lifespan was particularly high in the *Erica* forest (4.6 yr) with a 75 % higher value than in the savanna.

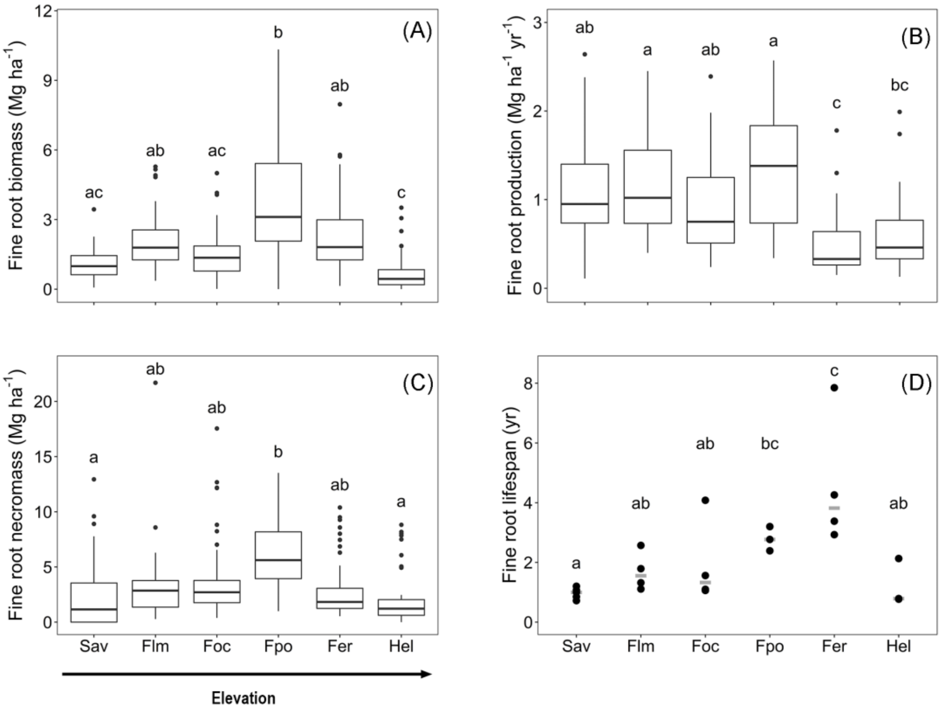

Figure 2.1: Fine root biomass (A), fine root production (B), fine root necromass (C) and mean fine root lifespan (D) in the six communities along the elevation transects on Mt. Kilimanjaro. Different lower case letters indicate significant differences between communities according to linear mixed effects models with Tukey HSD post-hoc comparison ($p < 0.05$). Box-whisker plots with median, 25- and 75-percentiles and extremes. Fine root turnover: dots are measurements, the gray line is the median. Sav, savanna; Flm, lower montane forest; Foc, *Ocotea* forest, Fpo, *Podocarpus* forest; Fer, *Erica* forest; Hel, *Helichrysum* heathland.

2.4.2 Community differences in fine root morphological and chemical traits

Along the slope, marked changes in the root morphological and chemical traits of the woody plants were observed that are caused by both elevation and community differences in species and functional type composition (Figure 2.2). Mean fine root diameter varied between 0.4 (in the *Erica* forest and *Helichrysum* heathland) and 0.9 mm (in the *Podocarpus* forest). It increased by more than 50% from the savanna to the *Podocarpus* forest, corresponding to minima of SRL and RTD.

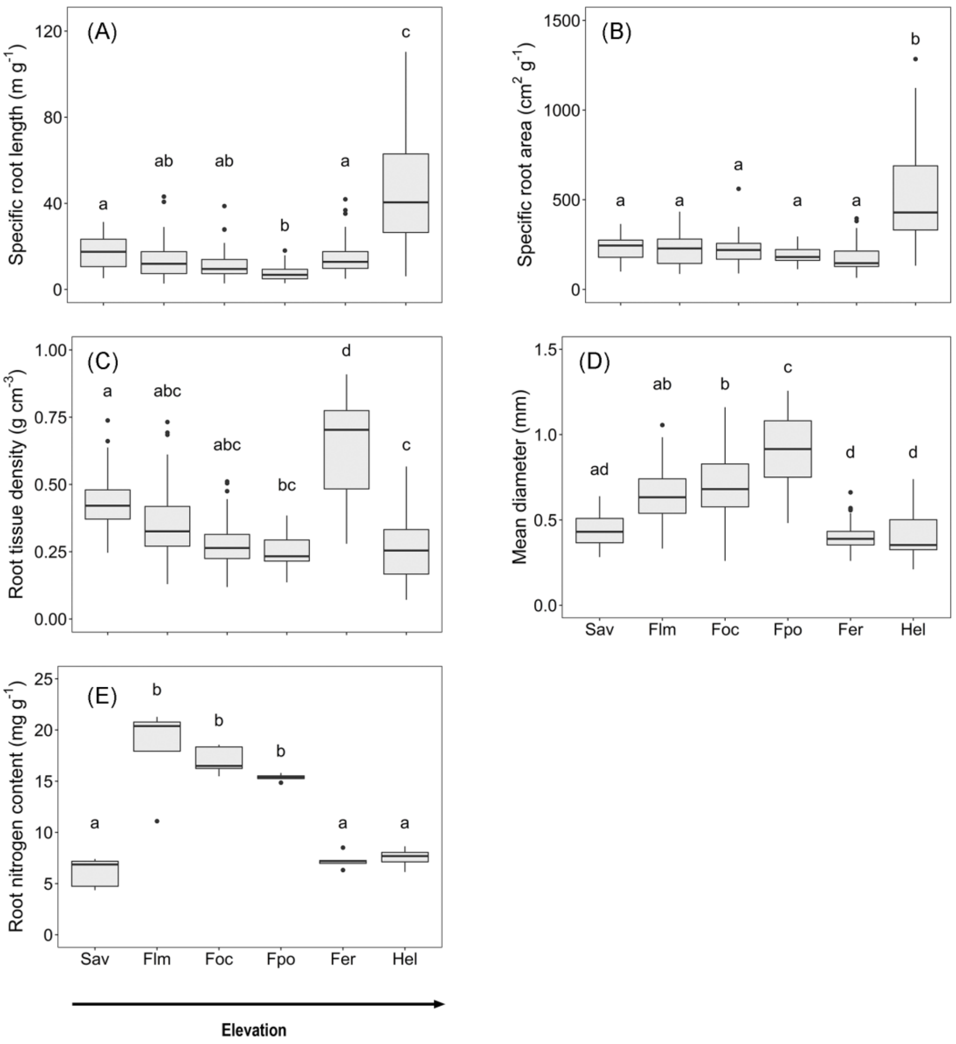

Figure 2.2: Root morphological and chemical traits for the six communities along the elevation transects on Mt. Kilimanjaro. (A) Specific root length, (B) specific root area, (C) root tissue density, (D) mean root diameter and (E) root nitrogen content. Different lower case letters indicate significant differences between communities according to linear mixed effects models with Tukey HSD post-hoc comparison ($p < 0.05$) (box-whisker plots with median, 25- and 75-percentiles and extremes). Sav, savanna; Flm, lower montane forest; Foc, *Ocotea* forest, Fpo, *Podocarpus* forest; Fer, *Erica* forest; Hel, *Helichrysum* heathland.

Community means of SRL and SRA were highest in the *Helichrysum* heathland (47 m g^{-1} and 517 cm^2 g^{-1}, respectively), which is dominated by dwarf shrubs. SRL gradually decreased by almost two third from the savanna woodland (18 m g^{-1}) to the upper montane *Podocarpus* forest, which holds the minimum mean value (7 m g^{-1}), followed by an increase by 50% towards the *Erica* forest (Figure 2.2). Fine root tissue density (RTD) decreased gradually from the savanna to the *Podocarpus* forest, where it reached its minimum (0.3 g cm^{-3}), and increased toward the *Erica* forest again with the maximum (0.7 g cm^{-3}), which exceeded the other ecosystems up to 2.5 fold. Root N content reached minima at both high and low elevation (savanna, *Erica* forest and *Helichrysum* heathland with 6.1, 7.3 and 7.5

mg g^{-1} respectively). It peaked in the lower montane forest (18.3 mg g^{-1}) and steadily declined toward the *Erica* forest higher up.

2.4.3 Relationships between fine root biomass, necromass and productivity, and elevation, climate and soil in the montane forest belt

Focusing on the ecosystems in the montane forest belt, neither fine root biomass or necromass, nor fine root production revealed a significant dependence on elevation (Table S 2.3), demonstrating the dominant influence of community composition on these variables. FRB and FRP showed a unimodal relation to mean annual precipitation with a peak at about 2000 mm (Figure 2.3), but both variables were unrelated to temperature and edaphic parameters (soil C:N and pH). Neither stem density nor aboveground biomass influenced FRB across the different tropical montane forest communities. Stand basal area, but not aboveground biomass, had a positive influence on FRP.

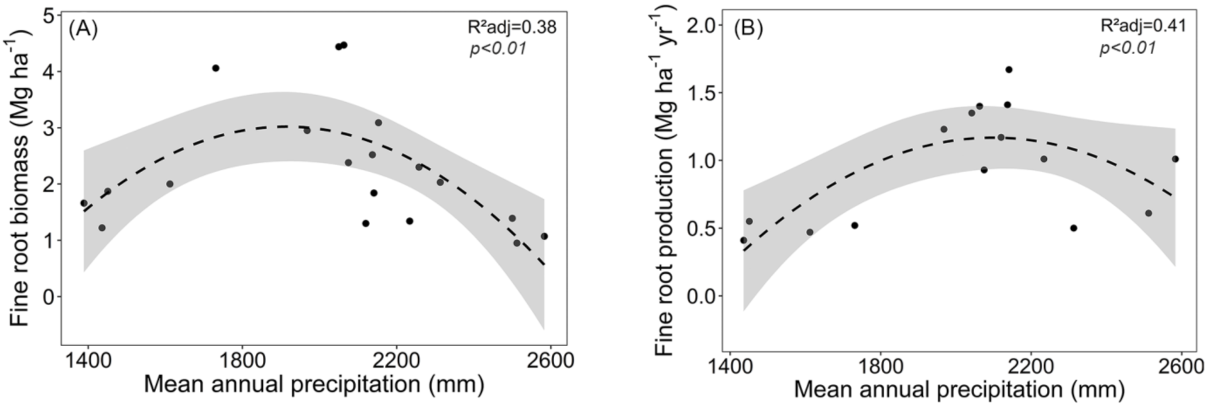

Figure 2.3: Dependence of (A) fine root biomass, and (B) fine root production on mean annual precipitation in the four forest communities on Mt. Kilimanjaro (lower montane forest, *Ocotea* forest, *Podocarpus* forest, *Erica* forest). Dashed lines indicate a 2nd order polynomial regression fitted to the data and gray areas display the 95% confidence interval.

Mean fine root lifespan significantly increased with elevation ($p < 0.01$) and decreased with increasing precipitation and temperature (Table S 2.3, Figure 2.4). Positive effects were also found for soil C:N ratio and stem density, while aboveground biomass and basal area correlated negatively with lifespan. The estimates of the simple linear and non-linear regression models among fine root related variables and abiotic and biotic factors are shown in Table S 2.4.

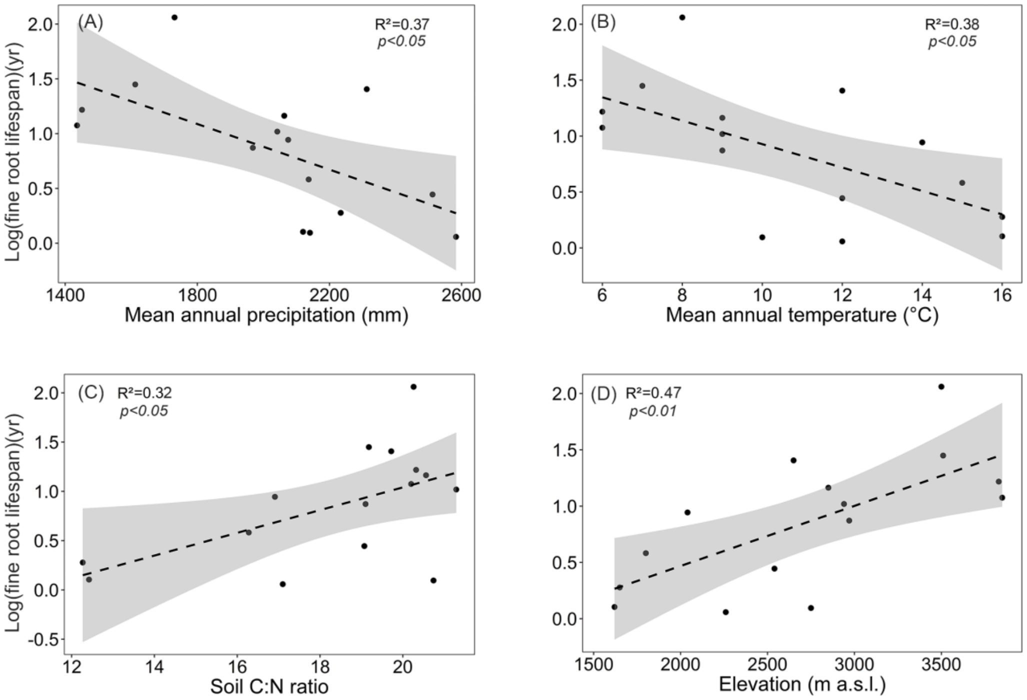

Figure 2.4: Dependence of log (mean fine root lifespan) on (A) mean annual precipitation, (B) mean annual temperature, (C) soil C:N ratio, and (D) elevation in the four forest communities. Dashed lines indicate the linear regression and gray areas the 95% confidence interval.

In contrast to the absolute values of fine root biomass and production, the ratios FRB and FRP to aboveground biomass (FRB:AGB, FRP:AGB) significantly increased with elevation (Figure 2.5A, B). Both ratios increased with soil C:N ratio and decreased with mean annual precipitation (Figure 2.5C-F).

2.4.4 Dependence of fine root morphology and chemistry on abiotic and biotic factors in the montane forest belt

Mean fine root diameter in its dependence on temperature and annual precipitation was best described with an optimum curve, which peaked at about 10 °C and 2100 mm yr^{-1}, corresponding to the upper montane *Podocarpus* forest, while SRL and SRA reached their minima roughly at these conditions. Mean fine root diameter increased with precipitation but showed a hump-shaped relation to temperature. It tended to be higher in communities with larger aboveground biomass and basal area, but decreased with stem density (Table S 2.5). SRL was negatively related to aboveground biomass, and SRA peaked at intermediate soil C:N ratios. The relation of SRL and SRA to temperature was generally weak, while SRA was significantly related to precipitation. Root tissue density tended to increase with decreasing precipitation and temperature.

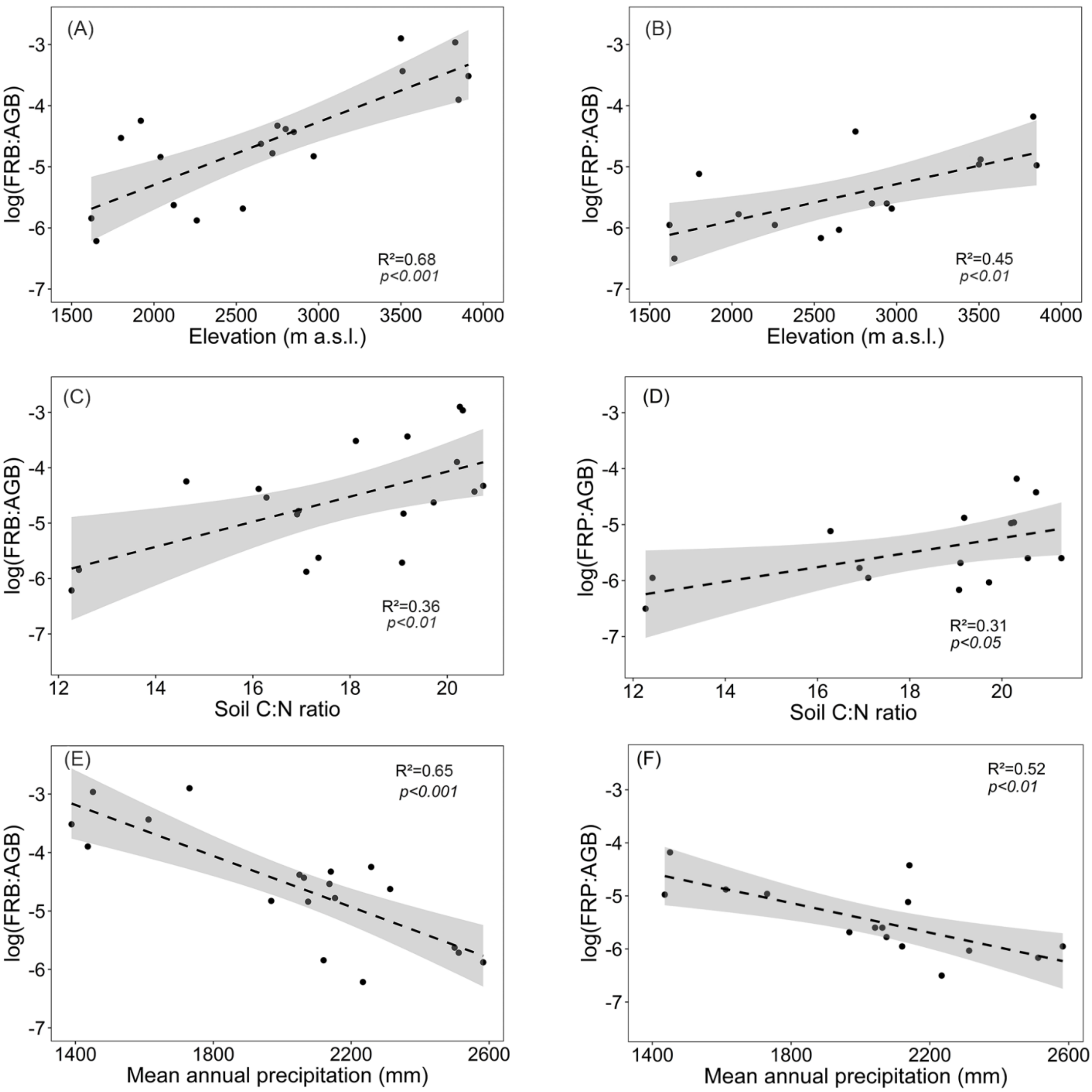

Figure 2.5: Dependence of fine root biomass to aboveground biomass ratio (log(FRB:AGB)) or fine root production to aboveground biomass ratio (log(FRP:AGB)) on (A, B) elevation, (C, D) soil C:N ratio and (E, F) mean annual precipitation for the four forest communities. Dashed lines indicate the linear regression and gray areas the 95% confidence interval.

Communities with higher aboveground biomass and basal area tended to have lower RTD, while stem density was positively related to RTD (Table S 2.5). Root N content was closely negatively associated with soil C:N ratio and decreased with decreases in temperature and precipitation. Communities with higher biomass and basal area had higher root N contents. The estimates of the simple linear and non-linear regression models among fine root morphological and chemical traits and abiotic and biotic factors are shown in Table S 2.6. Most of the abiotic and biotic factors were highly correlated (Table S 2.7).

As expected, various root traits were related to each other across our community sample, notably fine root lifespan negatively to root N content, and root tissue density negatively to mean root diameter, SRA and root N content (Figure S 2.1, Table S 2.8). The main traits affecting fine root lifespan were root N content (negative relation) and root tissue density (positive relation) (Figure 2.6). FRB increased with mean fine root lifespan, and, unexpectedly, N content increased with mean root diameter (Table S 2.8). SRL exhibited a significant relation only to SRA (positive) and to mean root diameter (negative). The estimates of these regression models are given in Table S 2.9.

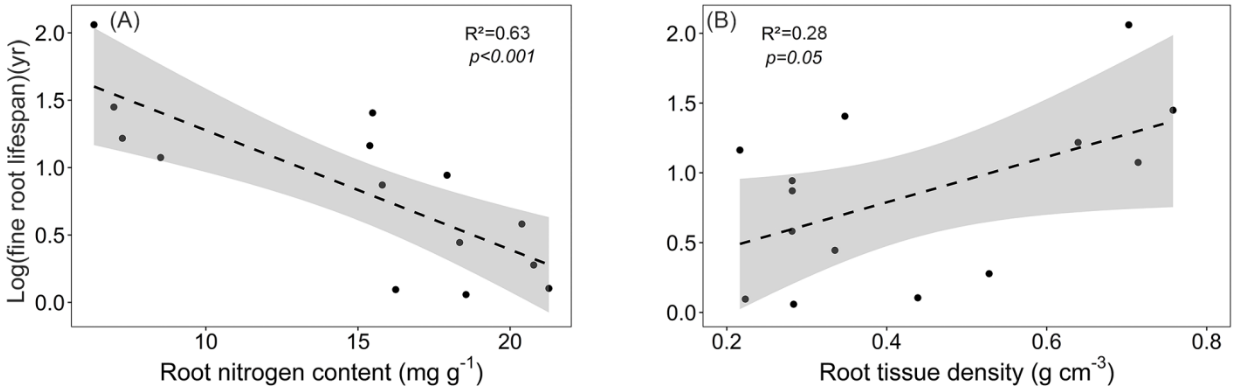

Figure 2.6: Dependence of log (mean fine root lifespan) on (A) root nitrogen content and (B) root tissue density. Dashed lines indicate the linear regression and gray areas the 95% confidence interval.

2.4.5 Principal components analysis on the inter-relationship between belowground, aboveground and abiotic variables

The ordination of the four mountain forest communities by means of principal components analysis (PCA) based on the fine root-related variables, elevation, climatic factors, stand structural characteristics and soil properties revealed the differentiation of the ecosystems along the elevational gradient (Figure S 2.2). Most of the fine root-related variables, elevation and all of the stand structural characteristics were related to the first axis, while none of the soil properties were associated with it (eigenvalue = 0.66) (Table 2.2). This first axis separated the ecosystems along the elevational gradient, grouping the lower montane forest, *Ocotea* forest and *Podocarpus* forest together but separating them from the *Erica* forest. The *Erica* forest was associated with high stem density, high FRB:AGB and FRP:AGB ratios and high root tissue density. The second axis (eigenvalue = 0.22) separated the *Podocarpus* forest from the remaining forest ecosystem types. Fine root biomass, necromass and soil C:N ratio were positively related to this axis.

Table 2.2: Results of a principal components analysis on the differentiation of the four tropical montane forest ecosystems along the elevation gradient with respect to fine root related variables, elevation, climatic, stand structure and soil properties.

	Axis 1 (EV 0.640)	**Axis 2 (EV 0.221)**	**Axis 3 (EV 0.139)**
Topograpical variable			
Elevation	**0.86**	0.49	0.16
Climatic factors			
MAT	**-0.79**	-0.58	-0.19
MAP	**-0.91**	-0.19	0.38
Soil properties			
C:N soil	0.52	**0.63**	0.57
pH	0.13	0.07	**-0.99**
Soil moisture	-0.25	0.53	**0.81**
Stand structural variables			
Aboveground biomass	**-0.97**	0.09	-0.22
Stem density	**0.99**	0.06	-0.11
Basal area	**-0.96**	0.25	-0.09
Root related variables			
Fine root production	**-0.91**	0.32	-0.27
Fine root biomass	-0.13	**0.87**	-0.48
Fine root necromass	-0.37	**0.92**	-0.12
Lifespan	**0.94**	0.34	-0.07
FRB:AGB	**0.99**	0.07	-0.10
FRP:AGB	**0.98**	-0.03	0.19
SRL	0.71	**-0.71**	-0.01
SRA	**-0.78**	-0.51	0.38
RTD	**0.95**	-0.27	-0.17
Mean root diameter	**-0.81**	0.59	-0.01
Root N content	**-0.98**	-0.22	0.01

Given are the loadings of the selected variables along the three explanatory axes. Numbers in brackets indicate the eigenvalues of the axes. Numbers in bold mark the variables with closest correlation to the respective axis.

2.5 Discussion

2.5.1 Elevational change in fine root biomass and productivity

Stand fine root biomass in the soil to 40 cm depth was generally lower in the six studied communities than corresponding values reported from other tropical ecosystems. The mean fine root biomass of woody plants in the savanna woodlands at the foot of Mt. Kilimanjaro (1.0 Mg ha^{-1}) was at the lower end of the range of values reported for other tropical savannas and dry forests (0.4 to 11.86 Mg ha^{-1}) (Roy and Singh, 1994; Chen et al., 2003; February and Higgins, 2010; Moore et al., 2018). In the present study, we only consider woody roots, whereas most of the cited studies did not differentiate between woody and non-woody roots. Nonetheless, this fact does not explain our low values, as the fraction of non-woody plant roots accounted on average for only 12% of total fine root mass in our savanna plots, the only ecosystem of the study with a significant cover of non-woody plants. More likely causes are the relatively low mean annual precipitation and low aboveground tree biomass at our savanna sites. Furthermore, fine root production in the savanna was also lower and fine root lifespan higher than the range of values given for other savannas and tropical dry forests (ca. 1.0 vs. 2.3 to 14.3 Mg ha^{-1} yr^{-1} and 0.97 yr compared to 0.07 to 0.38 yr, respectively) (Kummerow et al., 1990; Pandey and Singh, 1992; Chen et al., 2004; Moore et al., 2018).

In the tropical montane forest belt, fine root biomass and production (1.5-3.7 Mg ha^{-1} and 0.5-1.3 Mg ha^{-1} yr^{-1}) were also in the lower range of values reported in a review of tropical moist forest root data, corresponding closer to values found for tropical lowland forests (0.1-14.4 Mg ha^{-1} and 0.8-21.9 Mg ha^{-1} yr^{-1}, respectively, Hertel and Leuschner, 2010). Our values are also smaller than figures reported from a tropical montane forest in Rwanda (7.6 Mg ha^{-1} and 3.8 Mg ha^{-1} yr^{-1}, respectively) (Nyirambangutse et al., 2017). Whether this apparent difference is due to species identity effects, or the local climatic and specific edaphic conditions on Mt. Kilimanjaro, must remain open. The use of ingrowth cores, as is done in many other studies, is typically associated with relatively low fine root production estimates compared to other techniques such as the sequential coring or minirhizotron approaches (Hertel and Leuschner, 2002). This could partly explain the relatively low fine root production values reported in our study. Since all available methods for estimating fine root productivity suffer from specific drawbacks, comparing the results of different studies should be done with great care.

Fine root biomass more than tripled from the savanna at 900-1100 m to the montane *Podocarpus* forest at 2700-2900 m a.s.l., principally reflecting the large increase in aboveground biomass from the semi-arid foothills to the humid cloud forest belt. Even though the savanna had the lowest fine root biomass of all six ecosystems along the slope, the corresponding FRB:AGB ratio was two orders larger in this ecosystem than in the tropical montane forests higher upslope. The savanna sites at the foot of Mt. Kilimanjaro are characterized not only by seasonal soil moisture deficits, but also by slow N turnover and generally low NH_4^+ and NO_3^- concentrations in the soil liquid phase. Grass-dominated patches seem to be exposed to marked N limitation (Becker et al., 2016; Gerschlauer et al., 2016). Nutrient (N) shortage, together with periodic drought, should favor a vegetation with a high FRB:AGB ratio in order to increase nutrient and water uptake and reduce water loss (Kozlowski and Pallardy, 2002; Brunner et al., 2015). In the forest belt (~1800 to 2900 m), AGB changed only little with increasing elevation toward the *Podocarpus* forest, while FRB increased from 2.0 to 3.7 Mg ha^{-1}. The corresponding increase in the FRB:AGB ratio may be explained by the temperature decrease and its negative effect on nutrient supply. It appears that thermal constraints on soil microbial activity and thus N mineralization rate are fostering carbon investment into the root system at higher elevations on Mt. Kilimanjaro in a similar manner as was found in elevation gradient studies in the Ecuadorian and Peruvian Andes (Moser et al., 2011; Leuschner et al., 2013; Girardin et al., 2013) and other tropical mountain forests (Hertel and Leuschner, 2010).

However, other factors such as moisture seem also to be influential. FRB was somewhat lower in the mid-elevation montane *Ocotea* forest (2100-2800 m) than in the other three forest types, which may relate to the favorable growing conditions at this elevation. MAP and soil moisture, leaf litter nutrient concentrations, decomposition rate and N turnover all were highest in this forest belt (Hemp, 2006a; Appelhans et al., 2015; Becker et al., 2015; Becker and Kuzyakov, 2018; Gerschlauer et al., 2016), suggesting that growth limitation through low N and soil dryness is less important in this than in the other forest communities, while thermal conditions are still relatively favorable. Higher soil fertility is in forests commonly associated with a relatively low fine root biomass (e.g. Vogt et al., 1987; Leuschner and Hertel, 2003; Achat et al., 2008), in accordance with the prediction of resource balance hypothesis (Bloom et al., 1985).

Higher upslope in the upper montane *Podocarpus* and *Erica* forests, N limitation of plant growth is indicated by low leaf litter N contents, and low N:P and high leaf litter C:N ratios (Becker et al., 2015). *Podocarpus* species have been found to form root nodules with symbiotic mycorrhizal fungi (Becking, 1965; Khan, 1967) and also with N_2-fixing bacteria (Huang et al., 2007), but strong evidence for significant N_2 fixation in the nodules is lacking so far. Dickie and Holdaway (2010) suggested that nodule formation may be related to a root volume increase in the course of mycorrhizal infection. In the *Erica* forest, the dominant woody species possess specific adaptations to low N availability, notably the capability to access organic N compounds through their ericoid mycorrhiza (Cairney and Meharg, 2003).

Limitation by N or other nutrients in the high-elevation vegetation belts on Mt. Kilimanjaro is also indicated by the decrease in fine root N content with elevation. In apparent contradiction, Gütlein et al. (2018) reported relatively high rates of nitrate leaching in the soil of the *Podocarpus* forest. This could result from a relatively slow plant N uptake and uncoupling of supply and plant demand in the cold high-elevation climate.

With the shift of dominant plant life forms from trees to dwarf shrubs at the alpine tree line (~3900 m a.s.l.), plant strategies to cope with cold and N-poor soils change. The plants in the afroalpine heathland produce on average thinner fine roots with greater surface area development, and fine root biomass per aboveground biomas is much higher. The afroalpine *Helichrysum* heathland with its patchy vegetation structure, low decomposer activity and slow C and N cycling rates (Gütlein et al., 2017; Becker and Kuzyakov, 2018) may require an extended fine root system to access patches with higher nutrient availability.

2.5.2 Abiotic and biotic drivers of fine root biomass, productivity and fine root lifespan

When exploring the effects of elevation, climate, soil and stand structure on FRB and fine root dynamics for the four montane forest communities while excluding savanna and afroalpine scrub, we find a greater C allocation to the fine root system with increasing elevation and also with factors indicating N shortage, in support of our hypothesis.

At the stand level, the only environmental factor significantly influencing fine root biomass was MAP, displaying a hump-shaped relationship with a peak at about 2000 mm yr^{-1}. This contrasts with root studies along Andean mountain slopes (Moser et al., 2011; Girardin et al., 2013), where FRB increased with elevation and decreasing temperature, while MAP (which was high throughout the transects) was not influential. Studies in tropical, temperate and boreal forests have found positive, negative or no relationship between FRB and MAP in single- or multiple-species studies (Joslin et al., 2000; Green et al., 2005; Finér et al., 2007; Hertel et al., 2013). It appears that fine root system size and its relation to precipitation depends largely on species and the range of MAP investigated. It is possible that the optimum curve found for the FRB-MAP relation on Mt. Kilimanjaro is partly caused by species-specific differences in fine root system size, which varies considerably along the slope.

Other than FRB itself, the ratios of fine root biomass and production to aboveground biomass (FRB:AGB and FRP:AGB) were closely negatively related to MAP ($p < 0.01$). This suggests that rainfall reduction has a larger effect on plant-internal carbohydrate partitioning between root and shoot than on stand fine root biomass itself. However, the FRB:AGB and FRP:AGB ratios also increase with soil C:N ratio and elevation (and decrease with temperature) on Mt. Kilimanjaro, variables partly correlating with MAP, which makes it difficult to disentangle the role of these factors along the slope. Nevertheless, the general picture along the slope of Mt. Kilimanjaro suggests that decreases in temperature, soil moisture and also nitrogen availability all stimulate higher investment in the fine root system, as is predicted by optimal resource partitioning theory (Bloom et al. 1985). Not only N supply (and that of other nutrients) decreases with declining temperature, but nutrient diffusion in the soil and root N uptake activity as well, which is dependent on ATP and thus plant carbon gain. A fine root study along a 2000-m elevation transect in the Ecuadorian Andes reached at similar conclusions except for precipitation (Moser et al., 2011).

The significant relations of mean fine root lifespan to elevation and temperature, MAP and soil C:N ratio across the four forest communities show that lifespan increases under conditions of reduced nutrient and water availability. Under nutrient-poor and cold conditions, plants may be forced to produce root tissue with low nutrient content. Through its sclerenchymatic structure, such tissue is well protected against water loss, herbivory and pathogen attack. Since resource uptake is typically fairly low, roots can reach a favorable cost/benefit ratio in such resource-limited environments only, when they are long-lived (McComark and Guo, 2014). In accordance, Girardin et al., (2013) observed an increase in fine root lifespan with increasing elevation in the Peruvian Andes, whereas a decrease was reported in the Ecuadorian Andes (Graefe et al., 2008), where temporal soil anoxia occurred as an additional stressor at high elevations (Moser et al. 2011).

It appears that unfavorable growing conditions with pronounced resource limitation can act on root lifespan in two opposing ways, either by increasing or by decreasing longevity. Higher longevity increases the nutrient return on carbon and nutrient investment into root production, while reduced longevity could result in higher uptake rates, when young, physiologically more active fine roots with higher uptake capacity are replacing older, less active roots (Eissenstat et al., 2000). In addition, resource limitation in infertile or cold soils is often associated with physical and/or chemical stress to the root in form of cold damage and toxicity, which may reduce root lifespan through increased mortality, as is the case in the upper montane belt of the Ecuadorian Andes. We explain the increase in fine root lifespan with decreasing resource availability on Mt. Kilimanjaro with the species turnover along the gradients. Thus, more stress-tolerant taxa with a more conservative C economy of their roots replace taxa with more acquisitive, shorter-lived roots, thereby avoiding high root mortality under stress.

The spatial variation in root variables in the Mt. Kilimanjaro environmental matrix is caused by both global climate patterns (e.g. temperature effects on FRB, and FRB:AGB and FRP:AGB ratios) and local influences (e.g. effects of species, soil and local topography on fine root lifespan and productivity). Broadly similar fine root patterns as found on Mt.

Kilimanjaro might be expected also on other semi-humid African mountains as Mt. Kenya, Mt. Meru, Mt. Elgon and the Aberdare Mountains, which have a similar general climate with comparable vegetation zonation, and join a number of key species (e.g. *Ocotea usambarensis*, *Podocarpus latifolius, Erica* species) (Bussman, 2006). However, the local conditions likely will cause somewhat deviating rooting patterns in these mountains, as, for example, precipitation levels are relatively high on Mt. Kilimanjaro and a bamboo belt is absent, or, the *Erica* forest of Mt. Kilimanjaro belt is replaced by patchy *Erica* scrub on Mt. Elgon and the Aberdare Mountains (Bussman, 2006, Hemp, 2006b).

Working along temperature and precipitation gradients might allow some conclusions on the belowground response of these ecosystems to global change. However, a space-for-time approach (Blois et al., 2016) applied to a mountain with a linear temperature, but unimodal precipitation gradient along the slope suggests that predictions about the adaptation of root systems to a warmer and drier future can hardly be made.

2.5.3 Fine root morphology and chemistry as dependent on environment and species identity

Our transect covers a broad temperature (25 to 3 °C) and precipitation (620 to 2580 mm) range. The consequently large variation in vegetation types (forest, savanna, alpine scrubland and heathland) and dominant plant life forms (angiosperm and gymnosperm trees, shrubs and dwarf shrubs) should be associated with considerable variation in fine root morphology. Indeed, community means of fine root tissue density and root N content varied by a factor of three across the six communities (0.25-0.65 g cm^{-3} and 7-18 mg g^{-1}), while the relative constancy of SRL and SRA (with the exception of the higher values in the *Helichrysum* heathland) is remarkable, as it does not reflect the variability in environmental conditions among the ecosystems. The continuous decrease in RTD with increasing elevation up to the *Podocarpus* forest could relate to the increase in moisture availability (Weemstra et al., 2016), which may allow producing less xeromorphic fine roots. The associated increase in mean fine root diameter in upslope direction is best explained by the presence of root nodules in *Podocarpus*, which lack in the other species.

The root morphology change in the lower part of the transect had little influence on root surface development (SRL and SRA), as the root tissue density decrease is roughly compensated by the diameter increase in upslope direction. This suggests that plants reduce their mean fine root diameter toward more xeric, low-elevation sites primarily to increase drought tolerance and, to a lesser extent, for expanding the absorbing surface area and reaching water and nutrient patches (Bardgett et al., 2014). High root tissue density in the savanna and high-elevation *Erica* forest may further be needed to penetrate the hard, drought-affected soil in these communities and to be less susceptible to damage by rhizophagous soil animals (Eissenstat, 1991; Weemstra et al., 2016). A major change in root morphology is encountered in upslope direction from the *Podocarpus* forest to the *Erica* forest, as the dominant woody plants of both communities represent different growth strategies. The high SRL and SRA values of the patchy alpine *Helichrysum* heathland reflect the typically thinner, less suberized and more tender fine roots of dwarf shrubs, and may help to reach nutrient-rich patches in the generally nutrient-poor soil (Holdaway et al., 2011; Kramer-Walter et al., 2016). In conclusion, elevational changes in water availability, and most likely also N availability, together with species identity effects, seem to be more influential on fine root morphology than the temperature decrease itself.

2.5.4 Variation in fine root morphological traits: Is there evidence in support of a root economics spectrum (RES)?

Some of our results support the existence of a continuum of fine root trait syndromes along the four tropical montane forest ecosystems, suggesting a trade-off between resource acquisition and conservation. As expected, mean fine root lifespan correlated negatively with root N content and positively with RTD, and SRL (but not SRA) decreased with increasing mean fine root diameter. On the one end of the assumed trait spectrum are species with short-lived roots of low density and high N content and presumably high uptake capacity, on the other end taxa with long-lived, dense and N-poor roots, which are more resistant to herbivory and abiotic stress, but have lower uptake capacities. However, in our sample, root diameter was negatively related to RTD and positively to N content, i.e. species that produced on average thinner fine roots did this by increasing tissue density, while reducing N content. This is not what is expected from the resource acquisition-conservation trade-off, as thinner fine roots normally have a higher specific surface area, which should, according to the RES concept, be associated with a higher uptake activity (i.e. higher N content) and shorter lifespan (i.e. lower tissue density) to maximize resource acquisition per investment. Therefore, the conventional concept of a RES along an acquisitive-conservative continuum does not fit well to our results.

The recently proposed concept of fine root trait multi-dimensionality has opened new horizons in the study of RES (Kramer-Walter et al., 2016; Valverde-Barrantes and Blackwood, 2016). This concept that was tested in tree seedlings along a soil fertility gradient (Kramer-Walter et al., 2016), states that, while there are root traits associated along one axis in a root economics spectrum (especially RTD), other traits such as diameter and SRL can be associated to the RES in an orthogonal way (Kramer-Walter et al., 2016). Such a multi-dimensionality of the trait space entails that fine root morphology is less constrained than the construction of leaves, enabling different combinations of SRL and RTD within the root economics spectrum. In nutrient-poor soils, plants could either develop roots of high SRL to explore distant nutrient patches or operate roots with low SRL and long lifespan to reduce C investment in the fine root system. Our results from the Mt. Kilimanjaro environmental matrix seem to support a multi-dimensional RES, as SRL and RTD, contrary to the negative relation assumed in a one-dimensional RES, show indeed variable combinations. The large diversity in species and plant life forms in our ecosystems has also to be taken into account, as root morphology is strongly influenced by genotype, despite great plasticity in fine root traits (Hodge, 2004). This is visible when comparing the *Podocarpus* and *Erica* forests in our study, which both seem to be N-limited, while exhibiting different RTD values as seen in the PCA.

While our results are in line with a multi-dimensional RES at the community level, they do not confirm the assumption of RTD being the most decisive fine root trait along soil fertility gradients (Holdaway, 2011; Kramer-Walker et al., 2016). Phylogeny seems to overrule any dependency of RTD on soil fertility, as the conifer *Podocarpus* with root nodules pursues a largely different nutrient acquisition strategy than *Erica* with its ericoid mycorrhiza. Our data further suggest that even some of the more widely accepted root trait correlations may not be universally valid. For example, there is evidence that root lifespan can also increase, and not decrease, with a higher root N content (Gordon and Jackson, 2000; Hendricks et al., 2006).

2.6 Conclusions

To our knowledge, this is the first study on fine root system properties (morphology, biomass, and dynamics) in their dependence on elevation and associated environmental factors in African tropical forests. It complements a few earlier studies in tropical mountains of South America and South-east Asia, but differs from these in that the mountain base is semi-arid and that it extends beyond the alpine tree line. The study covers an altitudinal distance of ca. 3000 m, which is associated with a turnover in ecosystem types and plant life forms, ranging from low-elevation savannas to afroalpine scrub. This results in remarkably broad fine root trait spectra along the slope. Even when analyzing only the montane forest belt, where differences in vegetation structure are less pronounced, the variation in fine root biomass, productivity and fine root morphology was still large. This demonstrates that, beside elevation effects, species identity and phylogeny are playing an important role as factors controlling fine root system properties. The interplay between biotic and several abiotic drivers made it difficult to clearly disentangle the influence of temperature, moisture and nutrient availability on root properties in the Mt. Kilimanjaro environmental matrix. This is an unavoidable disadvantage of comparative studies along elevation and climatic gradients. Nevertheless, the study revealed important changes in plant carbon allocation patterns along the slope. When expressed in relation to aboveground biomass, FRB and FRP showed close relations to elevation (temperature), MAP and soil C:N ratio, indicating a general shift in carbohydrate allocation from shoot to root with decreasing resource availability and temperatures. This phenomenon seems to be widespread in tropical as well as extra-tropical regions (e.g. Leuschner et al., 2007; Hertel et al., 2008; Hertel and Leuschner, 2010; Hertel and Schöling, 2011), suggesting a significant role of nutrient availability in the elevational decrease of forest productivity in mountains around the globe.

The analysis of inter-relationships between important root morphological and chemical traits and mean root lifespan revealed several relations consistent with a root economics spectrum, but our data fit better with the concept of multi-dimensionality in the root trait spectrum, and they underpin that root responses to soil fertility are largely dependent on phylogeny and specific root symbionts. Moreover, the majority of species combine acquisitive and conservative root traits in a certain way, and the extremes of the trait spectrum are linked by a continuum of intermediate strategy types. Future studies on a RES and the dependence of root traits on the environment should use standardized root analysis methods, focus more on traits with closer link to physiology (e.g. root respiration, nutrient uptake capacity, root drought and cold resistance), address the effects of biotic interactions, and quantify linkages between aboveground and belowground traits for the dominant species.

2.7 Author contributions

DH developed the study design, NSC conducted the field work, data processing and analysis, JNB and AH contributed with soil and stand structure data. Data interpretation and paper writing were done by NSC, DH and CL with contributions of all authors.

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2.10 Abbreviations

FRB: fine root biomass
FRN: fine root necromass
FRP: fine root production
RES: root economics spectrum

2.11 References

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Conflict of interest statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.12 Supplementary material

Table S 2.1: Community means of fine root biomass (FRB), necromass (FRN), productivity (FRP), turnover and mean fine root lifespan, and ratios of FRB to aboveground biomass, FRP to aboveground biomass, and FRB to FRN in the six communities on Mt. Kilimanjaro (means and SE in parentheses).

	Fine root biomass (Mg ha^{-1})	Fine root necromass (Mg ha^{-1})	Fine root production (Mg ha^{-1} yr^{-1})	Fine root turnover (yr^{-1})	Fine root lifespan (yr)	FRB:AGB	FRP:AGB	FRB:FRN
Savanna	1.04 (0.06)	2.47 (0.47)	1.10 (0.08)	1.07 (0.10)	0.97 (0.08)	0.2075 (0.0856)	0.2365 (0.1057)	0.42 (0.11)
Lower montane forest	1.97 (0.27)	2.78 (0.88)	1.13 (0.11)	0.65 (0.11)	1.70 (0.32)	0.0076 (0.0023)	0.0033 (0.0010)	0.71 (0.27)
Ocotea forest	1.46 (0.21)	3.40 (1.04)	0.95 (0.27)	0.68 (0.16)	1.95 (0.72)	0.0065 (0.0021)	0.0048 (0.0024)	0.43 (0.12)
Podocarpus forest	3.74 (0.41)	6.51 (0.59)	1.33 (0.05)	0.36 (0.03)	2.78 (0.23)	0.0102 (0.0012)	0.0036 (0.0001)	0.57 (0.11)
Erica forest	2.16 (0.49)	2.90 (0.77)	0.49 (0.03)	0.25 (0.05)	4.60 (1.12)	0.0377 (0.0067)	0.0092 (0.0020)	0.74 (0.45)
Helichrysum heathland	0.80 (0.34)	2.30 (1.09)	0.69 (0.17)	1.01 (0.27)	1.23 (0.45)	0.1429 (0.0366)	0.1687 (0.0896)	0.35 (0.29)

Table S 2.2: Carbon stocks in aboveground biomass, fine root biomass and soil organic matter in the six communities on Mt. Kilimanjaro (means and SE in parentheses).

	*Aboveground biomass (Mg C ha^{-1})	Fine root biomass (Mg C ha^{-1})	Soil organic C (Mg C ha^{-1})
Savanna	3.81 (0.94)	0.49 (0.03)	109.65 (17.65)
Lower montane forest	173.58 (42.82)	0.90 (0.13)	241.22 (45.74)
Ocotea forest	135.18 (23.54)	0.70 (0.11)	278.96 (16.05)
Podocarpus forest	176.75 (1.67)	1.63 (0.16)	295.55 (16.12)
Erica forest	27.78 (2.95)	1.04 (0.24)	293.70 (5.87)
Helichrysum heathland	3.06 (0.98)	0.35 (0.15)	110.74 (52.86)

*Data on aboveground biomass C from Ensslin and others 2015 (DBH ≥ 10 cm) and Schellenberger-Costa unpublished (*Erica* forest DBH ≥ 5 cm) and data on soil organic C from Becker N. J. Unpublished

Table S 2.3: Regression analyses relating fine root biomass, fine root necromass, fine root production and turnover to elevation, climatic, edaphic and stand structural variables.

	Elevation			Mean annual precipitation			Mean annual temperature			Soil C:N ratio	
	r^2adj r^2	*P*		*r^2adj r^2*	*P*		*r^2adj r^2*	*P*		*r^2adj r^2*	*P*
Fine root biomass	0.12	n.s.	*	**0.38**	**<0.01**	*	0.07	n.s.	*	0.04	n.s.
Fine root necromass	0.17	<0.1	*****	0.06	n.s.	*	0.11	n.s.	*	0.09	n.s.
Fine root production	0.25	<0.1	*	**0.41**	**<0.05**	*	0.14	n.s.	-	0.02	n.s.
Log (fine root lifespan)	**0.47**	**<0.01**	**-**	**0.37**	**<0.05**	**-**	**0.38**	**<0.05**		**0.32**	**<0.05**

		pH (KCl)			Aboveground biomass			Stem density			Basal area	
		r^2	*P*		*r^2*	*P*		*r^2*	*P*		*r^2*	*P*
Fine root biomass		0.02	n.s.	-	$3.6\ 10^{-5}$	n.s.		0.08	n.s.		0.03	n.s.
Fine root necromass	-	0.01	n.s.	-	0.01	n.s.	-	0.09	n.s.		0.01	n.s.
Fine root production	-	0.03	n.s.		0.23	<0.1	-	0.24	<0.1		**0.28**	**<0.05**
Log (fine root lifespan)	-	0.05	n.s.	**-**	**0.33**	**<0.05**		**0.52**	**<0.01**	-	0.19	n.s.

Fine root biomass in Mg ha^{-1}, fine root necromass in Mg ha^{-1}, fine root production in Mg ha^{-1} yr^{-1}, mean fine root lifespan in yr, elevation in m a.s.l., mean annual temperature in °C, mean annual precipitation in mm, aboveground biomass in Mg ha^{-1}, stem density in n ha^{-1} and basal area in m² ha^{-1}. Significant relations are marked in bold ($P<0.05$), nonlinear relations are indicated by (*) and negative relations by (-).

Table S 2.4 Estimates of the simple linear and non-linear regression models of fine root biomass and dynamics with the abiotic and biotic factors along the tropical montane forest at Mt. Kilimanjaro.

Fine root variable	Factor	Intercept	Estimate (X)	Estimate (X^2)	P- value
Fine root biomass	Elevation	-5.26	$5.55\ 10^{-3}$	$-9.54\ 10^{-7}$	n.s.
	MAP	-17.03	$2.10\ 10^{-2}$	$-5.48\ 10^{-6}$	< 0.01
	MAT	-0.42	0.64	$-3.32\ 10^{-2}$	n.s.
	Soil C:N ratio	0.70	0.09		n.s.
	pH (KCL)	0.93	0.31		n.s.
	AGB	2.27	$-3.91\ 10^{-5}$		n.s.
	Stem density	2.53	$-6.76\ 10^{-4}$		n.s.
	Basal area	2.31	$-1.22\ 10^{-3}$		n.s.
Fine root necromass	Elevation	-13.39	$1.27\ 10^{-2}$	$-2.18\ 10^{-6}$	< 0.1
	MAP	-21.15	$2.70\ 10^{-2}$	$-7.02\ 10^{-6}$	n.s.
	MAT	-1.83	1.37	$-7.20\ 10^{-2}$	n.s.
	Soil C:N ratio	-0.91	0.26		n.s.
	pH (KCL)	2.12	0.31		n.s.
	AGB	4.12	$-1.38\ 10^{-3}$		n.s.
	Stem density	5.27	$-3.68\ 10^{-3}$		n.s.
	Basal area	3.81	$-1.16\ 10^{-3}$		n.s.
Fine root production	Elevation	$1.13\ 10^{-1}$	$1.16\ 10^{-3}$	$-2.64\ 10^{-7}$	< 0.1
	MAP	-7.20	$7.97\ 10^{-3}$	$-1.90\ 10^{-6}$	<0.05
	MAT	0.47	$4.47\ 10^{-2}$		n.s.
	Soil C:N ratio	1.34	$-2.14\ 10^{-2}$		n.s.
	pH	1.87	-0.22		n.s.
	AGB	0.65	$1.13\ 10^{-3}$		< 0.1
	Stem density	1.11	$-1.69\ 10^{-4}$		< 0.1
	Basal area	0.48	$1.13\ 10^{-2}$		< 0.05
Log (fine root lifespan)	Elevation	-0.60	$5.34\ 10^{-4}$		< 0.01
	MAP	2.96	$-1.04\ 10^{-3}$		< 0.05
	MAT	1.97	-0.11		< 0.05
	Soil C:N ratio	-1.27	0.12		< 0.05
	pH (KCL)	2.62	-0.38		n.s.
	AGB	1.36	$-1.90\ 10^{-3}$		< 0.05
	Stem density	0.53	$3.48\ 10^{-4}$		< 0.01
	Basal area	1.39	$-1.29\ 10^{-2}$		n.s.

Table S 2.5: Regression analyses relating five root morphological and chemical traits to elevation and climatic, edaphic and stand structural variables

	Elevation				Mean annual precipitation				Mean annual temperature				Soil C:N ratio			
		r^2_{adj}/r^2	*P*			r^2_{adj}/r^2	*P*			r^2_{adj}/r^2	*P*			r^2_{adj}/r^2	*P*	
SRL	-	0.15	<0.1	*	-	**0.32**	**<0.05**	*	-	0.14	n.s.	*		0.03	n.s.	*
SRA		0.09	n.s.		-	0.12	n.s.	*		0.04	ns.s			**0.39**	**<0.01**	*
RTD	-	**0.55**	**<0.001**	*	-	**0.48**	**<0.01**		-	**0.28**	**<0.05**	*	-	0.09	n.s.	*
Mean root diameter		**0.43**	**<0.01**	*		**0.44**	**<0.01**	*		**0.24**	**<0.05**	*	-	0.005	n.s.	
Root nitrogen content	-	**0.74**	**<0.001**			**0.62**	**<0.001**	*		**0.66**	**<0.001**		-	**0.30**	**<0.05**	

	pH (KCl)				Aboveground biomass				Stem density				Basal area			
		r^2_{adj}/r^2	*P*			r^2_{adj}/r^2	*P*			r^2_{adj}/r^2	*P*			r^2_{adj}/r^2	*P*	
SRL		0.03	n.s.	*	-	**0.21**	**<0.05**			0.03	n.s.	*	-	0.18	<0.1	
SRA	-	0.15	n.s.			0.04	n.s.	*	-	0.18	<0.1			0.05	n.s.	
RTD		0.18	n.s.	*	-	**0.65**	**<0.001**	*		**0.52**	**<0.001**		-	**0.50**	**<0.001**	
Mean root diameter	-	0.21	n.s.	*		**0.45**	**<0.01**	*	-	**0.35**	**<0.05**	*		**0.40**	**<0.01**	
Root N content	-	0.16	n.s.	*		**0.72**	**<0.001**	*	-	**0.62**	**<0.001**	*		**0.63**	**<0.001**	

SRL= specific root length in m g^{-1}, SRA= specific root area in cm^2 g^{-1}, RTD= root tissue density in g cm^{-3}, mean diameter in mm, root nitrogen content in mg g^{-1}, elevation in m a.s.l., mean annual temperature in °C, mean annual precipitation in mm, aboveground biomass in Mg ha^{-1}, stem density in n ha^{-1} and basal area in m^2 ha^{-1}. Significant relations are marked in bold ($P<0.05$), 2nd order polynomial relations are indicated by (*) and negative relations by (-).

Table S 2.6: Estimates of the simple linear and non-linear regression models of fine root morphological and chemical traits with the abiotic and biotic factors along the tropical montane forest at Mt. Kilimanjaro.

Fine root variable	Factor	Intercept	Estimate (X)	Estimate (X^2)	P- value
Specific root length	Elevation	41.06	-2.386 10^{-2}	4.522 10^{-6}	< 0.1
	MAP	100.3	-8.94 10^{-2}	2.196e-05	< 0.05
	MAT	32.11	-3.95	0.17539	n.s.
	Soil C:N ratio	-61.96	9.13	-0.2741	n.s.
	pH (KCL)	-113.09	57.52	-6.503	n.s.
	AGB	15.53	-0.01		< 0.05
	Stem density	8.58	7.87 10^{-3}	-1.702e-06	n.s.
	Basal area	16.69	-0.11		< 0.1
Specific root area	Elevation	250.91	-0.02		n.s.
	MAP	521.5	-3.98 10^{-1}	1.143e-04	n.s.
	MAT	170.25	2.75		n.s.
	Soil C:N ratio	-1064.32	157.62	-4.772	< 0.01
	pH	385.03	-42.00		n.s.
	AGB	168.5	3.18 10^{-1}	-5.453e-04	n.s.
	Stem density	215.54	-0.02		< 0.1
	Basal area	174.96	0.60		n.s.
Root tissue density	Elevation	1.47	-9.76 10^{-4}	1.99 10^{-7}	< 0.001
	MAP	1.11	-3.51 10^{-4}		< 0.01
	MAT	1.24	-0.15	6.25 10^{-3}	< 0.05
	Soil C:N ratio	3.16	-0.35	0.01	n.s.
	pH (KCL)	-5.78	2.67	-0.28	n.s.
	AGB	7.45 10^{-1}	-2.66 10^{-3}	3.63 10^{-6}	< 0.001
	Stem density	2.94 10^{-1}	1.22 10^{-4}		< 0.001
	Basal area	0.68	-6.94 10^{-3}		< 0.001
Mean root diameter	Elevation	-8.54 10^{-1}	1.27 10^{-3}	-2.47 10^{-7}	< 0.01
	MAP	-3.02	3.57 10^{-3}	-8.44 10^{-7}	< 0.01
	MAT	-0.35	0.19	-8.27 10^{-3}	< 0.05
	Soil C:N ratio	0.75	-5.69 10^{-3}		n.s.
	pH (KCL)	8.13	-3.33	0.37	n.s.
	AGB	3.01 10^{-1}	2.44 10^{-3}	-3.06 10^{-6}	<0.01
	Stem density	8.69 10^{-1}	-4.36 10^{-4}	7.65 10^{-8}	<0.05
	Basal area				<0.01
Root N content	Elevation	29.96	-5.75 10^{-3}		< 0.001
	MAP	-40.07	4.52 10^{-2}	-8.77 10^{-6}	< 0.001
	MAT	2.23	1.14		< 0.001
	Soil C:N ratio	33.18	-1.06		< 0.05
	pH (KCL)	177.54	-73.69	8.16	n.s.
	AGB	5.54	4.98 10^{-2}	-4.32 10^{-5}	< 0.001
	Stem density	21.01	-1.27 10^{-2}	2.19 10^{-6}	< 0.001
	Basal area	5.62	0.21		< 0.001

Table S 2.7: Results of correlation analyses between elevation, climatic, edaphic and stand structural variables for the six communities on Mt. Kilimanjaro. Pearson correlation coefficient r and P are given. Elevation in m a.s.l., mean annual temperature in °C, mean annual precipitation in mm, aboveground biomass in Mg ha^{-1}, stem density in n ha-1 and basal area in m^2 ha^{-1}.

	Elevation		MAP		MAT		Soil C:N ratio		pH		AGB		Stem density	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
MAP	- **0.8**	**<0.001**												
MAT	- **0.98**	**<0.001**	+ **0.76**	**<0.001**										
Soil C:N ratio	+ **0.75**	**<0.001**	- 0.37	n.s.	**- 0.74**	**<0.001**								
pH	+ 0.05	n.s.	- 0.3	n.s.	+ 0.03	n.s.	- 0.47	<0.1						
AGB	- **0.73**	**<0.001**	+ **0.66**	**<0.01**	+ **0.66**	**<0.01**	- **0.65**	**<0.01**	+ 0,00	n.s.				
Stem density	+ **0.52**	**<0.05**	- **0.51**	**<0.05**	- 0.44	n.s.	+ 0.33	n.s.	+ 0.1	n.s.	**- 0.5**	**<0.05**		
Basal area	- **0.71**	**<0.001**	+ **0.76**	**<0.001**	+ **0.63**	**<0.01**	- **0.46**	**0.049**	- 0.15	n.s.	+ **0.87**	**<0.001**	**- 0.53**	**<0.05**

Climatic data from Appelhans and others (2015), topographic and stand structure data from Hemp (unpublished data), aboveground biomass data from Ensslin and others (2015) and David Schellenberger-Costa (unpublished data), soil data from Becker (unpublished data). Values in bold indicate significant correlation ($p < 0.05$). Positive correlations are marked with (+) and negative ones with (-).

Table S 2.8 : Simple linear and nonlinear regression models on the relation between fine root lifespan (log-transformed) and various root morphological and chemical traits.

	Root nitrogen content		Mean root diameter		RTD		SRA		SRL		Fine root biomass	
	r²adj	*P*	*r²adj*	*P*	*r²adj*	*P*	*r²adj*	*P*	*r ² adj*	*P*	*r²adj*	*P*
Log (fine root lifespan)	**- 0.63**	**<0.001**	- 0.22	<0.1 *	0.28	<0.1	- 0.18	n.s.	1.27 10^{-5}	<0.1	**0.38**	**<0.05**
SRL	- 0.17	<0.1	**- 0.51**	**<0.001 ***	0.06	n.s.	**0.4**	**<0.01**				
SRA	0.03	n.s. *	0.06	n.s. *	**- 0.32**	**<0.05**						
RTD	**- 0.42**	**<0.01**	**- 0.58**	**<0.001**								
Mean root diameter	**0.29**	**<0.05**										

Mean root lifespan in years, SRL= specific root length in m g^{-1}, SRA= specific root area in cm^2 g^{-1}, RTD= root tissue density in g cm^{-3}, mean diameter in mm, and root nitrogen content in mg g^{-1}, fine root biomass in Mg ha^{-1}. Significant relations are marked in bold ($P<0.05$), 2nd order polynomial relations are indicated by (*) and negative relations by (-).

Table S 2.9: Estimates of the simple linear and non-linear regression models among fine root morphological and chemical traits and fine root lifespan along the tropical montane forest at Mt. Kilimanjaro.

Fine root variable	Factor	Intercept	Estimate (X)	Estimate (X^2)	P- value
Log (fine root lifespan)	Root N content	2.16	-0.09		< 0.001
	Mean root diameter	4.26	-10.30	7.02	< 0.1
	Root tissue density	0.14	1.63		< 0.1
	Specific root area	1.89	-5.59 10^{-3}		n.s.
	Specific root length	0.84	1.59 10^{-4}		< 0.1
Specific root length	Root N content	17.98	-0.41		< 0.1
	Mean root diameter	18.73	-2.69	-10.86	< 0.001
	Root tissue density	9.49	6.39		n.s.
	Specific root area	-0.87	0.06		< 0.01
Specific root area	Root N content	50.43	23.49	-0.82	n.s.
	Mean root diameter	8.35	626.43	-465.59	n.s.
	Root tissue density	259.25	-150.80		< 0.05
Root tissue density	Root N content	0.74	-0.02		< 0.01
	Mean root diameter	0.83	-0.67		< 0.001
Mean root diameter	Root N content	0.99	-0.86		< 0.05

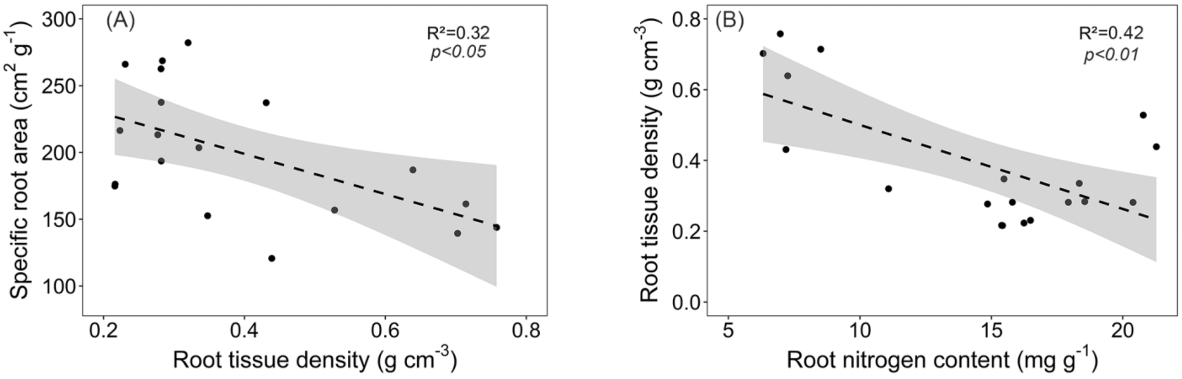

Figure S 2.1: Relationship between (A) specific root area and root tissue density, and (B) root tissue density and root nitrogen content. Dashed lines indicate the linear regression and grey areas the 95% confidence interval.

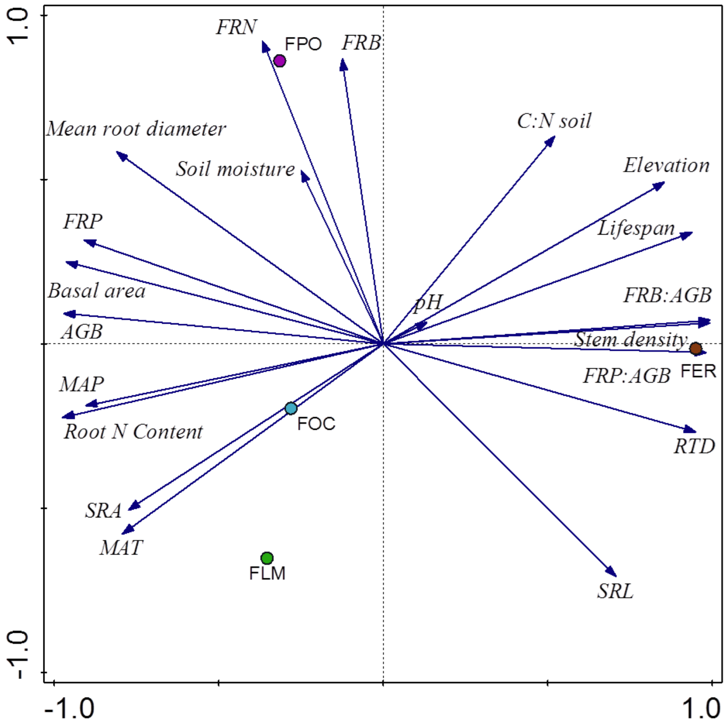

Figure S 2.2: Plot showing the distribution of the four tropical montane forest ecosystems plots along the elevational gradient (FLM = lower montane forest, FOC = *Ocotea* forest, FPO = *Podocarpus* forest, FER = *Erica* forest) in PCA axes 1 and 2 with fine root related variables, elevation, climatic, stand structural and soil properties. Vector length and angle are proportional to the direction and degree of their correlation with the ecosystem ordination scores.

CHAPTER

3

Climate implications on forest above- and belowground carbon allocation patterns along a tropical elevation gradient on Mt. Kilimanjaro (Tanzania)

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3.1 Abstract

Tropical forests represent the largest store of terrestrial biomass carbon (C) on earth and contribute over-proportionally to global terrestrial net primary productivity (NPP). How climate change is affecting NPP and C allocation to tree components in forests is not well understood. This is true for tropical forests, but particularly for African tropical forests. Studying forest ecosystems along elevation and related temperature and moisture gradients is one possible approach to address this question. However, the inclusion of belowground productivity data in such studies is scarce. On Mt. Kilimanjaro (Tanzania), we studied aboveground (wood increment, litter fall) and belowground (fine and coarse root) NPP along three elevation transects (c. 1800 - 3900 m a.s.l.) across four tropical montane forest types to derive C allocation to the major tree components. Total NPP declined continuously with elevation from 8.5 to 2.8 Mg C ha^{-1} yr^{-1} due to significant decline in aboveground NPP, while fine root productivity (sequential coring approach) remained unvaried with around 2 Mg C ha^{-1} yr^{-1}, indicating a marked shift in C allocation to belowground components with elevation. The C and N fluxes to the soil via root litter were far more important than leaf litter inputs in the subalpine *Erica* forest. Thus, the shift of C allocation to belowground organs with elevation at Mt. Kilimanjaro and other tropical forests suggests increasing nitrogen limitation of aboveground tree growth at higher elevations. Our results show that studying fine root productivity is crucial to understand climate effects on the carbon cycle in tropical forests.

Keywords

Africa, carbon cycle, fine roots, net primary production, tropical montane forest

3.2 Introduction

Tropical forests cover only 13% of the earth’s land surface but may account for one third of terrestrial net primary productivity (NPP) (Del Grosso 2008) and store 40-50% of the terrestrial biomass carbon (Houghton 2005; Lewis et al. 2009). Climate change has the potential to reduce the forests’ sink strength for carbon (C) in a hotter and drier climate through reduced CO_2 uptake, increased respiratory CO_2 release, or both (Lloyd and Farquhar 2008; Cernusak et al. 2013; Mitchard 2018). Changing temperature and moisture conditions may also affect the patterns of carbohydrate allocation to aboveground and belowground sinks, i.e. wood increment, the production of leaves, flowers and fruits, root production, root exudation, and C transfer to mycorrhiza. For example, warmer temperatures tend to accelerate soil nitrogen (N) cycling with the consequence of better nutrient supply and higher aboveground productivity at the cost of root productivity (Robertson and Groffman 2015; Moser et al. 2011). Yet, higher temperatures also increase the atmospheric vapor pressure deficit, which can induce drought stress with negative effects on forest productivity even in situations when soil moisture is not limiting (Yuan et al. 2019). On the other hand, climate warming could also lead to increased precipitation, as is predicted by climate change scenarios for parts of East Africa (Niang et al. 2014), possibly hampering root growth in already wet soils. Thus, wood production and timber yield may decrease or increase with climate change as a consequence of allocation shifts, independently of changes in total forest productivity. How long-term change toward a hotter climate with higher evaporative demand influences the C cycle of tropical forests is hence only poorly understood. One reason is that most productivity studies in tropical forests have considered only aboveground components of productivity (wood and leaf production), while ignoring belowground productivity. Measuring root productivity is labor-intensive, especially with respect to the fine root fraction (conventionally roots < 2 mm in diameter). The synchronization of root growth and root mortality, together with the high spatial heterogeneity of the root system makes the observation of root dynamics notoriously difficult (Eissenstat et al. 2000). Consequently, various authors have used estimates of root production to obtain total (above- and belowground) productivity, but these production figures should be considered with caution.

A second reason of this limited understanding is the existence of contradicting evidence with respect to the temperature dependence of net primary productivity (NPP) of tropical and subtropical forests, i.e. the balance of photosynthetic carbon gain and carbon loss through autotrophic respiration. The climatic control of forest NPP is still a matter of debate, as several studies provide evidence for a direct temperature dependence of productivity (Huxman et al. 2004; Leuschner et al. 2013, Lichstein et al. 2014; Chu et al. 2015), while other investigations point to a more prominent role of solar radiation, tree functional traits and stand structural attributes as principal drivers of forest NPP (Luyssaert et al. 2007; Michaletz et al. 2014; Fyllas et al. 2017).

Some insights on the importance of climatic drivers can be gained from the comparative study of forest productivity along climate or elevation gradients. Even though these gradients are often linked with change in other environmental factors, they have the advantage of informing about long-term acclimative and adaptive responses of trees to climate warming. We are aware of only two comprehensive studies along tropical elevation gradients that covered the major above- and belowground components of forest productivity, i.e. the study by Girardin et al. (2010) and Malhi et al. (2017) in the Peruvian Andes, and the study by Moser et al. (2011) and Leuschner et al. (2013) in the Ecuadorian Andes. Since root production was also investigated, these two pioneer studies allow conclusions on carbon allocation shifts along the elevation gradient that may relate to alterations in temperature, moisture and nutrient availability. Both studies found increased carbon allocation to root production with increasing elevation, but the extent to which the shift occurred, differed. Moreover, both studies report deviating elevational trends for total NPP, carbon partitioning and fine root carbon residence time, highlighting the regional character of the observed patterns. This suggests that local differences in climate and geology, and probably also in the biogeographical setting, may lead to deviating patterns of NPP and carbon allocation change with elevation in different tropical mountains (Malhi et al. 2011). Comprehensive studies of forest NPP and its components along elevational gradients are lacking in the African and Asian tropical forest realms so far.

Measuring aboveground and belowground productivity in combination with the corresponding biomass C pools allows to estimate rates of ecosystem carbon cycling and to distinguish ‘fast’ and ‘slow’ C pools, i.e. plant components with low or high carbon residence time (CRT). . Comparative analyses of CRT in different ecosystem types along climate or elevation gradients may deepen our understanding of abiotic and biotic controls of ecosystem C cycling (Girardin et al. 2010; Malhi et al. 2017).

The moist tropical lowland forests of Africa are on average less species-rich than neotropical forests, but mean aboveground biomass is higher and average tree height greater (Hemp et al. 2017; Sullivan et al. 2017). In fact, biomass in tropical montane forests on Mt. Kilimanjaro is higher than in Andean montane forests (Ensslin et al. 2015). However, it is unknown if this higher biomass is also reflected in a higher productivity. Less is known about the carbon cycle of African tropical forests compared to their American and Asian counterparts. Apart from very few NPP measurements in lowland forest plots (e.g. Moore et al. 2018), only one productivity assessment has been done in an African tropical montane forest (Rwanda; Nyirambangutse et al. 2017). Mount Kilimanjaro, in northern Tanzania, with a forest belt covering an elevation gradient of approx. 2000 m. from lower montane forest to the subalpine *Erica* forest, the highest forests of Africa (Hemp 2005), offers unique opportunities to study the variation in forest NPP and carbon allocation with temperature and moisture change, as soils are of similar origin (volcanic) and age. By determining all major components of NPP (aboveground wood increment, aboveground litter fall, fine root and coarse root production) in four characteristic forest types total NPP and aboveground/belowground carbon allocation patterns were derived and related to temperature, rainfall and soil chemistry patterns on the mountain. We further calculated nitrogen use efficiency of productivity (NUE), carbon residence time, and the transfers of C and N with aboveground and belowground (root) litter fall to the soil for the different forest ecosystems in order to characterize putative factors influencing productivity along the elevation gradient.

The following hypotheses guided our research: (1) total NPP declines and C allocation shifts to the fine root system as a consequence of increasing N limiting conditions with elevation; (2) nutrient use efficiency of productivity is higher at high elevations as a respond to the low soil N availability; (3) C and N fluxes from fine root mortality to the soil are more important than from leaf litter at high elevations as a result of plant species adaptation to the harsh conditions.

3.3 Material and Methods

3.3.1 Study site

The study was conducted on the southern and south-eastern slopes of Mount Kilimanjaro, northern Tanzania (3°4´33´´S, 37°21´12´´E), in the framework of the KiLi project, an interdisciplinary research project on biodiversity, biotic interactions and biogeochemical cycles in the main ecosystems of the mountain. From the foothills to the mountain top, a characteristic sequence of vegetation belts from savanna woodland through tropical montane forest to alpine heathland is found (Hemp 2006). The present study investigates four different types of tropical montane forest inside Kilimanjaro National Park at elevations from 1800 to 3910 m a.s.l. Along three elevation transects, twelve study plots of 0.25 ha were demarcated in the four forest types, resulting in threefold replication at the plot level (Table 3.1). Detailed information about the forest ecosystems and their plant species composition is given in Hemp (2006). The lower montane forest (1800 - 2000 m a.s.l.) is characterized by *Macaranga kilimandscharica*, *Agauria salicifolia* and, to a lesser degree, *Ocotea usambarensis*. The middle montane forest (*Ocotea* forest, 2100 - 2800 m a.s.l.) is dominated by *Ocotea usambarensis, Ilex mitis, Xymalos monospora* and the tree fern

Cyathea manniana, and contains a dense understory layer. The upper montane forest (*Podocarpus* forest, 2800 - 3000 m a.s.l.) hosts *Podocarpus latifolius* as the dominant tree species together with *Hagenia abyssinica* and *Prunus africana*. In the subalpine belt (i.e. the treeline ecotone sensu Körner (2012)) (3500 - 3900 m a.s.l), *Erica* bushlands with some remnants of *Erica trimera* forests are dominant, which form the forest line.

Mean annual temperature ranges from 15 °C in the lower montane forest to 4°C in the highest *Erica* forest (Appelhans et al. 2015). Rainfall exhibits a bimodal seasonal distribution on Mt. Kilimanjaro with a long rainy season from March to May and a short rainy season around November (Hemp 2006). Mean annual precipitation decreases from 2200 mm in the lower montane forest (1800 m a.s.l.) to 1000 mm in the subalpine *Erica* forest belt (3900 m a.s.l.) (Hemp 2006).

The soils on the southern slopes of Mt. Kilimanjaro developed from the same volcanic deposits and are thus of similar age (Dawson 1992). In the four forest types, andosols with folic, hystic or umbric properties are predominant, indicating high topsoil carbon contents (Zech 2006).

Table 3.1 Physiographic and stand structural characteristics of the plots studied in the four forest types on Mt. Kilimanjaro. Given are means ± SE (n = 3).

	Lower montane forest	*Ocotea* forest	*Podocarpus* forest	*Erica* forest
Elevation (m a.s.l.)	1800-2040	2120-2750	2800-2970	3500-3910
MAT (°C)[a]	14.7 ± 0.3	11.3 ± 0.7	9.3 ± 0.3	6.0 ± 1.2
MAP (mm)[b]	2227 ± 215	1995 ± 285	1545 ± 178	1051 ± 75
C:N (topsoil)[c]	15.9 ± 0.7	19.3 ± 1.0	18.6 ± 1.3	19.6 ± 0.7
pH (KCl) (topsoil)[c]	4.23 - 4.34	3.49 - 4.23	3.83 - 5.35	4.45 - 4.54
WFPS (%) [d]	21.6 ± 8.4	41.1 ± 13.2	36.1 ± 11.5	n.d.
AGB (Mg ha^{-1})[e]	267.4 ± 45.0	328.8 ± 83.6	404.4 ± 49.7	32.5 ± 7.2
Stem density (n ha^{-1})[e]	397 ± 39	311 ± 36	523 ± 33	2091 ± 1196
Basal area (m^2 ha^{-1})[e]	47.1 ± 9.8	46.1 ± 7.7	55.7 ± 5.1	14.9 ± 3.3
Mean tree height (m)[e]	15.6 ± 0.9	16.0 ± 1.8	15.5 ± 1.3	4.9 ± 0.1
LAI (m^2 m^{-2})[f]	6.25 ± 0.03	5.43 ± 1.16	5.09 ± (0.54)	2.15 ± 0.22
Leaf C:N ratio (mg mg^{-1})	19.2 ± 0.6	21.2 ± 1.3	29.4 ± 3.2	37.9 ± 0.8

[a]MAT: mean annual temperature (Appelhans et al. (2015)); [b]MAP: mean annual precipitation (Hemp (2006)); [c]soil C:N and pH data (0-10 cm of the topsoil) (Becker, unpublished data); [d]WPFS water-filled pore space (0-5 cm topsoil) (weighted mean of measurements in the long and short rainy seasons, and the long and short dry seasons) (Gütlein et al. (2018)); [e]AGB: aboveground biomass stand structural data of trees > 10 cm DBH (Hemp (unpublished data)), in case of *Erica* forest DBH ≥ 5 cm (Schellenberger Costa, unpublished data); [f]LAI: leaf area index (Rutten et al. (2015)).

3.3.2 Determination of NPP

In all 12 plots, we measured aboveground wood increment, aboveground litter fall production, coarse root production and fine root production as the main components of net primary productivity over a 24-month interval (Aug 2014 – Jul 2016) (Clark et al. 2001a). In case of fine root production, the measuring period covered only one year (Aug 2015 – Jul 2016). We assumed that a steady-state in the production of leaves, twigs, inflorescences and fruits, i.e. equality between the production of new organs and litter fall, did exist in the canopy (Aragão et al. 2009) and also in the fine root system (Graefe et al. 2008).

3.3.2.1 Aboveground productivity and coarse root production

The production of aboveground biomass was derived from stem increment measurements with dendrometer tapes (UMS, München, Germany) mounted permanently on 40 stems per plot (480 stems in total). In the 0.25 ha-plots, the 40 stems were selected from the more abundant tree species and from different diameter classes to include the whole spectrum of stem diameters above 10 cm DBH. In case of the *Erica* forest, the DBH threshold was set to ≥ 5 cm, as the 5-10 cm-class contributes significantly to total coarse wood biomass in this ecosystem. Dendrometer tapes were placed at 1.3 m height above ground. When buttresses or stem anomalies were present, we moved the dendrometers some centimeters higher or lower. Tape readings were conducted at monthly intervals from August 2014 to July 2016. Aboveground biomass production was calculated as the difference in biomass between the first and last reading, divided by interval length. To obtain aboveground biomass from DBH and tree height, we applied the pantropical allometric equation of Chave et al. (2014) (Eq. 1):

$$AGB = 0.0673(\rho D^2 H)^{0.0976} \qquad (1)$$

in which AGB is the aboveground biomass estimate (in kg per tree), ρ is the specific gravity of the wood (dry weight per fresh volume in kg m^{-3}), D the trunk diameter (DBH in cm), and H tree height (in m). AGB covers coarse wood biomass, and twig and leaf mass. This NPP component is termed hereafter NPP-aboveground wood. The procedures to measure specific wood gravity (wood density) and tree height in the plots are described in Ensslin et al. (2015) and Schellengerger Costa et al. (2017). When wood density data were not available from the plots (in 2 species and 16 stems), values were taken from the global wood density database (Zanne et al. 2009). For stems that were not equipped with dendrometer tapes in the plots, we used increment rates averaged per species and per plot. For tree species that were not monitored, we used increment rates averaged over all measured species in the plot.

The production of coarse roots, i.e. roots with diameter > 5 mm, was derived from measured aboveground biomass increment applying the equation given for tropical forests by Cairns et al. (1997) (Eq. 2).

$$BGB = \exp(-1.0587 + 0.8836 \ln(AGB)) \qquad (2)$$

with BGB being the belowground biomass and AGB the aboveground biomass, both in Mg ha^{-1}. The equation was primarily derived for root diameters > 5 mm, but some of the included studies considered also fine roots. Since fine roots contribute with only a few percent to belowground biomass, we used the measured AGB difference between first and last reading for estimating the production of coarse root biomass (hereafter: NPP-coarse roots), the main component of belowground biomass.

To express NPP in terms of carbon (C), we used a C content of the biomass of 48.2 % for all NPP components (Thomas and Martin 2012). Carbon residence time (CRT) of the NPP components was calculated by dividing the amount of C contained in the biomass by the C accumulated by the formation of new biomass (productivity) (Malhi et al. 2004). This was done for all NPP components except for aboveground litter fall, as its biomass was not measured.

3.3.2.2 Aboveground litter production and its components

Aboveground litter fall was measured with ten randomly placed litter fall traps (size: 1 m x 1 m) that were collected at monthly intervals from August 2014 to July 2016. PVC tubes were inserted at the corners of the traps and a nylon mesh of 1 mm mesh width placed 20 cm above ground between the four tubes. In the *Ocotea* forest the net was located at 80 cm height to collect the litter above the understory vegetation layer. In the laboratory, the collected material was separated for leaves, twigs (diameter < 20 mm) and other litter components (inflorescences, fruits, leaf fragments, unidentified material), oven-dried at 60 °C for one week, weighed and the mass expressed as litter fall per time interval and ha^{-1} (hereafter: NPP-aboveground litter fall). To obtain total aboveground NPP (NPP-aboveground), the NPP-aboveground litter fall was added to the NPP-aboveground wood.

The C and N content of all litter fractions was measured through gas chromatography with a CN elemental analyzer (Vario EL III, Hanau, Germany). We analyzed five samples per plot, each consisting of the mix of two samples from two sampling dates (May 2016 for the long wet season and July 2016 for the long dry season). For direct comparison with the fine root litter fraction, we calculated the leaf litter C:N ratio and the annual transfer of C and N with leaf litter fall to the soil, using plot-level means. The nitrogen use efficiency (NUE) of productivity was calculated in the different forest types after Vitousek (1982) by dividing annual aboveground litter mass by litter N content.

3.3.2.3 Fine root production

As all available methods for measuring fine root productivity (NPP-fine roots) are known to be error-affected (Majdi 2005; Vogt et al. 1998; Hertel and Leuschner 2002; Moser et al. 2010), it is advisable to combine at least two approaches to get an impression of the possible bias in the data (Clark et al. 2001a). In this study, we applied the sequential coring approach (Persson 1980; Majdi 1996) together with the ingrowth core method (Majdi et al. 1996). The first method has been widely used but is labor-intensive and may be problematic especially at sites with low root mass seasonality (Vogt et al. 1998; Hertel and Leuschner 2002). The second method has been found to underestimate fine root production, but it can be conducted with a relatively high number of replicates and may serve for comparing root production at different sites when root growth is fast, as is typically the case in tropical forests (Vogt et al. 1998). While the absolute productivity values obtained with the ingrowth core method seem to underestimate fine root production and may better be used to characterize the regeneration potential of the fine root system after disturbance, the figures from a large number of locations may nevertheless give an impression of relative differences in plot-level fine root productivity.

3.3.2.3.1 Sequential coring approach

Due to the high labor effort needed to collect and process the samples, the sequential coring approach was conducted in only one of the three elevation transects. On four occasions in the period August 2015 – July 2016 (Aug 2015, Jan 2016, May 2016, Jul 2016), 15 soil cores (3.5 cm in diameter) per plot and date were extracted down to 40 cm depth and stored at 5°C until analysis. Coring on the subsequent date was conducted at a distance of 20 cm to the last one, moving to the corners of a square. In the laboratory, samples were washed through a sieve of 200 µm mesh size and root fragments > 10 mm in length and ≤ 2 mm in diameter were picked out. Living and dead fine roots were distinguished under a stereomicroscope based on the degree of root elasticity, the cohesion of cortex, periderm and stele, and the turgidity of the cortex (Leuschner et al. 2001). Fine roots from woody plants were selected by means of root morphology and branching patterns and the lack of visible suberinization. Only these roots were considered for further analysis. Root samples were dried at 70°C for 48 h and weighed. Additionally, we applied the protocol proposed by Van Praag et al. (1988) and modified by Hertel and Leuschner (2002) to cover necromass fragments < 10 mm in length, which represent a large part of the necromass. Six samples per plot were selected, the larger root fragments (> 10 mm) extracted by hand, and the remaining of the sample spread homogenously on a filter paper (730 cm^2) subdivided in 36 squares. In six randomly chosen squares all root fragments (mostly necromass) were extracted under the stereomicroscope and the mass determined by drying and weighing. This small necromass fraction was multiplied by six to extrapolate to the entire sample and added to the necromass fraction > 10 mm in length to obtain an estimate of total fine root necromass. We then extrapolated the mass of the small root fraction to the fine root necromass of the remaining samples that were not included in this more detailed analysis, using linear regression equations between the masses of the small root fragments and the larger dead fine root fraction. A mean ratio of small to large root fractions was used in cases when a regression equation could not be applied.

Fine root production was calculated with the “minimum-maximum” approach (Edwards and Harris 1977; McClaugherty et al. 1982) by subtracting the lowest mean fine root mass (biomass plus necromass; n = 15 cores) from the highest mean in the 1-yr measuring period, considering only significant differences between dates (Vogt et al. 1986). As this method does not take into account the simultaneous occurrence of fine root production and root mortality in the study period, underestimation of fine root production is possible (McClaugherty et al. 1982). For the other two transects, where the sequential coring method was not conducted, fine root production was estimated from the fine root biomass data of these transects (Sierra Cornejo et al. 2020) using a regression between root biomass and root production determined in the first transect. NPP-fine roots and NPP-coarse root were added to obtain NPP-belowground.

3.3.2.3.2 Ingrowth core study

As a second independent method, an ingrowth core study was conducted in the 12 plots of the three transects. In September 2014 and February 2015 (dry season), ten ingrowth cores (0-40 cm soil depth) were placed at random locations in each plot. After soil extraction with a 3.5 cm-soil corer, all visible roots were removed in situ by hand and the original hole was refilled with the root-free soil. The original soil layer sequence and bulk density were restored as good as possible. The location of the core was precisely marked with three thin plastic sticks and a PVC tube on the surface of the same diameter of the core. No mesh was inserted in the soil to avoid growth barriers and further disturbance of the soil texture (Hertel et al. 2013; Kubisch et al. 2017). The locations were resampled after one year with the same corer. Samples were processed in the laboratory following the same protocol as used in the sequential coring approach but without the detail study on the very small root particles. This step was not necessary because the retrieved cores contained mostly newly ingrown living fine roots. Fine root production was calculated as ingrown fine root mass (living and dead roots) in relation to the length of the period between the start of recolonization and harvest (Vogt et al. 1998). To determine the recolonization starting point in the four forest types, we installed four additional ingrowth cores per plot and resampled them at monthly intervals during the subsequent four months. According to this side study, fine roots started to grow into the cores in the lower montane forest two months after exposure, and in the *Ocotea, Podocarpus* and *Erica* forests after three months. These periods were subtracted from the 1-yr-long exposure period. Fine root production was expressed at an annual basis (in Mg ha^{-1} yr^{-1}). Due to logistic problems, we could not retrieve the ingrowth cores from four of the 12 plots (one plot per forest type). The missing production values were estimated from the mean ratio between fine root production and fine root biomass of a forest type.

In analogy to aboveground nutrient use efficiency, we calculated the nitrogen use efficiency of fine root production for the four forest types by dividing fine root production by the N content of fine root biomass. Fine root growth is set equal with fine root litter production, assuming a steady state between fine root production and mortality (Graefe et al. 2008). This calculation uses the fine root N content data of the fine root biomass survey of Sierra Cornejo et al. (2020).

Carbon and nitrogen fluxes to the soil with root mortality were estimated for the four forest types by multiplying the C and N content of the fine root biomass (Table S1, Sierra Cornejo et al. 2020) with the calculated fine root productivity in the plot, assuming equivalence of productivity and mortality.

3.3.3 Statistical analysis

All statistical analyses were conducted in R-3.4.0 (R Core Team 2017). The influence of forest type on total NPP was analyzed with an ANOVA using plot-level means of productivity data. Differences between forest types were detected with a Tukey HSD post-hoc test. Linear and non-linear regressions were calculated to relate (1) the different NPP components, the partitioning of carbon to different sinks, carbon residence time, and nutrient use efficiency (fine root or aboveground production) to elevation, and (2) the NPP components to climatic, edaphic and stand structural variables. Again, plot means were used in the regression analyses. The normality and homoscedasticity of the residuals was visually inspected. We used a significance level of $p < 0.05$ throughout the study.

3.4 Results

3.4.1 Elevational change in NPP

Total NPP (NPP-aboveground plus NPP-belowground) decreased from the lower montane forest at 1800-2040 m a.s.l. (17.8 ± 1.4 Mg ha^{-1} yr^{-1}) to the subalpine *Erica* forest at 3500-3910 m to a third (5.8 ± 0.4 Mg ha^{-1} yr^{-1}) (which equals 8.5 to 2.8 Mg C ha^{-1} yr^{-1}) (Table 3.2, Figure 3.1). All NPP components except fine root productivity decreased continuously with elevation in size, with the steepest slope detected for NPP-aboveground litter fall (reduction to less than 10 %), a moderate slope in NPP-aboveground wood (reduction to about a quarter), and the slightest slope in NPP-coarse roots (Table 3.2, Figure 3.2). The relative proportion of NPP-aboveground litter fall and NPP-aboveground wood changed only little between 1800 and 3200 m to drop sharply toward the subalpine *Erica* forest (Table 3.2). In contrast, fine root productivity calculated with the sequential coring approach remained relatively constant across the elevation transect with 3.6-4.7 Mg ha^{-1} yr^{-1}.

Table 3.2: Net primary productivity (NPP) and its components, carbon residence time (CRT) in different biomass components, and nitrogen use efficiency of productivity (NUE) in the four forest types. Given are means ± SE (n=3). Values in brackets give carbon fluxes (in Mg C ha-1 yr-1). NPP-belowground = NPP-fine roots (Sequential coring) plus NPP-coarse roots.

	Lower montane forest	*Ocotea* forest	*Podocarpus* forest	*Erica* forest
Production (Mg ha^{-1} yr^{-1})				
NPP total	17.79 ± 1.41 (8.47)	13.24 ± 2.31 (6.43)	12.65 ± 0.87 (6.16)	5.75 ± 0.40 (2.78)
Aboveground production	12.06 ± 1.19 (5.83)	8.42 ± 1.89 (4.14)	8.23 ± 0.83 (4.04)	1.40 ± 0.47 (0.69)
Canopy litterfall	8.07 ± 0.42 (3.91)	4.60 ± 0.37 (2.30)	5.21 ± 0.64 (2.58)	0.54 ± 0.05 (0.28)
Leaves	6.14 ± 0.39 (2.98)	3.24 ± 0.34 (1.64)	3.18 ± 0.36 (1.61)	0.009 ± 0.003 (0.005)
Branches	0.95 ± 0.19 (0.45)	0.45 ± 0.09 (0.22)	0.59 ± 0.12 (0.29)	0.32 ± 0.05 (0.16)
Rest	0.99 ± 0.04 (0.48)	0.91 ± 0.18 (0.44)	1.44 ± 0.17 (0.69)	0.22 ± 0.01 (0.11)
Wood	3.99 ± 0.82 (1.92)	3.82 ± 1.63 (1.84)	3.02 ± 0.29 (1.45)	0.86 ± 0.42 (0.42)
Belowground production	5.73 ± 0.22 (2.64)	4.83 ± 0.49 (2.29)	4.42 ± 0.48 (2.12)	4.35 ± 0.08 (2.08)
Fine roots Sequential coring	4.65 ± 0.02 (2.12)	3.80 ± 0.34 (1.79)	3.57 ± 0.54 (1.71)	4.07 ± 0.20 (1.95)
Fine roots Ingrowth core	1.14 ± 0.14 (0.51)	1.12 ± 0.31 (0.54)	1.42 ± 0.11 (0.66)	0.47 ± 0.06 (0.22)
Coarse roots	1.08 ± 0.20 (0.52)	1.03 ± 0.39 (0.50)	0.85 ± 0.07 (0.41)	0.27 ± 0.12 (0.13)
% NPP Aboveground litterfall	46 ± 2	36 ± 4	41 ± 2	9 ±1
% NPP Aboveground wood	22 ± 3	27 ± 7	24 ± 2	14 ±6
% NPP fine roots	26 ± 2	30 ± 5	28 ± 4	72 ± 8
% NPP coarse roots	6 ± 1	7 ± 2	7 ± 1	5 ± 2
CRT Aboveground wood (yr)	68.8 ± 5.1	98.9 ± 15.6	133.8 ± 9.4	48.2 ± 10.8
CRT Coarse roots (yr)	45.5 ± 2.9	62.4 ± 8.8	81.6 ± 4.9	33.0 ± 6.7
CRT Fine roots (yr)	0.52 ± 0.01	0.36 ± 0.04	1.19 ± 0.28	0.64 ± 0.23
NUE (N) Aboveground litterfall (g g^{-1}	70.2 ± 4.8	72.1 ± 1.7	74.4 ± 7.1	111.9 ± 9.7
NUE (N) Fine roots (g g^{-1})	65.0 ± 12.7	58.9 ± 2.2	65.2 ± 1.2	145.05 ± 6.6
Fine root: leaf litter ratio (g g^{-1})	0.76 ± 0.04	1.19 ± 0.14	1.15 ± 0.19	535.60 ± 124.48

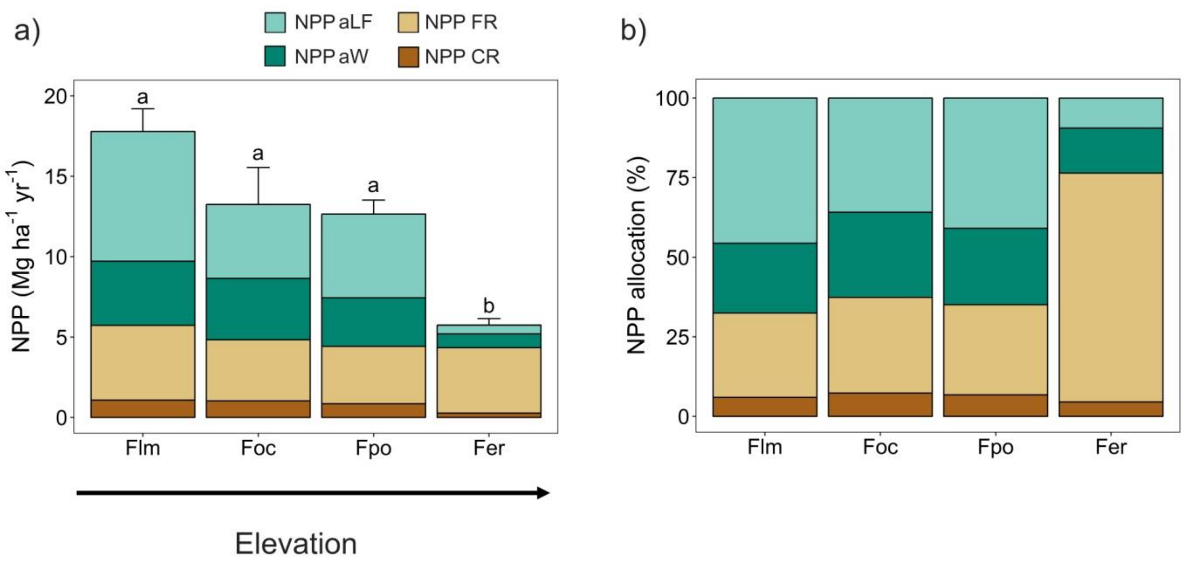

Figure 3.1: a) Net primary productivity (NPP) of the four tropical forest ecosystems by components and b) NPP allocation into the different components. All values are means ± SE (n=12). Lower case letters indicate significant differences between total NPP values of the different ecosystems according to Tukey HSD tests ($p < 0.05$).

In the lower montane, the Ocotea and the Podocarpus forest, 35-45% of total NPP was contributed by aboveground litter production (mostly leaves, twigs < 20 mm, and fruits), 25-30% by NPP-fine roots, and 20-25% by NPP-aboveground wood. NPP partitioning was different in the Erica forest at highest elevation, where about 70% of total NPP referred to fine root productivity, while aboveground NPP (NPP-wood and NPP-litter fall) contributed only with c. 25 % (Figure 3.1). Consequently, the proportion of NPP-fine roots to total NPP increased greatly at the transition from the Podocarpus to the Erica forest upslope, indicating a large belowground shift in carbohydrate allocation above 3000 m (Figure 3.3). This shift was associated with a dramatic increase in the fine root:leaf litter ratio, as leaf litter fall was very small and fine root productivity relatively high in the Erica forest (Table 3.2).

Fine root productivity estimated with the ingrowth core method gave three to four times (in case of the Erica forest even eight times) smaller annual productivity values than the corresponding sequential coring data (Table S 3.2). As for the sequential coring data, no change with elevation was visible for the NPP-fine roots (IC) data (1.1. – 1.4 Mg ha-1 yr-1), but a reduction to the half occurred in the Erica forest (Table 3.2).

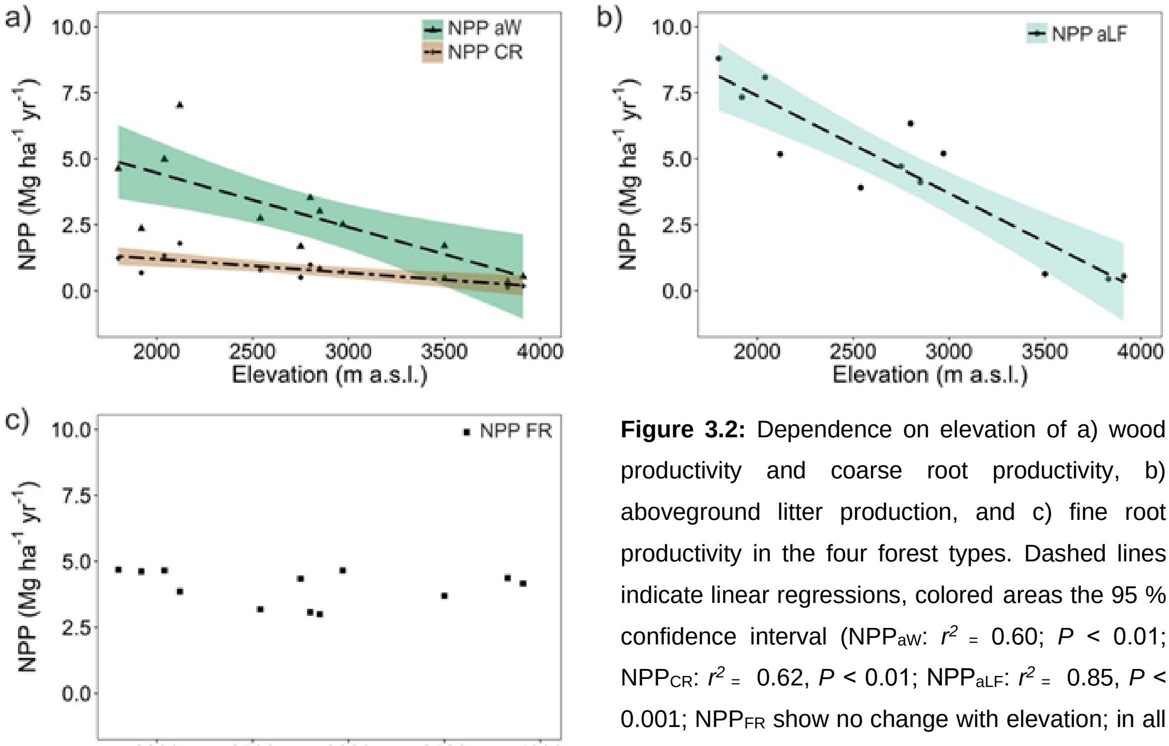

Figure 3.2: Dependence on elevation of a) wood productivity and coarse root productivity, b) aboveground litter production, and c) fine root productivity in the four forest types. Dashed lines indicate linear regressions, colored areas the 95 % confidence interval (NPP_{aW}: $r^2 = 0.60$; $P < 0.01$; NPP_{CR}: $r^2 = 0.62$, $P < 0.01$; NPP_{aLF}: $r^2 = 0.85$, $P < 0.001$; NPP_{FR} show no change with elevation; in all cases (n = 12). aLF – aboveground litter fall; aW – aboveground wood; CR – coarse roots; FR – fine roots.

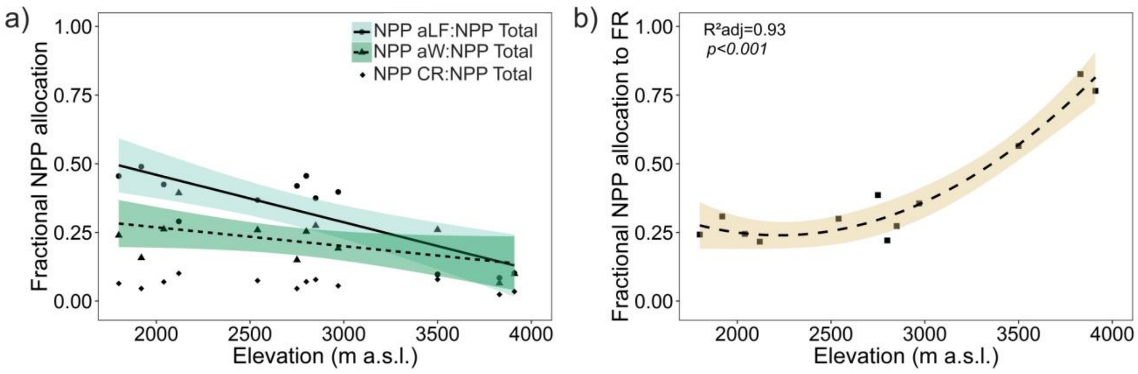

Figure 3.3: Change with elevation in the proportion of (a) aboveground litter (leaves, fruits, twigs), coarse wood productivity, and coarse root productivity, and (b) fine root productivity in total NPP in the four forest types. The different straight lines indicate linear regressions, the curved line a 2nd-order polynomial regression, the colored areas the 95% confidence intervals. (NPPaLF: NPPtotal: $r^2 = 0.68$, $P < 0.01$; NPPaW: NPPtotal showed no change with elevation: $r^2 = 0.31$, $P < 0.1$; NPPCR: NPPtotal showed no change with elevation: $r^2 = 0.23$, $P = 0.12$; NPPFR: NPPtotal: $r^2adj = 0.93$, $P < 0.001$; (n = 12). aLF – aboveground litter fall; aW – aboveground wood; CR – coarse roots; FR – fine roots.

3.4.2 Environmental drivers of NPP

Total NPP and its components aboveground litter, aboveground wood and coarse root productivity showed a tight positive relation to mean annual temperature (and a negative to elevation; Table 3.3). The positive relation to MAP was also tight, and in NPP-aboveground wood and NPP-coarse roots even closer than the relation to temperature. Total NPP and NPP-aboveground litter fall were negatively related to soil C:N ratio, which was not the case in the other NPP components. In contrast, fine root productivity was related neither to elevation, climatic or soil factors, nor to aboveground biomass or basal area (Table 3.3). Some of the biotic and abiotic factors were strongly correlated (Table S 3.3).

Table 3.3: Regression analysis relating the different productivity components to physiographic, edaphic and climatic, and stand structural variables across the four forest types on Mt. Kilimanjaro. Given is the adjusted r^2 and the P value of the relationships.

	Elevation		Mean annual temperature		Mean annual precipitation		Soil C:N ratio		pH (KCl)		Aboveground biomass		Basal area	
	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P	r^2/ r^2adj	P	r^2	P
NPP total	**- 0.87**	**<0.001**	**0.76**	**<0.001**	**0.61**	**<0.01**	**- 0.43**	**<0.05**	- 0.09	n.s.	**0.47**	**<0.05**	**0.54**	**<0.01**
NPP aLF	**- 0.85**	**<0.001**	**0.79**	**<0.001**	**0.53**	**<0.01**	**- 0.46**	**<0.05**	- 0.01	<0.1	**0.68**	**<0.01 ***	**0.47**	**<0.05**
NPP aW	**- 0.6**	**<0.01**	**0.46**	**<0.05**	**0.55**	**<0.01**	- 0.21	n.s.	- 0.21	n.s.	**0.32**	**<0.01**	**0.6**	**<0.01**
NPP CR	**- 0.62**	**<0.01**	**0.48**	**<0.05**	**0.55**	**<0.01**	- 0.21	n.s.	- 0.2	n.s.	**0.64**	**<0.01**	**0.63**	**<0.01**
NPP FR	- 0.04	n.s.	0.05	n.s.	0.01	n.s.	- 0.09	n.s.	- 0.01	n.s.	- 0.18	n.s.	- 0.11	n.s.

NPP in Mg ha^{-1} yr^{-1}, elevation in m a.s.l., mean annual temperature in °C, mean annual precipitation in mm, aboveground biomass in Mg ha^{-1} and basal area in m^2 ha^{-1}. Significant relations are marked in bold (P<0.05), nonlinear relations are indicated by (*) and the r^2_{adj} is given. Negative relations are indicated by (-). aLF – aboveground litter fall; aW – aboveground wood; CR – coarse roots; FR– fine roots.

3.4.3 Carbon residence time and nitrogen use efficiency

Carbon residence time (CRT) as the quotient of productivity and biomass in an NPP component ranged between 48 and 134 years in the aboveground biomass (wood) fraction, and between 33 and 82 years in the coarse root fraction (Table 3.2). CRT was highest in the conifer-dominated *Podocarpus* forest, and lowest in the *Erica* forest, resulting in a humped-shaped relation with elevation ($r^2adj = 0.80$, $p < 0.001$) (Figure S 3.1). We determined CRT values (mean lifespans) for the fine root fraction of 0.36 – 1.19 years with a peak in the *Podocarpus* forest and a minimum in the *Ocotea* forest.

Nitrogen use efficiency of productivity (NUE) was relatively similar for aboveground litter and fine root productivity in a given forest type (Table 3.2). Both efficiencies remained on a similar level in all forest types except in *Erica forest*, which showed 50 to 120 % higher values (Figure 3.4). NUE reached its highest value in the fine root productivity of the *Erica* forest (145 g g^{-1}). The NUE values were negatively related to NPP across the four forest types (Table S 3.4).

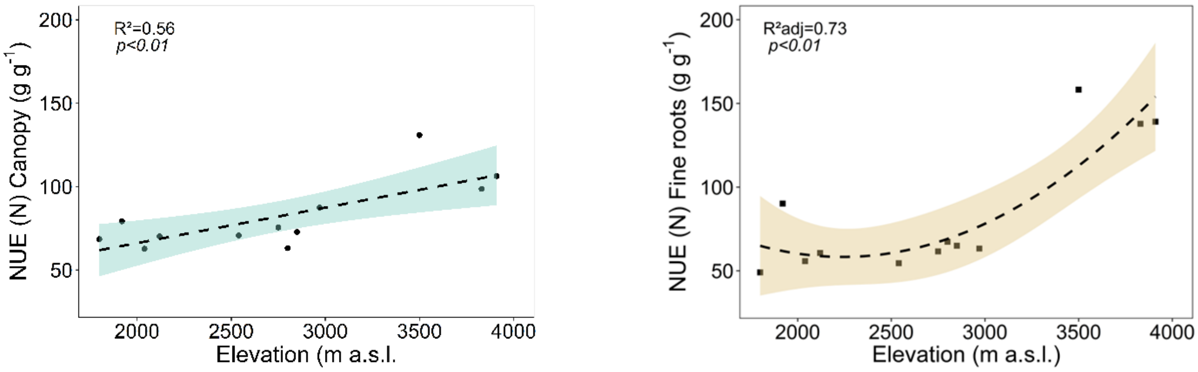

Figure 3.4: Change with elevation in the nitrogen use efficiency of aboveground productivity (a) and fine root productivity (b) in the four forest types. Dashed lines indicate linear regressions or 2nd-order polynomial regressions (n= 12), colored areas the 95 % confidence interval.

3.4.4 Carbon and nitrogen transfers to the soil via leaf and root litter

The leaf- and root-derived carbon and nitrogen fluxes were of similar size in the lower to upper montane forests, while the root litter flux greatly exceeded the leaf-born flux in the *Erica* forest (195.1 ± 9.7 g C m^{-2} yr^{-1} and 2.8 ± 0.3 g N m^{-2} yr^{-1} from fine roots vs. 0.5 ± 0.2 g C m m^{-2} yr^{-1} and 0.01 ± 0.005 g N m^{-2} yr^{-1} from leaves) (Table 3.4).

Table 3.4: Estimated annual transfers of carbon and nitrogen to the soil with leaf litter production and with fine root mortality (sequential coring approach) in the four forest types. Also given are the N content and the C:N ratio in the litter fractions. Assuming a steady state of fine root production and mortality, fine root production is equal to fine root litter production. Given are means ± SE (n=3).

		Lower montane forest	*Ocotea* forest	*Podocarpus* forest	*Erica* forest
C flux ($g\ m^{-2}\ yr^{-1}$)	Fine root litter	211.72 ± 7.44 a	178.91 ± 19.76 a	171.26 ± 27.79 a	195.13 ± 9.65 a
	Leaf litter	298.48 ± 16.23 b	163.88 ± 16.97 a	160.56 ± 16.39 a	0.50 ± 0.15 b
N flux ($g\ m^{-2}\ yr^{-1}$)	Fine root litter	7.67 ± 1.32 a	6.42 ± 0.35 a	5.51 ± 0.92 a	2.83 ± 0.25 a
	Leaf litter	8.53 ± 0.97 a	4.04 ± 0.61 b	3.84 ± 0.93 a	0.011 ± 0.005 b
N ($mg\ g^{-1}$)	Fine root litter	16.47 ± 2.78 a	17.02 ± 0.66 a	15.35 ± 0.27 a	6.92 ± 0.30 a
	Leaf litter	13.87 ± 1.21 a	12.37 ± 0.62 b	11.84 ± 1.68 a	12.80 ± 0.11 b *
C:N ratio ($g\ g^{-1}$)	Fine root litter	29.22 ± 4.71 a	27.71 ± 1.54 a	31.16 ± 0.40 a	69.48 ± 3.25 a
	Leaf litter	37.39 ± 2.86 a	45.26 ± 1.89 b	46.25 ± 6.51 a	48.73 ± 5.24 b

Different lower case letters indicate significant differences between fine root and leaf litter fluxes in a given ecosystem according to ANOVA ($p<0.05$). *Data from David Schellengerger Costa (unpusblished data).

3.5 Discussion

3.5.1 *Decreasing net primary productivity with elevation and its causes*

Our replicated study of key productivity components in four mountain forest types along a 2000-m elevation gradient in tropical East Africa shows a continuous decline of total NPP with increasing elevation, that was associated with a marked belowground shift in carbon allocation toward the fine root system. With a decrease in mean annual temperature from 14.7 to 9.3 °C, total NPP and its aboveground components NPP-aboveground litter fall and NPP-aboveground wood decreased by roughly 30% from the lower montane forest to the montane *Podocarpus* forest, contradicting the conclusions of Luyssaert et al. (2007) and Michaletz et al. (2014) on only minor or no temperature effect on forest productivity in warm-temperate to tropical climates with MAT > 10 °C.

Total NPP values recorded in the four montane forest types on Mt. Kilimanjaro (2.8 – 8.5 Mg C ha^{-1} yr^{-1}) were in the lower range of data from pantropical surveys of forest productivity (3.1 – 21.7 Mg C ha^{-1} yr^{-1}) (e.g. Clark et al. 2001b; Kitayama and Aiba 2002; Aragão et al. 2009), but similar to figures from tropical montane forests in Rwanda (9.2 ± 2.1 Mg C ha^{-1} yr^{-1}) (Nyirambangutse et al. 2017) and the Ecuadorian and Peruvian Andes (3.9 - 6.4 and 4.1 - 7.1 Mg C ha^{-1} yr^{-1}, respectively; Moser et al. 2011; Girardin et al. 2010). The subalpine *Erica* forest at the upper forest line (2.8 Mg C ha^{-1} yr^{-1}) had a 30% lower NPP than the corresponding upper montane forest at high elevations in the Ecuadorian Andes (3060 m a.s.l.) (4.1 Mg C ha^{-1} yr^{-1}) (Moser et al. 2011) and almost 65% lower NPP than the tree line forest in the Peruvian Andes (3500 m a.s.l.) (7.9 Mg C ha^{-1} yr^{-1}) (Malhi et al. 2017). Several factors may have contributed to this difference, among them the local species composition and related differences in stand structure, but also the regional climatic and edaphic conditions. The Ecuadorian upper montane forest has a larger stem density, basal area and mean tree height than the *Erica* forest in East Africa. The total NPP estimations in the Peruvian forest include measurements of small trees (2-10 cm DBH) productivity, leaf herbivory and carbohydrate transfer to the mycorrhiza (Malhi et al. 2017), which have not been quantified in our study. However, even when we increase our NPP value by 20 % to account for these NPP components, the Peruvian tree line forest is still more productive than its counterpart in eastern Africa. Both Andean upper montane forests grow under higher precipitation and temperature levels than the *Erica* forest on Mt. Kilimanjaro (Moser et al. 2011; Malhi et al. 2017), demonstrating the transition to a high-elevation climate with relatively low precipitation on top of the volcano (1050 mm yr^{-1}), whereas the uplift of moist air masses proceeds to higher elevations in the Andes.

Until recently, the change in forest productivity with elevation in tropical mountains was only studied with respect to aboveground NPP, while ignoring root productivity (Raich et al. 1997; Kitayama and Aiba 2002; Wang et al. 2003). Only the two studies from Peru and Ecuador by Girardin et al. (2010) and Moser et al. (2011) have investigated root production as well, allowing conclusions on elevational change in total NPP. The observed NPP trends differ between the three transects. While total NPP decreased more or less continuously from the lower montane to the upper montane forest in the Ecuadorian Andes (Moser et al. 2011; Leuschner et al. 2013), an abrupt productivity decline from the submontane (1500 m) to the cloud forest (1700 m) and nearly constant productivity higher upslope was found in the Peruvian Andes (Girardin et al. 2010; Malhi et al. 2017). In contrast, we found on Mt. Kilimanjaro a gradual NPP decline from the lower montane to the upper montane (*Podocarpus*) forest and an abrupt decrease toward the subalpine *Erica* forest. Carbon residence time in the wood increased from the more productive lower montane forest to the upper montane *Podocarpus* forest and sharply decreased to the *Erica* forest, revealing a hump-shaped curve along the slope. This pattern may partly be explained by the change from a forest with evergreen broadleaf trees to a conifer-dominated forest with *Podocarpus latifolia* and finally to an *Erica trimera* forest, which is exposed to more extreme minimum temperatures and of lower stature than the forests further downslope.

The data from Tanzania and Ecuador suggest that a major driver of the NPP decline is the reduction in leaf area index (LAI). In both transects, LAI decreased by more than 50 % over a 2000-m elevation gradient from lower montane to upper montane elevation (Leuschner et al. 2007; Rutten et al. 2015). A decreasing photosynthetic capacity (A_{sat}) with elevation may also contribute to the productivity decline. According to a pan-tropical A_{sat} data base, the light-saturated net photosynthesis of trees at ambient temperature decreases on average by 1.3 µmol CO_2 m^{-2} s^{-1} per 1 km altitude increase in tropical mountains (Wittich et al. 2012). The decrease is typically linked to an altitudinal decrease in mass-related foliar N content (Moser et al. 2011; Schellenberger Costa et al. 2017). The results from the elevation transects in the Andes and eastern Africa indicate that both leaf area and photosynthetic capacity are influenced by the N supply along the mountain slope with the consequence that local and regional differences in soil fertility may cause somewhat different elevational trends in LAI, A_{sat} and total NPP.

Soil chemical and biological analyses in the Tanzanian and Ecuadorian transects indicate that N availability decreases with elevation in tropical mountains. On Mt. Kilimanjaro, both soil and leaf C:N ratio increased with elevation toward the subalpine *Erica* forest, while fine root N content decreased, in conjunction with declining decomposition rate (Becker and Kuzyakov 2018). Moreover, we found a large increase in the N use efficiency of canopy and root productivity from the montane *Podocarpus* forest to the higher *Erica* forest on Mt.

Kilimanjaro, linked to a pronounced shift in C allocation toward the root system. These findings explain the significant negative relation that exists between total NPP and soil C:N ratio in our four studied forest types. The elevational temperature decrease can influence N cycling through a negative impact on various processes, among them ammonification and nitrification, the diffusion of inorganic N compounds in the soil, the activity of mycorrhiza, and root N uptake (Chapin 1980; Pendall et al. 2004; Marschner 2012; Robertson and Groffman 2015). We assume that the temperature effect on N cycling and N supply and resulting reductions in leaf area (and possibly photosynthetic capacity) represent a major pathway through which elevation is acting on total C gain. In addition, tree growth was found to be stimulated by low nutrient input at the tree line ecotone in the Swiss Alps (Möhl et al. 2018), supporting our assumption of nutrient limitation of aboveground tree productivity at high elevations. The replacement of macrophyllous, broadleaved trees by southern hemispheric conifers at the upper montane zone and ericoid trees with more sclerophyllous leaves and their specialized ericoid mycorrhiza at the subalpine zone can also be interpreted as an indication of increasing nutrient limitation at higher elevations.

Clearly, other environmental factors besides N supply must be responsible for the elevational NPP decrease as well, notably thermal limitation of meristem activity (Körner 2012). However, there is no physiological reason to assume that low temperatures should constrain aboveground meristematic activity more than belowground meristematic activity. Thus, a direct thermal effect on meristematic activity cannot explain the pronounced allocation shift toward root growth. Another factor possibly limiting forest productivity at higher elevations is reduced irradiance due to persistent cloud cover at montane elevation, as it was assumed for the Peruvian transect (Malhi et al. 2017). However, on Mt. Kilimanjaro, cloudiness was highest in the *Ocotea* forest belt (Hemp 2006), where total NPP was still comparatively high with 6.4 Mg C ha^{-1} yr^{-1}, exceeding the NPP of the *Erica* forest higher up more than twofold. This makes it unlikely that enhanced light limitation in the cloud belt plays an important role in this transect.

The regression coefficients suggest that precipitation (MAP) is influencing NPP, but to a lesser degree than temperature and related phenomena such as nutrient supply. Our results are in line with an analysis of large NPP data sets that revealed an increase in the NPP of tropical forests with MAP until fairly high values (> 2500 mm), followed by a decrease at even higher rainfall possibly due to reduced irradiance or reduced soil biological activity and N mineralization in wet soil (Schuur 2003; Del Grosso 2008). On Mt. Kilimanjaro MAP (not higher than 2200 mm) seems to promote forest NPP across the entire sequence of forest belts present on this mountain.

3.5.2 Fine root productivity and elevational shifts in carbon allocation

By including fine root production, our study is among the few that allows estimating the total NPP of tropical forest along elevation gradients and analyzing shifts in carbon allocation. The obtained productivity data have to be interpreted with caution, however. The sequential coring and ingrowth core methods yielded largely different results, as has been found in other studies (Vogt et al. 1998; Hertel and Leuschner 2002; Moser et al. 2010). Both approaches have their shortcomings. The sequential coring technique with the minimum-maximum calculation approach is believed to underestimate fine root productivity, when the seasonal variation in total fine root mass (live and dead) is larger than the difference between highest and lowest fine root mass count, and fine root decomposition is rapid (Vogt et al. 1998). The ingrowth core approach investigates root growth under more artificial conditions and starts from an injured root system. The growth rates estimated by this method may give an impression of the root system's regeneration potential rather than reflecting root productivity under natural conditions. As in the study of Moser et al. (2010), our ingrowth core productivity values were several times smaller than those obtained from sequential coring, especially at high elevations. Based on the ingrowth core data, we calculated fine root productivities in the range of 0.2 to 0.7 Mg C ha^{-1} yr^{-1} and a mean carbon residence time between 1.4 and 5.0 years for the fine root fraction in the four forest types (Table S2). This is higher than fine root longevities derived from mini-rhizotron observation in tropical montane forests in the Ecuadorian Andes (0.70-0.95 years; Graefe et al. 2008) and also exceeds the average fine root longevity of 1.32 years found by Gill and Jackson (2000) for tropical forests in a meta-analysis (mostly sequential coring studies). According to our sequential coring data, average fine root longevity was 0.4 to 1.2 years, which matches these figures better. We thus suggest that the sequential coring data may give a more realistic picture of fine root dynamics than the ingrowth core results, which have greater value for site comparison.

Three main conclusions may be drawn from our fine root productivity (sequential coring) data: (1) Fine roots are the most dynamic component in the studied tropical mountain forests, exceeding even the leaf fraction. (2) With 26 to 72 % of total NPP, fine root productivity consumes more carbon than wood production. (3) In contrast to the other productivity components, fine root growth seems to be controlled mostly by intrinsic factors (probably sink strength and carbohydrate availability), as significant relations to climatic, edaphic and stand structural parameters were lacking. With the resource balance hypothesis (Bloom et al., 1985), the pronounced shift in carbon allocation from leaf growth (NPP-aboveground litter fall) to fine root growth from the upper montane *Podocarpus* to the subalpine *Erica* forest is interpreted as a change from predominant light limitation to nutrient (or water) limitation of growth. In our study, decreasing N supply with elevation is a plausible explanation, but deficiency in other elements such as phosphorus (P) or potassium is possible at tropical high-elevation sites (Graefe et al. 2010). Yet, leaf mass N:P ratios suggest primarily N limitation of growth on Mt. Kilimanjaro (Townsend et al. 2007). A belowground shift of C allocation has been concluded from studies on productivity or biomass changes along elevation transects in other tropical mountains (Leuschner et al. 2007; Girardin et al. 2010; Moser et al. 2011; Leuschner et al. 2013) and temperate mountains (Hertel and Schöling 2011, Mao et al. 2015) as well. Fine root studies at the alpine and arctic tree line also indicate that the C allocation preference of trees shifts belowground in cold environments (Ruess et al. 2003; Kubisch et al. 2017). As these studies were conducted mostly in humid climates, it is unlikely that water limitation is a possible driver of the allocation shift. Even though Mt. Kilimanjaro receives relatively low precipitation on its top, the amount in the *Erica* forest belt (1050 mm) seems to be high enough to exclude a main role of water shortage.

Thus, we assume that high-elevation forests in tropical mountains are not only limited by low temperatures that restrict meristem activity, but their growth seems to be constrained by nutrient (in particular N) shortage as well. Whether reductions in N supply, N diffusion in the soil, or impaired N uptake and relocation in the plant are primarily limiting, has to be addressed in physiological and soil chemical studies that should include soil warming experiments.

Fine roots are not only large sinks for photosynthates, but also important sources of organic C and N which is transferred to the soil upon root death and via root exudation and transfer to the mycorrhiza (Rasse et al. 2005; Godbold et al. 2006). Our fine root productivity data suggest that the C and N flux to the soil with root litter is roughly of similar importance as the leaf litter flux in the lower montane and *Ocotea* forest, while it represents an important C and N source supplied by woody plants in the *Erica* forest. Comparable results have been obtained from Ecuadorian forests at high elevations (Röderstein et al. 2005). This highlights the need to include fine root dynamics in biogeochemical studies in forests of the tropics and elsewhere (Litton et al. 2007).

3.5.3 Conclusions

To our knowledge, this is the first study on forest productivity and its main above- and belowground components along an elevation transect on a paleotropical mountain. In accordance with earlier studies in neotropical elevation transects, we found a more or less continuous decrease of total NPP with elevation, confirming a pronounced temperature influence on forest productivity also in the temperature interval between 10 and 15 °C. Comparison with other tropical and non-tropical elevation transect studies reveals that the pronounced belowground shift in carbon allocation with elevation is a characteristic feature of mountain forests worldwide, suggesting increasing growth impairment through N (nutrient) limitation toward higher elevations, which seems to act independently from direct low-temperature effects on meristem activity. Inherent bias in the fine root productivity data warrants caution in the interpretation of the calculated fine root production data. Nevertheless, from the remarkable constancy of root productivity along the slope and the fairly good agreement with earlier neotropical fine root studies, we assume that the observed patterns are mostly valid. Future direct observational studies with mini-rhizotrons could provide a welcome independent estimate of fine root productivity. We conclude that initiatives to measure all major components of forest productivity along elevation gradients can generate valuable additional insights in the climate dependence of carbon cycling in woodlands.

3.6 Author contributions

DH developed the study design, NSC conducted the field work, data processing and analysis, JNB, DSC and AH contributed with soil and stand structure data. AH selected and established the research plots. NSC, CL and DH interpreted the data and the paper writing was done by CL, NSC and DH with contributions of all authors.

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3.9 Supplementary material

Table S 3.1: Carbon and nitrogen content in fine root biomass of four forest ecosystems along the elevation on Mt. Kilimanjaro (Sierra Cornejo et al. 2020).

Ecosystem	Content in fine roots (mg g^{-1})	
	Carbon	Nitrogen
Lower montane forest	455.2 ± 14.2	16.5 ± 2.8
Ocotea forest	470.3 ± 17.3	17.0 ± 0.7
Podocarpus forest	478.0 ± 4.8	15.4 ± 0.3
Erica forest	479.0 ± 2.3	6.9 ± 0.3

Table S 3.2: Net Primary Productivity (NPP), NPP allocation to the components, carbon residence time (CRT), carbon (C) and Nitrogen (N) fluxes from fine roots to the soil via root dead; and fine root:leaf litter ratio of four forest ecosystems along the elevation on Mt. Kilimanjaro calculated using the ingrowth core method for fine root NPP estimation. Given are means ± SE (n=3). Values in brackets refer to carbon sequestration (Mg C ha^{-1} yr^{-1}). FR – fine roots; aLF – aboveground litter fall; aW – aboveground wood; CR – coarse roots.

Ingrowth core method				
	Lower montane forest	*Ocotea* forest	*Podocarpus* forest	*Erica* forest
Production (Mg ha^{-1} yr^{-1})				
NPP total	14.28 ± 1.43	10.57 ± 2.26	10.50 ± 0.98	2.15 ± 0.61
Fine roots Ingrowth core	1.14 ± 0.14	1.12 ± 0.31	1.42 ± 0.11	0.47 ± 0.06
% NPP aLF	57 ± 3	46 ± 6	49 ± 3	28 ± 5
% NPP aW	27 ± 4	33 ± 8	29 ± 2	36 ± 8
% NPP FR	8 ± 1	11 ± 4	14 ± 1	25 ± 7
% NPP CR	7 ± 1	9 ± 2	8 ± 1	12 ± 2
BAR Fine roots (CRT) (yr)	2.16 ± 0.23	1.32 ± 0.13	2.77 ± 0.23	5.31 ± 1.33
BAR total (CRT) (yr)	51.54 ± 2.90	68.82 ± 11.60	90.18 ± 6.50	28.81 ± 5.08
C flux from fine roots	52.12 ± 7.33	53.21 ± 16.04	67.71 ± 4.96	22.73 ± 3.07
N flux from fine roots	1.92 ± 0.50	1.87 ± 0.46	2.17 ± 0.13	0.33 ± 0.04
Fine root:leaf litter ratio	0.19 ± 0.01	0.35 ± 0.11	0.45 ± 0.05	66.84 ± 21.87

C content of fine roots obtained by the ingrowth core approach was determined using a C/N elemental analyzer (Vario EL III, Hanau, Germany). Three samples per plot were analyzed, with each sample consisting of two root subsamples that were mixed.

Table S 3.3: Pearson correlation analysis between elevation, climatic, edaphic and stand structural variables for the four ecosystems along the elevation. Pearson correlation coefficient *r* and *P* are given. Elevation in m a.s.l., mean annual temperature (MAT) in °C, mean annual precipitation (MAP) in mm, aboveground biomass (AGB) in Mg ha^{-1}, and basal area in m^2 ha^{-1}**.**

	MAT		MAP		Soil C:N ratio		pH		AGB		Basal area	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Elevation	**- 0.97**	**<0.001**	**- 0.87**	**<0.001**	**0.61**	**<0.05**	0.37	n.s.	**- 0.62**	**<0.05**	**- 0.66**	**<0.05**
MAT			**0.81**	**<0.01**	**- 0.6**	**<0.05**	- 0.27	n.s.	0.5	n.s.	0.57	<0.1
MAP					- 0.34	n.s.	- 0.46	n.s.	**0.75**	**<0.01**	**0.72**	**<0.01**
Soil C:N ratio							- 0.25	n.s.	- 0.25	n.s.	- 0.31	n.s.
pH									- 0.27	n.s.	- 0.2	n.s.
AGB											**0.95**	**<0.001**

Climatic data: MAT from Appelhans et al. (2015) and MAP from Hemp (2006), topographic and stand structure data from Hemp (unpublished data), *Erica* forest stand structural data from Schellenberger Costa (unpublished data), soil data from Becker (unpublished data). Values in bold indicate significant correlation (p< 0.05). Positive correlations are marked with (+) and negative ones with (-).

Table S 3.4: Relation of NUE from aboveground litter fall and fine roots to elevation. NPP total in Mg ha^{-1} yr^{-1} and NUE in g g^{-1}.

	NUE Canopy litter		NUE Fine roots	
	r²adj	*P*	*r²adj*	*P*
NPP total	**- 0.65**	**<0.01**	**- 0.70**	**<0.001**

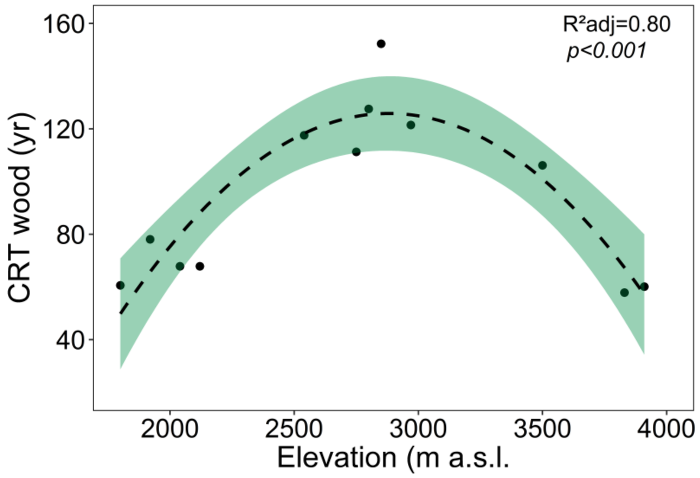


Figure S 3.1: Change with elevation in the carbon residence time (CRT) of aboveground wood in the four forest types. The dashed line indicates a 2nd-order polynomial regression (n= 12), the colored area the 95 % confidence interval.

CHAPTER

4

Effects of land-use change and disturbance on the fine root biomass, dynamics and related root traits of forest and savanna ecosystems at different elevations at Mt. Kilimanjaro

Natalia Sierra Cornejo, Joscha N. Becker, Andreas Hemp and Dietrich Hertel

4.1 Abstract

Tropical forests and savanna ecosystems are threatened by anthropogenic activities such as conversion into agricultural land, logging and fires. Land-use change and disturbance affect ecosystem properties like the fine root system functioning, with consequences on the ecosystems' carbon (C) and nitrogen (N) cycle, as fine roots are one of the physiologically most important tree components. We studied the effects of different types of land-use change and disturbance on the fine root biomass, dynamics, morphology and chemistry as well as on the C and N fluxes to the soil via fine root litter across a variety of natural and disturbed ecosystems distributed in four elevational zones at Mt. Kilimanjaro (Tanzania). We found a decrease of the fine root biomass (80%), necromass (80-90%), production (50-60%) and on C and N fluxes (60-80%) to the soil via fine root mortality with land-use change in almost all the elevation zones. In the lower montane zone, the traditional agroforestry homegardens are highlighted for their high fine root turnover rates and for their similar stand fine root production, C and N fluxes and leaf litter quality compared to the natural lower montane forest. The decrease of C and N fluxes with forest disturbance was markedly strong in the upper montane zone (60 and 80% decrease respectively), where patches of *Podocarpus* forest have been disturbed by fire. We conclude that changes on species composition, stand structure and land management practices caused by land-use change and disturbance have a strong impact on the fine root system. Consequently, fine root C stocks, fine root production and the C and N supply to the soil from root litter are modified, which strongly affects the ecosystems' C and N cycle.

Key words

Agroforestry, East Africa, fine root biomass, fine root dynamics, fine root morphological traits, forest disturbance, land-use change, root litter.

4.2 Introduction

Up to 85 % of the world's forests is subject to different kinds of anthropogenic use (IPCC, 2019). Conversion of forests by human activities plays a major role for climate change as this is the second largest driver of greenhouse gas emissions (25 % of the global emissions) (IPCC, 2019). The increase of population and higher resource demands, together with the response to economic opportunities, depending on institutional factors, are among the main drivers of land-use change (Lambin et al., 2001). In addition, ecosystem disturbance can be intensified by climate change, e.g. through more aggressive fires caused by drier conditions (Hemp, 2005). Among the terrestrial biomes, tropical forests are the most intensively used by humans, with 70% of their surface being fragmented or degraded by different activities, from intensive permanent agriculture to logging and attendant fire (Meiyappan and Jain, 2012; Lewis et al., 2015; Mercer, 2015). In particular, the total area of forest cover losses in Africa for the period 2000-2005 accounted for 11.5 Mio ha (Hansen et al. 2010), the rates of forest loss in sub-Saharan Africa being among the fastest in the world (Fisher, 2011).

Land-use change has strong effects on biodiversity, plant community composition and stand structure characteristics, as well as on climate regulation, physical properties of ecosystems and biogeochemical cycles (Foley et al., 2005; Canadell et al., 2007; Vitousek et al., 2008; Gerschlauer et al., 2016; Peters et al., 2019). Influential factors of the carbon (C) and nitrogen (N) cycle such as C storage, C allocation to the fine root system, soil N availability and fluxes of matter from plants to the soil (e.g. plant litter) are consequently affected. Studying the impact of land-use change on plant components and related activity, such as fine root dynamics, is crucially important to understand its effects on ecosystem functioning and processes.

Fine roots (Ø < 2 mm) are the responsible organs for nutrient and water uptake. At the same time, they hold rapid turnover rates, release exudates, establish symbioses with mycorrhiza and bacteria and their litter contributes as substrate for the soil fauna and microorganisms (Bardgett et al., 2014; Jackson et al., 1997; Jones et al., 2005; Gill and Jackson 2000). They also shape the soil, affecting soil stability, porosity and bulk density (Bardgett et al., 2014). Fine root functions are tightly linked to their morphological and chemical traits. For instance, nutrient uptake is related to the absorptive surface as well as to the length of the roots to reach nutrient patches, reflected in the specific root area (SRA) and specific root length (SRL); the ability to penetrate compact soils and the storage capacity are related to root diameter; the carbon and nutrient storage, as well as the defense against herbivory and drought are linked to root tissue density (RTD); and the metabolic activity to root N content (RNC) (Freschet and Roumet, 2017). Besides, these traits influence fine root lifespan (i.e. the inverse of turnover), which is a determinant factor in fine root activity, young roots being more active than older ones (Eissenstat et al., 2000; McCormack and Guo, 2014; Weemstra et al., 2016).

The alteration of species composition, stand structure and soil properties caused by land-use change affects the fine root biomass, dynamics and morphological traits (Leuschner et al., 2009; Rajab et al., 2016; Cai et al., 2019). In addition, land-use management of agricultural systems entails the use of different practices (e.g. fertilizers, tillage, removal of plant litter) modifying the C and N cycle of the previous natural ecosystems (Allison and Vitousek, 2004; Balesdent et al., 2000; Beer et al., 1998; Gerschlauer et al., 2016) and thus, the fine root system.

Although fine root traits are genotypically determined, they are highly plastic and present a large intra and interspecific variability. This characteristic enables plants to respond to changes in soil nutrient availability, as well as to coexisting species with a wide range of strategies of C investment into the fine root system (Pregitzer et al., 1993; Chapman et al., 2012; Hodge, 2004; Valverde-Barrantes et al., 2013; Bardgett et al., 2014), which are based on a cost-benefit approach (Eissenstat et al., 2000). An acquisitive strategy invest resources on a high metabolic activity, entailing high nutrient uptake rates and short lifespan, together with a high specific root length (biomass per length unit) to explore and exploit more soil volume (Eissenstat et al., 2000; Weemstra et al., 2016). On the other hand, a conservative strategy holds lower metabolic rates together with long lifespan and high root tissue density to protect fine roots, e.g. against herbivory.

Another important component of the C and nutrient cycling is plant litter, as it represents a major source of organic matter to the soil. Land-use conversion affects fluxes of plant litter through changes in quantity and quality (N content, C:N ratio, lignin) of litter production. Both of these characteristics influence the soil microbial community composition and activity (Wardle et al., 2004; De Deyn et al., 2008; Bardgett et al., 2014) and thus, the soil N availability and C stocks. Fluxes of C and N to the soil via fine root mortality have not received as much attention as the ones from leaf litter, especially in tropical ecosystems (Matamala et al., 2003). The study of fine roots is crucial to understand the changes on the C and N cycle along the plant-soil interface in a context of land-use change.

Forests at Mt. Kilimanjaro play a key role in the regional climate regulation, the provision of water, firewood as well as fertile land for food cultivation, among other benefits for the local communities (Hemp, 2005; Agrawala et al., 2003;). Mt. Kilimanjaro harbors a wide range of natural and disturbed ecosystems distributed in vegetation belts across the elevation (Hemp, 2006a). In the foothills, 85 % of the savanna was converted to maize, millet and bean fields during the period from 1968 to 1985 (Soini, 2005; Hemp and Hemp, 2018). Upwards, from the lower montane forest until the border of Kilimanjaro National Park, around 1800 m a.s.l., two agroforestry systems (among others) give sustain and income to the local population. The shade coffee plantations as an intensive system and the traditional “Chagga homegardens”, as a sustainable land-use model which consist on a multilayer system with autochthonous trees, banana plants, coffee trees and other shadow tolerant crop species (Soini 2005; Hemp, 2006b). In the middle montane forest zone (until 2600 m a.s.l.), selective logging of the dominant species, *Ocotea usambarensis*, took place until the year 1984, these forests patches still being under regeneration process (Rutten et al., 2015). In the case of the upper montane zone, areas of the forest dominated by *Podocarpus latifolius* have been replaced by *Erica excelsa* forest due to uncontrolled anthropogenic fires (honey collectors and poachers) and intensified by the drier conditions consequence of climate change, (Hemp, 2005).

The wide range of natural and anthropogenic ecosystems occurring at Mt. Kilimanjaro gives the opportunity to assess the impact of different types of land-use conversion and disturbance on ecosystems processes such as C and N cycle. Therefore, we study fine root biomass and dynamics, C and N fluxes to the soil via fine root mortality and fine root morphological and chemical traits at four elevation zones (three in the case of fine root morphological and chemical traits) covering different natural and disturbed ecosystems. The specific objectives of this study are: (1) to determine the differences on fine root biomass, dynamics and other traits among natural/semi-natural and disturbed ecosystems in each elevation zone; and (2) to assess the effects of land-use change on the C and N fluxes to the soil via fine root litter.

4.3 Methods

4.3.1 Study area

This study is part of the KiLi project (DFG-FOR1246), an interdisciplinary framework with the aim of studying the effects of a climate and land-use gradient on biodiversity, biotic interactions and biogeochemical processes at the southern and south-eastern slopes of Mt. Kilimanjaro, in northern Tanzania (3°4´33´´S, 37°21´12´´E). This free standing mountain exhibits a vertical zonation of vegetation belts (see detailed description by Hemp, 2006a). In each elevation zone, natural and semi-natural ecosystems together with areas of human-induced disturbance are present. Our study includes four of these elevation zones (colline, lower, middle, and upper montane forest), covering four natural (or semi-natural) ecosystems and five disturbed ones (Table 4.1). At the foothills of the mountain, in the colline zone (800 - 1100 m a.s.l.), savanna woodlands, with Acacia-Commiphora trees dominating the landscape, represent the semi-natural ecosystem type, as they are object of occasionally grass cutting and fire by farmers. Their conversion into maize fields (together with sunflowers and beans), for local and regional food supply, has increased during the last decades (Soini, 2005). In the lower montane zone (1200 - 2000 m a.s.l.), the lowland montane forest characterized by *Macaranga kilimandscharica*, *Agauria salicifolia* and, to a lesser degree, *Ocotea usambarensis*, is subject of low intensity logging of small stems for fire wood by local people. At this elevation zone, there is also a traditional multicrop system called “Chagga homegarden”. It consists of a mixed system of banana and coffee as dominant crops, together with cultivated fruit trees (e.g. *Persea americana*) and shaded tolerant crops as taro, yams and beans under remnant natural forest trees (e.g. *Albizia schimperiana*, *Grevillea robusta*) (Hemp, 2006b). The small holder farmers obtain food, woody and medical products for subsistence and some income for coffee sales. The only fertilizer used is dung, and its application is low to no existent. Weed control is done by digging the first 20- 30 cm of soil several times per year. In this vegetation belt, also commercial coffee plantations are present. This intense agroforestry system consists of coffee trees organized in lines separated by approx. 2 m together with several shade trees (e.g. *Grevillea robusta, Albizia sp.*). Hose and aerial irrigation is used, and organic (urea) or/and inorganic (NPK) fertilizer is applied several times per year. Litter from pruning is removed from the system. In the middle montane zone (2100 – 2800 m a.s.l.), the natural *Ocotea* forest is dominated by *Ocotea usambarensis*, *Ilex mitis*, *Xymalos monospora* and the tree fern *Cyathea manniana* (Hemp, 2006a). Due its high commercial value, *Ocotea usambarensis* has been a target for selective logging until the year 1984, when logging was banned (Agrawala, 2013), the disturbed forest areas still being under regeneration process (Rutten et al., 2015). The upper montane zone (2800 - 3100 m a.s.l.) hosts *Podocarpus latifolius* as the dominant tree species, together with *Hagenia abyssinica* and *Prunus africana*. In this zone, human-induced fires entailed the replacement of *Podocarpus latifolius* as the dominant species by *Erica excelsa*, which re-sprout from stumps (Hemp, 2005).

Our study design consists of 5 replicates of each natural and disturbed ecosystem type in their corresponding elevation zone. In total, 45 plots with 0.25 ha size were sampled. The tropical montane forest, *Ocotea* and *Podocarpus* disturbed forest plots are located inside Kilimanjaro National Park, while the savanna, maize, homegarden and coffee plantation plots are outside the protected area. The colline zone (savanna and maize fields) was left out from the fine root morphological analyses as maize, being a grass, holds very different root morphological traits than the woody plant roots in which we focused.

Table 4.1: Characteristics of natural and disturbed ecosystems along the land-use gradient at Mt. Kilimanjaro. Mean ± SE (n=5)

	Savanna (Sav)	Maize field (Mai)	Lower montane forest (Flm)	Homegarden (Hom)	Coffee plantation (Cof)	*Ocotea* forest (Foc)	*Ocotea* forest logged (Fod)	*Podocarpus* forest (Fpo)	*Podocarpus* forest disturbed (Fpd)
Land use category	semi-natural	agricultural	semi-natural	agroforestry	agroforestry	natural	disturbed	natural	disturbed
Elevation (m a.s.l.)	871-1130	860-1020	1620-2040	1150-1840	1120-1360	2120-2750	2220-2560	2720-2970	2770-3060
MAT (°C)	23.8 ± 0.5	23.8 ± 0.4	15.2 ± 0.4	18.6 ± 0.9	20.5 ± 0.9	11.6 ± 0.4	12 ± 0.5	9.4 ± 0.2	9.8 ± 0.4
MAP (mm)	820 ± 94	840 ± 49	2114 ± 139	1836 ± 233	1609 ± 136	2042 ± 192	1995 ± 156	1474 ± 109	1426 ± 102
SOC (g kg^{-1})	26.5 ± 3.6	15.1 ± 2.35	137.9 ± 1.3	54.6 ± 10.7	36.6 ± 7.77	194.6 ± 12	188 ± 16	213 ± 11.2	199 ± 19.9
C:N soil	14.44 ± 0.90	11.57 ± 0.42	14.50 ± 0.96	12.19 ± 0.49	11.82 ± 0.54	18.80 ± 0.70	18.25 ± 1.21	18.80 ± 1.00	21.50 ± 1.18
pH (KCL)	5.38 - 7.27	4.56-6.34	4.23 - 5.30	4.45-5.93	4.01-5.64	3.49 - 4.25	4.32-4.39	3.83 - 5.35	4.00-4.85
VWC (%)	12.9	11,8	11.2	16.4	16.8	22.9	13.1	19.3	16.8
BD (g cm^{-3})	1.01 ± 0.06	1.09 ± 0.03	0.45 ± 0.06	0.71 ± 0.08	0.89 ± 0.11	0.31 ± 0.02	0.33 ± 0.03	0.26 ± 0.02	0.32 ± 0.03
Texture (%) clay/silt/sand	27/34/39	36/44/20	45/41/14	44/39/17	46/39/15	48/41/10	47/41/11	44/47/9	42/46/13
AGB (Mg ha^{-1})	10.0 ± 2.1 *	16.6 ± 2.5 *	360.1 ± 88.8	92.5 ± 17.2	58.0 ± 28.5	280.5 ± 48.8	356.6 ± 22.3	366.7 ± 3.5	…
Stem density (n ha^{-1})	45 ± 12	…	388 ± 22	424 ± 23	226 ± 23	309 ± 20	598 ± 87	516 ± 76	1598 ± 378
Basal area (m^2 ha^{-1})	0.92 ± 0.24	…	49.50 ± 6.30	21.91 ± 3.97	11.84 ± 3.82	46.96 ± 5.26	62.58 ± 2.59	58.66 ± 3.62	40.54 ± 8.63
DBH (cm)	15.88 ± 0.94	…	35.43 ± 3.17	21.64 ± 1.96	24.78 ± 1.19	22.58 ± 1.19	23.47 ± 1.27	36.74 ± 3.11	18.87 ± 3.97
Mean tree height (m)	4.63 ± 0.26	…	17.68 ± 1.64	7.33 ± 0.70	9.14 ± 0.67	12.04 ± 0.89	13.96 ± 0.89	16.16 ± 1.25	8.94 ± 1.95
LAI (m^{-2} m^{-2})*	0.77 ± 0.10	…	5.87 ± 0.30	2.64 ± 0.06	1.97 ± 0.30	5.32 ± 0.76	6.49 ± 0.43	5.30 ± 0.35	4.01 ± 0.38
LUI	0.13 - 0.35	0.53 - 0.79	0.08 - 0.17	0.45 - 0.63	0.95-1.00	0.00 - 0.01	0.07 -0.11	0.00 - 0.01	0.21 - 0.25

MAT: mean annual temperature, MAP: mean annual precipitation, SOC: soil organic carbon, VWC: volumetric water content, AGB: aboveground biomass, LAI: leaf area index, LUI: land-use index. AGB refers only to tree woody biomass except for (*) that refers total aboveground biomass. In homegardens and coffee plantations, stand structure variables refers to woody plant of all DBH sizes. In *Ocotea* and *Podocarpus* forest natural and disturbed, stand structure variables refer to woody plants with DBH > 10 cm. MAT data from Appelhans et al. (2015), MAP, topographic and stand structure data from Hemp (2006), Hemp (unpublished data), soil data for mineral topsoil (0-10 cm) from Becker (unpublished data), Pabst et al. (2015) and Gütlein et al. (2018), LAI data from Rutten et al. (2015), LUI data from Peters et al. (2019).

Mean annual temperature along our elevational zones ranged from 25°C in the savanna to 9°C in the burned *Podocarpus* forest (Appelhans et al., 2015). Rainfall is characterized by a bimodal distribution with a long rainy season from March to May and a shorter one around November (Hemp, 2006a). Along the slope, mean annual precipitation exhibits an unimodal pattern with minimum values around 620 mm at the foothills and maximum values around 2600 mm at 2200 m a.s.l in the middle montane forest, followed by a decrease to 2050 mm in the upper montane zone (Hemp, 2006a; Appelhans et al., 2015). The soils on the Kilimanjaro mountain all have roughly a similar age and developed from the same volcanic deposits (Dawson, 1992). In the savanna, Vertisols have developed, while in the tropical montane forest, Andosols are predominant (Zech et al., 2014).

4.3.2 Fine root biomass inventory

At each plot, 10 soil samples were taken at random locations down to 40 cm depth with a soil corer of 3.5 cm diameter and stored in plastic bags at 5°C until processing. Nine of the 20 natural plots were focused of a more intensive study; thus, 15 samples were collected in these plots instead. In the laboratory, samples were washed under running water over a sieve of 200 µm mesh size and all root fragments greater than 1 cm in length and ≤ 2 mm in diameter were selected. Under the stereo-microscope, fine roots were separated into biomass (living) and necromass (dead) fractions by means of the degree of root elasticity, the cohesion of cortex, periderm and stele, and the turgidity of the cortex (Leuschner et al., 2001). We further separated herb, grass and fern roots from woody tree (and shrub) roots by the lack of suberinization. To compare the fine root bio- and necromass of the natural and disturbed ecosystems in the colline zone (savanna and maize field plots) we join together the data of woody and herbaceous roots. In the rest of the ecosystems, we only used woody fine roots data to develop the analyses.

In order to estimate the root necromass of fragments lower than 1 cm in length, we followed the method introduced by Van Praag and others (1988) and modified by (Hertel and Leuschner, 2002). Six samples per plot were selected, and after extracting the larger root fragments (> 1 cm length) as described above, they were spread homogenously on a filter paper (730 cm2) subdivided into 36 squares. From six randomly selected squares, root fragments were extracted under the stereoscope, dry and weight. We then developed linear regression equations between the masses of the small root fragments and the larger dead fine root fractions. Finally, we applied these equations to the remaining samples that were not included in this more detailed analysis to obtain their small dead fine root fraction. When it was not possible to apply a regression equation, a mean ratio of small to large root fractions was used. The root material was dried at 70°C for 48 hours, weighed and the fine root biomass and necromass were expressed in Mg ha^{-1} to 40 cm depth.

4.3.3 Fine root morphological and chemical traits

Morphological traits of the living woody fine roots were investigated prior to drying. Each root sample was scanned using an EPSON perfection V700 scanner (EPSON America Inc.). Specific root length (m g^{-1}), specific root area (cm^2 g^{-1}), mean root diameter (mm) and root tissue density (g cm^{-3}) were calculated from data obtained through the scans using WinRhizo software (Regent Instruments Inc., Québec, Canada) and the respective fine root biomass data. In addition, we combined the information about specific root length and specific root area with data on fine root biomass to obtain the root length index (RLI) (root length per soil surface) and root area index (RAI) (root area per soil surface) per plot. We determined the C and N content of the living fine root fraction with a CN elemental analyser (Vario EL III, Hanau, Germany). Three samples per plot were analyzed, with each sample consisting of two collected samples in the field and mixed. We did not report values of fine root morphological and chemical traits for the ecosystems at the colline zone as this kind of traits cannot be compared between maiz (a grass species) and the woody fine roots from the other systems.

4.3.4 Fine root production and turnover

Ingrowth cores were used to estimate annual woody fine root production (Majdi 1996). This method is very useful for studying differences in root production between sites when root growth is fast, as it happens in tropical forests (Vogt et al., 1998), as well as in studies comprising a large number of plots, as it is the case in our study at Mt Kilimanjaro. Therefore, in our case it was not feasible to obtain enough replicates for each ecosystem by using other methods, which are more time consuming in the field and in the laboratory (e.g. minirhizotrons, sequential coring). In September 2014 and February 2015 (dry season) we installed 10 ingrowth cores per plot at random locations in the topsoil down to a depth of 40 cm. After extraction of the soil with a corer of 3.5 cm in diameter, we removed all visible roots by hand and refilled the holes with the original root-free soil. We restored the original soil horizon sequence and soil bulk density as good as possible. Each location was precisely marked with a plastic tube with the same diameter as the soil core on top of the soil as well as with three thin plastic sticks. We resampled these locations after one year. Processing of the samples was done in the laboratory as described in the previous section and fine root production was calculated as fine root biomass growth into the cores related to the length of the period between the start of recolonization and harvest (Vogt et al., 1998). To determine the start of recolonization in the different studied ecosystems we placed four additional ingrowth cores in every plot and resampled one of them every month (last core resampled after 4 months). Accordingly, recolonization started in the savanna, lower montane forest, homegarden and coffee plantation plots roughly two months after core installation; and in the middle and upper montane zones after three months. Fine root production values were extrapolated to one year and expressed as Mg ha^{-1} yr^{-1}. In the case of the maize field plots, we assume that fine root production is equal to the total fine root mass (living plus dead), as maize is an annual crop. Fine root turnover was calculated at the plot level by dividing annual fine root production by mean standing fine root biomass (Gill and Jackson, 2000). We assume a steady state between fine root mortality and productivity (Graefe et al., 2008).

Finally, we estimated for each ecosystem type the carbon and nitrogen fluxes to the soil via root mortality (equal to production) by multiplying the carbon and nitrogen concentrations in the fine root biomass by the corresponding fine root mortality rate, and expressed the flux in g m^{-2} yr^{-1}.

4.3.5 Coarse root C stocks

Coarse root C stocks were estimated indirectly from the aboveground C stocks reported in Ensslin et al. (2015) applying the equation by Cairns et al. (1997) (equation 1) for tropical sites.

$$BGB = \mathrm{Exp}\,(-1.0587 + 0.8836 \times \ln(AGB)) \qquad \text{(eqn 1)}$$

BGB is the belowground biomass (in our case C stocks) in coarse roots and root stock in Mg ha^{-1} and AGB is the aboveground biomass (C stocks) in Mg ha^{-1}.

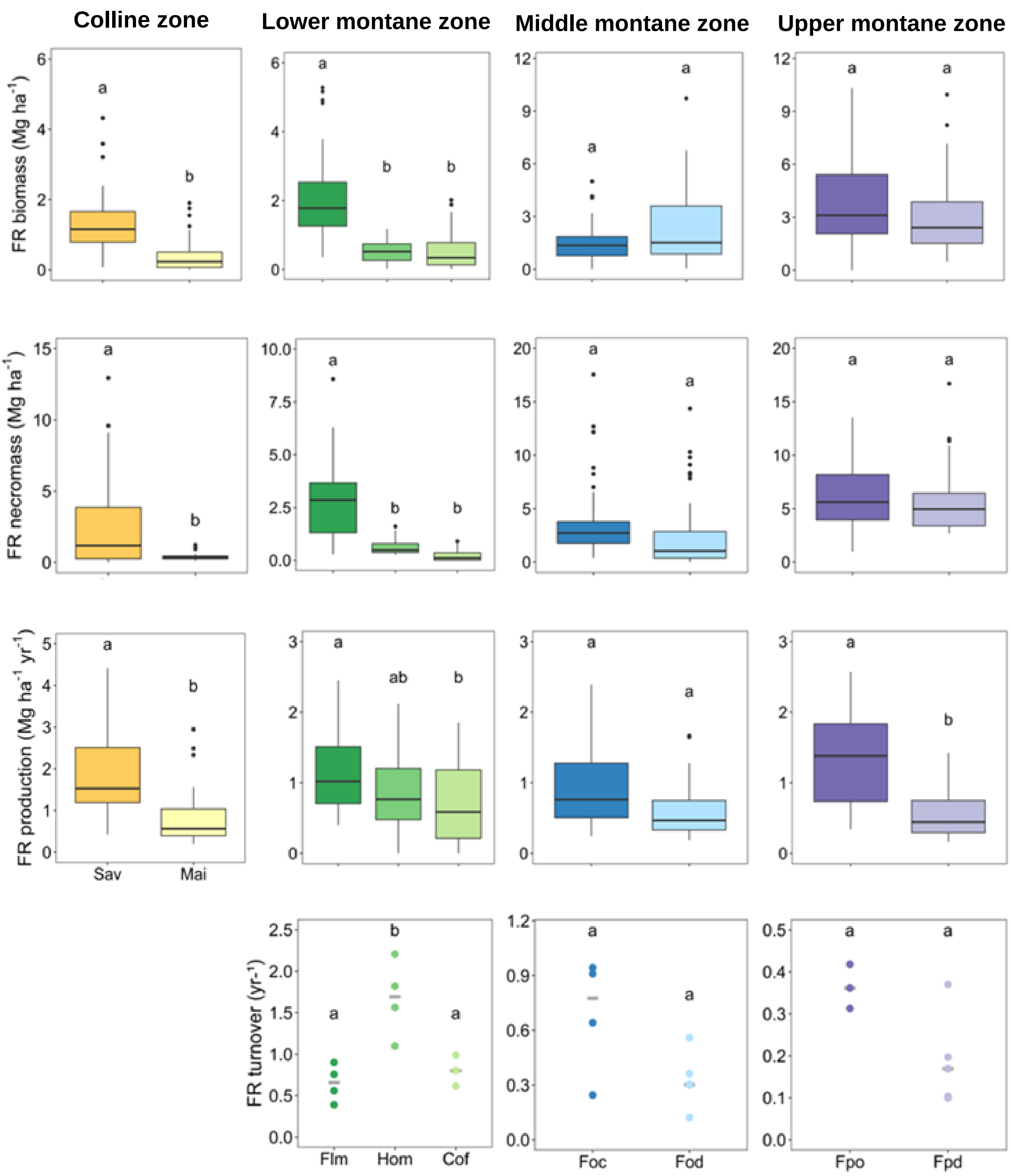
4.3.6 Statistical analysis

To determine differences on fine root biomass, necromass, productivity and root morphological traits among the natural and disturbed ecosystems, linear mixed effects models (LME) were applied in each elevational zone separately. We used all data points and designated “ecosystem” as fixed effect and “plot” as random effect. The Satterthwaite approximation of degrees of freedom was applied to correct for unbalanced sample numbers. In the case of fine root turnover, RLI, RAI, C and N fluxes and fine root N content, we used mean values per plot and applied ANOVA. For the lower montane zone, we used Tukey’s HSD post-hoc adjustment for multiple comparisons to detect differences between ecosystems types. A significance level of $p < 0.05$ was used throughout the analyses. When normality and homoscedasticity of model residuals were not found, log-transformation was applied to meet these criteria. In the case of samples with 0 values, we added one unit to all the data before log transformation. Principal component analysis (PCA) was used to assess the interrelation of the fine root related variables, stand structure and soil properties across the different natural and disturbed ecosystem types (only for the montane forest zones). Statistical analyses were conducted in R 3.4.0 software (R Development Core Team, 2017), using “lmerTest” and “multcomp” packages (Kuznetsova et al., 2017; Hothorn et al., 2008), and CANOCO software, version 5.02 (Biometris, Wageningen, the Netherlands).

4.4 Results

4.4.1 Fine root biomass, necromass and dynamics of natural and semi-natural vs. disturbed ecosystems

Conversion of colline savanna woodland to maize fields resulted in a three and six fold decrease of total fine root biomass (FRB) and necromass (FRN) respectively and a 50% decline of fine root production (FRP) (lFigure 4.1). In the maize fields, no woody fine roots from adjacent trees were present. In the lower montane belt, all fine root variables were affected by the transformation of the lower montane forest to agroforestry systems (lFigure 4.1). Fine root biomass and necromass decreased around 70% and 75 - 90% respectively from lower montane forest to the agroforestry systems (Table S 4.1). Lower montane forest and homegardens presented values of FRB: FRN close to 1, while fine root necromass in coffee plantations accounted for one third of fine root biomass (Table S 4.1). Fine root production decreased by 50% from lower montane forest to coffee plantations, with no significant change compared to homegardens. However, homegardens exhibited the highest value for fine root turnover. At the middle montane zone, we did not observe any differences between *Ocotea* forest and selectively logged *Ocotea* forest. The upper montane zone showed a decrease of threefold on fine root production from the natural *Podocarpus* forest to the burned *Podocarpus* forest, and a similar marginal decrease on fine root turnover ($p = 0.05$).



lFigure 4.1 Fine root biomass and necromass, fine root production and turnover in natural and disturbed ecosystems grouped by elevational zones at the southerns slopes of Mt. Kilimanjaro. Different lower letters indicate significant differences between ecosystems following linear mixed effects models with Tukey HSD post-hoc comparison ($p < 0.05$). Fine root turnover: dots are plot means and the gray line is the median. Sav = savanna, Mai = maize field, Flm = lower montane forest, Hom = homegarden, Cof = coffee plantation, Foc = *Ocotea* forest, Fod = *Ocotea* forest logged, Fpo = *Podocarpus* forest, Fpd = *Podocarpus* forest disturbed.

4.4.2 Root morphological and chemical traits of natural and semi-natural vs. disturbed ecosystems

The traditional agroforestry homegardens showed a higher specific root length and specific root area, together with a lower mean root diameter and root tissue density (marginal difference p = 0.05) compared to the lower montane forest (Figure 4.2). Contrary, coffee plantations showed lower root N content than the lower montane forest and homegardens, but did not differ from both ecosystems in the other fine root traits. In the middle montane zone (*Ocotea* forest), disturbance did not affect fine root traits. The burned *Podocarpus* forest presented a higher SRL and RTD, together with a lower mean root diameter and N content compared to its natural counterpart.

4.4.3 Principal component analysis

Ordination of the ecosystems following principal component analysis (PCA) based on fine root related variables, stand structural characteristics and soil properties established the differentiation of the ecosystems along the three elevation zones and distinguished natural from disturbed ecosystems (Figure 4.3). Axis 1 separate the elevational zones and axis 2 the land use. Most of the fine root related variables, stand structural characteristics and soil properties were related to the first axis (eigenvalue = 0.59) (Table 4.2, Figure 4.3). This first axis markedly separated the agroforestry systems from the rest and associated them positively to SRA, SRL and bulk density. Soil organic carbon (SOC), soil C:N and FRB were the most influential variables associated negatively with the first axis. The second axis (eigenvalue = 0.26) separated *Podocarpus* forest disturbed on one side and *Podocarpus* forest in the opposite side, from the rest of ecosystems. Fine root production, mean root diameter, DBH and height were negatively interrelated to this axis in the direction of *Podocarpus* forest, while stem density, RTD and root C:N ratio where positively interrelated to the axis in the direction of burned *Podocarpus* forest. *Ocotea* forest and *Ocotea* forest disturbed were very close in respect to both axes, indicating strong similarities between them.

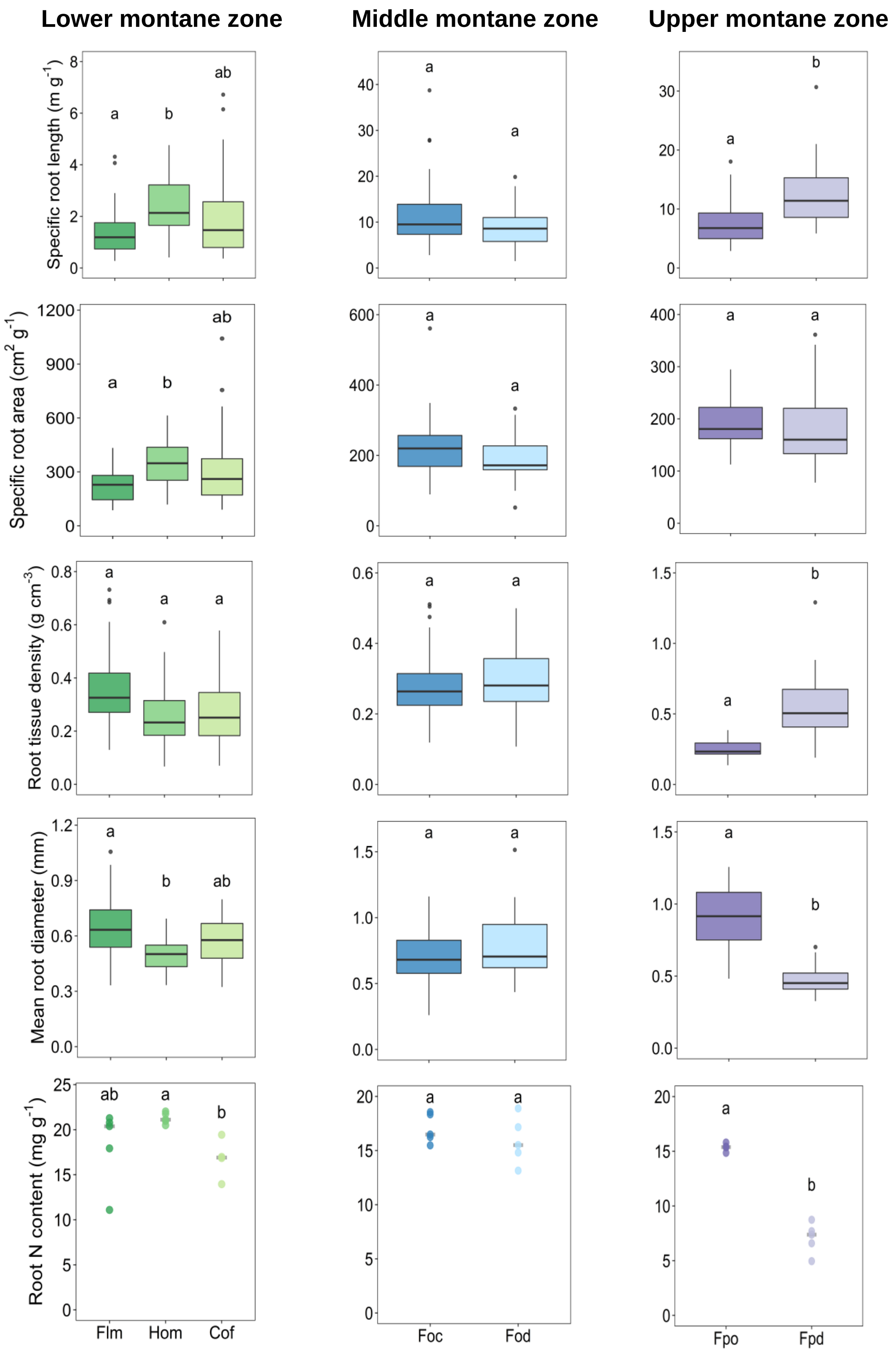


Figure 4.2: Fine root morphological and chemical traits in natural and disturbed ecosystems grouped by elevational zones at the southern slopes of Mt. Kilimanjaro. Different lower letters indicate significant differences between ecosystems following linear mixed effects models with Tukey HSD post-hoc comparison ($p < 0.05$). Fine root N content: dots are plot means and the grey line is the median. Flm = lower montane forest, Hom = homegarden, Cof = coffee plantation, Foc = Ocotea forest, Fod = Ocotea forest logged, Fpo = Podocarpus forest, Fpd = Podocarpus forest disturbed

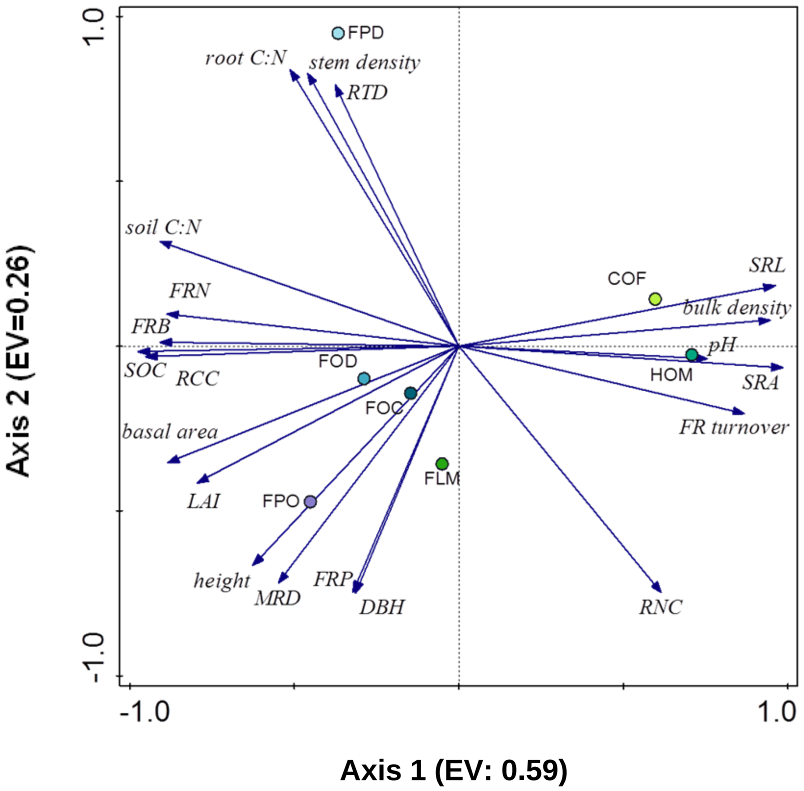


Figure 4.3: Plot showing the distribution of three natural forest ecosystems (FLM = lower montane forest, FOC = Ocotea forest, FPO = Podocarpus forest), two agroforestry ecosystems (HOM = homegarden, COF = coffee plantation) and two disturbed forest ecosystems (FOD = Ocotea logged, FPO = Podocarpus disturbed) at the southern slopes of Mt. Kilimanjaro, in relation to the PCA axes 1 and 2 (EV = eigenvalues of the axes) and their association with fine root related variables, soil properties and stand structural characteristics. Vector length and angle are proportional to the direction and degree of their correlation with the plot ordination scores.

4.4.4 Root area index and root length index of natural vs. disturbed forest ecosystems

The transformation of natural ecosystems did not affect the root length index (root length per soil surface) (RLI) nor the root area index (root area per soil surface) (RAI) in any of the different elevation belts (Table 4.3). However, the change of lowland montane forest to agroforestry systems results on a marginally significant decrease of RAI from natural forest to homegardens and to coffee plantations (p = 0.085 and 0.084, respectively).

Table 4.2: Results of a Principal Components Analysis (PCA) on the differentiation of three natural ecosystems and 4 disturbed ecosystems with respect to fine root biomass, dynamics and traits, soil properties of the topsoil and stand structural characteristics. Given are the loadings of the selected variables along the four explanatory axes. Numbers in brackets indicate the eigenvalues of the axes and numbers in bold mark the variables with closest correlation to the respective axis.

	Axis 1 (EV 0.59)	**Axis 2 (EV 0.26)**	**Axis 3 (EV 0.07)**
Soil properties			
SOC	**-0.97**	-0.02	0.09
Soil C:N ratio	**-0.91**	0.32	0.11
pH	**0.76**	-0.04	-0.54
Bulk density	**0.95**	0.08	-0.05
Stand structural variables			
Stem density	-0.46	**0.83**	-0.25
Basal area	**-0.88**	-0.35	0.11
Height	-0.63	**-0.67**	-0.15
DBH	-0.32	**-0.75**	-0.53
LAI	**-0.80**	-0.41	0.22
Fine root variables			
Fine root biomass	**-0.91**	0.01	-0.35
Fine root necromass	**-0.89**	0.10	-0.32
Fine root production	-0.31	**-0.75**	-0.44
Fine root turnover	**0.87**	-0.20	-0.07
Fine root traits			
Specific root length	**0.96**	0.18	-0.11
Specific root area	**0.98**	-0.06	-0.01
Mean root diameter	-0.55	**-0.72**	0.08
Root tissue density	-0.38	**0.79**	-0.24
Root carbon content	**-0.95**	-0.03	0.25
Root nitrogen content	**0.62**	-0.75	0.12
Root C:N ratio	-0.51	**0.84**	-0.18

Table 4.3: Root length index (RLI) and root area index (RAI) of natural and disturbed ecosystems in three elevation zones along the southern slopes of Mt. Kilimanjaro. Lower case letters indicate significant differences on RLI or RAI among natural and disturbed ecosystems in the same elevational zone according to ANOVA and afterwards Tukey HSD post-hoc comparison ($p < 0.05$).

	Lower montane			Middle montane		Upper montane	
Ecosystem	Lower montane forest	Homegarden	Coffee plantation	*Ocotea* forest	*Ocotea* forest logged	*Podocarpus* forest	*Podocarpus* forest disturbed
RLI ($m\ m^{-2}$)	2763.0 ± 781.9 a	1344.1 ± 166.9 a	1096.9 ± 283.7 a	1540.8 ± 175.5 a	1964.0 ± 778.3 a	2803.7 ± 608.6 a	3641.7 ± 599.8 a
RAI ($m^2\ m^{-2}$)	4.5 ± 1.1 a	2.0 ± 0.3 a	1.8 ± 0.5 a	3.1 ± 0.4 a	4.3 ± 1.5 a	7.1 ± 1.0 a	5.3 ± 0.9 a

4.4.5 Differences on aboveground and belowground C stocks with land-use change and disturbance

Land-use change entailed a decrease of the above-and belowground ecosystems C stocks, except for the middle montane zone and for the aboveground biomass in the colline zone, where an increase was reported with ecosystem disturbance (Table 4.4). Soil C stocks experienced the smallest change for all types of land disturbance, although, as they hold the highest soil organic C values, the magnitude of its decrease is large. Among all, the highest decrease on total reported stocks (plant and soil) was from lower montane forest to coffee plantations (63%). On the other hand, the decrease from the semi-natural forest to homegardens is slightly lower, but similar regarding fine roots C stocks. At the upper montane forest belt we only have available data for fine root and soil C stocks. A moderate decrease on fine roots happened (20% decrease) in comparison to the agricultural systems, whereas no decrease was reported in soil C stocks.

Table 4.4: Soil C stocks and its difference between natural and disturbed ecosystems across the distinct elevational zones at Mt. Kilimanjaro.

Elevation zone	Ecosystem	Aboveground C stocks	Coarse roots C stocks	Fine roots C stocks	Soil C stocks	Total C stocks
Colline	Savanna woodlands	5.02 ± 1.00	1.02 ± 0.23	0.50 ± 0.05	109.65 ± 17.65	116.19 ± 16.52
	Maize fields	8.01 ± 1.18	0.20 ± 0.16	0.15 ± 0.02	70.38 ± 9.77	77.84 ± 10.69
Lower montane	Lower montane forest	173.58 ± 42.82	29.98 ± 6.55	0.90 ± 0.13	241.22 ± 45.74	445.67 ± 51.79
	Homegarden	44.60 ± 8.27	9.07 ± 1.50	0.24 ± 0.03	168.21 ± 24.15	222.13 ± 25.33
	Coffee plantation	27.94 ± 13.74	5.86 ± 2.53	0.26 ± 0.10	128.85 ± 19.63	162.91 ± 22.13
Middle montane	*Ocotea* forest	135.18 ± 23.54	24.17 ± 3.77	0.70 ± 0.11	278.96 ± 16.05	439.01 ± 22.27
	Ocotea forest logged	171.89 ± 10.73	30.06 ± 1.66	1.12 ± 0.34	300.90 ± 3.19	503.07 ± 67.70
Upper montane	*Podocarpus* forest	176.75 ± 1.86	30.84 ± 0.29	1.81 ± 0.43	295.55 ± 16.12	503.35 ± 17.09
	Podocarpus forest disturbed	…	...	1.44 ± 0.20	293.86 ± 2.66	…

Elevation zone	Ecosystem	Aboveground	Coarse roots	Fine roots	Soil	Total
Colline	Savanna woodlands - Maize fields	+ 59.5%	- 80.4%	- 70.72%	- 35.8%	- 32.2%
Lower montane	Lower montane forest - Homegarden	- 74.3%	- 69.8%	- 72.9%	- 30.3%	- 50.2
	Lower montane forest - Coffee plantation	- 83.9%	- 80.5%	- 70%	- 46.6%	- 63.4%
	Homegarden - Coffee plantation	- 37.4%	- 35.4%	-9.2 %	- 23.4%	- 26.7%
Middle montane	*Ocotea* forest - *Ocotea* forest logged	-27.2%	+ 24.4%	+ 60.5%	+ 7.9%	+14.6%
Upper montane	*Podocarpus* forest - *Podocarpus* forest disturbed	…	…	- 20.4%	- 0.6%	…

(+) indicates an increase on C stocks with land-use change or ecosystem disturbance while (-) indicates a decrease.

4.4.6 Carbon and nitrogen fluxes to the soil via fine root mortality

Most of the land-use changes and ecosystem disturbances along Mt. Kilimanjaro resulted on a decrease on C and N fluxes to the soil via fine root mortality (Table 4.5). At the lower montane zone, both fluxes decreased around a half from lower montane forest to coffee plantations, whereas homegardens maintained the fluxes and fine root N content values compared to the semi-natural forest. Both agroforestry systems (traditional homegardens and intensive coffee plantations) differ in their N fluxes and fine root N content, with lower values reported for the coffee plantations. Upward the mountain, in the middle elevation zone, no significant differences were found among selective logging and the natural *Ocotea* forest. The strongest decrease on N fluxes among all ecosystems were reported in the upper montane zone, with an 81% lower value in the burned *Podocarpus* forest compared to its natural counterpart. The fine root N content also decreased a half, whereas the C: N ratio increased more than threefold in the *Podocarpus* disturbed forest.

Table 4.5: Estimated C and N fluxes to the soil through calculated fine root mortality (=production), N content and C:N ratio of fine roots in natural and disturbed ecosystems in three elevational zones in the southern slopes of Mt. Kilimanjaro.

Elevation zone	Ecosystem	C flux ($g\ m^{-2}\ yr^{-1}$)	N flux ($g\ m^{-2}\ yr^{-1}$)	N ($mg\ g^{-1}$)	C:N ratio
Colliine	Savanna woodlands	72.8 ± 59.5 a	1.0 ± 0.6 a	6.1 ± 0.6 a	71.4 ± 8.1 a
	Maize fields	30.4 ± 14.8 b	0.9 ± 0.4 a	10.8 ± 0.9 b	37.2 ± 3.5 b
Lower montane	Lower montane forest	52.1 ± 5.2 a	2.3 ± 0.3 a	18.3 ± 1.9 ab	27.5 ± 3.7 a
	Homegarden	34.6 ± 2.5 ab	1.8 ± 0.2 a	21.3 ± 0.3 a	20.4 ± 0.4 a
	Coffee plantation	19.6 ± 5.4 b	0.8 ± 0.2 b	17.1 ± 0.9 b	27.8 ± 2.9 a
Middle montane	*Ocotea* forest	46.3 ± 13.4 a	1.6 ± 0.4 a	17.0 ± 0.6 a	28.9 ± 1.3 a
	Ocotea forest logged	28.4 ± 4.3 a	0.9 ± 0.1 a	15.9 ± 1.0 a	32.5 ± 1.8 a
Upper montane	*Podocarpus* forest	63.1 ± 3.0 a	2.1 ± 0.1 a	15.4 ± 0.2 a	31.9 ± 0.5 a
	Podocarpus forest disturbed	24.1 ± 3.6 b	0.4 ± 0.1 b	7.1 ± 0.6 b	72.5 ± 7.6 b

4.5 Discussion

Our investigation, based on a wide range of land-use change types at different elevation zones at Mt. Kilimanjaro, showed a decrease of the fine root biomass, production and turnover with anthropogenically-induced land-use change or disturbance in almost all studied ecosystems. A decrease in the C and N fluxes to the soil via fine root death was also observed, especially in the disturbed *Podocarpus* forest (upper montane zone).

4.5.1 Effects of land-use change and disturbance on the fine root biomass, dynamics and related traits of different tropical ecosystems

4.5.1.1 Colline zone: intensive agriculture

The conversion of savanna woodlands to maize fields represents a big impact on the fine root C stocks as it entails a 70% decrease of the fine root biomass. Our fine root biomass values for savanna were at the lower end of the range of values reported for other tropical savannas and dry forests (0.4 to 11.86 Mg ha^{-1}) (Roy and Singh, 1994; Chen et al., 2003; February and Higgins, 2010; Moore et al., 2018). Differences might be due to the lower mean annual precipitation, the lower number of trees at our savannas and to its semi-natural condition, as they are subject to logging and burning pressure that has been intensifying during the last decades (Agrawala et al., 2003; Hemp and Hemp, 2018). The values found for maize fields are similar to values reported in south Senegal and in Morogoro region in Tanzania (0.32 and 0.30 Mg ha^{-1} respectively) (Jonsson et al., 1988; Manlay et al., 2002).

4.5.1.2 Lower montane zone: traditional and intensive agroforestry

The decrease of fine root biomass, necromass and production from semi-natural forest to agroforestry systems has also been reported in other studies in tropical regions (Hertel et al., 2009; Hundera et al., 2013). Our range of values for the fine root biomass and dynamics in the agroforestry systems is lower compared to estimations for shade coffee plantations and cacao agroforests: FRB: 1.6-2.2 Mg ha^{-1}, FRP: 1.5-4.5 Mg ha yr^{-1} (Hertel et al., 2009, Leuschner et al., 2009, 2013; Abou Rajab et al., 2015; Defrenet et al., 2016). These studies were carried in systems with much higher number of stems and mean annual precipitation compared to our study sites.

Land-use conversion entails changes in plant community composition together with stand structural and soil characteristics. Regarding the effects of stand structure, a lower aboveground biomass (AGB) and leaf area index (LAI) entails less available photosynthates leading to a decrease of the stand fine root biomass and production, as reflected in the results of the PCA (as we did not have data on AGB for all ecosystems we included basal area and height, which are determinant factors of AGB), in line with results reported in coffee plantations (Defrenet et al., 2016). At the stand level, values of fine root biomass and production are positively associated with soil C:N (indicator of N shortage). However, the two order higher fine root production to aboveground biomass ratio (FRP:AGB) in the agroforestry systems (Table S1), which also hold higher soil fertility values (lower soil C:N) compared to the lower montane forest, might indicate a higher investment of carbon in the fine root system when there is more N available, as reported in other studies (Pregitzer et al., 1993; Nadelhoffer, 2000).

Our results point to homegardens as high dynamic systems with high turnover rates and more pronounced acquisitive traits than the lower montane forest, which might be a result of the plant species composition together with the effects of management practices. Although root traits are highly plastic in the adaptation to their environment, they are also phylogenetically driven, having the different species their particular set of features and strategies (Valverde-Barrantes et al., 2013, 2015). For example, much higher turnover rates have been reported for banana trees (2, 6 and 17 yr^{-1} for cord, secondary, tertiary and root hairs (Araya, 2005)) than for coffee plantations (1-1.3 yr^{-1}) and tropical mountain forest (~0.8 yr-1) (Gill and Jackson, 2000; Defrenet et al., 2016). At the same time, *Musa sp.* (banana) is known for being a fast growing species able to hold very high SRL (150 m g^{-1}) (Turner and

Barkus, 1981). Acquisitive traits are related to high turnover rates (inverse of lifespan) (Eissenstat et al., 2000; Weemstra et al., 2016) in line with our results. In fact, the PCA separated homegardens from the other ecosystems based on the SRA, SRL and fine root turnover. Moreover, a rapid fine root turnover is advantageous when competing for resources, as young fine roots are considered to be more active in nutrient uptake than old ones (Eissenstat et al., 2000). Although *Albizia sp.,* a characteristics species in homegardens, has been found to compete with coffee lateral roots, complementary niche use between species in similar agroforestry systems has also been reported (Dossa et al., 2008; van Asten et al., 2011; Defrenet et al., 2016). Acquisitive morphological traits are also beneficial in the homegardens root competition environment as they facilitate soil exploration (high SRL) and provide more area for a possible symbiosis with microorganisms (high SLA).

Management practices, for instance the density of shade trees, leaf litter and tillage, might also have a strong influence on fine root dynamics and traits. In homegardens, the reported high turnover rates might be indirectly facilitated by the presence of trees and leaf litter; through an effect on microbial activity and thus on N availability (García-Palacios et al., 2013; Horwath, 2015; Gerschlauer et al., 2016). Fine root turnover in homegardens might also be triggered by tillage, as it enhances fine root mortality (Schroth, 1998) and the consequent replacement of dead roots. The lower FRB:FRN ratio and high turnover rates found in homegardens compared to coffee plantations agree well with this idea. Tillage also destabilizes soil organic matter (SOM) facilitating N mineralization and improving the root system (Balesdent et al., 2000; Blomme, 2002; Chen et al., 2009). High SRL might be advantageous after this practice as it enables fine roots to recover faster after disturbance, as it has been shown for arbuscular mycorrhizal trees (Eissenstat et al., 2015).

On the other hand, coffee plantations hold a less dynamic fine root system. Apart from the difference in species composition, both agroforestry systems are object of different management practices. Coffee plantations present lower number of shade trees (also *Albizia* and *Grevillea sp.*), removal of pruned parts and addition of fertilizers (NPK). The lower leaf litter values and removal of pruned parts result on lower C substrate for microorganisms, which have to rely on dead fine roots and other C sources (e.g. root exudates, dead soil fauna and microorganisms, SOM) leading to a low gross N turnover (Gerschlauer et al., 2016).

The fine root length and surface per land area (RLI and RAI) are not affected by land-use conversion, as fine root morphological traits (SRL and SRA) compensate the low fine root biomass. LAI follows the same trend with land-use intensity than RAI does, in line with a land-use disturbance gradient in Indonesia (Leuschner et al., 2009).

4.5.1.3 Middle montane zone: selectively logged forest

Effects on fine root system (biomass, necromass, morphological and chemical traits) are not detectable 30 years after the end of the logging activity in *Ocotea* forest. These results indicate a complete recovery of the fine root system. In a study on fine root biomass regeneration of primary and secondary tropical forest, Hertel et al. (2007) suggested that the fine root system was recovered after 1-2 years since disturbance and did not find differences among fine root biomass of primary and secondary forest pulling data from different studies together. Fine root biomass recovery depends on stand age (Cavelier et al., 1996), which agrees well with our data, as forest disturbance happened more than 30 years ago. However, the higher density of late successional species in the disturbed ecosystems (Rutten et al., 2015) can be noticed in the tendency of the fine root system to hold a higher fine root biomass, lower fine root dynamics and more conservative morphological and chemical traits, characteristic of slow growing species (Weemstra et al., 2016).

4.5.1.4 Upper montane zone: regenerated forest after burning

Fires at the upper montane zone of Mt. Kilimanjaro have led to a change of the plant species community, becoming *Erica excelsa* the dominant species instead of *Podocarpus latifolius* (Hemp, 2005), with consequences for the fine root system. The major effect is observed on fine root dynamics, decreasing fine root production and turnover a 50% in the disturbed ecosystem. Although we assume that the disturbed forest present a lower aboveground biomass due to the dominance of *Erica excelsa*, fine root biomass did not change. This fact indicates the high investment of carbon on the fine root system characteristic of *Erica sp.* together with the high SRL and SRA (Sierra Cornejo et al., 2020). The decline of fine root N content might indicate lower mineralization rates in the disturbed ecosystem (Hobbie et al., 2006). Both, *Podocarpus* and *Erica sp.* have adaptations to N shortage conditions. The distinctive fine root traits of the plant communities where they are the dominant species follow a mixture of conservative and acquisitive strategies. *Erica sp.* present high RTD, lifespan and low N content (conservative) and on the other hand, high SRL and fine root diameter (acquisitive), whereas patterns are the opposite in plant community dominated by *Podocarpus latifolius* (Sierra Cornejo et al., 2020). A high N content indicates high litter quality (Silver and Miya, 2001), which entails easier decomposition by the microbial community. Both species present mycorrhiza symbiosis: arbuscular in the case of *Podocarpus sp*. and ericoid in *Erica sp.* (Khan, 1967; Cairney and Meharg, 2003). The opposite strategies of these species affect the C and N cycles through the nutrient uptake capacity, the fine root system size, and the quantity and quality of root litter. There might be a slow-down of the C and N cycle with the replacement of *Podocarpus latifolius* forest by

Erica excelsa following events of fire, as already indicated by the lower fine root production, turnover, litter quality (N content) and the lower soil C:N values in the disturbed ecosystem.

4.5.2 Impact of land-use change and disturbance on ecosystem plant and soil C stocks

Land-use conversion to agricultural and agroforestry systems has a strong impact on all C stocks, whereas the selectively logged *Ocotea* forest seems to be recovered after 30 years since disturbance and *Podocarpus* disturbed forest only shows, for the moment, a low impact on its fine roots and soil C stocks. The most affected studied component was plant C stocks, due to the change on stand structure and species composition (Ensslin et al., 2015). Although the percentage of soil C stocks decline is small, soils contain the highest amounts of sequestered C among the studied ecosystem components. Thus, their reduction implies a large loss. Decrease in plant litter and soil microbial biomass with land-use conversion, both characteristic of agricultural ecosystems, drives soil C stocks reduction, (Pabst et al., 2016; Post and Kwon, 2000). A detailed explanation about the effects of land-use change on the aboveground and soil C stocks can be found in Ensslin et al. (2015) and Pabst et al. (2015) respectively.

The biggest difference on C stocks is displayed between lower montane forest and coffee plantations, reflecting the impact of intensive production systems. Management practices in agriculture and agroforestry systems are a crucial factor for soil C sequestration potential, as tillage, addition of fertilizers, removal of litter and release of Cu as fungicide affect the microbial activity and mineralization of SOM, as well as the fine root dynamics (Oikeh et al., 1999; Gale and Cambardella, 2000; Tian et al., 2010; Pabst et al., 2016). The small decrease on fine root C stocks in the upper montane belts is due to the large fine root system of *Erica sp*. which dominates the disturbed forest. Changes on soil C stocks at this elevation zone might be seen in future years, as C fluxes from leaf litter will be extremely reduced (*Erica sp*. holds a low litter fall (Sierra Cornejo et al., under review)) and the fine roots of *Erica sp.* have a long lifespan and low quality (Sierra Cornejo, et al., 2020), which may reduce C supply to the microbial community and decomposition.

Decrease of C stocks with conversion to maize fields in the colline zone will probably be extended across a larger area as a result of the predicted increase of the African population until the year 2050 (United nations, 2013). Land-use change will also depend on the economic opportunities offered by the institutions (Lambin et al., 2001). In addition, climate modulates the effects of land-use change on ecosystem functions, being arid ecosystems less resistant to alterations in a climate change context (Peters et al., 2019). In the case of homegardens, this ecosystem is endangered by the intensification of its production (there is no space for expansion), crop diversification and substitution for grasslands (Soini, 2005; Maghimbi, 2007). In coffee plantations, the yield production is decreasing as a consequence of higher temperatures, lower precipitations and management practices (Kumburu, 2012; Craparo et al., 2015). Coffee cooperatives incentivize the use of shade trees, which might improve C stocks (Kumburu, 2012; Pabst et al., 2016). Further studies in homegardens and coffee plantations are necessary to assess the effects of these new challenging conditions on ecosystem C stocks. Regarding the tropical montane forest, its protection under the Kilimanjaro National Park is crucial for the maintenance of ecosystem processes and interactions as well as for the population settlements in the entire region. It entails essential functions as regulation of watershed and climate and provides resources for the local communities, as firewood and non-timber products (Agrawala et al., 2003; Hemp, 2005). The control of fires at the upper montane zone is crucial,to avoid changes on the water regime and C sequestration capacity.

4.5.3 Effects of land-use change and disturbance on the C and N fluxes to the soil vial fine root mortality

Soil C and N inputs via fine root mortality decrease with land-use conversion and disturbance in almost all elevation zones on Mt. Kilimanjaro, especially in the upper montane zone. Similar effects were found in a study on the conversion of tropical rain forest to shaded cacao plantations and to different intensities of timber extraction in Indonesia (Hertel et al., 2009). This decrease entails a lower contribution to soil C stock and lower substrate amount for the decomposer community.

Although the fluxes from fine root litter to the soil are lower compared to leaf litter in the studied ecosystems (Table S2) and generally in tropical forests and agroforestry systems (Hertel et al., 2009), the byproducts of root decomposition might play a key role in soil C storage (Rasse et al., 2005; Clemmensen et al., 2013). In addition, specific land-use practices and vegetation characteristic can increase the relative importance of belowground C inputs. At maize fields and *Podocarpus* forest disturbed, the strong decrease of leaf litter due to its removal in the first case and to the low quantity of litter fall in the second, entails a large reduction of the plant contribution to soil carbon stocks. Thus, the microbial community has to rely on fine roots, exudates, dead fauna and SOM among others as substrate. Moreover, the decline of the plant substrate amount (leaf and fine root litter) might entail microorganisms to decompose SOM (Kramer and Gleixner, 2006), which leads to the destabilization of this important carbon pool. Besides, decomposition of plant litter is strongly influenced by its quality, which affects soil microbial community composition and activity (Makkonen et al., 2012; See et al., 2019; Wardle et al., 2004). At *Podocarpus* forest disturbed, the lower amount of available substrate, its lower quality and higher soil C:N compared to natural *Podocarpus* forest are other indicators of the possible slowing down of decomposition with forest disturbance, with consequences on soil C stocks and N availability.

Savannas and homegardens hold higher C and N fluxes to the soil via fine root litter mortality than maize and coffee plantations. This difference among land-use change is added to the high potential of this ecosystems for soil C sequestration, as they hold lower decomposition rates and lower labile C decomposition compared to maize and coffee plantations, respectively (Mganga and Kuzyakov, 2014; Becker and Kuzyakov, 2018).

Specifically, homegardens highlight as a dynamic ecosystem, with high fine root turnover rates and high litter quality, high substrate availability and microbial efficiency (Pabst et al., 2016). Despite the changes on species composition and stand structure, homegardens keep some of the properties from the lower montane forest. Their multilayer vegetation structure and crop diversity contribute to maintain high biodiversity, leaf litter production and high gross N turnover rates, (Hemp, 2006b; Becker et al., 2015, Gerschlauer et al., 2016). To these processes, we add the maintenance of the C and N fluxes from fine root mortality to the soil and the root litter quality, which plays an important role on the plant-soil interface pathway of the C and N cycle.

4.6 Conclusions

The increase of land-use transformation worldwide, especially in tropical ecosystems, urges assessment of its effects on ecosystems components. Fine roots, being typically underrepresented in this kind of studies, play a remarkable role on ecosystem processes (e.g. C and N cycles). Different types of land-use conversion or disturbance at Mt. Kilimanjaro generally decrease stand fine root biomass and production as well as the C and N fluxes to the soil via fine root mortality, leading to a decrease on soil C stocks. Moreover, in the upper montane zone, these changes might lead to a slow down of the C and N cycle and soil N availability. The variation on plant species composition, stand structure and, to a lesser extent, management practices such as leaf litter removal, tillage and the use of fertilizers or cow manure are among the responsible factors of the changes on ecosystem processes. In addition, land-use change entails a shift of the fine root litter quality, which is considered to drive the microbial community composition and activity, playing, indirectly, a major role in C and N cycles.

Change on plant species composition leads to a different matrix of fine root morphological traits, as we observe in the acquisitive fine root traits at homegardens, driven by the community species composition, and the mixture of traits at *Podocarpus* disturbed forest, driven by the dominance of *Erica excelsa*. We highlight the agroforestry system "Chagga homegarden" as it maintains properties and processes from the semi-natural forest, such as high fine root turnover rates, similar C and N fluxes from fine root mortality to the soil and fine root litter quality, while providing sustain and resources to the local communities. We encourage the development of further studies on this agroforestry system focused on e.g. the complementary niche use of plant species, fine root system under different management practices, variation on fine root traits between coffee in homegardens and in coffee plantations, as well as differences on fine root litter decomposition, exudates and microbial activity and composition among these two agroforestry systems. These studies would contribute to a better understanding of the plant strategies and the implications of land-use change for ecosystems processes related to the C and N cycle.

4.7 Author contributions

DH developed the study design, NSC conducted the field work, data processing and analysis, JNB and AH contributed with soil and stand structure data. NSC and DH interpreted the data and the paper writing was done by NSC and DH with contributions of all authors.

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4.10 Supplementary material

Table S 4.1: Means of fine root biomass (FRB), necromass (FRN), productivity (FRP), and turnover, and ratios of FRB to aboveground biomass, FRP to aboveground biomass, and FRB to FRN in the different natural, semi-natural and disturbed ecosystems on Mt. Kilimanjaro (means and SE in parentheses).

	Fine root biomass (Mg ha^{-1})	Fine root necromass (Mg ha^{-1})	Fine root production (Mg ha^{-1} yr^{-1})	Fine root turnover (yr^{-1})	FRB:AGB	FRP:AGB	FRB:FRN
Savanna woodlands	1.22 ± 0.11	2.51 ± 0.49	1.78 ± 0.28	0.74 ± 0.07	0.14 ± 0.04	0.22 ± 0.07	0.56 ± 0.11
Maize fields	0.39 ± 0.04	0.42 ± 0.04	0.80 ± 0.08	…	0.03 ± 0.01	…	0.94 ± 0.07
Lower montane forest	1.97 ± 0.27	2.53 ± 0.71	1.13 ± 0.11	0.65 ± 0.11	0.008 ± 0.002	0.003 ± 0.001	1.06 ± 0.26
Homegarden	0.57 ± 0.07	0.61 ± 0.05	0.82 ± 0.06	1.67 ± 0.23	0.007 ± 0.001	0.011 ± 0.002	0.94 ± 0.12
Coffee plantation	0.62 ± 0.24	0.22 ± 0.03	0.46 ± 0.13	0.80 ± 0.08	0.018 ± 0.009	0.012 ± 0.005	2.75 ± 0.76
Ocotea forest	1.46 ± 0.21	3.40 ± 1.04	0.95 ± 0.27	0.68 ± 0.19	0.007 ± 0.002	0.005 ± 0.002	0.54 ± 0.12
Ocotea logged	2.29 ± 0.72	2.65 ± 1.52	0.58 ± 0.08	0.33 ± 0.07	0.006 ± 0.002	0.002 ± 0.000	1.67 ± 0.55
Podocarpus forest	3.74 ± 0.32	6.51 ± 0.59	1.33 ± 0.05	0.36 ± 0.03	0.010 ± 0.001	0.004 ± 0.000	0.60 ± 0.11
Podocarpus forest disturbed	3.01 ± 0.41	5.63 ± 0.79	0.50 ± 0.07	0.19 ± 0.05	…	…	0.56 ± 0.08

Table S 4.2: Carbon and nitrogen fluxes to the soil through leaf litter and its N content and C:N ratio in semi-natural and agroforestry systems in the lower montane zone at Mt. Kilimanjaro.

Elevation zone	Ecosystem	C flux (g m^{-2} yr^{-1})	N flux (g m^{-2} yr^{-1})	N (m g^{-1})	C:N ratio
Leaf litter*	Lower montane forest	216.9 ± 0.7	4.9 ± 0.2	1.1 ± 0.1	44.9 ± 0.5
	Coffee plantation	223.1 ± 16.0	11.0 ± 0.8	2.4 ± 0.1	20.4 ± 0.6
	Homegarden	270.1 ± 124.7	16.03 ± 7.33	2.9 ± 0.1	17.0 ± 0.1

* Data from Becker et al. (2015)

CHAPTER

5

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

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5.1 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

5.2 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 and 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 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.

5.3 Material and Methods

5.3.1 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 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 5.1). Thus, we defined three vegetation cover classes: low-vegetation (low-veg), herbal vegetation (herb) and shrub vegetation (shrub) (Figure 5.1). 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 5.1) 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).

Figure 5.1: Picture of the tropical alpine *Helichrysum* site (A) characterized by different vegetation classes (B: low-vegetation, C: herb and D: shrub).

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 5.1, Table 5.2).

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 5.1: 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).

5.3.2 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.

Table 5.2: 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 *($p \leq 0.05$).*

5.3.3 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 60 ml 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 (N2O: 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, CH4-C or 0.6 µg N_2O -N m^{-2} h^{-1}, respectively.

5.3.4 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).

5.3.5 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).

5.3.6 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).

5.4 Results

5.4.1 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 5.2). 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 5.2).

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 5.2). 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 5.2) and in the same range than VWC measurements calculated from soil samples used for quantification of gross N turnover rates (Figure 5.3).

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 5.2).

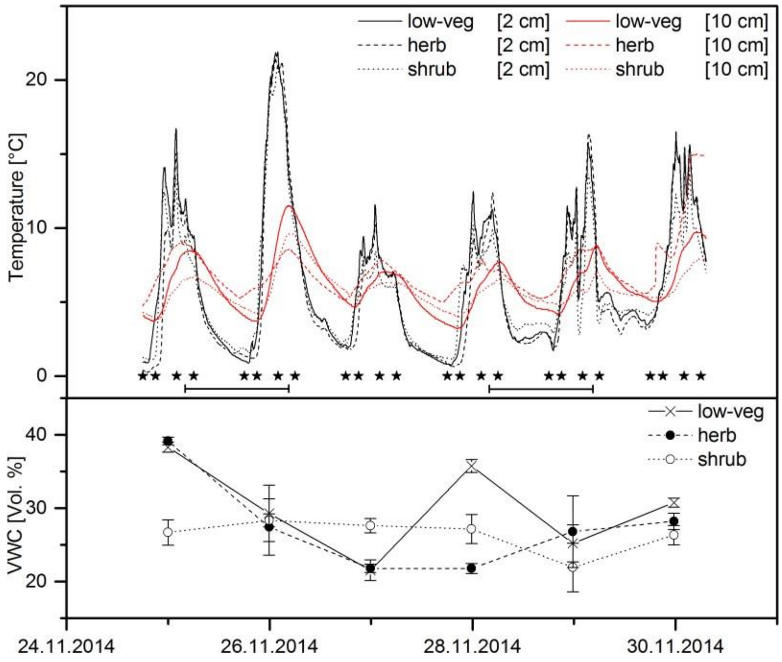

Figure 5.2: Course of soil temperature (2 and 10 cm) and volumetric soil water content (0-5 cm) at three vegetation classes of a tropical alpine *Helichrysum* site. Stars represent gas sampling times and lines below the stars the incubation time for the ^{15}N labeled soil.

5.4.2 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 5.3A). 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 5.3B).

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 5.3E). 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.

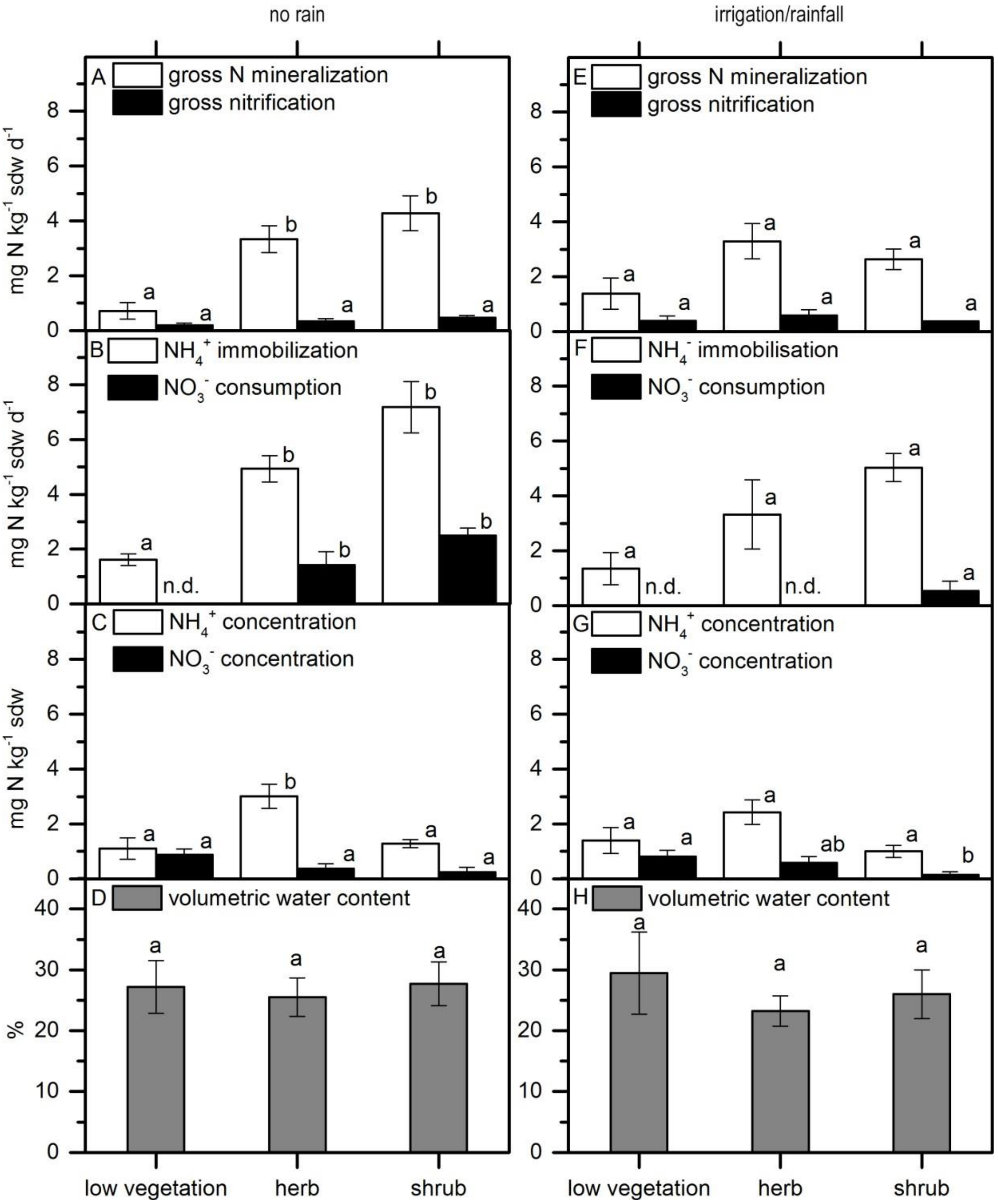

Figure 5.3: Gross N-turnover rates, soil N concentration and water content at three vegetation classes of a tropical alpine *Helichrysum* site. A-D represent measurements for no-rain, E-H represent measurements after irrigation (rain). Stars indicate times of GHG chamber measurements, lines indicate 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 5.3C) 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 Nconcentrations were slightly lower but showed the same trends compared to the no-rain sampling. (Figure 5.3G). Across all vegetation classes NO^- concentrations were persistently lower than NH_4^+ concentrations, irrespective of irrigation/rainfall (Figure 5.3C 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 5.2).

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 5.3).

Table 5.3: N turnover indicators for the three vegetation classes for no-rain, irrigation/rain and combined conditions. Nretrel: relative N retention; ImmNH4 $^+$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. Nretrel: relative N retention; ImmNH4 $^+$rel: relative NH_4 $^+$ immobilization.

5.4.3 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 5.4), and for evaluation of diurnal patterns divided into four classes representing different hours of the day (Figure 5.4).

Soil CO_2 emissions were low and ranged between 3.3 and 28.3 mg C m^{-2} h^{-1}. Emissions were significantly higher on the herb and shrub plots compared to the low-veg plots (Table

5.4). 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 5.4A). 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 5.4). At the herb and shrub plots, uptake rates were significantly higher (app. 50%) than on the low-veg plots (Table 5.4).

Table 5.4: Compilation of minimum, mean, maximum and area weighted mean fluxes of CO2 (mg C m^{-2} h^{-1}), CH4 (µg C m^{-2} h^{-1}) and N2O (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 ($p \leq 0.05$).

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 5.4B). 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 5.4C, Table 5.4).

5.4.4 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 5.2). 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 5.2).

5.4.5 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 5.5). 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 5.6). NO $^-$ 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 (r^2<0.5). Note that nitrification, N_2O and CH_4 emissions could not be explained by any of the parameters.

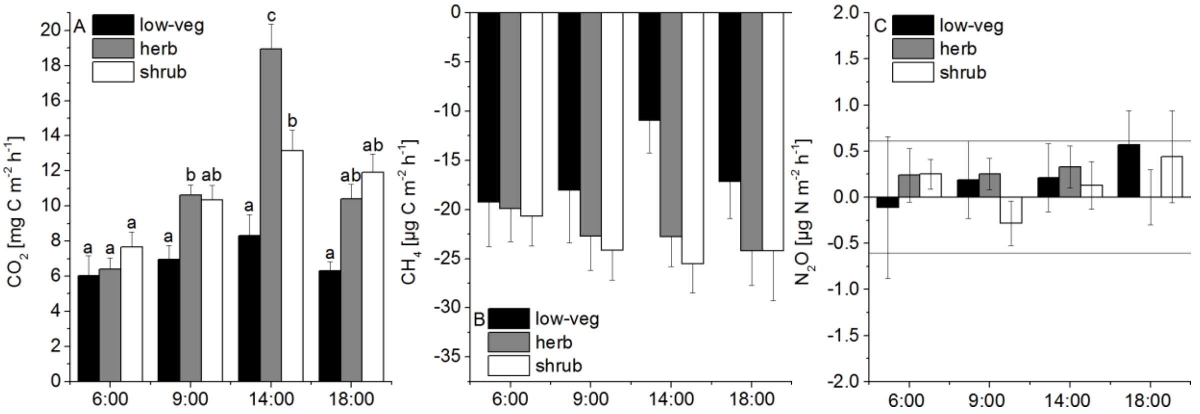

Figure 5.4: Diurnal patterns of soil GHG exchange (A: CO_2, B: N_2O, C: CH_4) at three vegetation classes of a tropical alpine Helichrysum site. Error bars represent standard error of the mean. Letters indicate significant ($p < 0.05$) temporal differences of fluxes within a vegetation class. Note no letters are presented for CH_4 and N_2O since no significant differences were detected. Lines at 0.6 and -0.6 in (Figure 5.4C), 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.

5.5 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 5.7). 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.

5.5.1 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 5.5 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 5.7) 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 5.7), including all vegetation classes, is much lower, but in the same range as rates reported for an N-limited beech forest$_4$ 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 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 5.2), 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 5.7). 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.

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 and 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 5.5, Table 5.6) 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).

Table 5.6: Multiple regression analysis for identification of main environmental controls on gross N processes and greenhouse gas emissions.

	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

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 5.7) and N retention capacity (Table 5.3) 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 5.3). 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 5.7: 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 5.7), 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.

5.5.2 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 and Gavrichkova, 2010). This is supported by our findings with more pronounced diurnal patterns of soil CO_2 emissions at the vegetated plots (Figure 5.4A). 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 5.1; Figure 5.2). 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 5.4A). 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 5.2). In addition, during the observation period, soil moisture was highest at the low- veg plots (Figure 5.2) 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 5.4B). 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 5.3, Table 5.7), 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.

5.6 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.

5.7 Acknowledgements

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CHAPTER

6

Legume and non-legume trees increase soil carbon sequestration in Savanna

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6.1 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 C4 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

6.2 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 et al., 2007; Lambin et al., 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 et al., 2005; Meyer et al., 2009; Schleicher et al., 2011). Other approaches estimated carbon (C) and nutrient stocks or fluxes in the ecosystem as a whole (Varella et al., 2004; Veenendaal et al., 2004; Grace et al., 2006; Werner et al., 2014; Chen et al., 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 et al., 1993; Hibbard et al., 2001; Ludwig et al., 2004; Perakis and Kellogg, 2007; Rascher et al., 2012; Otieno et al., 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 et al., 2015). Legume trees such as Acacia species can resolve N limitation in African savanna grasslands (Ludwig et al., 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 et al., 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 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 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.

6.3 Methods

6.3.1 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 et al., 2014). Rainfall mainly occurs over a short rainy season around November and a longer rainy season from April to June.

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.

6.3.2 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 6.1). 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.

Table 6.1: 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

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, 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 H_2O 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.

6.3.3 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 et al., 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 et al., 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.

6.3.4 *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).

6.4 Results

6.4.1 *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 6.2). 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 6.2), we pooled data from all trees of both species for further comparisons.

6.4.2 *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 6.1). 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.

Table 6.2: 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
δ^{13}C [‰]	-14.6 ± 0.22	-14.6 ± 0.3	0.9334
δ^{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

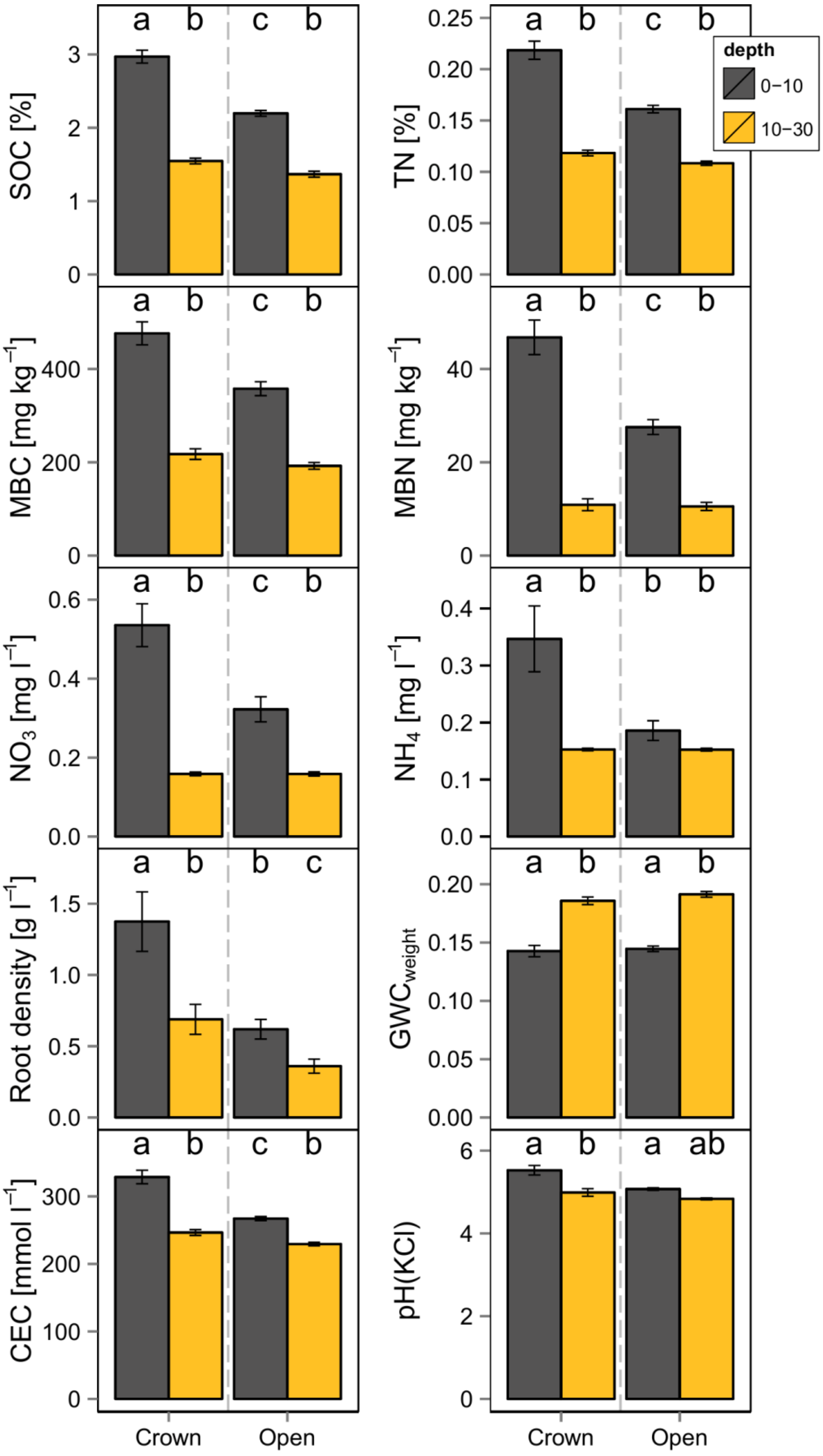

Figure 6.1: Soil properties at 0-10 and 10-30 cm depth, under the crown (n=18) and open area (n=30). Standard error of the mean is shown as error bars with significance levels (a-c) derived from mixed effect model ANOVA for nested designs ($p \leq 0.05$).

Compared to the upper 10 cm of soil, the values of most parameters were lower at 10-30 cm soil depth (Figure 6.1). 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 S 6.1). 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).

6.4.3 Isotopic composition

The abundance of ^{13}C in soil under the crown was shifted towards the values of tree litter (Figure 6.2). The δ^{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), δ^{13}C values increased by about 1.0‰ on average. Mean δ^{13}C values in the top 10 cm were more negative under the crown than in open area (-16.8‰ and -14.5‰, respectively). δ^{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 δ^{13}C, which is equivalent to a 15% mass contribution of tree leaf litter to SOM (Figure 6.2).

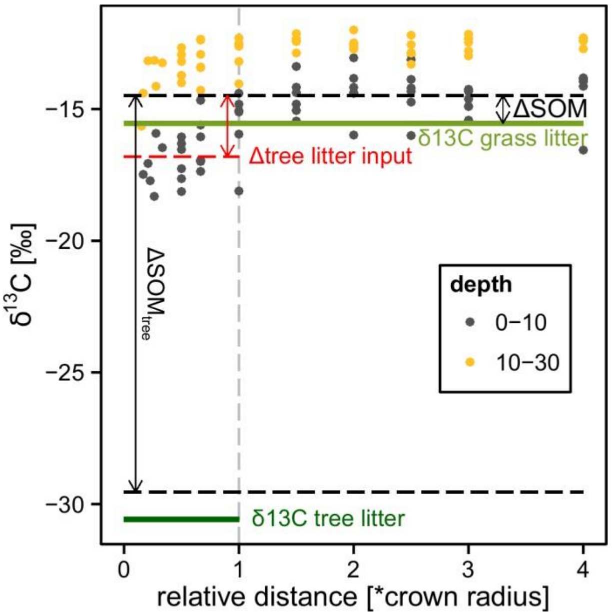

Figure 6.2: δ13C at 0-10 and 10-30 cm soil depth with increasing distance from the stem. Solid lines indicate average δ13C composition of tree and grass litter. Dashed lines show mean δ13C values in 0-10 cm soil under the crown (black) and outside the crown (red) and potential value at 100% tree litter contribution. Black arrows indicate 13C 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.

6.4.4 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 6.3). 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 6.3). 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 6.3: 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

6.5 Discussion

6.5.1 *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 et al., 2004). Nonetheless, the underlying mechanisms of this effect are under debate.

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 et al., 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 6.2). 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 et al., 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.

The higher soil C and N content is limited to the area under the crown and to the upper 10 cm of soil (Figure 6.1). 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 et al., 1997). This agrees with previous findings that the species composition in savanna herb-grass layers changes with varying tree cover (Belsky, 1994; Ludwig et al., 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 6.2) 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 6.1) 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.

6.5.2 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 et al., 2010). However, we found no differences in water contents under and outside the tree crowns at the end of the dry season (Figure 6.1). 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 6.3).

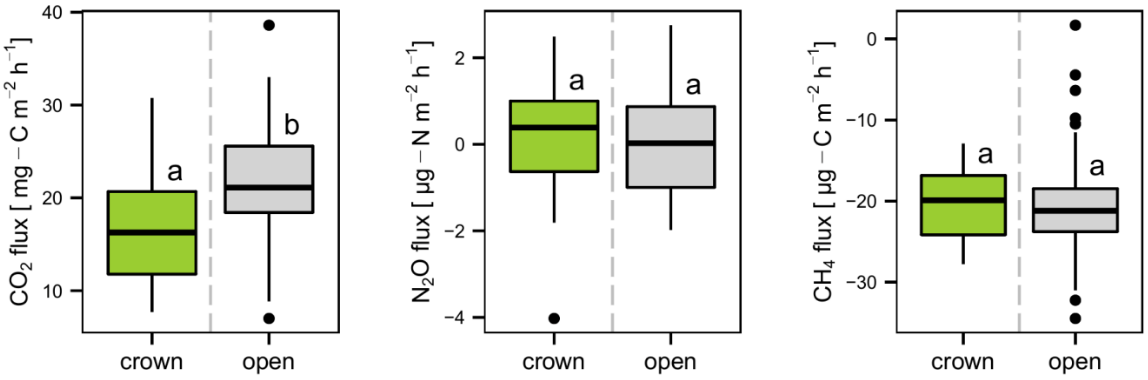

Figure 6.3: Soil greenhouse gas emissions under tree crowns and in open savanna area. Medians, interquartile range (IQR) and extreme values (>1.5 x IQR deviation) are displayed as bold lines, boxes with whiskers and dots, respectively. Significance levels derived from mixed effect model ANOVA for nested designs are shown as letters a-b ($p \leq 0.05$).

This efflux trend was negatively related to fine root density. Because these variables are usually positively correlated under dry conditions (Ceccon et al., 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 6.3). Since NO_3^- addition is known to reduce microbial C mineralization (Burton et al., 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 et al., 2001) and reflect a low carbon use efficiency (Sinsabaugh et al., 2013; Blagodatskaya et al., 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 et al., 2007; Spohn, 2015).

6.5.3 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 et al., 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 et al., 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 6.4). The potential of savanna ecosystems to act as a C sink, as proposed by Grace et al. (2006), is very variable and directly depends on how the vegetation structure affects N availability.

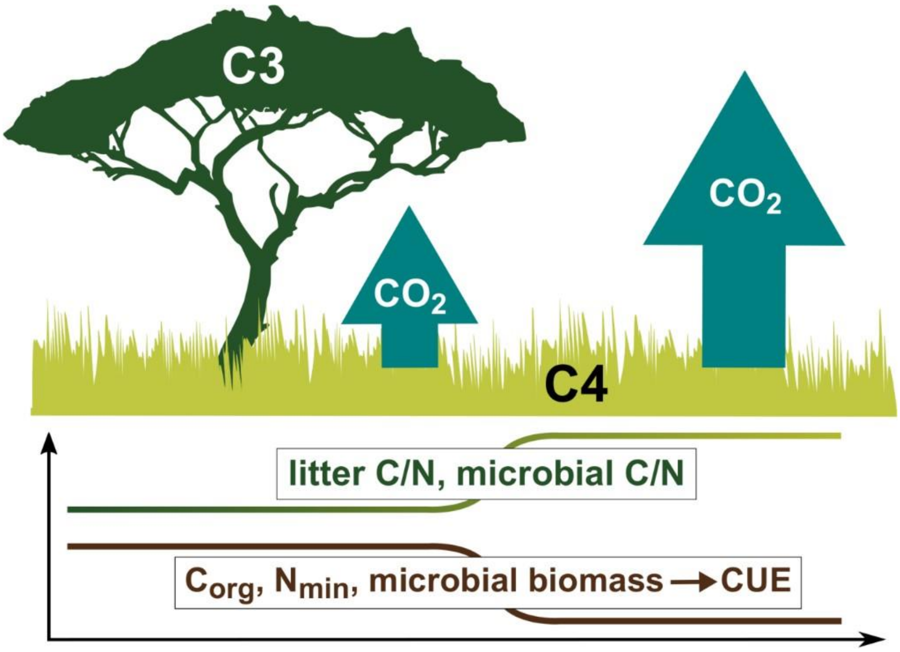

Figure 6.4: Effect of savanna trees on soil C and nutrient pools and related changes in soil respiration under dry conditions. The wide C:N ratio of C4 plant litter reduces N availability (Nmin) and microbial biomass (MBC). Soil microbial C:N ratios and respiration increase due to low carbon use efficiency (CUE).

6.6 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 6.4). 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.

6.7 Acknowledgements

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6.9 Appendix

Table S 6.1: 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$_2$O)	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}

CHAPTER

7

Synopsis

7. Synopsis

Gradients displaying different environmental conditions are very useful to deepen our knowledge about ecosystem functioning in a context of global change. Elevational transects offer a unique opportunity to better understand ecosystem processes and plant adaptation strategies under varying climatic conditions. In addition, land-use gradients entail the alteration of ecosystems processes, leading to modifications on plant community species composition and stand structure characteristics. Besides, ecosystems with high spatial vegetation heterogeneity reflect the close interaction among above and belowground processes (e.g. C and N fluxes to the soil, soil N turnover rates, GHG fluxes). Fine roots constitute the plant-soil interface, processing high amounts of C and N fluxes and influencing above as well as belowground processes. The study of the fine root system, from an ecological perspective, contributes with valuable information to a better understanding of ecosystem processes and plant strategies. Thus, the main objectives of the present study were (1) to determine the effects of elevation (climate) and assess the impacts of land-use gradients on the fine root system (biomass, dynamics and morphological and chemical traits) (*chapter 2 & 4*); (2) to explore the covariation of fine roots in the framework of a root economic spectrum (RES) *(chapter 2)*; (3) to quantify above and belowground NPP and determine carbon allocation patterns along the elevation (*chapter 3*); (4) to address the influence of the vegetation on belowground processes and soil properties in patchy and resource limiting ecosystems (*chapter 5 & 6*). This chapter summarizes and discusses the results of the studies included in this thesis dissertation, linking them to further results of the KiLi project as well as to studies in the same field in tropical ecosystems and placing them in a broader context.

7.1 Fine root adaptive strategies along the elevational gradient

At Mt. Kilimanjaro, fine root biomass increased from savanna woodlands until *Podocarpus* forest along the elevational gradient, declining towards *Helichrysum* heathlands. However, when relating the fine root biomass and production to aboveground biomass, we found very high values for the extreme ecosystems, savanna and *Helichrysum* heathlands, and an increase of the carbon allocation to the fine root system with increasing elevation along the tropical montane forest.

7.1.1 Ecosystems with extreme conditions: savanna woodlands and *Helichrysum* heathlands

The large fine root biomass and production in relation to the aboveground biomass in savanna woodlands is an adaptive strategy to the strong water limitation conditions and to the patchy vegetation structure *(chapter 2)*. Savanna sites at Mt. Kilimanjaro show low values of soil moisture, nitrogen dynamics, ammonium and nitrate concentrations, as well as nitrogen limitation (Gerschlauer et al., 2016, *chapter 6*). A large fine root system enables the plants to reach water and nutrient patches at the same time that water loss is minimized by transpiration with the restriction of aboveground growth, with high FRB:AGB ratios being characteristic for water limited ecosystems (Kozlowski and Pallardy, 2002; Metcalfe et al., 2008; Brunner et al., 2015). We also identified adaptive strategies to water shortage on fine root morphological traits of woody plants in savanna. The high root tissue density prevents from drought stress and herbivory, whereas the high specific root length enables the reaching of new soil volume (Eissenstat, 1991; Weemstra et al., 2016; Freschet and Roumet, 2017). In addition, the observed short fine root lifespan enhances the creation of young, more active roots to replace the dead ones, supporting higher nutrient uptake rates (Eissenstat, 2000). Other strategies against drought have been reported in savanna trees, such as deep tap and long superficial roots, which can reach down to 9 m depth in e.g. *Acacia* sp. (Schenk, 2005). Savannas at Mt. Kilimanjaro hold lower stand values for FRB and FRP compared to other savannas worldwide. This fact might be due to the low biomass of woody fine roots at the top soil layers, the low tree density and low precipitation rates in our plots (maximum FRB value was found in Ghana: 11.86 Mg ha^{-1} (Moore et al., 2018) and maximum FRP value in Australian savanna 14 Mg ha^{-1} yr^{-1} (Chen et al., 2004)).

The afroalpine *Helichrysum* heathlands also present high fine root biomass and production in relation to their aboveground biomass as a respond, in this case, to N limiting conditions *(chapter 2)*. Low temperatures entail slow mineralization as well as low fine root nutrient uptake rates, with large fine root systems representing a good adaptation to these conditions (Holdaway et al., 2011). Thinner, less suberized fine roots with high surface enable the plant to explore new soil areas while facilitating higher nutrients uptake rates. This strategy is also indicated by the high amount of living roots in low cover vegetation plots where, despite the lower N turnover rates, there are important nutrients and water contents *(chapter 6)*. Besides, low decomposition rates (Becker and Kuzyakov, 2018) together with the high fine root turnover observed in our study lead to the accumulation of fine root necromass, which constitute an important contribution of carbon to the soil *(chapter 2)*.

7.1.2 Elevational gradient along the tropical montane forest ecosystems

In line with the resource partitioning theory, carbon allocation to the fine root system increased as soil N availability became limiting along the elevation (*chapter 2 & 3;* Bloom, 1985). Nitrogen limitation at high elevations is indicated by low N content in leaves and fine roots, low N:P in fresh leaves, high NUE in canopy and roots and high soil C:N *(chapter 3)* (Vitousek, 1982; Tanner et al. 1998; Townsend et al., 2007). The decrease of temperature along the slope causes the decline of the decomposition rates and the consequent increase of N shortage (Becker and Kuzyakov, 2018). Colder temperatures also result in lower nitrogen diffusion and N uptake rates (Müller et al., 2016) a process depending on ATP and thus, on C gain, which also declines with elevation *(chapter 3).* Similar results have been reported along forest elevational gradients in other tropical (Leuschner et al., 2007; Hertel and Wesche, 2008; Hertel and Leuschner, 2010; Moser et al., 2011) as well as temperate regions (Hertel and Schöling, 2011). Nevertheless, the increase of the C investment into the belowground compartment along the elevation highlights the key role of fine roots in the strategy of plants to cope with their environment.

Based on our observations, we assume that the identity of the dominant tree species determines the strategy of the fine root system traits at ecosystem level to respond to N limiting conditions (*chapter 2*). The upper montane *Podocarpus* forest (2720 -2970 m a.s.l.) and the *Erica* forest at the treeline (3500 – 3900 m a.s.l.) experienced high N limitation (more pronounced in the case of *Erica* forest). However, they presented a combination of fine root trait strategies (conservative and acquisitive) with an opposite trend: whereas *Podocarpus* forest exhibited low values of SRL and RTD, *Erica* forest showed high values of these both traits (*chapter 2*). In fact, the majority of the fine root related variables displayed an abrupt change from *Podocarpus* forest to the *Erica* forest, at the subalpine zone, indicating the key role of species identity and phylogeny in the control of fine root traits and plant strategies (*chapter 2 & 3*) (Valverde-Barrantes et al., 2015). However, our data set of fine root traits also responded to soil fertility conditions, mean annual precipitation and biotic interactions with mycorrhiza and herbivorous e.g. root tissue density decreased from savanna woodlands, where high RTD is an adaptation against drought, to *Podocarpus* forest, where less xeromorphic roots can be built due to the high water availability; N content decreased in *Erica* fine roots due to lower soil fertility; and *Podocarpus* forest showed higher SRA due to nodules holding mycorrhiza. Ultimately, plant community composition depends on the climate and soil properties (Kricher, 2011). Thus, plant communities are successful in their environment because they have the appropriate characteristics to survive in it. Therefore, fine root related conditions determined by species identity show strategies to adapt to their environmental conditions.

Another relevant fine root trait in the carbon economics strategy of plants is fine root lifespan, which is also driven by species identity and soil fertility. Along the tropical montane elevational gradient, the increase of this fine root trait corresponds to a conservative strategy as N limitation increases (*chapter 2,* Girardin et al., 2013). Low fine root turnover rates entail lower respiration costs and avoid C investment in the construction of new roots (Pregitzer et al., 1993; Eissenstat, 2000). However, fine root lifespan can also decrease due to cold and physical damage at high elevations, as well as a respond to low soil N availability (Moser et al., 2011; Kubisch et al., 2017). Thus, changes in the values of this fine root trait might be also consequence of species composition turnover along the elevation, being taxa with acquisitive traits and short lifespans downslope replaced by more conservative species at the upper zones. We should be cautious while interpreting patterns in fine root lifespan, as its values depend on the methodology used to estimate fine root production (ingrowth cores or sequential coring in our study) *(chapter 3)*.

7.1.3 Multidimensional root economic spectrum (RES)

The root economic spectrum states that fine root traits covariate along an acquisitive-conservative resource axis to develop the strategy that better fits to the environmental conditions at species and community level (Weemstra et al., 2016; Li et al., 2019), similar to the leaf economics spectrum (LES). Several studies have reported results that agree with the existence of a RES at species level in temperate and artic ecosystems (Freschet et al., 2010; Liese et al., 2017) and at community level along fertility, land-use and elevational gradients (Prieto et al., 2015; Kramer-Walter et al., 2016; Li et al., 2019). However, while many leaf traits span along one axis, fine root traits have been observed to be multidimensional (Laughlin et al., 2010). Plants cannot construct tough, long-lived leaves with high SLA, but they can build fine roots with SRL of any density (Kramer-Walter et al., 2016). Thus, a multidimensional concept of a RES entails a lower constrain in building different combinations of fine root traits to success in distinct environmental conditions (Laughlin et al., 2010; Kramer-Walter et al., 2016). In the case of our dataset, it fits better to a multidimensional approach *(chapter 2).* Thus, we can explain the occurrence of fine roots holding a high RTD in *Erica* forest, which might be beneficial against herbivory and pathogens (Eissenstat, 1991; Laliberté et al., 2015; Weemstra et al., 2016), at the same time that they present a high SRL, which enables the reaching of nutrient patches.

In this study, we reported patterns of fine root traits that have not been observed before. We found a positive relation between lifespan and root tissue density and a negative relationship between lifespan and fine root N content, which were only explored in one study so far (Valverde-Barrantes et al., 2007). Lifespan is rarely incorporated in RES studies. This fine root trait is a key factor to improve the understanding of the C and N return to the soil via plant litter decay *(chapter 3)*. Current inconsistences of some of the fine root traits in a RES context might be caused by the more diverse responses of fine roots to resource shortage than the respond of leaves and their symbiosis with mycorrhiza, which can influence specific root traits (Hodge, 2009; Weemstra et al., 2016). Additionally, at the ecosystem level, one species may lead the responses of the entire community, masking the characteristics of other species which might have different strategies to cope with the same environmental conditions (Eissenstat, 2000). Besides, even some of the more widely accepted root trait correlations may not be universally valid e.g. root lifespan can also increase, and not decrease, with a higher root N content (Gordon and Jackson, 2000; Hendricks et al., 2006). The study of RES is a fascinating research field, with a large number of new questions entering the scene (*section 7.8*). The existing empirical evidence for a RES needs further research, as numerous relations remained to be tested and new traits can be incorporated to the spectrum (e.g. mycorrhiza symbiosis) (Roumet et al., 2016; Weemstra et al., 2016).

7.2 Effects of climate on the net primary productivity and carbon allocation patterns

Focusing on the tropical montane forest, we found a decrease of total NPP and a shift of the carbon allocation from above to belowground components along the elevation *(chapter 3)*. The NPP values at tropical montane forests in Mt. Kilimanjaro are similar to values in the neotropics and other tropical montane forest in Africa (Moser et al., 2010; Nyirambangutse et al., 2017; Malhi et al., 2017), with the exception of the lower values of the subalpine *Erica* forest. Net primary productivity values depend on the balance between photosynthesis and plant respiration. An increase in respiration towards higher elevation with lower air temperatures is unlikely, whereas the photosynthetic capacity is positively related to the leaf area index (LAI). At Mt. Kilimanjaro, LAI decreased along the slope by a factor of 3. We suggest that the decrease of total NPP along the elevational gradient might be caused by the increase of N limitation, which result on a decline of LAI and, consequently, of photosynthesis capacity (*chapter 3*). N limitation at high elevations at Mt. Kilimanjaro is indicated by low soil and fine root C:N ratio, low fine root N content, low decomposition rates as well as high N use efficiency (NUE) of canopy and fine root productivity (*chapter 3*, Becker and Kuzyakov, 2018; Schellenberger Costa, unpublished data). The decrease of temperature with elevation affects the N cycling through a decline on N diffusion in the soil, ammonification and nitrification rates, mycorrhiza activity and N uptake rate by roots (Chapin, 1980; Pendall et al., 2004; Marschner, 2012; Robertson and Groffman, 2015).

Other environmental factors can also trigger the decrease of NPP at high elevations, for instance, temperature. Low values of this climatic factor limit meristem activity (Körner 2012). However, we rule out temperature as the main driver of NPP as fine root productivity did not experience any decline despite the colder conditions at high elevations. The regression coefficients suggest that precipitation (MAP) is influencing NPP, but to a lesser degree than temperature and related phenomena such as nutrient supply

We assume that the main constraining factor of forest growth at high elevations is N limitation, in line with the resource balance hypothesis (Bloom et al., 1985). The pronounced shift in carbon allocation from leaf growth (NPP-aboveground litter fall) to fine root growth along the slope is caused by a change from predominant light limitation to nutrient limitation. This shift of C allocation to the belowground components seems to be a worldwide feature of mountain forests (Leuschner et al. 2007; Girardin et al. 2010; Hertel and Schöling 2011; Moser et al. 2011; Leuschner et al. 2013; Mao et al. 2015; Kubisch et al. 2017) Our conclusions agree with similar studies along elevation gradients in the Ecuadorian and Peruvian Andes (Girardin et al., 2010; Moser et al., 2011; Leuschner et al., 2013; Malhi et al., 2017).

Carbon residence time is a key factor of the C cycle controlling carbon pools over NPP (Malhi, 2012; Friend et al., 2014). The increase of the carbon residence time of aboveground wood from lower montane until *Podocarpus* forest, indicates a change from fast to slow carbon pools along the slope. The upper montane *Podocarpus* forest, holding the highest carbon residence time and aboveground biomass, constitutes the most important vegetation carbon pool in the tropical montane forest. The uncertainties on CRT in global vegetation models point to the necessity of carrying out studies on vegetation dynamics and demographic processes (Friend et al., 2014).

Fine roots are the most dynamic compoment of the Mt. Kilimanjaro tropical forest, their productivity exceeding the one from aboveground wood. Their relevant role in the plant C partitioning makes their incorporation into NPP studies essential for assessing plant strategies and improving estimates of total NPP. Beside increasing the understanding of ecological processes, this data contributes to the improvement of terrestrial ecosystem models as well as predictive models in a context of global change.

7.3 Carbon and nitrogen return to the soil via leaf and fine root mortality along the elevation

Fluxes of C and N via fine root and leaf mortality represent an important source of these elements for the soil and organisms living in it. These fluxes decline gradually along the slope until *Podocarpus* forest, within the range for other tropical montane forests

(Lisanework and Michelsen, 1994; Röderstein et al., 2005; Becker et al., 2015; Kotowska et al., 2016). At *Erica* forest, a sharp decrease of these fluxes takes place, the root litter flux greatly exceeding the leaf-born flux values. This higher contribution of fine root litter was also shown in other studies in the tropics in sites with soil resource limitation (Sanford and Cuevas 1996; Röderstein et al., 2005). Additionally, *Erica sp.* fine roots exhibit traits assumed to confer more resistance against herbivory and decomposition (RTD and low N content) *(chapter 2 & 3).* Hence, at high elevations, C and N cycles are slowed down due to the decrease of C and N fluxes to the soil via leaf and fine root mortality and low decomposability of fine roots, which represent the main source of C and N to the soil. All these factors, together with the low decomposition rates (Becker and Kuzyakov, 2018) reinforce the low N availability for plants in the system, playing a feedback on NPP, NUE and carbon allocation *(chapter 2 & 3)*. Species identity, in addition to the fine root traits, plays a major role on C and N fluxes from plants to the soil. We highlight the need of including fine root dynamics in biogeochemical studies in forests ecosystems worldwide (Litton et al., 2007).

7.4 Methodological impacts on fine root production estimations

Estimations of fine root production differ very often depending on the method used to calculate them (Vogt et al., 1998; Hertel and Leuschner, 2002; Moser et al., 2010), as they do in our study *(chapter 2 & 3)*. For example, at an elevation gradient at the Ecuadorian Andes, estimations from ingrowth cores and sequential coring differed by a factor of 6 at high elevations (Moser et al., 2010). Both methods have positive and negative aspects, and the decision of which one to use depend on the kind of study and aim. A good explanation of the benefits and disadvantages of these methods is compiled in different studies (Hertel and Leuschner, 2002; Vogt et al., 1998; Majdi et al., 2005). Depending on the chosen methodology, different conclusions on the dimension of ecosystem processes and plant strategies might be developed. In the present study, our main results, i.e. the shift of C allocation to fine roots and the decline of total NPP, increase of fine root NUE and decrease of C and N fluxes with root mortality along the slope are not altered by the use of the sequential coring or the ingrowth core method (although the slope of the linear models and the explained variance differ). However, there are important implications of favoring one approach over the other: (1) different values of total NPP reflect distinct capacity of the studied ecosystems on sequestering atmospheric C and fixing it in their biomass; (2) a variance on the proportion of C allocation to fine roots influences their importance in the C and N cycle at N limiting conditions and the strategy plants follow to cope with harsh environments; (3) contrasting fine root CRT affect belowground C stock and turnover as well as the fine root strategy in different environmental conditions; and (4) the great difference of the magnitude of C and N fluxes with fine root litter to the soil influence the relevance of the role of fine roots in this pathway of the C cycle. All these aspects together affect our understanding of the functioning of tropical montane forest at Mt. Kilimanjaro and their implication on the addressed pathways of C and N cycle.

7.5 Effects of land-use change and ecosystem disturbance on the fine root system

The numerous land-use and disturbance regime modifications in the different elevation zones at Mt. Kilimanjaro lead to a consistent decrease of nearly all investigated properties and processes namely fine root biomass, fine root production, fine root litter quality and C and N fluxes to the soil via fine root mortality *(chapter 4)*. Changes on fine root biomass and C and N fluxes have also been observed in land-use conversion of tropical forests to coffee, cacao and other plantations (Hertel et al., 2009; Leuschner et al., 2009; Hundera et al., 2013; Kotowska et al., 2016). The impacts of land-use change on the fine root system are a consequence of the alteration of the plant species composition and stand structure (e.g. lower aboveground biomass and basal area). It might be that management practices in agricultural systems also play a role on these changes.

The conversion of savanna to maize fields in the colline zone entails the 70% decrease of fine root C stocks, probably leading to impacts on other fine root functions as soil stability and rhizodeposition.

At the lower montane zone, the decrease on aboveground biomass and change of species composition led to the rather steep decline of stand fine root biomass from lower montane forest to agroforestry systems. Homegarden agroforest, with its multilayer vegetation structure and crop diversity, stands out for being a high dynamic ecosystem able to retain properties from the lower montane forest such as a high stand fine root production, C and N fluxes from fine root mortality to the soil and fine root litter quality. We add these properties to the high biodiversity, leaf litter production and high gross N turnover rates reported in other studies (Hemp, 2006; Becker et al., 2015, Gerschlauer et al., 2016). This ecosystem also presents higher turnover rates and more pronounced acquisitive traits than lower montane forest. The mixture of species (*Musa sp*., *Coffea sp*., *Albyzia schimperiana*, *Grevillea robusta*) might be the responsible of these high values through species specific characteristics, whereas management practices may also play a role. High presence of shade trees allows for large amounts of leaf litter, which enables high gross N turnover rates and high soil fertility (Horwath, 2015; Gerschlauer et al., 2016) and helps to reduce chemical fertilizer application among other benefits (Beer et al., 1998; Dossa et al., 2008; Rajab et al., 2016; Tscharntke et al., 2011). Despite the fact that it might also involve root competition, niche complementary use among some of the species present in homegardens (*Musa sp*., *Coffea sp*. and *Albyzia sp*.) has been reported in similar agroforest systems but not in the case of other species (e.g. *Grevillea sp*.) (Defrenet et al., 2016; van Asten et al., 2011; Dossa et al., 2008). Acquisitive morphological fine root traits and high fine root turnover are also advantageous in a root competition context (Valverde-Barrantes et al., 2013) as well as under tillage practices to replace dead or damage roots. Contrary, coffee plantations are more static agroforest ecosystems, with much lower C and N inputs to the soil via fine root mortality than tropical montane forest. This system holds a much lower number of shade trees than homegardens, removal of a fraction of litterfall and pruned material and addition of NPK fertilizer, leading to low gross N turnover rates and an open N cycle (Gerschlauer et al., 2016). The number of shading trees have a main role on C and N processes as their litterfall acts as substrate for the soil microorganisms. In monocultures at Mt. Kilimanjaro (maize, coffee), the decrease of the C stocks and microbial biomass is related to a reduction of the input of plant litter and rhizodeposition (Dinesh et al., 2003; Pabst et al., 2016), which is in line with the reduction of the C and N fluxes to the soil from fine root mortality, the removal of the litter in maize fields and pruned parts and the decrease on fine root biomass in coffee plantations.

The fine root system at the middle montane forests seems to be recovered from selective logging of *Ocotea* forest occurred 30 years ago, as no differences were found in any of the studied fine root related variables. A recovery of the fine root biomass in secondary tropical forest is expected after 1-2 years (Hertel et al., 2007) and depends on stand age (Cavelier et al., 1996). However, regeneration of the aboveground components of these forests is still in process, as mature *Ocotea usambarensis* trees are missing in some areas of the eastern slopes of Mt. Kilimanjaro (Rutten et al., 2015).

The replacement of *Podocarpus latifolius* as dominant species by *Erica excelsa* after events of fire results on a less dynamic system with lower fine root production, turnover and fluxes of C and N to the soil via fine root mortality. We also expect a higher fine root:leaf litter ratio, typical from *Erica sp.* in the disturbed plots (*chapter 3*). The low C and N fluxes to the soil from fine root and leaf litter in these plots (*chapter 3 & 4*), together with low fine root litter quality might affect microbial activity, slowing down decomposition and increasing N shortage at the disturbed forest (Chapman et al., 2006). This slowdown of the C and N cycling is already reflected in higher soil C:N. Thus, species identity has a large impact on the fine root system and on C and N cycling. Despite high soil C:N and lower decomposition (what could lead to increase of C stocks), it is probable that soil C stocks decrease in the future in the disturbed forest as a result of the low litter input, reaching values close to the

Erica forest upwards (*chapter 3*). Thus, the disturbance by fire of *Podocarpus* forest entails strong impacts on the soil C stocks and dynamics.

7.6 Effects of spatial vegetation heterogeneity on belowground processes

The patchy vegetation of alpine *Helichrysum* heathlands and savanna woodlands enables to study the tight relation among above and belowground processes such as gross N turnover, GHG fluxes and soil C sequestration *(chapter 5 & 6)*. Both ecosystems, at the lower and upper extremes of the elevational gradient at Mt. Kilimanjaro respectively, present stressful conditions (e.g. seasonal water limitation in savanna; wide daily variation in temperature in *Helichrysum* heathlands; N limitation in both). Thus, they offer a good opportunity to study the effects of vegetation on ecosystem processes as the C and N cycle, through their linkage to the microbial community.

The patchy structure of the vegetation in *Helichrysum* heathlands has a strong influence on gross N turnover rates and greenhouse gas fluxes through plant litter, rhizodeposition, and their effects on microbial biomass and microbial activity *(chapter 5)*. High amounts of substrate can maintain high microbial biomass and gross N turnover rates (Gerschlauer et al., 2016). Thus, high vegetation cover plots presented high soil organic carbon, microbial biomass and high mineralization and NH_4^+ immobilization rates and CO_2 fluxes, in line with other studies (Hodge et al., 2000; Schimel and Bennett, 2004; Phillips et al., 2011; Kuzyakov and Blagodatskaya, 2015). Additionally, high NH_4^+ immobilization rates, high relative N retention by microbes and low N_2O fluxes indicate N limiting conditions at these plots. This situation leads to strong competition among plants and nitrifying microorganisms for NH_4^+, which might, in the short term, reduce plant N availability. However, internal N recycling through microloops enables N retention, which can lead to sustainable N provisions to plants as, in the long term, they compete better against microbes due to their higher and long-term storage capacity (Hodge et al., 2000; Kuzyakov and Xu, 2013). The competition for NH_4^+ at vegetated plots, together with N limitation, may enhance the reported high fine root mortality rates and, in consequence, the high fine root turnover rates *(chapter 2)*.

Multiple regression analysis showed that mineralization and NH_4^+ immbolization are driven by microbial N and SOC, which depends on C and N supply through litterfall, dead roots and rhizodeposition, fine roots playing a key role in the input of C and N to the soil. Although mineralization entails a decline of SOC, the high amounts of substrate led still to high values of soil C stocks. Mineralization is related to CO_2 release via soil respiration.

Soil microorganisms, roots and mycorrhiza are responsible for soil respiration, which is also related to temperature and the amount of substrate available to the microbial community (Raich and Schlesinger, 1992). Although high and medium cover vegetation plots presented higher respiration rates than lower cover vegetated plots, unexpectedly, the first ones showed slightly lower CO_2 fluxes compared to the medium cover sites. This observation is related to the lower temperatures in the high cover vegetated areas consequence of larger shading. Thus, the C and N cycles at *Helichrysum* heathlands are driven by temperature. It was not possible to observe any effect of soil moisture on gross N turnover rates and green house emissions due to high percolation rates.

Regarding the other estimated GHG fluxes, the lower uptake rates of CH_4 in the low cover vegetated plots are a consequence of high bulk density, which reduces soil aeration, and high soil moisture, limiting gas exchange through the soil-atmosphere interface. In the case of N_2O emissions from the soil to the atmosphere, they are originated indirectly through nitrification or directly via denitrification (Conrad, 1996; Butterbach-Bahl et al., 2013). High dissolved organic carbon (DOC) entails heterotrophic microbial NH_4^+ immobilization over gross autotrophic nitrification (Butterbach-Bahl and Dannenmann, 2012). The very low nitrification rates and the lack of anaerobic conditions for denitrification explain the undetectable values of N_2O fluxes. Thus, the N cycle at this afroalpine ecosystem is very tight due to coupled ammonification-NH_4^+ immobilization, with no losses to the atmosphere, characteristic of undisturbed systems.

As movement of vegetation in the past has been reported in paleoclimate and paleo environmental studies (Zech, 2006; Schüler et al., 2012) and N_2O fluxes are positively related with vegetation, future increase of the vegetation cover with climate change will entail higher N turnover rates. However, it will not open the cycle through losses to the atmosphere as long as soil C:N is not constrained. The expected increase of N deposition in African tropical regions (Dentener et al., 2006; Vitousek et al., 1997) might destabilize this cycle, as narrower soil C:N may trigger nitrification, with the consequent N losses to the atmosphere and waters.

The characteristic spatial vegetation heterogeneity of savanna woodlands consisting of a matrix of grassland with scattered trees has a strong influence on belowground properties and processes *(chapter 6)*. The positive effects of trees on soil fertility N availability, C pools and understory growth confers them the status of “islands of fertility” (García-Moya and McKell, 1970). In our study, we tried to disentangle the mechanistic behind the effects of this patchy structure on belowground processes such as soil fertility and C sequestration. Under the tree crown, we found an increase of soil C and N content, soil nutrient content, fine root density, increase of microbial C and N, vegetation biomass, and lower C:N ratios in vegetation and microbial biomass and CO_2 fluxes compared to the open grassland. These changes were reported only in the first 10 cm of soil. Tree N fixing capacity affected neither soil properties nor gas fluxes in our study site, contrary to some studies (Vitousek and

Walter, 1989; Sitters et al., 2015), but in line with others (Bernhard-Reversat, 1982; Belsky et al., 1989). We relate this lack of N fixing effect to the extremely low density of tree roots and the absence of nodulation in both, tree and herb roots. Savanna trees have very deep as well as long lateral roots, nodulation shifting to deeper soil zones in dry conditions (Vetaas, 1992). Thus, we point to the tree leaf litter as the major driver of belowground processes and supplier of C in the limited space under the crown area. Whereas inputs of grass litter did not differ on $\delta^{13}C$ under the crown and in open grassland, the $\delta^{13}C$ values in soil under the crown shifted to values toward tree leaf litter, pointing to this component as a major driver of soil C stocks and nutrient content in this area. The 15% of SOM is originated from tree leaf litter. Savanna trees act as nutrient pumps (Scholes, 1990), taking up nutrients and water from deep soil layers in the weathering zone, and returning them to the soil as litter fall (as in our study), root litter and exudates. Tree fine roots were almost negligible in the topsoil, thus, we dismissed lateral pumping and highlight a preferential vertical flow.

Concerning soil respiration rates, higher values were found in open grasslands, despite the high fine root density and microbial biomass under the crown. As water content did not differ under and outside the crown and soil respiration showed a negative relation with fine roots, we ruled out these two factors as drivers of soil respiration. The soil C sequestration potential of an ecosystem depends on the microbial activity, microbial carbon use efficiency and plant litter quality (de Deyn et al., 2008; Bradford and Crowther, 2013). Grass litter presented lower quality than tree litter (higher C:N), as it contained 50% less N. This wider C:N value of the grass litter was, at the same time, reflected on a wider microbial C:N value in open grassland, (Nicolardot et al., 2001), which indicates low carbon use efficiency (Sinsabaugh et al., 2013; Blagodatskaya et al., 2014). Thus, the microbial community needs to release more CO_2 to use the super C content of the substrate and SOM to meet their C and N requirements.

Savanna trees promote soil fertility and C storage in two ways: (1) directly by contributing to the C inputs to the soil through leaf litter and acting as a nutrient pump; (2) indirectly by avoiding soil C mineralization. The spatial abundance of trees in savannas has a large influence on soil C pools and fluxes, the effect of tree density being higher than vertical growth (no effect of tree size related to age). Two possible scenarios might appear in the future: an increase of tree cover due to decrease of large herbivory mammals and prevention of wildfires (Staver et al., 2011) or a decrease due to increase on temperature uncertainties with climate change (IPCC, 2013) or with land-use conversion to agricultural land (Soini, 2005; *chapter 4*). The last scenario would entail a decline on the capacity of savanna soils to store C.

7.7 Conclusions

The present study dealt with the effects of a climate (elevation) and land-use gradient on the fine root system and the response of the above and belowground processes to elevation and spatial vegetation heterogeneity at Mt. Kilimanjaro, leading to the following conclusions:

I. Along the elevation of the tropical montane forest, there is a shift of the C allocation from canopy to fine roots as a respond to increasing N limiting conditions at high elevations due to low temperatures triggering low mineralization rates. A large fine root system enables the plant to meet its resource requirements under these conditions. This phenomenon seems to be a feature of montane forest worldwide.

II. A multidimensional RES fits well with our data from a soil fertility gradient along the tropical montane forest elevation, as it gives more possibilities on trait combination to respond to the different environmental stress factors affecting the fine root system. Along the slope, there are different combinations of acquisitive and conservative traits, species identity being an important driving factor of fine root traits.

III. The decrease on NPP along the elevation gradient at the tropical montane forest is due to increasing soil N shortage, which result in lower LAI and consequently a decline on photosynthesis capacity. Besides, C and N fluxes to the soil vial leaf and fine root mortality also strongly decrease with elevation, slowing down the C and N cycles.

IV. At the subalpine *Erica* forest, the abrupt change of the investigated variables such as the strong decrease of NPP and C and N fluxes to the soil vial leaf and fine root mortality takes place. These changes are a consequence of low soil fertility conditions together with species identity and its inherent properties. At the same time, the values of these variables reinforce soil N limitation for plants through the feedback that vegetation plays on belowground processes. The upper montane *Podocarpus* forest stands out as a high carbon pool driven by the high CRT of aboveground coarse wood.

V. Species identity is an important factor controlling fine root system as well as aboveground properties and processes, the presence of a species on a specific site being driven by the climatic and soil characteristics. Therefore, climate has a strong effect on the above and belowground components and processes.

VI. Land-use conversion for agriculture and agroforestry decreases fine root biomass and dynamics as well as the inputs of C and N to the soil via fine root litter due to change on species composition and stand structure. Management practices such as tillage or the maintenance of shade trees triggering plant litter and root competition might also affect fine root dynamics and morphology, altering C and N cycling. Disturbance of upper montane *Podocarpus* forest by fire and replacement by *Erica excelsa* as dominant species leads to an extremely decrease of C and N fluxes, slowing down belowground processes and the probable decrease of soil C stocks in a close future.

VII. The traditional agroforestry system “Chagga homegardens” stands out for maintaining some of the properties from the semi-natural lower montane forest as a high stand fine root production and high C and N inputs to the soil via fine root mortality, contributing to soil C stocks and as substrate for the microbial community.

VIII. Spatial vegetation heterogeneity plays a strong influence on belowground processes such as gross N turnover rates, GHG fluxes, microbial activity, and soil C and N content through plant litter and rhizodeposition. Plant litter amount and quality, affecting microbial activity, as well as the microbial carbon use efficiency are major drivers of soil C sequestration.

IX. The incorporation of field-base fine root data is crucial for studies on terrestrial carbon cycle to better understand ecosystem functioning, plant strategies, the influence of fine roots on above and belowground processes, as well as to predict ecosystems responses to disturbances. Special attention should be focus on the approach used to estimate fine root production, taking into account the purpose of the study and characteristics of the ecosystem, as different methods might lead to different conclusions.

7.8 Implications, uncertainties and perspectives

The different pathways of the terrestrial C and N cycle will be affected by changes in temperature and increasing metabolic rates until a physiological limit. Furthermore, shifts on precipitation patterns, which are essential for ecosystem processes such as NPP and changes on species composition are expected to happen as a consequence of global change (IPCC, 2013). Vegetation at Mt. Kilimanjaro has experienced driven-climate movements during the last glacial period, major shifts being reported in upper vegetation zones (Schüler et al., 2012; Zech, 2006). Wetter and cooler climate led to an uplift of the *Erica* belt and montane forest of 1500 m. Precipitation is considered to be the major driver of these vegetation changes at Mt. Kilimanjaro (Schüler et al., 2012). Ongoing climate change can also result on shifts of vegetation at this volcano, as it has occurred with climate variation in the past. Although the confidence of an increase of the temperatures along this century is high, there is much uncertainty about precipitation patterns in East Africa (IPCC, 2013) and thus, on the changes the vegetation zones will experience with climate change. Shifts of species or groups of species will have large effects on C and N cycles. In addition, it is crucial to take into account changes on N such as current N deposition, as it plays a major role on the C cycle (IPCC, 2013). Land-use change and ecosystem disturbance have further implications on plant communities composition, affecting therefore biogeochemical cycles, ecosystem functioning and climate change.

Climatic models predict drier conditions for arid areas where savanna woodlands stand, as well as higher differences between dry and rainy season (IPCC, 2013). In addition, land-use conversion to open grasslands and croplands also entails drier conditions, enhancing fire frequency and a higher loss of trees, which, at the same time, plays a feedback on climate by the increase of aridity (Hoffmann and Jackson, 2000). Savannas hold a high potential for soil C sequestration (Pabst et al., 2016). However, with tree loss and conversion to croplands, this potential, together with the C and N inputs through plant litter to the soil, decreases *(chapter 4 & 6)*. In a warmer climate, the montane forest is expected to move upwards, although it strongly depends on precipitation rates. This shift might entail loss of plant species at the lower montane forest, as they cannot be replaced by lowland species (Colwell, 2008). Another strong impact might be the constraint of the upper montane *Podocarpus* forest if *Ocotea* forest moves upwards and *Erica* forest downwards (as a result of enhanced fires by climate change (Hemp, 2005)). The replacement of *Podocapus latifolius* for *Erica excelsa* as dominant species leads to a decrease of carbon sequestration in plant biomass and soil and a slower C and N cycling *(chapter 3 & 4)*. Even if *Podocarpus* moves upwards, it might not be able to grow on the steep slopes where part of *Erica* forest stands. The higher presence of *Erica* sp. in the afroalpine zone will accelerate gross N turnover and might increase soil C stocks through plant litter. However, alpine ecosystems are in danger due to the lack of physical space to migrate upwards (Körner, 2003), species being forced to adapt to the new environmental conditions to avoid extinction. Nevertheless, these are all suppositions based on past and present vegetation changes. Future climate-driven vegetation shifts strongly depend on precipitation regimes, which are currently uncertain, as well as on N disposal. The prediction of changes in ecosystem C and N cycle with climate change is very complex, as it depends on multiple factors such as species shifts, interactions with soil properties, biotic interaction and topography of the area among many others.

Mount Kilimanjaro acted as a refugee for species during past climate variation and might act as well as one in the future (Schüler, 2015). The protection of part of the forest on the south slope of Mt. Kilimanjaro under the figure of National Park is crucial to provide refugee for species, as well as for protecting other important ecosystem functions as C sequestration and storage, the maintenance of the cloud zone and watersheds for the entire region, regulation of the local climate and provision of resources as non-timber products and firewood to the local population in its lowlands (Agrawala et al., 2003; Hemp, 2009).

In a changing world, data about ecological processes, together with socio-economic and cultural factors are crucial in the process of ecosystem decision-management. All these aspects together contribute to ecosystems conservation, food security and the sustain of local populations. Maize fields and croplands at the foothills of Mt. Kilimanjaro are expanding, replacing the savanna woodlands and bushes (Soini, 2005). Current changes on agroforestry systems at Mt. Kilimanjaro also present uncertainties on how new circumstances affect their functioning. Coffee yield in Kilimanjaro region has decreased due to higher temperatures and new management practices have been recommended by coffee cooperatives, as the use of shade trees (Kumburu, 2012), which might improve carbon stocks and N availability (Dossa et al., 2008; Tully et al., 2012), and may lead to a lesser use of fertilizers. In the case of the “chagga homegardens”, these ecological and cultural valuable systems currently experience different changes (e.g. the remove of shade trees, decrease of the plot area and change to other crops) with consequences on C and N cycling and ecosystem functioning, but also probably on socio-economic aspects as the derived from yield production and costs. Studies of the impact of these changes on above and belowground processes might bring important conclusions to incorporate to socio-economic and political studies and to management practices together with a better understanding of ecosystem functioning.

Although hidden in the soil and accounting for a small fraction of ecosystem biomass, fine roots are a crucial piece of ecosystem functioning, holding the key to many ecological questions. In the last decades, ecological research on fine roots has largely increased (e.g. RES, drivers of decomposition, fine root system along different gradients) (Weemstra et al., 2016; Prieto et al., 2015; de la Riva et al., 2016; Cai et al., 2019; See et al., 2019). However, we need further knowledge on the following ecological research topics on fine roots: (1) the relation of fine root physiological functions (e.g. nutrient uptake, defense against drought) and morphological and chemical traits (Weemstra et al., 2016); (2) the relations among biotic factors as herbivory and mycorrhiza symbiosis to morphological traits in a context of RES; (3) the covariation of fine root and leaf traits along elevation gradients and the incorporation of other fine root traits to multidimensional RES (Kramer-Walter et al., 2016); (4) the relation of morphological traits and root competition in the context of a competition instead of spatially segregation (Valverde-Barrantes et al., 2013) (e.g. coffee trees in monoculture plantations and in homegardens); (6) the effects of fine root litter quality on gross N turnover rates, its contribution to SOM and the comparison of fine root litter to leaf litter decomposition in different environmental conditions; (7) carbon allocation to fine roots in harsh environmental conditions.

The present work contributes to the understanding of the role of fine roots and ecosystem functioning along a climate (elevation) and land-use gradient at Mt. Kilimanjaro. In addition, the data collected in this research can support broader studies on ecosystem functioning at Mt. Kilimanjaro and in tropical regions and contribute to develop more accurate predictions on terrestrial carbon models. Doing research with fine roots takes us closer to the exciting task of deepen our understanding of how nature works, which makes the laborious work more than worth it.

7.9 References

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sounds, warm and cold, for making me feel happy in its slopes.

Tanzania Tanzania,
Nakupenda kwa moyo wote (Tanzanian song)

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Other publications

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PRESENTATIONS

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Declaration of originality and certificate of ownership

I, Natalia Sierra Cornejo, hereby declare that I am the author of the present dissertation entitled ‘The role of the fine root system in carbon fluxes and carbon allocation patterns of tropical ecosystems along a climate and land-use gradient at Mount Kilimanjaro’. All references and sources that were used in the dissertation have been appropriately acknowledged and cited. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure.

Göttingen, October 2020

(Natalia Sierra Cornejo)