FRIEDRICH-SCHILLER-
UNIVERSITÄT
JENA
Chemisch-Geowissenschaftliche Fakultät

MASTERARBEIT

zur Erlangung des akademischen Grades Master of Science (M.Sc.)

Effect of forest gaps on soil enzymatic activity and tea bag decomposition index in the Hainich-Dün Biodiversity Exploratory

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Jena, 15.12.2021

Danksagung

Diese Masterarbeit wurde in Zusammenarbeit mit dem Institut für Geographie der Friedrich-Schiller-Universität Jena und dem Max-Planck-Institut für Biogeochemie Jena durchgeführt. Ich bin beiden Institutionen dankbar, dass sie diese Arbeit ermöglicht und mir die Gelegenheit gegeben haben, mein theoretisches und praktisches Wissen auf dem Gebiet der Waldökologie und Bodenbiogeochemie zu erweitern.

An erster Stelle möchte ich meiner Betreuerin und meinen Betreuern danken: Frau Dr. Marion Schrumpf für die Bereitstellung des Forschungsthemas und des konzeptionellen Rahmens trotz pandemiebedingter Unsicherheiten zu Beginn dieses Jahres; Herrn Dr. Alexander Tischer für zahlreiche Literaturempfehlungen, hilfreiche Ratschläge bei der statistischen Analyse und den freundlichen Zuspruch; und Herrn Dr. Ingo Schöning für seine Planung und Unterstützung im Feld und bei statistischen Analysen. Ich bin ihnen dankbar, dass sie ihre Zeit und ihr fundiertes Wissen und Erfahrung in wissenschaftlichen Diskussionen und persönlichen Gesprächen mit mir geteilt haben.
Theresa Klötzing danke ich für die Einführung in die Enzymanalyse und ihre umfassende Unterstützung bei der Laborarbeit. Ich danke Iris Kuhlmann für die Einführung in die CFE-Analyse und die praktischen Laborhinweise. Weiterhin möchte ich mich bei Jessica Heublein und Stephanie Strahl für ihre Hilfe im Labor bedanken, sowie bei Aliza Hilman, Paulina Hornung und Kyla Sehner, die mich beim Wiegen und Reinigen der über 1000 Teebeutel unterstützt haben. Meinem Kommilitonen Felix Lauer danke ich für seine Unterstützung mit RStudio, sowie für die kritische Begleitung meiner Arbeit. Ich bedanke mich bei dem gesamten Team der Bodenbeprobungskampagne im Hanich-Dün Biodiversitätsexploratorium für drei Wochen harte Arbeit, bereichernde Gespräche und das herzliche Lachen im verregneten und pandemiegeprägten Mai 2021.

Mein großer Dank geht an meine Eltern Elżbieta und Béla Enyedi für ihren starken Glauben an mich - Köszönöm Apu, hogy mindig bízol bennem!- und meinen Bruder Alexander Enyedi für seine Motivation und die besondere Unterkunft während der Probennahme. Außerdem danke ich meiner Großmutter Lotte Diesterheft, für die ermutigenden Lebensweisheiten an jedem Sonntagabend. Ich danke meinen Freunden im Karl-von-Hase Haus für ihr ehrliches Interesse an meiner Arbeit und die stärkende Gemeinschaft. Schließlich danke ich meinem Liebsten Martin Kschenka, der mich in den letzten sechs Jahren meines Studiums und nun beim Schreiben dieser Arbeit mit viel Zuspruch unterstützt hat.

Jena, 15.12.2021 Brigitta Enyedi

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a_g	decomposed fraction of green tea
ANCOVA	analysis of covariance
a_r	predicted labile fraction of rooibos tea
BexIs	Biodiversity Exploratories Information System
BG	β-glucosidase
C	control plot
$CaCl_2$	calcium chloride
C_f	carbon content of fumigated sample
CFE	chloroform fumigation extraction
C_{inorg}	inorganic carbon
C_{mic}	microbial biomass carbon
C_{nf}	carbon content of non-fumigated sample
C_{org}	organic carbon
D	deadwood
d	dilution factor
df	degrees of freedom
DMC	dry matter content
EP	experimental plot
F	*F*-value
FOX	forest gap experiment
G	gap
GD	gap with deadwood
HEW	Hainich-Dün Exploratorium Wald
Hg	hydrolysable fraction of green tea
Hr	hydrolysable fraction of rooibos tea
IW	initial weight
K_2SO_4	potassium sulfate
k	decomposition rate
k_{ec}	extractable part of microbial biomass carbon
MAP	mean annual precipitation
MAT	mean annual temperature
MES	2-(*N*-Morpholino)ethanesulfonic acid
MS	mean squares
MUF	4-methylumbelliferone
N	nitrogen
NAG	N-acetyl-glucosaminidase
N-E-S-W	north-east-south-west
Nf	nitrogen content of fumigated sample
N_{mic}	microbial biomass nitrogen
Nnf	nitrogen content of non-fumigated sample
P	phosphorus
Phos	phosphatase
S	sulfur
S	stabilisation factor
Sulf	sulfatase
TBI	Tea Bag Index
TOC	total organic carbon
TN_b	total bound nitrogen
WRB	World Reference Base for Soil Resources
Wt	remaining fraction

Abstract

As a result of the ongoing global climate change, forests and their ecosystem functions and services are highly vulnerable. Human-induced interference with ecosystems and global biogeochemical cycles have also led to a loss of biodiversity. Since forests are important regulators of climate and biodiversity and the basis for silviculture, a sustainable forest management is crucial for future maintenance of ecosystem functions and services. Canopy openings are considered a sustainable management measure. By altering the ambient environment, small forest gaps have the potential to regenerate the forest, increase structural complexity at stand level and promote biodiversity. Since microorganisms are involved in various biogeochemical processes, it is important to better understand the impact of forest gaps on microbial activity. This study investigated the effect of canopy openings on soil properties, microbial activity, and decomposition as part of the forest gap experiment (FOX) in the Hainich-Dün Biodiversity Exploratory. Microbial biomass carbon (C_{mic}) and the activity of four extracellular enzymes β-glucosidase (BG), N-acetyl-glucosaminidase (NAG), sulfatase (Sulf) and phosphatase (Phos) were measured in 36 mineral soil samples under different treatments including control plots (C), deadwood plots (D), forest gaps (G) and gaps enriched with deadwood (GD). The globally used Tea Bag Index (TBI) was applied to study decomposition in leaf litter and mineral soils under the treatments. Analysis of covariance and linear models were used to examine the effect of forest gaps and the correlation between the parameters. The results of the study showed no clear effect of the treatments on soil microbial activity and decomposition after one year of gap formation. Only BG activity was significantly promoted by forest gaps. All enzyme activities were significantly related to C_{mic} and physico-chemical soil properties (pH, C_{org}, N, S, estimated clay and water content). Decomposition rates *k* were higher in the litter layer (0.03) than in mineral soils (0.02) and mass loss of tea was significantly related to water content, which was higher in forest gaps. The results found spatial heterogeneity of soil related properties and decomposition indices. Although predominantly no significant effects of forest gaps were observable, this study suggests that small forest gaps are beneficial for microbial measures and highlights the importance of the investigation of relationships of abiotic soil properties, microbial activity and decomposition indices that are crucial for the understanding of biogeochemical cycles. Further long-term research of the impact of forest gaps is needed to develop optimal forest management practices.

1 Introduction

1.1 Motivation

The Paris Agreement on climate change, adopted in December 2015, emphasises the important role of forests as carbon sinks especially in achieving the long-term climate objectives of the international community (UNFCCC, 2015). As young regenerating forests are considered the largest carbon sink worldwide by storing carbon in soils and plants (Pugh et al., 2019), a sustainable management of these forests is essential. This political and ecological responsibility is particularly relevant to Germany, which is one of the most densely forested countries in the European Union (Gerstengarbe and Welzer, 2013). However, German forests will be increasingly affected by the predicted climatic changes due to global warming (Gerstengarbe and Welzer, 2013; Gessler et al., 2004; Sutmöller et al., 2008; Umweltbundesamt, 2021). Especially European beech forests are considered highly vulnerable (Sutmöller et al., 2008), as frequently occurring extreme weather events like droughts present a major threat to these ecosystems (Bosela et al., 2016; Gessler et al., 2004; Hartmann, 2011).

In addition to climate change, human-induced land use changes and interference with ecosystems and global biochemical cycles have led to a loss of biodiversity (Bartelt-Ryser, 2004; Penone et al., 2019). Even at the microbial level, the impact of forest management through altered environmental conditions is evident, for instance on microbial community composition and soil enzymatic activity. Since microorganisms are involved in numerous processes such as decomposition and nutrient cycling, environmental changes of both, natural and anthropogenic origin can affect ecosystem functions and thus forestry (Horwath, 2017; Srinivasarao et al., 2017).

The selection of the main tree species and the type and intensity of silvicultural management essentially determines the physical structure and composition of habitats for flora and fauna in forests (Umweltbundesamt, 2021). A sustainable forest management is also profitable for silvicultural yields. Thus, the aim of a long-term sustainable forest management is to maintain relevant ecosystem functions and contribute to biodiversity by creating valuable habitats and structures (Schulze et al., 2016; Umweltbundesamt, 2021). Many practices are discussed as a way to manage forests sustainably in order to counteract the consequences of climate change (D'Amato et al., 2011). The formation of canopy openings by selective thinning of forests is an approach to reduce forest density (Yang et al., 2017) and positively alter environmental conditions in forests to support biogeochemical processes. As a type of disturbance, gaps modify various ecological variables in forests (Muscolo et al., 2014). There are several observations that depending on the size of the disturbance gaps, can alter the microclimate and enhance biodiversity in forests (Brunet et al., 2010; Penone et al., 2019; Perreault et al., 2020; Yang et al., 2017), and promote the provision of ecosystem services (Felipe-Lucia et al., 2018). Open canopies provide a basis for natural regeneration of the forest, and also for the growth of different tree species (Bartsch and Röhrig, 2016; Zhu et al., 2003). In this way, multi-layered

and multi-aged forests are created at stand level, and spatial heterogeneity as well as compositional and structural complexity are increased (D'Amato et al., 2011). Leaving behind deadwood as a management option further supports biodiversity and is considered a robust measure against environmental disturbances (Umweltbundesamt, 2021).

As the management of forests under changing conditions presents a long-term ecological and economical challenge (Chapin et al., 2011), it is highly relevant to determine whether and how forest management has the potential to counteract the negative consequences of climate change (Bosela et al., 2016). There is a lack of detailed information on the effect of gaps in the forest (Muscolo et al., 2014) and it is not yet fully understood if a rich biodiversity in beech forests is crucial for the protection of ecosystem functions. These aspects support the need for further ecosystem research on soil biota and litter decomposition in order to better understand biogeochemical cycles and the relationship between biodiversity and different forest management practices (Tóth et al., 2018; Umweltbundesamt, 2021).
With the intention of closing this knowledge gap on forest manipulations and improving mechanistic information on the interplay between biodiversity and land use, a Forest Gap Experiment (FOX) was implemented as part of the Biodiversity Exploratories (Ammer et al., 2020). In 29 forests of different management types and functions, treatments of forest gaps (G) with and without deadwood removal (GD) and plots with deadwood enrichment (D) were created in 2020. Within the scope of the FOX, this master's thesis was realised at the Hainich-Dün Biodiversity Exploratory.

1.2 State of art

1.2.1 European beech forests

The European beech (*Fagus sylvatica*) is a deciduous tree species dominating a large number of forest types in Central Europe (Röhrig, 2006). Since its migration from south-eastern Europe starting from about 12,000 years B.P. (Anhuf et al., 2003), the European beech has been an important mixed tree species (Czajkowski et al., 2006; Röhrig, 2006) and later on relevant for forestry use as timber and firewood (Bartsch and Röhrig, 2016). During the Subboreal, about 4,000 years B.P., *Fagus sylvatica* was able to spread extensively as a consequence of the progressive large-scale clearing of mixed oak forests (Anhuf et al., 2003). Additionally, declining July temperatures and increasing precipitation favoured the growth of European beech forests, displacing mixed oak forests and spruce mountainous forests (Bartsch and Röhrig, 2016). European beech forests are often accompanied by *Acer, Betula, Carpinus, Fraxinus, Quercus, Ulmus* and *Sorbus* (Wäldchen, 2010), creating mixed deciduous forests. Forests that are rich in European beech are considered the potential natural vegetation of large parts of Central Europe (Ellenberg and Dierschke, 2010). Its dominating character and abundance results out of various ecological properties. *Fagus sylvatica* is mainly naturally rejuvenated and does not require thinning-out of forests for regeneration (Röhrig, 2006). As a

typical shade-tolerant plant species the European beech has a slow (Bartsch and Röhrig, 2016) but long-lasting growth up to an age of 150-250 years (Röhrig, 2006). It can extend its canopy steadily into old age and thus inhibit the growth of other shade-intolerant plants (Lemée, 1987). Even seedlings of *Fagus sylvatica* can develop under low light conditions. Thus a low light supply on rich and fresh soils is less inhibiting to growth for beech seedlings than the growth on nutrient-poor and drier sites (Bartsch and Röhrig, 2016).

However, seedlings are sensitive to late frost and can suffer from competition from vigorous ground vegetation (Bartsch and Röhrig, 2016; Röhrig, 2006). Uncovered beechnuts can be damaged by moderate frost and can die back in dry springs (Röhrig, 2006). Especially in the first week after germination beechnuts are often eaten by pigeons, bramblings and mice. During winter and spring the fungus *Rhizoctonia solani* can cause major losses in seedling numbers and sucking insects can weaken one- to two-year-old plants as well (Röhrig, 2006). Regarding water balance, old trees are also highly sensitive to prolonged droughts (Gessler et al., 2004), low water availability (Ellenberg and Dierschke, 2010), water-logging and flooding (Geßler et al., 2006), affecting growth, competitiveness and productivity of the European beech by such changing environmental conditions (Gerstengarbe and Welzer, 2013; Peuke et al., 2002). As another possible threat to *Fagus sylvatica* Sutmöller et al. (2008) refer to the beech bark disease with woolly beech scale (*Cryptococcus fagisuga*) and a fungus called *Neonectria coccinea*. Extreme weather conditions in combination with a thinning of forest stands by windthrow or clearings, lead to a reduced beech vitality, often accompanied by *Agrilus viridis* (Bressum, 2008).

There are conflicting views on the climatic adaptability of *Fagus sylvatica*. Czajkowski et al. (2006), Sutmöller et al. (2008) and Bressum (2008) describe that the European beech is considered to be site adaptable to changing environmental conditions, but numerous scientists have underestimated its tolerance to extreme drought or humidity. Especially in recent years, different diseases and damage patterns haven been increasingly observed in European beech forests (Bressum, 2008; Umweltbundesamt, 2021).

Past research, especially over the last 20 years, focusing on biodiversity in European beech forests, shows the relevance of this type of forest for economy and ecology (Schulze et al., 2016). In particular, the effect of forest management has been studied in various regions in Central Europe. As the Hainich-Dün area comprises three forest management types (even aged, uneven aged and unmanaged forests), numerous studies were conducted in this region. The management of even-aged forests is a common and traditional system of silvicultural practice (Messier et al., 2015), but is associated with low biodiversity. As an alternative, uneven-aged forests, which are characterised by the removal of individual trees or groups of trees, can counteract a loss of biodiversity (Puettmann et al., 2015). Structural richness can be achieved by dividing forest stands horizontally through small-scale tree removals as it is practiced in selectively cut forests (D'Amato et al., 2011; Röhrig, 2006). To investigate empirically which management type is beneficial for biodiversity within a forest stand of European beech, Schall et al. (2018) analysed the impact of even-aged and uneven-aged management on regional

biodiversity in the Hainich-Dün Biodiversity Exploratory. They included taxa of animals, plants, fungi and bacteria and calculated diversity indices showing that the type of forest use has a regional but not a local impact on biodiversity. Moreover, Schall et al. (2018) discovered that the even-aged management system had higher biodiversity indices than the uneven-aged forest for all taxonomic groups except birds. The effect of different forest management practices was also studied by Purahong et al. (2015), who conducted a litter bag experiment in the Hainich-Dün area. Their results show, that microbial communities, were significantly influenced by different management types and sampling dates. In contrast to Schall et al. (2018), Purahong et al. (2015) found that, for example, fungal richness in leaf litter was higher in unmanaged and selectively harvested forests compared to even-aged forests. Furthermore, the management type was found to influence biogeochemical factors such as pH, quality of leaf litter and microbial macronutrients (Purahong et al., 2015). According to Brunet et al. (2010) only forests with an uneven age structure and selective logging can achieve biodiversity as rich as in old growth forests. Especially beech forests with trees older than 180 years are valuable habitats for organisms (Brunet et al., 2010).
Heinrichs et al. (2020) argue, in contrary to the previously described studies, that large felling in age class forests in the Hainich increases regional species diversity, as it leads to different age phases in stands. Hence, small-scale selection-cutting procedures or the abandonment of forest use do not necessarily lead to an increase in biodiversity. With regard to Carbon storage in European beech forests, conclusions of Mund (2004) show that the management of age class forests or selection cutting forests has no significant impact. Only the abandonment of forest use in unmanaged forests leads to an increase in carbon stocks, which also supports the finding that reducing timber harvesting is crucial to increasing above-ground Carbon storage (D'Amato et al., 2011). The impact of deadwood that accumulates in unused forests plays an important role in Carbon cycling (Felipe-Lucia et al., 2018; Mund, 2004). The amount of deadwood is therefor regulated by forest management (Felipe-Lucia et al., 2018). Decaying deadwood affects nutrient cycles and creates habitats for numerous organisms that have a key role in ecosystem functioning (Krajick, 2001; Vilhelmsson, 2013).

It can be concluded, that a large number of studies have been conducted to observe different management practices in European beech forests, but as the results are partly controversial, there is a demand for further studies with robust data.

1.2.2 Forest gaps

Forest gaps or canopy openings are a type of disturbance that play a fundamental role in forest ecology (Muscolo et al., 2014; Yang et al., 2017). These holes in the canopy can occur as a consequence of diebacks of a single tree or a small group of trees due to natural impacts, such as storms, heavy snowfall, lightning strokes, droughts or pest infestations (Bartsch and Röhrig, 2016; Muscolo et al., 2014). But also the felling of trees as a silvicultural measure to initiate the regeneration of the forest creates patchy gaps, usually with a diameter of 30 m (Bartsch and

Röhrig, 2016). As the introduction of gaps affects different levels and factors in the forest, they can contribute positively to long-term productivity and nutrient cycling (Chapin et al., 2011), and promote different services even at the stand level (Felipe-Lucia et al., 2018). Gaps are known to increase solar radiation and light conditions that have the highest impact on tree regeneration and forest communities (Penone et al., 2019; Röhrig, 2006). Solar radiation is a regulating force in biological processes, e.g. photosynthesis, temperature regulation, carbon cycling and evapotranspiration (Fournier and Hall, 2017). The amount of light reaching the ground varies within forest gaps. Since the northern edge of gaps receives more solar radiation than the southern edge (Bartsch and Röhrig, 2016), there is also a small-scale variability within the forest gap. Openings in the canopy also alter the environment concerning soil and air temperature and moisture (Bartsch and Röhrig, 2016; Röhrig, 2006), and also decomposition and nutrient contents (Zhu et al., 2003). Moisture and temperatures are particularly high in the centre of the gap (Bartsch and Röhrig, 2016), which further promotes spatial heterogeneity on a small scale. Natural regeneration shows similar patterns. Especially in old beech forests, the creation of forest gaps by patchy clearances is necessary to enable natural regeneration and to establish a vertical structure (Röhrig, 2006). A horizontal differentiation of forests can also be achieved through thinning (D'Amato et al., 2011; Röhrig, 2006), as is often practised by selective harvesting as a type of forest management.

Over the last 30 years various studies have investigated the dynamics of forest gaps and their effect on microbial biodiversity (Muscolo et al., 2014). Thereby, the size of the gaps plays a critical role, e.g. for tree species composition and regeneration (Muscolo et al., 2014). Yang et al. (2017), for example, reported increased microbial activity in small gaps (40-50 m^2) in a Chinese pine forest, while large gaps had the lowest activities (100-120 m^2). This can be attributed to the fact that large gaps, especially those larger than 1000 m^2, have other characteristics than small gaps (Muscolo et al., 2014). On large clear-cut sites, there is evidence of reduced decomposition rates (Jerabkova et al., 2011), a loss of soil organic Carbon and total soil Nitrogen levels (Pennock and van Kessel, 1997) and a loss of soil organism diversity, as a consequence of the reduction of floor biomass and organic matter (Keenan and Kimmins, 1993). On small gaps where timber and deadwood remains due to harvesting, microbial activity is particularly stimulated (Perreault et al., 2020), because deadwood significantly improves habitat quality for various organisms (Umweltbundesamt, 2021). Thus, microbial community structures (Wang et al., 2021), natural regeneration (Muscolo et al., 2010) and understorey plant richness and abundance (Brunet et al., 2010; Penone et al., 2019) benefit from altered environmental conditions as a result of gap creation. Therefore, many aspects underline the potential of small gaps to increase biodiversity. Nevertheless, it is recommended to create gaps of different sizes in order to provide a high structural diversity in forests (Muscolo et al., 2014).

There are controversial reports regarding the temporal scale of the impact of forest gaps. Lin et al. (2015) found that canopy openings in European beech forests did not affect litter decomposition rates in the first few years after silvicultural treatment, but gaps accelerated fine-

root and litter decomposition in a long term after 23 years. After eight years following gap formation in a European beech forest Bauhus et al. (2004) found no significant impact on soil Carbon an Nitrogen stocks and decomposition of leaf litter and fine-roots. However, Yang et al. (2017) measured increased microbial activity after only one year of gap creation. The results show that there is still a demand for research on the temporal influence of forest gaps on soil properties and processes.

It can be concluded that the creation of gaps as a forest management mechanism influences various abiotic and biotic factors and processes. Disentangling the effects of gaps and interactions between the altered variables is an important challenge in forest ecology research and therefore relevant to sustainable forest management.

1.2.3 Microbial biomass and microbial activity

Chemical and physical soil parameters such as pH, nutrient and water content or grain size distribution provide important information about the quality and functionality of soils (Doran and Parkin, 1996). However, the investigation of microbial parameters, which are significantly influenced by abiotic factors (Frey, 2007), is also particularly important to determine soil fertility and thus ecosystem functioning.

Since the beginning of the 20th century, soil microbiology has been established as a scientific discipline that investigates microorganisms and their activities in soils (Schinner and Sonnleitner, 1996). The central objective of soil microbiology is to investigate the qualitative and quantitative distribution of soil microbes, their interactions and influence by environmental parameters, and their impact on the biochemistry of soils, as well as on material cycles and energy flows (Paul, 2015; Schinner and Sonnleitner, 1996; Tate, 2017). Microorganisms decompose and mineralise complex molecules in the soil by breaking down organic matter into inorganic compounds that serve as nutrients for other organisms (Bartelt-Ryser, 2004).

Microbial biomass is an important component of soil nutrient cycling, which in turn significantly influences ecosystem functions (Horwath, 2017).

The CFE-method (chloroform fumigation extraction) has become a common analysis to determine the microbial biomass Carbon in soil samples (Powlson et al., 2017). The method is applicable to a number of soil types (Vance et al., 1987) and is based on the principle that cell membranes of soil microorganisms (bacteria, fungi, algae and protozoa) are destroyed by chloroform fumigation. During this process high-molecular structures (polymers) are reduced by enzymatic autolysis to extractable oligomers that can be measured and represent the microbial biomass Carbon (Joergensen, 1996).

Soil microbial biomass is influenced by various factors. Among soil parameters parent material, pH value, water balance and grain size have an effect on microbial biomass (Powlson et al., 2017). In addition, environmental factors such as precipitation, temperature, exposure and landscape history are also influencing components (Horwath, 2017). Vegetation cover and history as well as animal and human activities should also be considered for the content of

microbial biomass. Solly et al. (2014) describe the influence of land use on microbial biomass in their study that shows that microbial biomass in soil is higher in grasslands than in forest ecosystems. Land use changes are also reflected in the microbial biomass, as for example the content of microbial biomass can increase through the conversion of forest to pasture (Tischer et al., 2015).

Information about the microbial activity in soils can be provided by the analysis of soil enzyme activities, as they are mostly of microbial origin (Baldrian, 2014; Wang et al., 2021). Soil enzymes are proteins with catalytic properties that enable the catalysis of several biochemical reactions, without a permanent alteration (Beck and Beck, 1996; Tabatabai, 1983). This function makes them key components in processes such as nutrient cycling, environmental quality, energy transformation and especially the decomposition of soil organic matter (Beck and Beck, 1996; Srinivasarao et al., 2017). Thus, the central function of extracellular enzymes is the breakdown of polymeric compounds and mineralisation of organic matter (Baldrian, 2014; Herold et al., 2014b). Enzymes are also involved in the formation of certain soil organic compounds, like humic matter (Schinner and Sonnleitner, 1996).

The high relevance of soil enzymes in decomposition and transformation processes was already recognised by scientists more than a century ago (Beck and Beck, 1996). Since then, researchers have been particularly concerned with the influences on enzymatic activity, e.g. through land use management, the use of agrochemicals or climate change in different ecosystems (Beck and Beck, 1996; Burns et al., 2013; Herold et al., 2014b; Srinivasarao et al., 2017; Tischer et al., 2019). The analytical procedure of enzyme analysis has been continuously developed. A frequently used technique for analysing enzymes is the fluorometric microplate enzyme assay of Marx et al. (2001). The method uses the fluorescent compound 4-methylumbelliferone (MUF) that is bonded to enzyme specific substrates. By using a microplate fluorimeter it is possible to quantify the fluorescent emission due to the release of MUF product for different enzymes over time (Marx et al., 2001). The comparison with standard concentrations allows a quantification of enzyme activities. The method provides information about microbial activity and allows drawing conclusions about nutrient turnover and soil functional diversity (Baldrian, 2014; Marx et al., 2001).

There are several enzymes involved in different nutrient cycles with varying functions (Wang et al., 2019). There is β-glucosidase (BG), an enzyme involved in Carbon cycling that hydrolyses cellobiose and water-soluble oligosaccharides to glucose (Beck and Beck, 1996; Berg and McClaugherty, 2008; Srinivasarao et al., 2017). N-acetyl-glucosaminidase (NAG) is important for the degradation and hydrolysis of chitin (fungi) and N mineralisation and therefore part of the Carbon- and N-cycling (Beck and Beck, 1996; Srinivasarao et al., 2017). Sulfatases (Sulf) are a crucial part of S-cycling and the mineralisation of sulfur-containing compounds, and catalyse the hydrolysis of organic sulfates, providing plant-available sulfur (Schinner and Sonnleitner, 1996; Srinivasarao et al., 2017; Strobl et al., 1996). Phosphatase is responsible for the hydrolysis of esters and anhydrides of phosphoric acids in P-cycling and

provides plants with phosphate (Srinivasarao et al., 2017).

The activity of these four enzymes can be influenced to varying degrees by different factors, yet they are all strongly related to the amount of organic matter, physical and biochemical soil properties and microbial biomass (Srinivasarao et al., 2017; Strobl et al., 1996). Enzymes are also regulated by environmental factors, such as temperature, humidity and solar radiation (Baldrian, 2014; Horwath, 2017).

Furthermore, there is evidence that microorganisms can react sensitively to environmental changes, e.g. land use/cover changes, which affect the catalytic properties of extracellular enzymes (Štursová and Baldrian, 2011; Tischer et al., 2015). For example, in a recent study in a semi-arid region in India, Meena and Rao (2021) found that cultivated land had lower soil C_{org} and N contents and consequently less C_{mic} and lower enzyme activities of BG and Phos than sites under forest cover. They describe the amount of soil organic matter, regulated by land use management, to be one of the most influential factors for microbial activities and functions. Furthermore, the intensity of land use and the associated changes in soil properties play a crucial role in enzyme kinetics (Tischer et al., 2019).

As described in chapter 1.2.1, different forest management practices, including canopy openness (Wang et al., 2021), have an impact on soil biogeochemical factors and microbial biodiversity and thus activity (Purahong et al., 2015). Especially the formation of small gaps in forests were found to increase the activity of several enzymes (acid phosphatase, urease, catalase and sucrose) (Wang et al., 2019), whereas clear cutting and large gaps limited the activity of β-glucosidase, L-leucineaminopeptidase and acid phosphatase in a Chinese pine forest (Yang et al., 2017). In beech forests of the Hainich-Dün region, various studies of enzyme activity have been carried out (Andersson et al., 2004; Herold et al., 2014a; Purahong et al., 2015). The results from Herold et al. (2014a) show that forest management had a rather minor impact on the activity of e.g. BG, NAG and Phos, compared to soil properties, which are significantly related to soil enzyme activity. Nevertheless, an indirect effect through the management intensity can be assumed (Herold et al., 2014a).

It should therefore be noted that the influences of e.g. different land use practices or climate change have not yet been clearly identified (Burns et al., 2013; Srinivasarao et al., 2017). Especially in forests ecosystems, information on functional microbial activity and enzymatic systems are largely lacking (Lang et al., 2016). Further research is needed in the field of forest and soil ecology.

In order to characterise microbial activity on forest sites under different treatments in the Hainich-Dün Biodiversity Exploratory and to provide more insights on functional relationships, this work is based on the analysis of microbial biomass C_{mic} and enzymatic activity of BG, NAG, Sulf and Phos in mineral soils.

1.2.4 Tea Bag Index

Decomposition is defined as the physical and chemical breakdown of dead organic matter (Chapin et al., 2011), that causes the emission of Carbon to the atmosphere (Djukic et al., 2018). Since decomposition as a soil process plays an important role in the Carbon cycle, and also provides nutrients to plants (Bartelt-Ryser, 2004; Chapin et al., 2011), the understanding of litter decomposition is highly relevant for climate research (Djukic et al., 2018).

As a way to study litter decomposition, a litter bag method has been used in many studies, in which bags of leaf litter are prepared and exposed in the habitat (Bärlocher, 2005). Litter bag approaches are based on the mass loss of plant material, e.g. leaves and needles, as a function of time (Bärlocher, 2005; Berg and McClaugherty, 2008). In addition to litter degradation, litter bag studies can also be used to investigate enzyme activity in the litter, as shown by a study of Andersson et al. (2004) in the Hainich.

As a further development of the litter bag approach, the cost-efficient and simple Tea Bag Index (TBI) was established by the Dutch ecologist Joost Keuskamp within the scope of a research project that started in 2010 (teatime4science, 2016). The TBI is an internationally standardised method to collect comparable, globally distributed data on decomposition rate (k) and litter stabilisation (S) (Keuskamp et al., 2013). The data collected worldwide provides information on soil health and enables the comparison of environmental influences on Carbon cycling. Thereby climate models can be improved that contribute to climate change research (teatime4science, 2016). The TBI method uses standardised litter substrates of green and rooibos tea having different decay times (Keuskamp et al., 2013). The relationship of k in the soil to S that depends on environmental conditions during the conversion of chemically labile compounds into persistent ones is investigated by the method (Dossou-Yovo et al., 2021). In early 2017, more than 2000 sites on all continents except Antarctica had already been investigated (teatime4science, 2016). This method was already used in different studies focussing on the investigation of, for example, the effect of pesticide seed dressings and herbicides on soil organisms and decomposition (van Hoesel et al., 2017), the assessment of Carbon decomposition rates in cranberry agroecosystems (Dossou-Yovo et al., 2021) or the effects of adding multiple nutrients on decomposition in grassland sites (Ochoa-Hueso et al., 2020).

The results of numerous studies have shown that the TBI is controlled by various factors. Generally, there are environmental, e.g. humidity and temperature and chemical properties affecting decomposition rates and k also depends on the presence of soil (micro-)organisms (teatime4science, 2016). Especially the quality of the litter controls k, while land-use was found to be less critical (Djukic et al., 2018). This is confirmed by a study of Tóth et al. (2018), which shows that pH and nutrient content have a stronger impact on k than agricultural management. Also, k is critically affected by water content (Mori et al., 2021).

The TBI method can be performed on different experimental treatments (teatime4science, 2016) to determine differences in degradation behaviour and other dependencies. In this work, the investigation of the TBI should help to better understand decomposition processes in

relation to microbial activity in European beech forests under different management practices. In addition, the decomposition data will help the scientific TBI network to create a global soil map that can be used to adjust climate models.

1.3 Objectives

The overall objectives underlying this master's thesis are to:

i) determine the effect of forest gaps on soil properties, microbial biomass, enzymatic activity, and tea bag decomposition in mineral soils under different treatments (D, G, GD)
ii) quantify tea bag decomposition indices in leaf litter and mineral soils
iii) analyse the relationships between abiotic soil properties, microbial activity and decomposition indices

The general assumptions of this thesis are that: (1) the formation of forest gaps would lead to enhanced microbial activity and decomposition indices in forest gaps compared to non-gaps; (2) increased microbial biomass content and enzymatic activity can be linked to high tea bag decomposition indices; and (3) changes in microbial activity and decomposition are related to changes in physico-chemical soil properties.

2 Material and methods

2.1 Study area

2.1.1 Hainich-Dün Biodiversity Exploratory

The Hainich-Dün study region with a total area of about 1,300 km^2 is located in the north-west of Thuringia in the centre of Germany and includes the forest region "Hainich" in the south, the "Dün" region in the north and the "Obere Eichsfeld" in between (BEO, 2021b). With an elevation of 285-550 m a.s.l. (Fischer et al., 2010), the Hainich is an extensive, forested ridge bordering the Thuringian Basin and surrounding hilly farmland (BEO, 2021b). The average annual temperature varies between 6.5 and 8 °C and precipitation is around 500 to 800 mm per year, indicating a cool mid-latitude climate with low precipitation (Fischer et al., 2010).
The parent rock in the Hainich-Dün region is predominantly Triassic limestone, which is overlaid with loess in large areas (Fischer et al., 2010). The predominant soil types in the Hainich include Luvisols and Stagnosols and on shell limestone sites, Leptosols occur on steep slopes (BEO, 2021b). The soil pH is weakly acidic (5.1 ± 1.1; mean ± SD) and the litter layers have a thickness of 2–5 cm (Purahong et al., 2015), and soil texture is clay loam to loamy clay (Wäldchen, 2010).
The Hainich comprises Germany's largest coherent deciduous forest area with 16,000 ha (BEO, 2021b). In addition to the European beech (*Fagus sylvatica*) as the dominant tree species, other deciduous tree species such as maple (*Acer*), ash (*Fraxinus excelsior*) and lime (*Tilia*) occur (Schall et al., 2018). The proportion of coniferous trees in the national park accounts for only about 3 % of the total area (Hainich Nationalpark, 2021). The deciduous forest stands in Germany, which are comparatively rich in species and structure, are characterised by their high proportion of deadwood (BEO, 2021b). Already in 1997, part of the hardwood forest area was given national park status until it was designated a World Heritage Site in 2011 (Hainich Nationalpark, 2021).

In terms of forest management, a variety of management practices of European beech forests can be found in the Hainich area (Fischer et al., 2010). A typical form of regional management is the selection-cutting forest, the so-called "Plenterwald" with an uneven-age forest structure organised in forest cooperatives (BEO, 2021b; Purahong et al., 2015). Selection-cutting forests are managed in single trees to small groups for about 140 years in the Hainich and up to 250 years in the Dün area (Heinrichs et al., 2020). Secondly, there are age-class forests, known as "Bauernwald", which were formerly used by farmers and are characterised by a variety of different tree types (BEO, 2021b). These forests have been managed for about two centuries with rotation periods of ca. 140 years by practicing shelterwood felling techniques (Heinrichs et al., 2020). The age-class forest has an even-age forest structure and is described as a "semi-natural forest with natural regeneration, [whereas selection-cutting forests are considered] close-to-nature forest management [types]" (Purahong et al., 2015). The third management type

is the unmanaged deciduous forest, which has an uneven-age forest structure and comprises the Hainich national park as a unique habitat for rare species (Hainich Nationalpark, 2021; Purahong et al., 2015). The three categories are referred to as management systems because they represent different concepts of forest management in the study region (Heinrichs et al., 2020).

The Hainich-Dün is one of the three Biodiversity Exploratories, along with the Schorfheide-Chorin and Schwäbische Alb (Figure 1). Since 2016, different ecological parameters, e.g. soil temperature, diversity of plants and soil (micro-) organisms or biomass, as well as ecological processes and relationships, are investigated and monitored on 50 experimental plots (EP) in each forest and grassland sites (BEO, 2021b). The central aim of the investigation of the functional long-term biodiversity research is to identify the impact of land-use differing in intensity on different organisms, their interactions and to detect how different components of biodiversity influence ecosystem processes and ecosystem services (BEO, 2021a; Fischer et al., 2010).

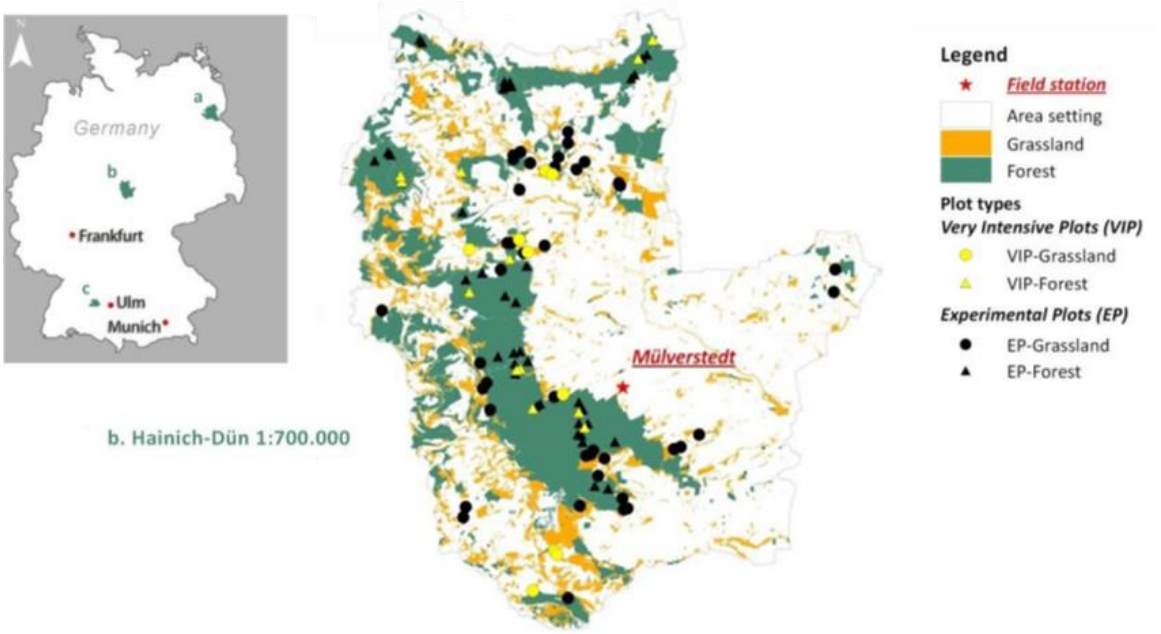

Figure 1 Left: Map of Germany showing the three Biodiversity Exploratories a) Schorfheide-Chorin, b) Hainich-Dün and c) Schwäbische Alb. Middle: Overview of the study area with grassland and forest sites and the location of the plots in the Hainich-Dün Biodiverstiy-Exploratory (modified after BEO (2021b))

2.1.2 Forest gap experiment

Within the framework of core project 6 of the functional biodiversity research, a new "FOrest gap eXperiment" (FOX) was established for a research period from 2020 to 2023 (BEO, 2021c). The central aim of the experiment is to identify how canopy openings and deadwood and their interaction impact biodiversity and ecosystem services in forests of different management types (Ammer et al., 2020). Therefor several forest gaps were created by felling trees in all three Biodiversity Exploratories in early spring 2020. The experimental design of the FOX is based

on four different treatments, shown in Figure 2. Besides the regular EP serving as control plot (C), there are three treatments: a gap in the forest canopy without deadwood (G), a gap with deadwood (GD) and no gap with deadwood enrichment (D) (Ammer et al., 2020). The G and GD treatments have a diameter of approx. 30 m depending on the mean top stand height (Ammer et al., 2020). On sites with deadwood enrichment pieces of felled trees (4-5 m) were placed evenly distributed. On these different treatments researchers of the Biodiversity Exploratories apply various investigation methods, e.g. recording and sampling deadwood logs by drilling, monitoring the development of roots and tree regeneration and observing the canopy closure by laser scanning (BEO, 2021c).

Table 1 Forest management and soil type of the experimental plots (C) (Fischer et al., 2010; Ostrowski et al., 2016).

Plot ID	Forest management type	Soil type (WRB)
HEW05	Age-class forest	Luvisol
HEW06	Age-class forest	Luvisol
HEW19	Age-class forest	Luvisol
HEW21	Age-class forest	Luvisol
HEW29	Selection-cutting forest	Luvisol
HEW30	Selection-cutting forest	Luvisol
HEW32	Selection-cutting forest	Luvisol
HEW47	Age-class forest	Stagnosol
HEW48	Selection-cutting forest	Stagnosol

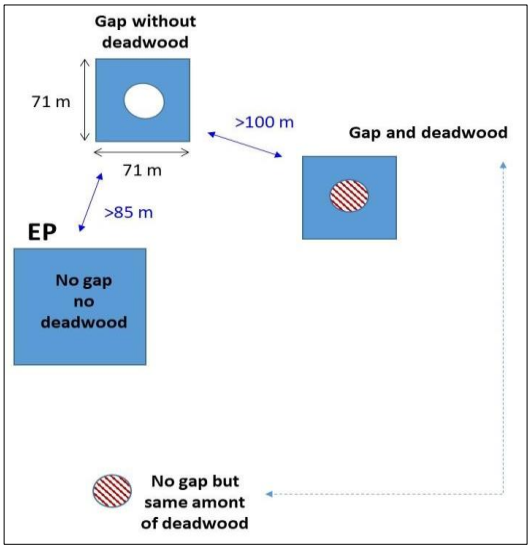

Figure 2 Scheme of experimental design with treatments G, GD, D and control plot EP (Ammer et al., 2020).

In the Hainich-Dün Biodiversity Exploratory nine forest sites, shown in Table 1, were selected to conduct the multi-site full-factorial experiment. The sites are characterised by different forest management practices and soil types. There are five age-class forest sites and four selection-cutting sites. According to World Reference Base for Soil Resources Luvisols and Stagnosols were identified on the experimental plots (Ostrowski et al., 2016). Similar characteristics can be assumed for treatments G, GD and D, as they are located in the immediate vicinity of the EP. Detailed maps of the locations of the EP and associated treatments can be found in the appendix (App. Figure I). The following four photos show the control plot and treatments with canopy openings and deadwood enrichment (Figure 3). The photographs were taken in August 2021.

Figure 3 Photographs of different treatments a) control plot (C), b) gap treatment (G), c) gap treatment with deadwood (GD) and d) deadwood treatment (D) (Enyedi, 2021).

2.2 Soil sampling

Within the scope of the soil sampling campaign in May 2021, in total 50 forest and grassland experimental plots were sampled in the Hainich-Dün Biodiversity Exploratory.

On each forest EP, 14 locations were sampled using a split tube auger (5 cm diameter, 40 cm length) along two 40 m transects (Solly et al., 2014). Prior to the retrieval of the mineral soil, litter and the three organic layers (Oi, Oe, Oa) were removed at each site. The uppermost 10 cm of the mineral soil samples were mixed to obtain a composite sample for each EP. Figure 4 shows a split tube auger with sampled soil.

In addition to the EPs, the three treatments (G, GD, D) of each plot of the FOX were sampled. Mineral soil samples were taken at 4 soil sub-plots (2 x 2 m) which are located in the geographical directions (N-E-S-W) from the plot centre. Plot-charts provided by the BExIS database were used to determine the location of the 4 soil sub-plots. As shown in Figure 5, in the 1 x 1 m south-eastern quarter (orange squares) of each soil sub-plot (green squares), two mineral soil samples (purple squares) were taken with 50 cm between the sampling points. Afterwards composite samples of the uppermost 10 cm of the two mineral soil samples were prepared for each sub-plot.

All samples of the forest EPs and FOX-plots were taken under similar weather conditions. The mineral soil samples were stored in cool boxes and transported to the field lab facility. Composite samples were homogenised and sieved to <4 mm and roots and stones were removed in the field lab. Due to a large number of samples taken at different locations, the soil samples were frozen to – 20 °C to ensure better comparability of the results of the subsequent enzyme and microbial biomass analysis (Herold et al., 2014b; Solly et al., 2014).

Figure 4 Split tube auger with soil sample (Schöning, 2021).

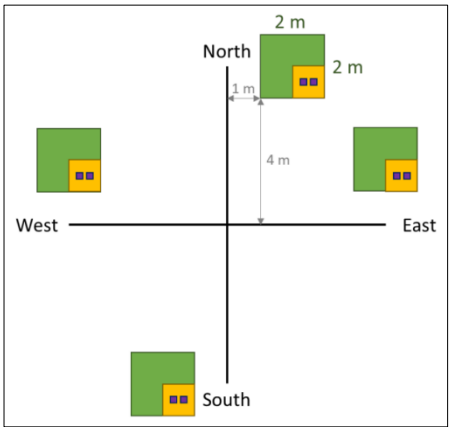

Figure 5 Sampling design for FOX-plots (protocol provided by Schöning, 2021).

2.3 Soil preparation

The mineral soil samples were prepared and analysed at the Max Planck Institute for Biogeochemistry in Jena. For better feasibility of analyses and to ensure a robust comparability of soil data from samples of the FOX-treatments and composite samples of the EPs, composite samples of the FOX-treatments were obtained as well. Therefore 20 g of each soil sample of the sub-plots (N-E-S-W) were thawed, mixed, and homogenised thoroughly. All soil samples were sieved to <2 mm and stored at 2 °C. A total number of 36 mineral soil samples were analysed, of which 9 samples were from the control plots (C) and 27 from the FOX treatments (G, GD, D). Each sample was divided into sub-samples for different analyses.

2.4 pH value

Soil pH analysis was performed according to Herold et al. (2014b). Therefor 10 g of each airdried (see chapter 2.5 for drying) soil sample were weighed into FALCON tubes and 25 ml of 0.01M $CaCl_2$ were added. The samples were shaken for 120 min using a overhead shaker (GFL 3040, Gesellschaft für Labortechnik mbH, Burgwedel, Germany). Soil pH was measured in the supernatant of a 1:2.5 mixture of soil and 0.01 M $CaCl_2$ using a pH-Meter with a glass electrode (pH 538, WTW, Xylem Analytics Germany Sales GmbH & Co. KG, Germany).

2.5 Water content

The gravimetric water content of the soil samples was determined after Lambe and Whitman (2009). Field moist soil samples were dried for at least 48 h at 40 °C in a drying chamber. The water content (w) is then determined by subtracting the dry mass (m_d) of the soil sample from the wet mass (m_w) and relating it to m_d. The water content is given in % and was later corrected for residual water content.

$$w = \frac{m_w - m_d}{m_d}$$

To determine the residual water content of the samples that were dried at 40 °C, ca. 2-3 g of each soil sample were weighed into beakers and dried again in a drying oven for at least 48 h at 105 °C. The residual water content is calculated following the same equation as above, using the initial weight and the output weight after drying and given in % of the weight of the oven-dried soil samples.

2.6 Estimated clay content

The estimated clay content was calculated after Wäldchen et al. (2012). Therefore, the residual water content of each soil sample was inserted into the following equation. The estimated clay contents are given in g kg^{-1}.

$$estimated\ clay\ content\ \left(\frac{g}{kg}\right) = 13{,}2 \times (residual\ water\ content \times 10) + 15{,}7$$

2.7 CNS analysis

Soil subsamples were ground in a ball mill for 3 min at a vibrational frequency of 30 Hz (RETSCH MM200, Retsch, Haan, Germany). Total C, N and S concentrations were determined by dry combustion with an elemental analyser (VarioMAX cube, Elementar Analysensysteme GmbH, Hanau, Germany).

To obtain the proportion of organic carbon (C_{org}), the total amount of inorganic carbon was determined first. Then 250 mg of the subsamples were muffled at 450 °C for 16 h (Nabertherm L9/11/B180, Thermo Fisher Scientific Inc., Schwerte, Germany) and thus the organic carbon was removed. C_{org} concentrations were calculated as the difference between total C and C_{inorg}. Furthermore, C, N, and S concentrations were corrected for residual water content. The C/N ratio was obtained by dividing C_{org} content by N content.

2.8 Microbial biomass

The microbial biomass was determined by chloroform fumigation extraction (CFE) according to (Joergensen, 1996). The method was carried out on all 36 mineral soil samples with one replicate for each sample and two blank sample per run for better accuracy. Briefly, the samples were divided into three sub-samples and 5 g were weighed into a beaker for the DMC determination, 6 g into Falcon tubes for the extraction of the non-fumigated sample and 6 g into another beaker for the fumigation with chloroform. To determine the DMC, the sub-samples were dried at 105 °C for at least 24 h and kept in a desiccator for cooling. The dry samples were weighed to calculate the DMC in relation to the initial sample weight. For the extraction of the non-fumigated samples 30 ml of 0.05M potassium sulfate K_2SO_4 (1:5) were added to the centrifuge tubes. The samples were shaken for 30 min horizontally with 130 U/min (LABOSHAKE heavy-load shaker, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) and centrifuged directly afterwards for 5 min at 3500 U/min (Megafuge 3.0R, Heraeus Holding GmbH, Hanau, Germany). Each sample was filtrated through funnels with Whatman N°1 filter paper which were previously with 60 ml of 0.05M K_2SO_4.

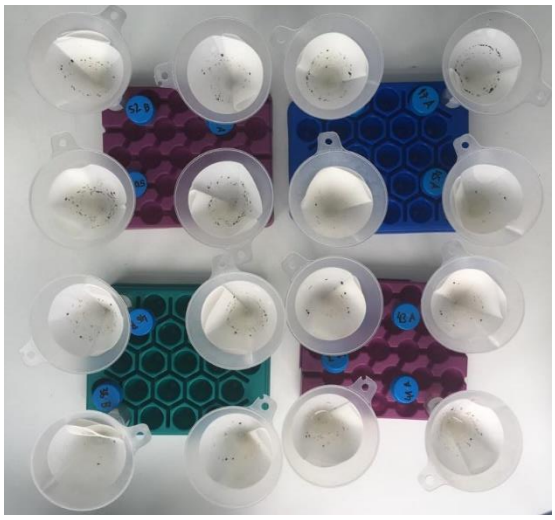

Figure 6 Filtration of unfumigated samples (Enyedi, 2021).

Figure 7 Desiccator with fumigated samples (Kuhlmann, 2021).

For fumigation, the samples were placed in a desiccator with 150 ml of ethanol free chloroform and boiling stones in an Erlenmeyer flask. Then the desiccator was connected to a vacuum pump and the vacuum was gradually increased until the chloroform began to boil. After boiling for 1 min, the desiccator was ventilated until the pressure was equalised. The procedure was repeated twice, but the boiling lasted 3 min at the last run. Without further ventilation, the samples were fumigated in the desiccator covered with dark foil for exactly 24 h (Meena and Rao, 2021).

After fumigation the desiccator was ventilated and the Erlenmeyer flask remoted. The desiccator was connected to the vacuum pump, vacuumed and ventilated 5 times, evacuated for 3 min starting at 200 mbar in order to evaporate the chloroform completely. After the last ventilation the samples were removed from the desiccator and treated with 0.05 M K_2SO, shaken, centrifuged and filtrated according to the extraction procedure described above.

All non-fumigated and fumigated extracts and blanks were deep-frozen to - 20 °C until analysis in the laboratory for Chemical Routine Measurements and Analysis (RoMA) at the Max Planck Institute for Biogeochemistry.

Total organic carbon (TOC) was measured three times in each supernatant using a sum parameter vario TOC cube (Elementar Analysensysteme GmbH, Hanau, Germany) and expressed in mg/L. The TOC values of the samples were corrected by subtracting the TOC content of the blank samples. The mean TOC content of the blanks was 1,32 mg/L.

To calculate the C_{org} content of the fumigated (C_f) and non-fumigated (C_{nf}) CFE samples, the dry matter content was multiplied by the weight of the CFE samples. Subsequently, C_f and C_{nf} are calculated in mg/g dry soil by relating the mean TOC value to the extraction volume (30 ml) and the dry weight of the CFE sample (Joergensen, 1996):

$$C\left(\frac{mg}{g}\right) = mean\ TOC\left(\frac{mg}{L}\right) \times \frac{extraction\ volume\ (ml)}{dry\ weight\ sample\ (g)} \times \frac{1}{1000}$$

The difference between Carbon content in the fumigated and non-fumigated samples is the chloroform-solvable Carbon content (EC) that is proportional to microbial biomass Carbon (C_{mic}) in the samples. A soil-specific conversion k_{ec}-factor of 0,45 was used to estimate the total amount of microbial biomass C_{mic}, as this is the extractable part of Carbon in microbial biomass (Joergensen, 1996):

$$C_{mic}\left(\frac{mg}{g}\right) = \frac{C_f\ -\ C_{nf}}{k_{ec}} = \frac{EC}{k_{ec}}$$

Total bound Nitrogen (TN_b) was measured in the same extracts as for C_{mic} using a sum parameter TN-100 (al envirotech, Düsseldorf, Germany). TN_b contents were expressed as ppm or mg/L respectively. Since the TN_b values of the blank sample were all below the limit of quantification of <0.379, no further correction of the TN_b contents was necessary. The biomass

N content of the fumigated and non-fumigated samples could be calculated similar to the formula of organic Carbon (Powlson et al., 2017):

$$N\left(\frac{mg}{g}\right) = mean\ TN_b\left(\frac{mg}{L}\right) \times \frac{extraction\ volume\ (ml)}{dry\ weight\ sample\ (g)} \times \frac{1}{1000}$$

N_{mic} was determined by subtracting the biomass N content of the non-fumigated from the biomass N content of the fumigated samples:

$$N_{mic}\left(\frac{mg}{g}\right) = N_f - N_{nf}$$

2.9 Enzyme analysis

As described in chapter 2.2 and 2.3 mineral soil samples were frozen to – 20 °C, thawed, sieved and stored overnight at 2 °C prior to enzyme activity analysis. Enzyme analysis was carried out within 3 weeks after sampling. To determine the microbial enzyme activity in the soil samples, the activity of β-glucosidase, N-acetyl-glucosaminidase, sulfatase and phosphatase was measured using the Multi-substrate-assay according to Marx et al. (2001). The method uses the fluorescent compound 4-methylumbelliferone (MUF) which is coupled to enzyme specific substrates.

In short, for each sample 1 g field-moist soil was dispersed in 50 ml sterile autoclaved double distilled water using an ultrasonic disaggregator with a low energy input (1:33 min at 35%) (450-D Digital Sonifier, BRANSON Ultrasonics Corporation, Danbury, U.S.A). The soil suspension was continuously stirred while an aliquot of 50 µl was transferred via multichannel pipette into rows of twelve wells on black PP-microtiter plates. Since it is likely that the phosphatase activity of some samples is too high for detection in the subsequent measurement, the soil solution for this enzyme was diluted 1:2 after all other positions were pipetted. Figure 8 shows the instruments of the preparation and the pipettor.

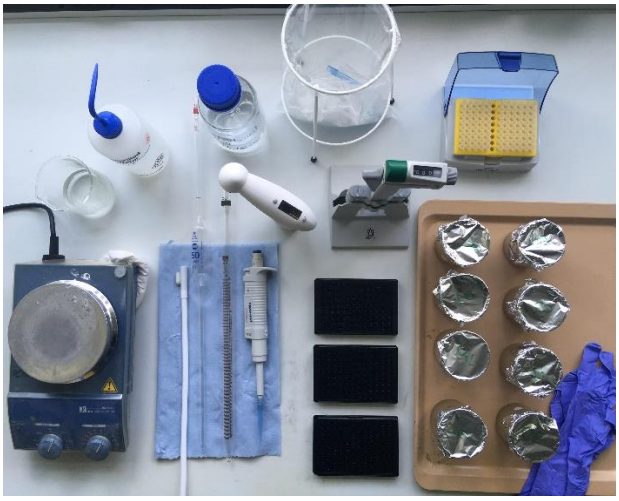

Figure 8 Instruments and equipment for enzyme analysis (Enyedi, 2021).

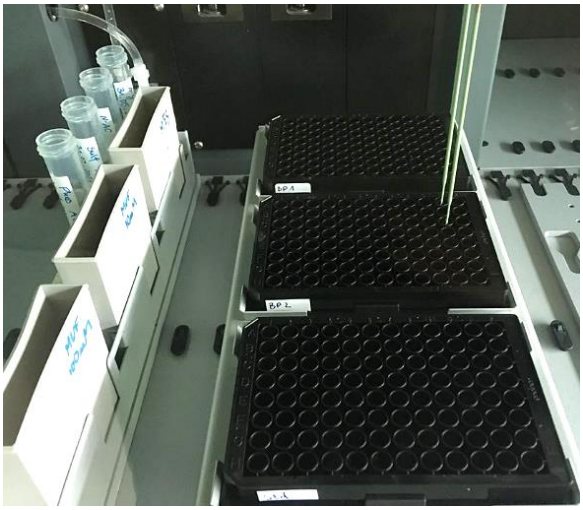

Figure 9 Pipetting substrate solutions for BG, NAG, Sulf and Phos into microtiter plates with TECAN freedom evo automated pipettor (Enyedi, 2021).

Then 50 µl of autoclaved 0,1 M MES-buffer and 100 µl of 1 mM substrate solution containing the fluorescent compounds 4-methylumbelliferone (4-MUF-β-D-glucoside for BG, 4-MUF-N-Acetyl-β-D-glucosaminide for NAG, 4-MUF-sulfate for Sulf and 4-MUF-phosphate for Phos) were pipetted to the enzyme plates via automated pipettor in each respective well (TECAN freedom evo, Tecan Group Ltd., Männedorf, Switzerland) (Figure 9). The MUF standard plate was pipetted with different concentrations of 10 µM and 100 µM MUF-standard (0, 10, 20, 50, 80, 120 µl) and MES-buffer solution (150, 140, 130, 100, 70, 30 µl).
The microtiter plates were covered and incubated in the dark at 30°C while they were shaken on a microplate shaker at 300 rpm. In order to ensure a linear increase in microbial activity, the microtiter plates were pre-incubated for 30 min prior to the first measurement. The fluorescence is measured after 0, 30, 60, 120 and 180 min with 360 nm excitation and 460 nm emissions using a microplate reader (Infinite 200, Tecan Group Ltd., Crailsheim, Germany).

To determine the enzymatic activity, the fluorescence was calculated first into pmol*well^{-1}*min^{-1} by dividing the mean slope of the soil samples by mean slope of the MUF standard:

$$fluorescence\left(\frac{pmol}{well * min}\right) = \frac{Slope\_soilsample}{Slope\_std.-curve}$$

Subsequently, potential enzyme activities expressed as nmol g^{-1} dw h^{-1} are calculated according to Herold et al. (2014b) by including the fluorescence in pmol well^{-1} min^{-1} (x), a conversion to h^{-1} (60), a dilution factor *d* (1 for β-Glu, N-Ac, Sulf; 2 for Phos), a conversion from pmol to nmol and relating to the dry matter content DMC of the initial weight IW of the soil sample:

$$fluorescence\left(\frac{nmol}{g\ dw * h}\right) = \frac{x \times 60 \times d \times 100}{IW \times DMC}$$

Specific enzyme activities were determined by dividing total enzyme activities by microbial biomass C_{mic} and expressed as nmol µg^{-1} C_{mic} h^{-1}.

2.10 Tea Bag Index

For the preparation and realisation of the tea bag experiment the protocol of Keuskamp et al. (2013) was applied. Therefor the initial weight of 576 nonwoven polypropylene tea bags of each Lipton green tea (Unilever, EAN: 8714100770542) and Lipton rooibos tea (Unilever, EAN: 8722700188438) was measured at the Max Planck Institute of Biogeochemistry in Jena. The labels of the tea bags were marked with a permanent marker with numbers indicating the subsequent burial location. For a better assessment 10 empty tea bags and tea bag labels were weighed to be able to subtract the corresponding weight later. The tea bags were stored in zip-lock bags until the burial.

A total number of 1152 tea bags were installed during the soil sampling campaign in May 2021 in the Hanich-Dün biodiversity exploratory. In detail, 32 tea bags were buried on each of the nine experimental plots and the corresponding treatments of the FOX plots in two depth layers

(litter and soil) with one replicate (576 tea bags per tea type).

On the C plots the installation sites were at 3 and 39 m of the two transects. The green tea bags were always placed 25 cm north and the rooibos tea bags 25 cm south of the soil sampling point, while leaving a distance of 50 cm between the replicates (A and B). Tea bags with an even number on the label were buried in the mineral soil to a depth of about 5 cm (Figure 10) and those with an odd number were placed between leaves in the litter layer while the labels were left visible above the ground. On the FOX plots the tea bags were buried in the south-eastern quarter of each soil sub-plot in the geographical directions N-E-S-W, 25 cm north and south of the soil sampling location. Figure 11 shows an example of placing the tea bags at the northern sub-plot of HEW48-C using a folding rule, an ice scoop, and a compass. A blue bamboo stick was placed between the tea bags to mark the burial site.

Figure 10 Burial of a rooibos tea bag in the mineral soil (Enyedi, 2021).

Figure 11 Placement of eight tea bags on HEW48-C (Enyedi, 2021).

The tea bags were retrieved after ca. 90 days (Figure 12) and stored separately in zip-lock bags. Out of 1152 tea bags, 1039 were found, of which 40 tea bags had holes or were broken. Some tea bags had strong ingrowth of roots and were excluded from weight measurement (Figure 13). There was a sample loss of about 13.28 %. 96 tea bags were lost in the litter, and 17 in the mineral soil The field-moist tea bags were stored at 4 °C. After the removal of adhered soil particles, roots and remaining labels the moist tea bags were weighed and dried for at least 48 h at 60 °C in a drying chamber (Keuskamp et al., 2013), as shown in Figure 14. The dried tea bags were weighed again to determine the water content by the weight difference between the wet and dry tea bags.

Figure 12 Field-fresh tea bags retrieved in August 2021 (Enyedi, 2021).

Figure 13 Root in-growth in tea bag (Enyedi, 2021).

Figure 14 Tea bags in a drying chamber at 60 °C (Enyedi, 2021).

To determine the decomposition indices, mass loss in g and % was calculated first. the weights were subtracted in the process. Thereby, the weights of label, cord and empty tea bag were subtracted from initial and final tea bag weights. The difference between initial and final weights thus represents the mass loss of tea material. Water contents of the tea bags and decomposition measures were aggregated for the four replicates per treatment plot.

The calculation of the stabilisation factor (*S*) and decomposition rate (*k*) was carried out according to Keuskamp et al. (2013). The two tea types differ in decomposition rates, because rooibos tea decomposes slower in the first decomposition phase, while labile material in green tea has already been completely consumed. That is why hydrolysable fractions must be taken into account in the calculation. Fractions are 0.842 g g^{-1} for green tea (H_g) and 0.552 g g^{-1} for rooibos tea (H_r).

The fraction decomposed of green tea (a_g) results from the initial and final weights:

$$a_g = 1 - \left(\frac{final\ weight_{green}}{initial\ weight_{green}} \right)$$

As parts of labile compounds of organic material stabilise during decomposition (Prescott, 2010), the stabilisation factor (*S*) can be used to describe the degree to which litter decomposed. *S* is calculated considering the hydrolysable fraction for green tea:

$$S = 1 - \left(\frac{a_g}{H_g} \right)$$

Subsequently, the predicted labile fraction of rooibos tea (a_r) was estimated:

$$a_r = H_r * (1 - S)$$

Similar to a_g, the weight difference of initial and final weights of rooibos tea is calculated as the remaining fraction (Wt):

$$Wt = \left(\frac{final\ weight_{red}}{initial\ weight_{red}} \right)$$

Finally, the decomposition rate (*k*) of the labile fraction of the tea substrate is obtained by a logarithmic function with known Wt and a_r and by referring to the incubation time in days (t):

$$k = \ln(\, a_r \,/\, (Wt \,-\, (\,1 \,-\, a_r\,)))\,/\,t$$

2.11 Statistical analysis

To obtain data on forest management types and soil types on the study sites, the project's internal database BExIS was used. Data set 1000 contains general information and coordinates of field plots of the Biodiversity Exploratories project (Ostrowski et al., 2016). All statistical analyses and visualisation of diagrams and graphs were conducted with RStudio, version 4.0.4 (R Core Team, 2021). Mineral soil and tea bag parameters were examined descriptively by means and standard error using the *psych* package. Linearity, homogeneity and normality of the variances of the residuals were visually assessed with Quantile-Quantile-Plots (Q-Q-Plots) (Dormann and Kühn, 2009). The following soil variables were log-transformed to meet the assumptions of ANOVA: C_{org}, N, S, C_{mic}, N_{mic}. General relationships between soil properties and enzyme activities were investigated by fitting linear models. To assess a correlation of individual soil parameters and avoid collinearity in regression models, *Pearson* correlation coefficients and pairwise partial correlations for each pair of variables were calculated using the *ppcor* package (Kim, 2015). T-Statistic and p-values ($p < 0,05$) of several variables showed significant relationships between soil parameters. Additionally, the variance inflation factor (VIF) of the *mctest* package was used to detect multicollinearity (Imdadullah et al., 2016). Multicollinearity was detected due to VIF values >10 for C_{org}, N, S, C_{mic}, N_{mic}. Collinear and redundant variables were excluded by stepwise removal until VIF values were <5 and VIF diagnostics failed to detect multicollinearity in the model. Due to strong correlation of C_{org} with many other soil parameters, only C_{org} and pH were selected as soil parameters for subsequent modelling. In the evaluation of the models, it is considered that C_{org} values also reflect N, S, C_{mic}, N_{mic}, clay and water content. General linear models (ANCOVA) were used to assess the impact of C_{org}, pH, Treatment and Plot ID on enzyme activities, and the influence of water content, C_{mic}, Treatment and Plot ID on decomposition indices and to test significance interactions. The Plot ID was included in the models, because it reflects site-related information between the nine individual HEW sites.

For the calculation of *S* and *k*, a data sheet for non-woven bags was used as a template (teatime4science, 2016), but the calculation itself was carried out in *RStudio*. The data was examined for outliers, which were removed from the data set if evidence was present in remarks (e.g. hole in tea bag). The tea bag indices were aggregated by using mean values for the replicates and sub-plots. Also, for the tea bag indices, the conditions of the ANOVA were checked, outliers removed and linear models used to examine the relationships between the parameters. The visualisation of functional relationships is represented by the regression line and the corresponding regression equation using the *ggplot* package.

3 Results

3.1 Soil properties

Results of measurements of abiotic soil properties of control plots and different treatments are shown in Table 2. A detailed overview of soil data can be found in the appendix (App. Table I). Soil properties are described in more detail in the following sections.

Table 2 Means (n = 9) and standard errors of soil properties in control plots (C), deadwood (D), gap (G) and gap enriched with deadwood (GD) treatments.

	C	D	G	GD
pH	4.29 ± 0.14	4.54 ± 0.19	4.21 ± 0.14	4.37 ± 0.21
Water content (%)	41.18 ± 1.76	44.76 ± 2.72	49.88 ± 44.2	49.41 ± 3.08
Estimated clay content (g kg^{-1})	225.87 ± 29.71	260.49 ± 38.77	270.75 ± 75.34	245.97 ± 61.94
C_{org} (g kg^{-1})	36.51 ± 3.12	41.23 ± 5.65	45.37 ± 9.12	42.22 ± 6.97
N (g kg^{-1})	2.76 ± 0.22	3.26 ± 0.48	3.18 ± 0.51	3.27 ± 0.62
S (g kg^{-1})	0.34 ± 0.03	0.39 ± 0.07	0.38 ± 0.06	0.40 ± 0.06
C/N ratio	13.18 ± 0.27	12.86 ± 0.40	14.25 ± 0.85	13.32 ± 0.36

The overall pH value of the mineral soil samples was between 3.75 and 5.67. Average pH value was higher on the D treatment compared to C, G, and GD. Figure 15 shows that single treatments have noticeably high pH values. According to Ad-hoc-AG Boden (2005) the investigated soil can be classified into buffer areas. With a pH value of above 5.0, the sites HEW05-C, HEW21-D, HEW32-D and GD are in the weakly acidic silicate buffer range. The majority of soils are in the moderately acidic exchange buffer range with a pH between 4.2 and 5.0. Soils of HEW6-GD, HEW21-C, all treatments of HEW29, HEW32-C and G, HEW47-G and all treatments of HEW48 had a pH value between 3.0 and 4.2 and are categorised as highly acidic belonging to the aluminium buffer range.

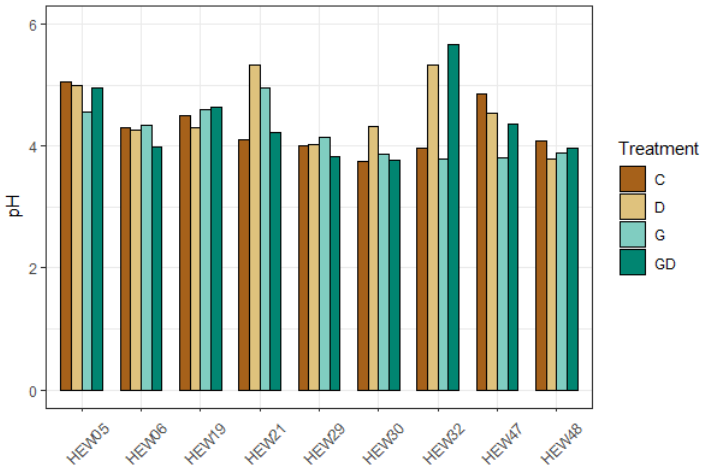

Figure 15 pH values for mineral soil samples on control plots (C), deadwood (D), gap (G) and gap with deadwood (GD) treatments.

The water content of the soil samples ranged from 33.61 % to 70.81 %. On average, the water content of soils on the gap sites was higher than on the C and D sites (Table 2). Figure 16 shows

that especially the G treatments on HEW5 and HEW6 had a high moisture content. It should be noted that the water content of soils is strongly influenced by the weather conditions at the time of sampling and these values are only a temporal measure.

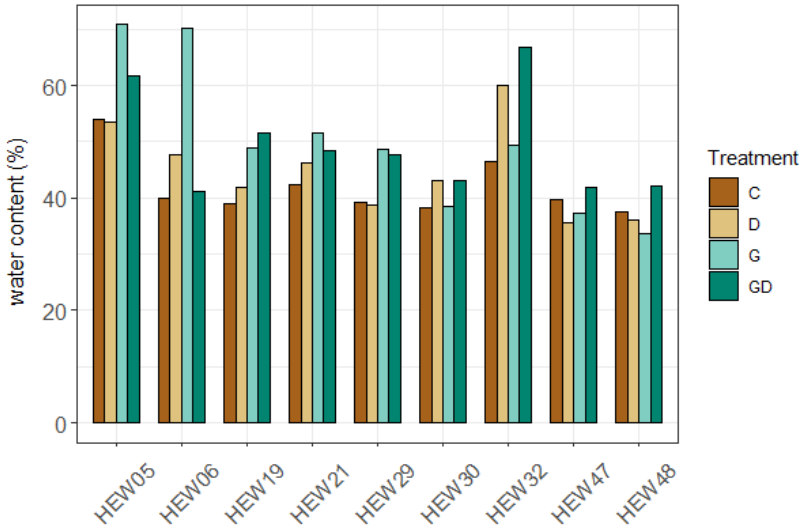

Figure 16 Water contents (%) of mineral soil samples on control plots (C) and treatments (D, G, GD).

Figure 17 presents the estimated clay content calculated according to Wäldchen et al. (2012). Estimated clay content ranged between 15.7 g kg^{-1} and 691.54 g kg^{-1}. Highest values were reached on the G treatment of HEW06 and lowest values on G and GD of HEW48.

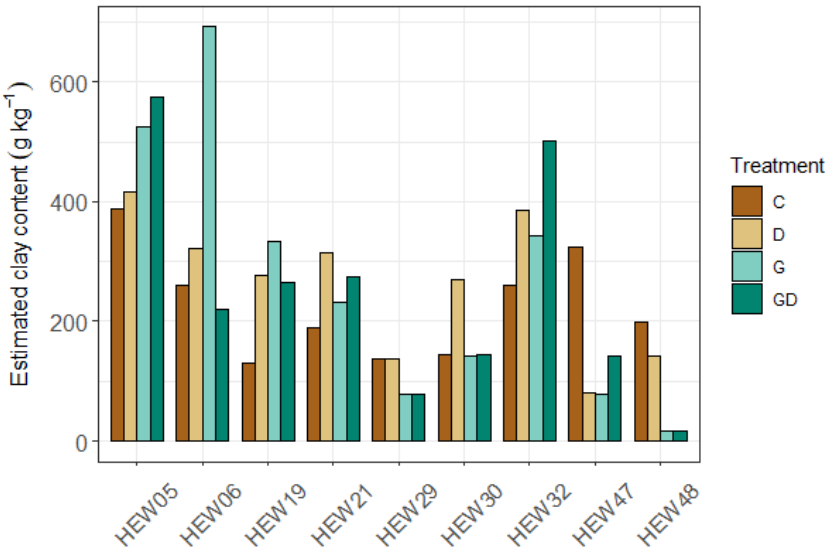

Figure 17 Estimated clay content (g kg^{-1}) of mineral soil samples on control plots (C) and treatments (D, G, GD).

The concentration of the C_{org}, N and S for each treatment of each plot are presented in Figure 18. The overall C_{org} concentrations varied between 23.2 g kg^{-1} and 107.6 g kg^{-1}. On average, there was a higher C_{org} content on G and GD. However, especially G treatments had a high standard error (Table 2). The G of HEW06 records the highest C_{org} concentration. All treatments of HEW05 and the D and GD treatments on HEW32 also showed comparatively high C_{org} contents. The N concentration of the samples ranged from 1.3 g kg^{-1} to 7.4 g kg^{-1}. S contents

varied between 0.2 g kg^{-1} and 0.8 g kg^{-1}. The mean N and S concentration was slightly higher on the treatments than on the control plots (Table 2). N and S concentrations on all treatments of HEW05, G on HEW06 and the D and GD treatments on HEW32 are noticeable with comparatively high contents (Figure 18). Thus, all three elements show a similar pattern.

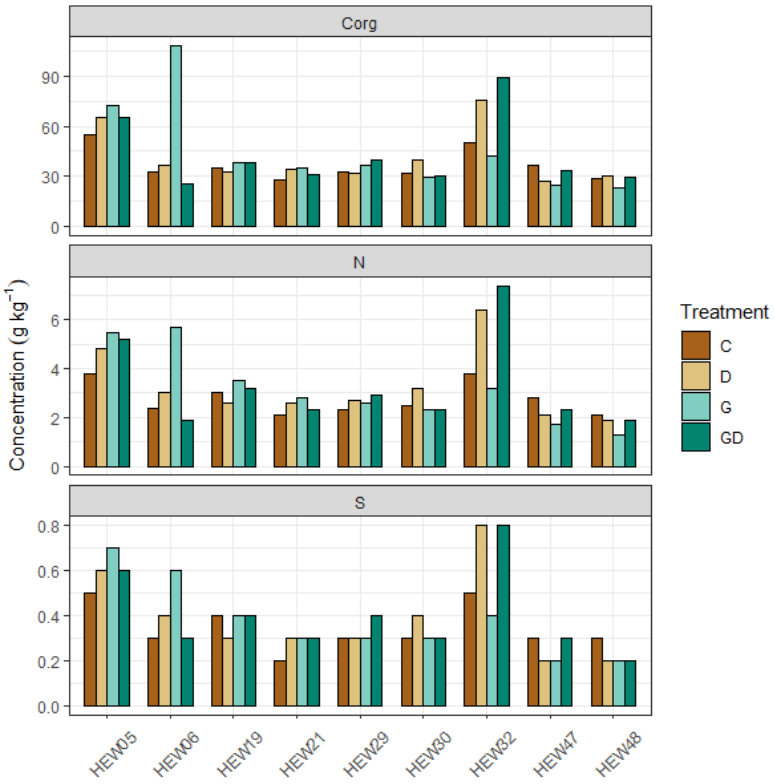

Figure 18 Element concentration (g kg^{-1}) of C_{org}, N and S of mineral soil samples on control plots (C) and treatments (D, G, GD).

Figure 19 shows the C/N-ratio. It varied between 10.87 and 18.9. Noticeable is G on HEW06, with the highest C/N-ratio. Treatments D, G and GD on HEW48 had a C/N-ratio above 15.

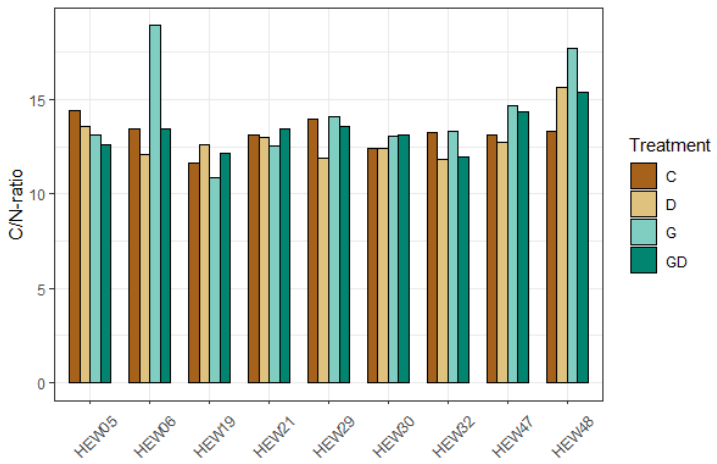

Figure 19 C/N-ratio of mineral soil samples on control plots (C) and treatments (D, G, GD).

3.2 Microbial biomass

Microbial biomass C_{mic} contents ranged from 161 µg g^{-1} to 1306 µg g^{-1}. Contents of N_{mic} varied between 6 µg g^{-1} and 78 µg g^{-1}. Table 3 shows that C_{mic} and N_{mic} contents were higher on all treatments in comparison to the control plots.

Table 3 Means (n = 9) and standard errors of C_{mic} and N_{mic} in control plots (C), deadwood (D), gap (G) and gap enriched with deadwood (GD) treatments.

	C	D	G	GD
C_{mic} (µg g^{-1})	468.78 ± 41.38	594.0 ± 89.91	485.11 ± 92.44	560.22 ± 120.19
N_{mic} (µg g^{-1})	23.78 ± 2.51	32.44 ± 5.26	25.89 ± 5.33	30.0 ± 7.47

Figure 20 presents the content of C_{mic} on each treatment of all investigated plots. The highest C_{mic} concentrations were recorded at GD and D treatment of HEW32, followed by all treatments of HEW05. The G treatment of HEW48 showed the lowest C_{mic} content. Generally, no clear pattern can be observed. According to simple linear models, treatments had no significant effect on C_{mic} (R^2= 0.03933, p-value= 0.7283). Since the patterns of N_{mic} and C_{mic} concentrations are very similar, N_{mic} contents are not visually represented here. Both indicators are significantly correlated with r=0.97, p<0.001 (App. Figure II).

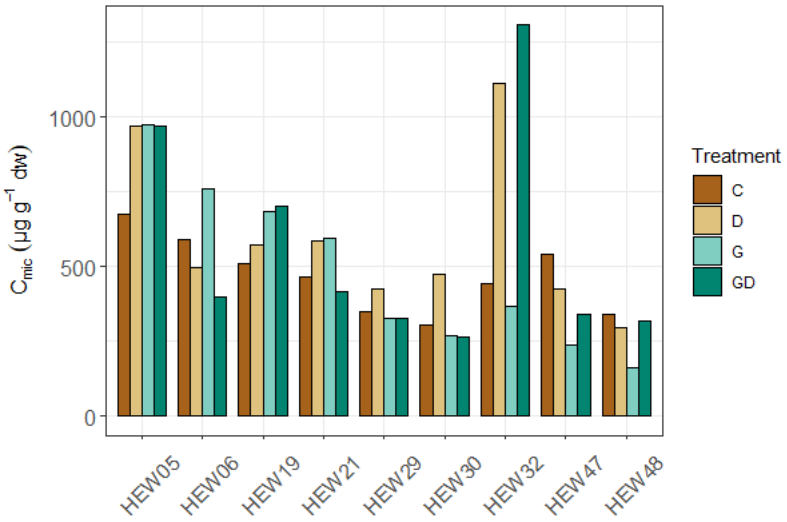

Figure 20 Microbial biomass C_{mic} (µg g^{-1}) content of mineral soil samples on control plots (C) and treatments (D, G, GD).

3.3 Soil enzymatic activity

BG activities ranged from 99.08 to 1251.26 nmol g^{-1} dw h^{-1}, NAG activities were between 37.44 and 837.48 nmol g^{-1} dw h^{-1}, Sulf comprised between 114.3 and 1213.42 nmol g^{-1} dw h^{-1} and Phos activities amounted to between 2199.68 and 10481.69 nmol g^{-1} dw h^{-1}. Thus, the lowest enzyme activity was recorded for NAG, followed by BG and Sulf. Phos had by far the highest enzyme activity. The results of mean enzyme activity measurements for control plots and treatments are presented in Table 4. It is shown that all mean enzyme activities on the treatments (D, G, GD) were higher compared to the control plots. However, spatial variability can be observed in Figure 21. It is noticeable that especially on the G sites of HEW05 and

HEW06 all four enzyme activities were comparatively high. BG, NAG and Sulf are increased on the GD and D treatment of HEW32.

Table 4 Means (n = 9) and standard errors of soil enzyme activities in control plots (C), deadwood (D), gap (G) and gap enriched with deadwood (GD) treatments.

	C	D	G	GD
BG (nmol g^{-1} dw h^{-1})	398.14 ± 75.62	470.74 ± 69.29	528.70 ± 133.43	617.47 ± 124.19
NAG (nmol g^{-1} dw h^{-1})	336.48 ± 42.85	400.87 ± 32.31	389.11 ± 86.4	403.63 ± 60.46
Sulf (nmol g^{-1} dw h^{-1})	456.69 ± 79.08	540.19 ± 34.06	524.03 ± 129.7	546.76 ± 73.9
Phos (nmol g^{-1} dw h^{-1})	3951.22 ± 478.45	4043.26 ± 309.2	5175.04 ± 1064.16	4414.15 ± 557.84

Abbreviations: BG: β-glucosidase; NAG: N-acetyl-glucosaminidase; Sulf: sulfatase; Phos: phosphatase

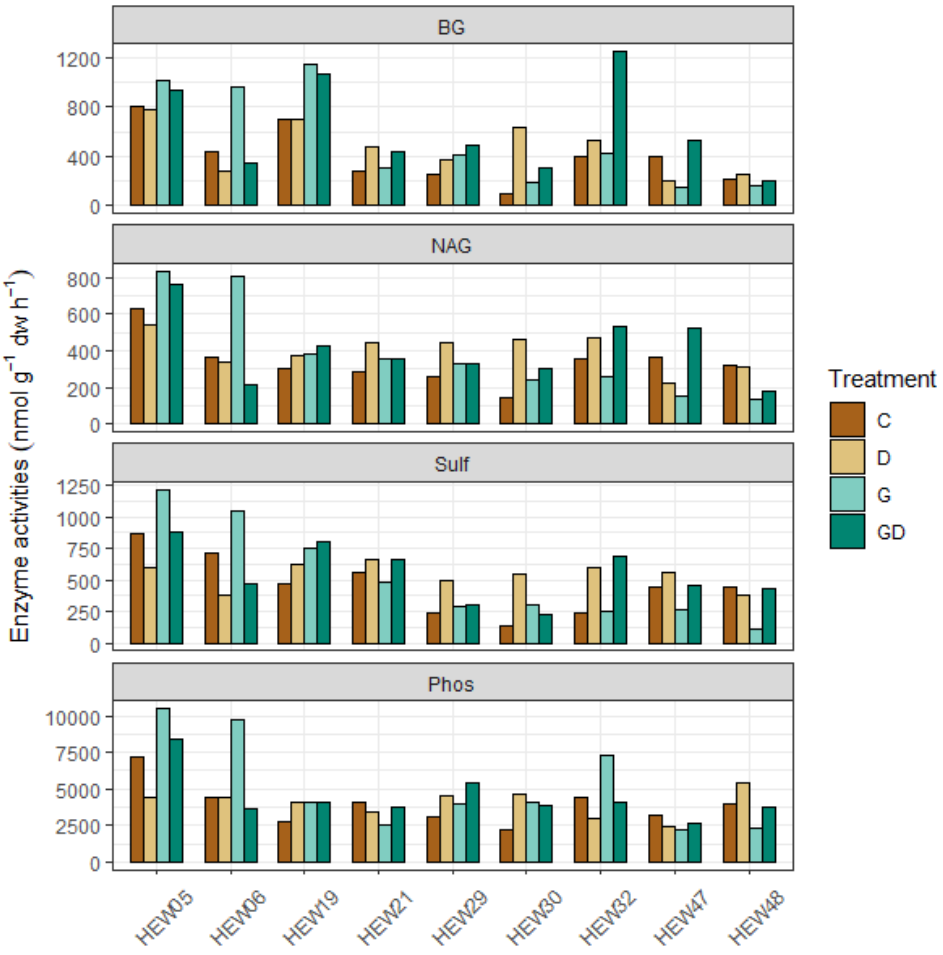

Figure 21 Potential enzymatic activity of β-glucosidase (BG), N-acetyl-glucos-aminidase (NAG), sulfatase (Sulf) and phosphatase (Phos) in nmol g^{-1} dw h^{-1} in mineral soil samples on control plots (C) and treatments (D, G, GD).

The specific enzyme activity is normalised to C_{mic} and indicated with s in prefix to the enzyme name. sBG ranged from 0.32 to 1.68 nmol μg^{-1} C_{mic} h^{-1}. sNAG varied between 0.41 and 1.54 nmol μg^{-1} C_{mic} h^{-1}, sSulf activity was between 0.45 and 1.6 nmol μg^{-1} C_{mic} h^{-1} and sPhos ranged from 2.69 to 19.95 nmol μg^{-1} C_{mic} h^{-1}. Similar to the potential enzyme activity of Phos, sPhos reached the highest values compared to the other enzyme activities. Table 5 shows the specific enzyme activity averaged for treatments and control plots. Again, except for sPhos

activity on the D treatment, all averaged specific enzyme activities were higher on the treatments especially on G and GD than on the C plots.

Table 5 Means (n = 9) and standard errors of specific (s) soil enzyme activities in control plots (C), deadwood (D), gap (G) and gap enriched with deadwood (GD) treatments.

	C	D	G	GD
sBG (nmol μg^{-1} C_{mic} h^{-1})	0.8 ± 0.1	0.83 ± 0.1	1.03 ± 0.12	1.13 ± 0.11
sNAG (nmol μg^{-1} C_{mic} h^{-1})	0.71 ± 0.05	0.74 ± 0.08	0.8 ± 0.06	0.83 ± 0.12
sSulf (nmol μg^{-1} C_{mic} h^{-1})	0.93 ± 0.11	1.01 ± 0.1	1.1 ± 0.08	1.09 ± 0.11
sPhos (nmol μg^{-1} C_{mic} h^{-1})	8.51 ± 0.71	8.22 ± 1.54	11.6 ± 1.59	9.63 ± 1.4

Abbreviations: BG: β-glucosidase; NAG: N-acetyl-glucosaminidase; Sulf: sulfatase; Phos: phosphatase

Although the specific enzyme activity of, for example, sBG was higher on the gaps (G and GD) in several plots compared to C and D (Table 5), no consistent pattern can be identified. For sBG and sNAG, the high activity on the GD treatment of HEW47 stands out in particular (Figure 22). In addition, sBG activity was increased on HEW19 and HEW29. The activities of sSulf were on a similar level with single exceptions, like HEW32 and HEW21-GD. For sPhos, HEW32-G reached the highest activity.

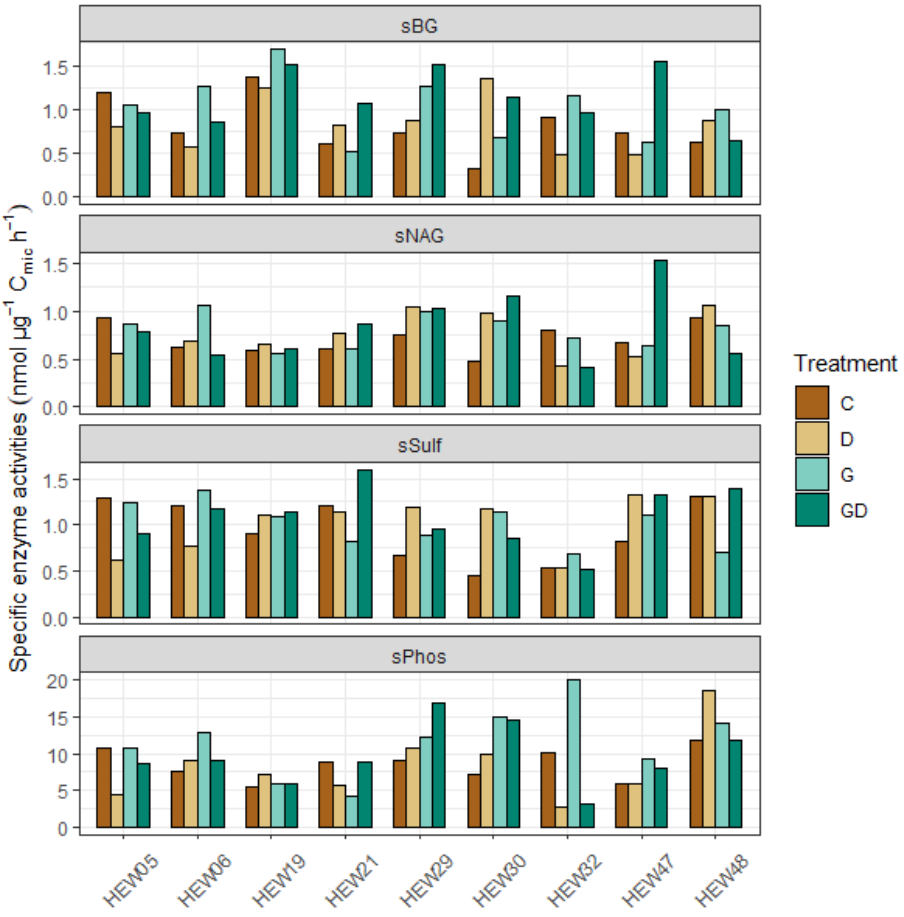

Figure 22 Specific (s) enzyme activities of β-glucosidase (sBG), N-acetyl-glucosaminidase (sNAG), sulfatase (sSulf) and phosphatase (sPhos) in nmol μg^{-1} C_{mic} h^{-1} in mineral soil samples on control plots (C) and treatments (D, G, GD).

3.4 Interaction of soil properties and microbial activity

The relationships of the abiotic and microbial parameters and the respective interactions between them are described in the following.

App. Figure II shows a correlation matrix of abiotic and microbial parameters. The matrix illustrates the relationships between the parameters by giving a scatter plot with a fitted line, the *Pearson* correlation coefficient and significance levels. All soil parameters are significantly and positively related to each other. Only the C/N-ratio correlates significantly negatively with C_{mic} (r= -0,38) and N_{mic} (r= -0,46).

To investigate the relationship between C_{org} and C_{mic}, a linear regression was calculated and visualised in Figure 23. C_{org} contents explained 65 % of the variance of the C_{mic} contents across all treatments. As C_{org} and C_{mic} are significantly positively correlated ($r = 0.80$; $p < 0.001$) with each other (App. Figure II), subsequent multiple models were calculated for relationships with C_{org} only.

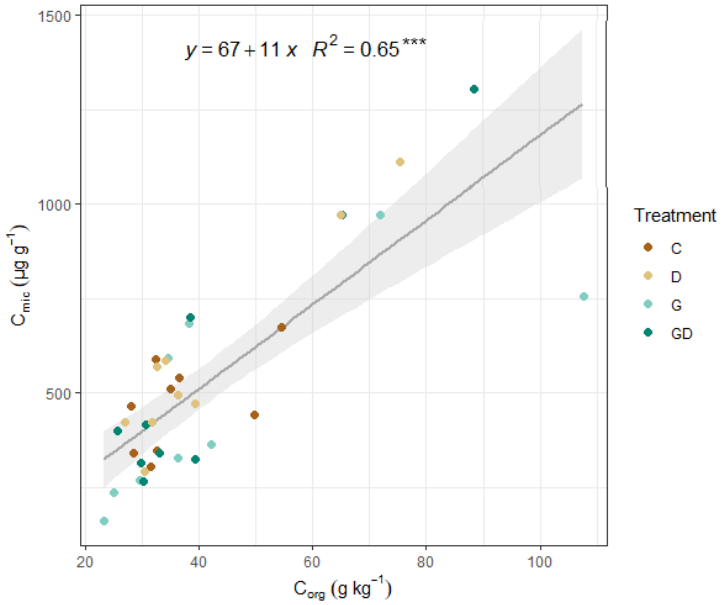

Figure 23 Linear model of C_{org} and C_{mic} with 36 observations and the regression equation. Significance is assessed by $^{+}$ < 0,1; *p<0,05; **p<0,01; ***p<0,001.

All potential enzyme activities are positively related to C_{org} and C_{mic} as it is shown in the correlation matrix (App. Figure II). These relationships are confirmed by multiple linear models of enzyme activities and C_{org} (App. Table III) and C_{mic} (App. Table IV) as presented in the appendix. Although the relationship of enzyme activities and microbial biomass was consistently positive, they differed for individual enzymes. For example, the relationship of Phos activity and C_{mic} was considerably weaker than for BG, NAG, and Sulf. Furthermore, there was a strongly significant and positive relationship between enzyme activities and pH, except for Phos.

Prior to calculation of multiple linear models, abiotic soil parameters were tested for multicollinearity. Finally, C_{org} and pH remained as non-collinear abiotic soil parameters for

modeling. ANCOVA was used to examine the influence of deadwood and forest gap treatments, considering C_{org} content, pH value and Plot ID, on the different potential (a) and specific (b) enzyme activities (Table 6).

For all potential enzyme activities (a), C_{org} was a highly significant influencing variable. The pH value had a significant effect on all enzymes except NAG. The different treatments show no direct significant impact on enzyme activity, only for BG there was a significant influence of the treatment effects**.** The Plot ID, which is a measure of spatial variability of the nine HEW sites, was significant for all enzymes. This site-specific differences are particularly strongly significant for BG, only weakly significant for NAG and Sulf, and only marginally significant for Phos. Linear models showed that across all treatments C_{org} and pH together explained 57 % of the variance of BG activities, 66 % of NAG activities, 43 % of Sulf activities and 53 % of Phos activities (App. Table III).
In the linear models for specific enzyme activity, C_{org} was excluded as a covariate, since C_{mic} information is already contained in the response variable. The pH value only had a strongly significant influence on the sPhos activities and was only marginally significant for sNAG activities. A linear regression showed that sPhos activities declined significantly with increasing pH value ($y = 34.9 – 5.38x$; $R^2 = 0.51$; $p < 0.001$) (App. Figure V). A treatment effect was again only observed for sBG, while the HEW-location (Plot ID) only had a very weak influence on sBG and sSulf.

Table 6 Analysis of covariance (ANCOVA) for (a) enzyme activities and (b) specific enzyme activities as response variables. C_{org} (organic Carbon concentration), pH, Treatment and Plot ID are explanatory covariables and given in rows in the order of entering the analysis. Degrees of freedom (df), mean squares (MS) and *F*-values (F) are presented in the table. Significance is assessed by $^{+} < 0,1$; $^{*}p<0,05$; $^{**}p<0,01$; $^{***}p<0,001$.

(a)		BG		NAG		Sulf		Phos	
	df	MS	F	MS	F	MS	F	MS	F
C_{org}	1	1830296	110.067***	695302	96.793***	827634	35.069***	56504087	38.419***
pH	1	181373	10.907**	11300	1.573	176013	7.458*	19405127	13.194**
Treatment	3	62515	3.759*	1873	0.261	5858	0.248	429120	0.292
Plot ID	8	102112	6.141***	20533	2.858*	80481	3.410*	3264898	2.220 $^{+}$
Residuals	22	16629		7183		23600		1470737	

(b)		sBG		sNAG		sSulf		sPhos	
	df	MS	F	MS	F	MS	F	MS	F
pH	1	0,00023	0.003	0,24148	4.049^{+}	0,07218	1.026	305,94	43.285***
Treatment	3	0,22655	2.602^{+}	0,02647	0.444	0,04490	0.638	10,84	1.534
Plot ID	8	0,18577	2.133^{+}	0,03969	0.666	0,14921	2.120^{+}	11,80	1.669
Residuals	23	0,08708		0,05964		0,07038		7,07	

Abbreviations: BG:β-glucosidase; NAG: N-acetyl-glucosaminidase; Sulf: sulfatase; Phos: phosphatase

3.5 Tea bag decomposition indices

In the following, the results on decomposition and water content of green and rooibos tea as well as stabilisation factor (*S*) and decomposition rate (*k*) are presented. A detailed overview can be found in the appendix (App. Table II).

The boxplot diagram in Figure 24 shows the decomposition given in % for each tea type and treatment in the litter and mineral soil. It highlights that green tea was decomposed stronger than rooibos tea. The mean decomposition of green tea over all treatments was 57.92±0.35 % in the litter layer and 57.25±0.26 % in the mineral soil. In contrast, rooibos tea was decomposed on average 34.67±0.42 % in the litter and 31.78±0.32 % in the mineral soil. The decomposition of both tea types did not differ remarkably between the individual treatments, as decomposition was on a similar level (Figure 24). A slightly less degradation can be observed for the G treatment in the litter layer. In addition to the differences in the degradation behaviour of the two tea types, a minor variation in decomposition can be observed concerning the depth. Averaged for both tea types, 46.43±0.79% were decomposed in the litter layer and 44.51±0.78% in the mineral soil.

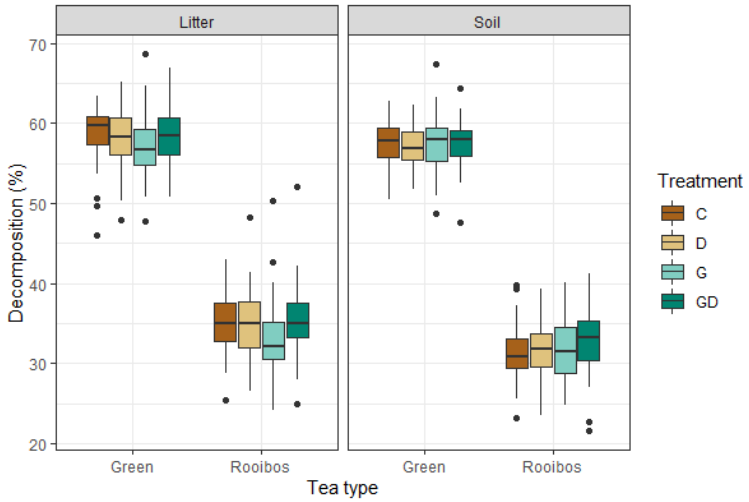

Figure 24 Decomposition (%) of green tea and rooibos tea in litter and mineral soil for aggregated treatments.

The water content of the tea bags is visualised in Figure 25. It shows the differences of the water contents for each tea type and treatment in the litter layer and mineral soil. The mean water content of tea bags in the litter layer was 143.89±8.33 % for green tea and 155.57±6.37 % for rooibos tea. In the mineral soil, the water content amounted to 201.98±3.73 % for green tea and 163.49±3.2 % for rooibos tea. Thus, the mean water content of both tea types was higher in the soil than in the litter. Regarding differences between the treatments, it is noticeable for both tea types that especially the gaps (G, GD) had a higher water content in the soil. In comparison, the gaps for green tea in the litter seem to have had a lower moisture content than C and D. If gaps (G, GD) and non-gaps (C, D) were grouped, the mean water content was 200.32±3.48 % for the gaps and 165.39±3.6 % for non-gaps in the mineral soil. In the litter there was a mean water contents of 138.48±6.91 % in the gaps and 161.11±7.86 % in non-gaps. In comparison to water contents in the soil, interquartile ranges and whiskers in the litter are clearly larger for both tea

types (Figure 25). It can therefore be assumed that there is a stronger variation of the water content in the litter than in the mineral soil.

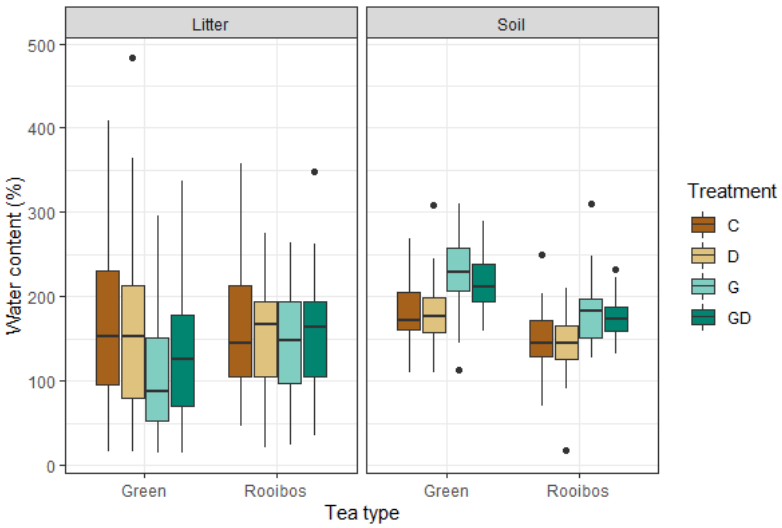

Figure 25 Water content (%) of green tea and rooibos tea in litter and mineral soil for aggregated treatments.

Figure 26 shows the interaction of decomposition and water content of the tea bags. In the litter layer, there is a significant relationship between degradation and water content, in both tea types. Thus, the water content explained 24 % of the variance of the decomposition of green tea and 22 % of the variance of the decomposition of rooibos tea. In the mineral soil, a significant relationship of decomposition and water content was not observed for green tea, but weakly for rooibos tea.

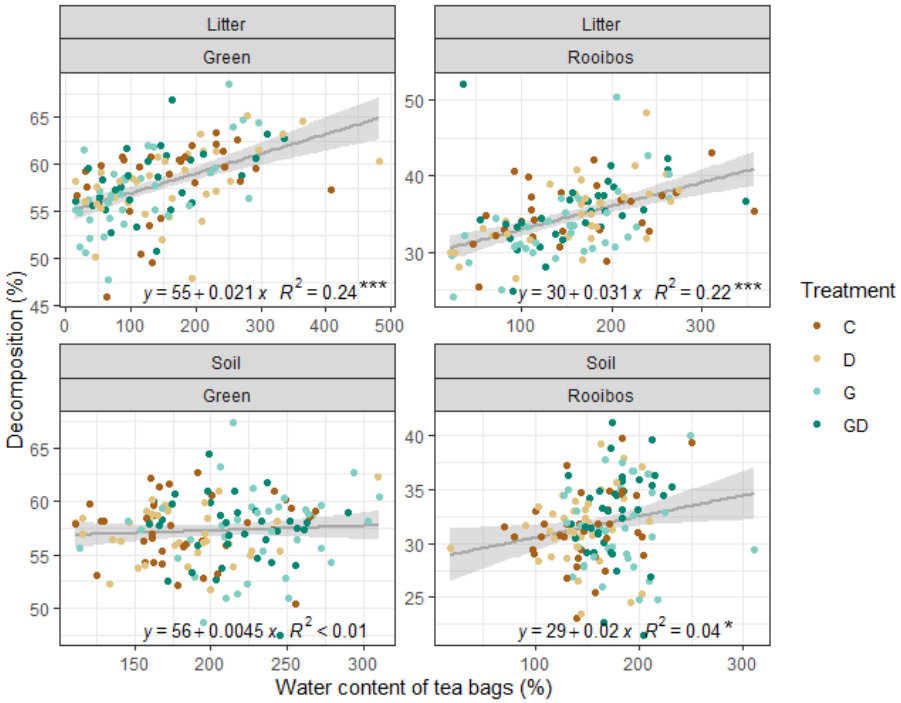

Figure 26 Relationship of water content of tea bags (%) and decomposition via mass loss (%) for green tea and rooibos tea in litter and mineral soil per treatment. The regression equation is given with significance assessed by $^{+} < 0,1$; $^{*}p<0,05$; $^{**}p<0,01$; $^{***}p<0,001$.

The stabilisation factor (*S*) and decomposition rate (*k*) calculated according to Keuskamp et al. (2013) are shown as follows (Table 7). The table presents that *S* and *k* factors are at similar levels across the treatments with minor variations.

Table 7 Means (n=9) of stabilisation factor (*S*) and decomposition rate (*k*) in control plots (C), deadwood (D), gap (G) and gap enriched with deadwood (GD) treatments.

	Depth	C	D	G	GD
S	Litter	0.24	0.25	0.26	0.25
	Soil	0.26	0.27	0.26	0.27
k	Litter	0.03	0.03	0.02	0.03
	Soil	0.02	0.02	0.02	0.02

S ranged from 0.18 to 0.31 in the litter and from 0.21 to 0.36 in the mineral soil. The box plot diagram (Figure 27) shows as well that *S* is at a similar level in litter and soil. In the litter, however, it is noticeable that higher stabilisation occured on the G treatments. In the mineral soil, hardly any differences are observed between the treatments.

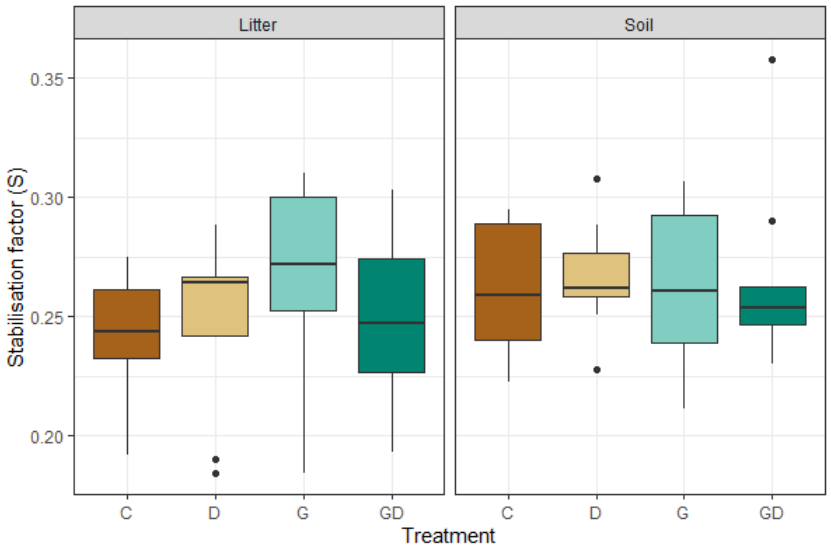

Figure 27 Stabilisation factor *S* in litter and mineral soil for aggregated treatments.

A more detailed visualisation of the stabilisation factor for each treatment and Plot ID shows that no consistent trend can be identified (Figure 28). However, some values are remarkable, e.g. the *S* factor of 0.36 on the GD treatment of HEW21 in the mineral soil.

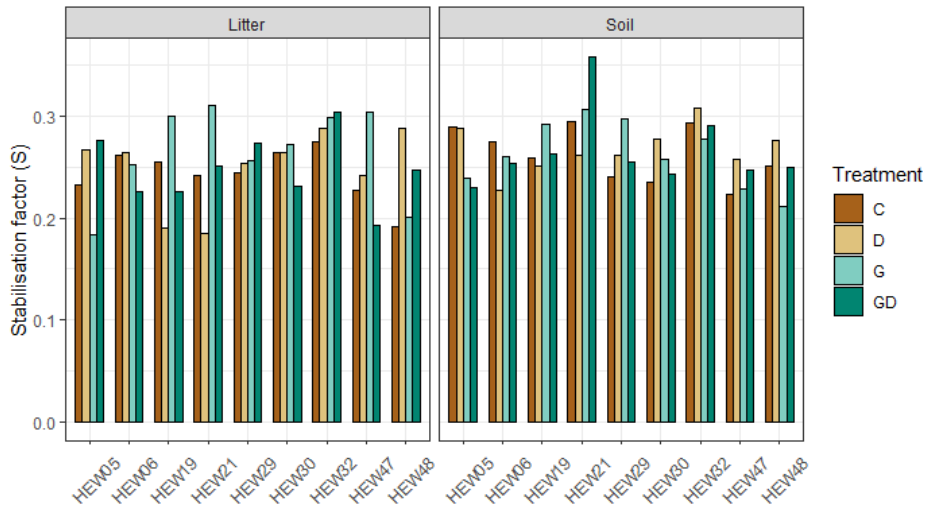

Figure 28 Detailed visualisation of stabilisation factor (*S*) in litter and mineral soil for each treatment.

Decomposition rate *k* ranged from 0.2 to 0.4 in the litter and from 0.1 to 0.4 in the mineral soil. Figure 29 shows that the decomposition rate in the litter is generally higher than in the soil. In the litter, *k* was at a similar level across the treatments, only the G treatment had a lower *k* than other treatments. In the mineral soil, slightly higher rates were achieved, especially on the GD treatment.

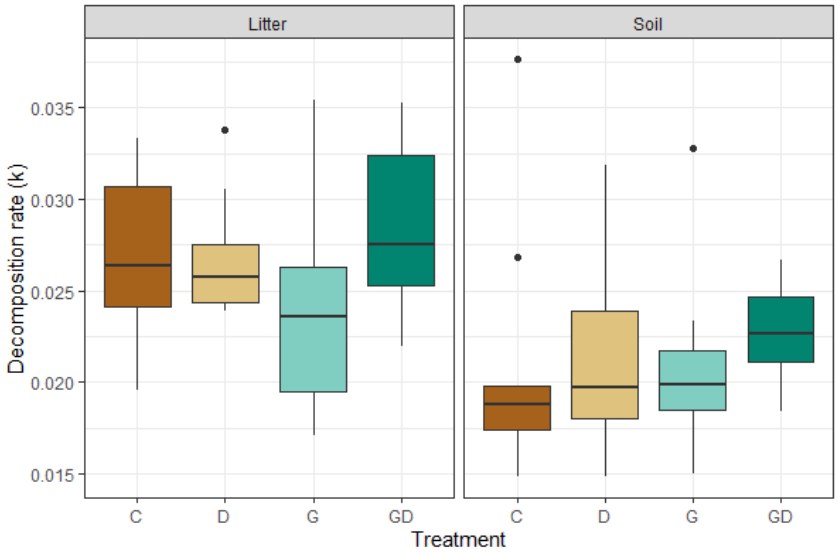

Figure 29 Decomposition rate (*k*) in litter and mineral soil for aggregated treatments.

The bar chart illustrates the decomposition rate for each treatment and Plot ID (Figure 30). Due to missing tea bags in the litter layer, two *k* values could not be calculated and are therefore not displayed in Figure 30. Similar to Figure 28. above, no clear trend could be identified. The *k* rate in litter and soil does not seem to be closely related, as there are no clear similarities. The highest *k* value was obtained in the mineral soil on the C plot of HEW32.

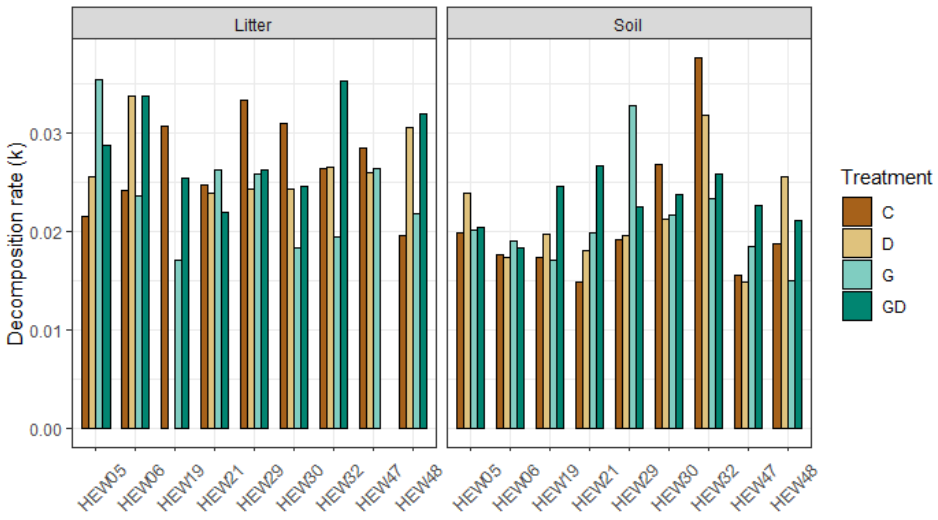

Figure 30 Detailed visualisation of decomposition rate (*k*) in litter and mineral soil for each treatment.

The relationship of *S* and *k* is shown in Figure 31. There is a positive interaction between *S* and *k* in litter and soil. Most observations in litter and mineral soil were between a stabilisation factor of 0.2 and 0.3. The scatterplot in the mineral soil is rather dense than in the litter, where observations show a higher variance of *k*.

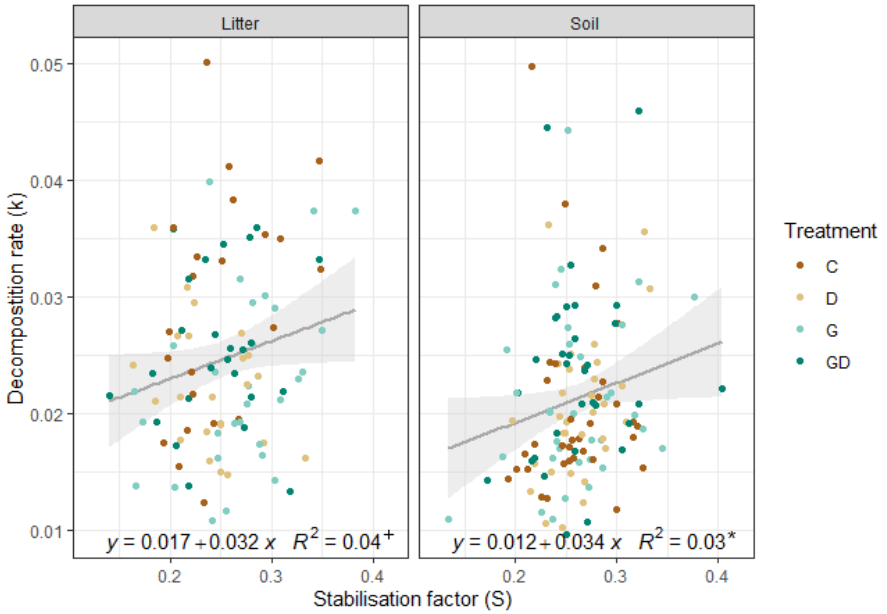

Figure 31 Relationship of stabilisation factor (*S*) and decomposition rate (*k*) for each treatment.

General linear models for the decomposition of green and rooibos tea and *S* and *k* indices were calculated to determine the influence of the treatments, taking into account the water content and the Plot ID as influencing variables. The results are shown in Table 8 for the litter (a) and the mineral soil (b).

Table 8 Analysis of covariance (ANCOVA) for tea bag decomposition indices in the litter layer (a) in the mineral soil (b) as response variables. Water content of tea bags (%), Treatment and Plot ID are explanatory covariables and given in rows in the order of entering the analysis. Degrees of freedom (df), mean squares (MS) and F-values (F) are presented in the table. Significance is assessed by + < 0,1; *p<0,05; **p<0,01; ***p<0,001.

(a)		Decomposition of green tea (%)		Decomposition of rooibos tea (%)		Stabilisation factor (*S*)		Decomposition rate (*k*)		
	df	MS	F	MS	F	MS	F	df	MS	F
Water content	1	73.12	16.447 ***	22.092	3.395 +	0.014239	21.850 ***	1	2.730e-06	0.099
Treatment	3	0.18	0.040	9.667	1.486	0.000085	0.131	3	3.217e-05	1.171
Plot ID	8	8.74	1.965	2.857	0.439	0.001473	2.260 +	8	1.054e-05	0.384
Residuals	22	4.45		6.507		0.000652		21	2.747e-05	

(b)		Decomposition of green tea (%)		Decomposition of rooibos tea (%)		Stabilisation factor (*S*)		Decomposition rate (*k*)	
	df	MS	F	MS	F	MS	F	MS	F
Water content	1	0.621	0.248	7.016	3.781 +	0.0000755	0.122	3.812e-05	2.298
Treatment	3	0.213	0.085	2.318	1.249	0.0000494	0.080	9.050e-06	0.546
Plot ID	8	8.883	3.547 **	12.795	6.895 ***	0.0020164	3.268 *	5.781e-05	3.485 ***
Residuals	23	2.505		1.856		0.0006170		1.659e-05	

The decomposition of green tea and stabilisation is significantly related to the water content of the tea bags in the litter. Decomposition increases with greater water content (Figure 26) and stabilisation decreases, as linear models have shown. In the decomposition of rooibos tea, the influence of the water content is only marginally significant. There was a significant positive relationship between water content and *k* in the litter layer ($y = 0.025+6.6\cdot10^{-6}x$, p-value = 0.031), but not in the mineral soil. Furthermore, an influence of the Plot ID on the decomposition of rooibos tea is observed. This relationship is particularly evident in the mineral soil, where all tea bag indices are significantly influenced by the Plot ID. In the mineral soil, there is a marginally significant interaction of water content and rooibos tea decomposition. For the other tea bag indices, there seems to be no correlation with water content in the soil. In both litter and mineral soil, there is no significant interaction between the different treatments and tea bag decomposition indices.

3.6 Relationships between soil parameters and decomposition indices

Linear models were used to investigate the correlation between soil properties and soil decomposition indices. A correlation for all soil and tea bag parameters (App. Figure II) revealed a significant relationship of water content measured in soil samples and water content of green (r = 0.39) and rooibos tea bags (r = 0.50). Tea bag decomposition indices for litter were not included in analysis with soil data. Soil parameters influenced tea bag decomposition indices to different extents.

Concerning abiotic soil parameters, linear models with decomposition of green tea and *k* showed the following relationships. There is a significant negative relationship of pH and the

decomposition of green tea in the mineral soil (y = 63-1.3537x, R^2 = 0.13, p-value = 0.0328) (App. Figure VI). No relationship was observed for estimated clay content and C/N-ratio with all decomposition indices. In the mineral soil, *k* was positively related to the element concentration of N (y = 0.018+0.0012x, R^2 = 0.10, p-value = 0.057) (App. Figure VII) and S contents (y = 0.017+0.011x, R^2 = 0.13, p-value = 0.028) (App. Figure VIII).

Regarding the interaction of decomposition indices with microbiological parameters, weakly significant correlations with enzyme activities and microbial biomass were found. For enzymes, only a weakly negative correlation between BG and the decomposition of green tea was identified (y = 58-0.002x, R^2 = 0.07, p-value = 0.114) (App. Figure II). Multiple linear models revealed no to marginally significant (for BG) influences of enzyme activities on decomposition, also under consideration of Treatment and Plot ID.
However, the ANCOVA for tea bag indices with C_{mic} as a biological parameter, Treatment and Plot ID showed in Table 9, that there is a significant relationship of C_{mic} and the decomposition of green tea. This negative relationship is visualised in App. Figure IX (y = 58-0.0024x, R^2 = 0.10, p-value = 0.0541). It is remarkable that soil samples with a high C_{mic} content (HEW32-GD, HEW32-D) had a comparatively low decomposition of green tea. Across all treatments C_{mic} explained 10 % of the variance of the decomposition of green tea.

Moreover, there is a marginally significant interaction of C_{mic} and stabilisation factor (*S*). Linear models revealed that this relationship is positive. The Plot ID as a measure of the variability of the study sites had a significant influence on the decomposition, especially of rooibos tea. The ANCOVA table finally shows that different treatments with gaps and deadwood had no significant impact on all decomposition indices.

Table 9 Analysis of covariance (ANCOVA) for tea bag decomposition indices in the mineral soil as response variables. C_{mic}, Treatment and Plot ID are explanatory covariables and given in rows in the order of entering the analysis. Degrees of freedom (df), mean squares (MS) and F-values (F) are presented in the table. Significance is assessed by + < 0,1; *p<0,05; **p<0,01; ***p<0,001.

		Decomposition of green tea (%)		Decomposition of rooibos tea (%)		Stabilisation factor (S)		Decomposition rate (k)	
	df	MS	F	MS	F	MS	F	MS	F
C_{mic}	1	13.608	4.689 *	0.123	0.044	0.0022282	3.230 +	1.541e-05	0.792
Treatment	3	0.066	0.023	3.079	1.104	0.0000047	0.007	7.300e-06	0.375
Plot ID	8	6.172	2.127 +	10.688	3.833 **	0.0015546	2.253 +	5.309e-05	2.730 *
Residuals	23	2.902		2.789		0.0006899		1.945e-05	

4 Discussion

4.1 Effect of forest gaps on soil properties, microbial activity and decomposition

Abiotic soil properties were not significantly influenced by different treatments with gap creation and deadwood enrichment. However, minor tendencies could be observed in Table 2. The average pH value on plots with deadwood (D and GD) was slightly higher than on control and gap plots. Although there is evidence that deadwood can increase soil pH (Perreault et al., 2020), it should also be noted that at the time of sampling, the deadwood had only been there for about a year and tree trunks have barely decayed. Panayotov (2016) reports, that even after about 20 years accumulated deadwood had no significant effect on the pH of mineral soils. In this context, the impact of deadwood is considered negligible. The formation of gaps had no effect on the soil pH value, as Wang et al. (2021) and Yang et al. (2017) have discovered, too.

With regard to the mean water contents, it is noticeable that moisture was higher in mineral soils in forest gaps than on C and D treatments. Not only the soil samples taken in May 2021 showed a higher moisture content in the gaps but also the tea bags buried in the mineral soil and retrieved in August 2021 (Table 2, Figure 25). The water contents are a momentary record and are not representative for long periods of time, but the assumption that forest gaps are usually wetter is confirmed by this study and is in line with findings of Muscolo et al. (2014). As a possible reason for this result, Zhu et al. (2003) state that more precipitation reaches the ground in gaps and less transpiration occurs than under closed canopies. In addition, weather conditions promotive for vegetation growth are indicated by the observation of touch-me-not balsam (*Impatiens noli-tangere*) at most forest gap sites, especially on HEW30-G, HEW32-GD, HEW47-GD and HEW48-G (shown in Figure 3c). Since *Impatiens noli-tangere* is an indicator for moisture (Oberdorfer, 2001), its presence on the gaps emphasizes the humid conditions in the soil. Concerning the contents of the elements C_{org}, N and S, it is noticeable that although the mean element contents on all treatments are slightly increased compared to the control plots (Table 2), there is a high standard error especially for C_{org} on G. Figure 18 also suggests a high spatial heterogeneity, with HEW06-G and HEW32-D and -GD being most striking for element concentrations. The C/N-ratio is the highest on HEW06-G.
Generally, many studies investigated the impact of gaps and deadwood on nutrient cycling and turnover of soil organic matter. Increased solar radiation and higher temperatures in soils of gaps cause changes in the nutrient cycle due to enhanced microbial activity and mineralisation of organic matter (Keenan and Kimmins, 1993; Muscolo et al., 2014; Zhu et al., 2003). But depending on the silvicultural method and the extent of gap creation, the removal of biomass can also result in the loss of certain nutrients (Pennock and van Kessel, 1997). By various mechanisms, deadwood leads to increased C_{org} and N concentrations in the soil up to a depth of 30 cm (Panayotov, 2016). Although the predominance of nettle (*Urtica dioica*) as an indicator plant for nitrogen-rich and moist soils (Oberdorfer, 2001) was also observed on the

sites with gaps especially on those of HEW05 and HEW06, no significant influence of the treatments on the nutrient contents C_{org}, N and S as well as the C/N-ratio could be detected in this study. This result is consistent with the findings of Ritter (2005), who also found no significant effect of the gaps on Carbon content and C/N ratio one year after the formation of the gap. According to Bauhus (1996), after 21 months, the C_{org} and N levels were on a similar level on gap sites and under closed canopy in a European beech forest. C_{org} contents do not react sensitively to short-term management measures because soil Carbon is aggregated over long periods of time through complex accumulation and stabilisation processes (Gleixner et al., 2005).

Due to conspicuously increased values of individual plots (HEW06-G, HEW32-GD, -D), which indicate high variability of soil conditions, site and soil characteristics were investigated in more detail during the second field inspection in August 2021. It was discovered that the HEW06-G is a site with black Carbon in the soil. The existence of black Carbon is indicated by the dark colour, which according to Eckmeier et al. (2007) is significantly related to the charcoal and C_{org} content, and a possible result of a past local small-scale fire event (Ansley et al., 2006). The comparatively high C_{org} content on HEW06-G (Figure 18) can be explained by the sequestration of charcoal and recalcitrant organic matter (Johnson and Curtis, 2001) and the vertical incorporation of aboveground charcoal into the soil by soil mixing animals (Eckmeier et al., 2007). The mortality of belowground roots may have also led to an increase of charcoal and C_{org} in the soil (Ansley et al., 2006; Hobley et al., 2017). Increases in soil C_{org} content as a result of fires events (Johnson and Curtis, 2001), which were mostly human-induced during the Holocene (Eckmeier et al., 2007), has an important impact on pedogenesis in temperate deciduous forests. Since there is evidence of an historical disturbance on plot HEW06-G, certain soil properties of this plot may biase the statistical assessment of treatment effects. For instance, in the linear regression in Figure 23, the value of HEW06-G represents an outlier. Hence, for following interpretations of the abiotic and also the microbial values on HEW06-G the influence by increased C_{org} contents due to black Carbon should be considered.

As the second field inspection also showed, there seems to be a different soil type on the GD and D treatment of HEW32 than on C and G. Soils on GD and D were shallow, rich in humus and had a rather granular structure. Particularly large limestone rocks were observed close to the soil surface (App. Figure III). Since the parent material was obviously limestone rather than loess, as in downslope plots C and G, it can be assumed that the soil type can be classified as Leptosol rather than Luvisol (Ad-hoc-AG Boden, 2005). This assumption is supported by comparatively higher C_{inorg} contents on GD and D which strongly depend on the parent material (App. Table I). The mineral soil samples of the two treatments GD and D, located higher on the slope, show striking abiotic properties, e.g. comparatively higher values of pH (Figure 15), water content (Figure 16), estimated clay content (Figure 17), C_{org}, C_{inorg}, N and S values (Figure 18, App. Table I). Theses increased values indicate that the soils were formed on carbonate-rich parent material (Bartsch and Röhrig, 2016). Also in this case of HEW32-GD and D, a bias in statistical models due to the impact of the parent material should be taken into

account. For the comparison of the treatments of the FOX with each other, caution is required in the interpretation of the three described sites regarding treatment effects.

In the following section, the question of whether forest gaps as a treatment have an effect on microbial activity will be discussed.

Despite slightly increased mean contents of C_{mic} on D, G and GD (Table 3), treatments had no significant direct effect on microbial biomass C_{mic} as indicated by an ANCOVA (App. Table V). The C_{mic} contents of this study are in line with the mean C_{mic} content of 449±214 µg g^{-1} detected by Solly et al. (2014) in the Hainich biodiversity exploratory. A comparison of the results of potential enzyme activities measured by Herold et al. (2014a) in the same study region showed that enzymatic activities in the mineral soil are mostly consistent. Mean potential activities of BG and NAG were at a higher level in Herold et al. (2014a), but were consistent with the range of the results of this study (Table 4). The mean Phos activity detected by Herold et al. (2014a) is in line with measurements of this study. Further, in line with Herold et al. (2014a), Phos reached by far the highest activity, followed by BG and then by NAG. The activity of Sulf was not studied by Herold et al. (2014a), but it can be stated that Sulf activity was at a similar level to BG in this study.

Regarding the influence of forest gaps and deadwood addition on the microbial activities, all potential and specific enzyme activities showed higher mean values on gaps than on the control sites (Table 4, Table 5). However, the ANCOVA (Table 6) detected a significant treatment effect only for the potential and specific activity of BG, including soil properties as covariables. On average, BG (Table 4, Figure 21) and sBG (Table 5, Figure 22) activities are remarkably higher on the gaps than on the control soil. On some G and GD treatments the BG activity was twice as high than on C and D treatments (Figure 21). The effect that forest gaps lead to increased potential and specific BG activity can be attributed to various aspects. There is great evidence that forest gaps cause a change in the microbiome and microbial community structures through an altered microclimate and physico-chemical properties (Wang et al., 2021), i.e. by increased soil temperature and humidity (Muscolo et al., 2010; Zhu et al., 2003). The felling of trees promotes microbial activity, since competition for nutrients and water exerted by trees is reduced (Mosca et al., 2007). Timber harvesting and the felling of single trees results in root mortality that increases the amount of organic matter and Carbon (Lewandowski et al., 2015; Mosca et al., 2007) that serves as a food source for microbes (Loeppmann et al., 2020). Because BG is involved in Carbon cycling by hydrolising the breakdown of cellulose (Perreault et al., 2020; Schinner and Sonnleitner, 1996; You et al., 2014), the significantly stimulated activity of BG observed in this study can be attributed to the additional organic material from the dead roots of the felled trees in the forest gaps (Mosca et al., 2007). The exact relationship between C_{org} and enzyme activity is further discussed in 4.2. Another possible explanation for the increased BG activity in the gaps is the increased solar radiation, as it is generally known that light conditions have a crucial impact on forest communities (Penone et al., 2019), and thus affect microbial activity. In forest gaps it was found that solar radiation leads to greater litter

decomposition (Lin et al., 2015) and turnover of Carbon (Méndez et al., 2019). The altered properties of litter due to increased radiation favour microbial activity (Lin et al., 2015). Thus, Méndez et al. (2019) reported increased BG activity in litter at sites with strong solar radiation, and in mineral soils with additional litter. More studies show increased BG activities in forest gaps: Perreault et al. (2020) investigated sites in a North American hardwood forest, where gaps of various sizes and degrees of deadwood decay were created by group selection harvest. Especially on forest gaps with highly decomposed deadwood, they found a high potential BG activity compared to the control with closed canopy after ten years of gap formation. Regarding gap size, Yang et al. (2017) found in a forest in northeast China that especially on small gaps, where 1-3 trees were felled, BG activity was higher, while large gaps caused enzyme activity to decrease. Mosca et al. (2007) investigated the effect of silvicultural treatments on enzyme activities and found increased BG activity after thinning in a forest in northern Italy as well.

However, since there was a significant treatment effect only on BG activity, but not on C_{mic} (Figure 20), and the activity of NAG, Sulf and Phos (Table 6), it can be concluded that forest gaps have predominantly no direct impact on microbial parameters one year after opening the canopy. Apparently the above-mentioned possible gap-induced changes, e.g. additional organic matter, do not necessarily lead to the colonisation by certain microbial drivers and the stimulation of enzymes involved in the N, S, and P cycle (Schinner and Sonnleitner, 1996). According to the assumptions of this study, it would be expected that forest gaps promote microbial activity. Since all mean enzyme activities tended to be higher on the gaps than on the control, the results of this work are consistent with several previous studies: Yang et al. (2017) found increased NAG activity on gaps, Wang et al. (2021) found the highest Phos activity on small gaps, and NAG and Phos activities were the highest on gaps with deadwood according to Perreault et al. (2020). Although Muscolo et al. (2010) state that small gaps have a significant impact on Carbon, N and P cycling with higher availability and enzyme activity, other studies highlight that microbial activity is unaffected or only minimally influenced by forest management. For example, silvicultural practices in age-class or selection cutting forests do not differ significantly in terms of Carbon storage (Gleixner et al., 2005; Mund, 2004). Lewandowski et al. (2015) described that the size of the forest gap had only minimal effects on the microbial community in the soil and Herold et al. (2014a) also found that forest management does not have a large and long-term effect on enzyme activity, but that rather soil conditions explain differences in the variance of enzyme activity.
Since the ANCOVA did not show a significant effect of the treatments on most enzyme activities (Table 6), the slightly increased mean values on the gaps in Table 4 and Table 5 should not be overinterpreted. In this aspect, the results of this work are rather consistent with the studies of Herold et al. (2014b), Lewandowski et al. (2015) and Mund (2004) that have identified forest management as a subordinate factor for microbial activity. Reasons for variations in microbial activity patterns are strongly related to certain soil properties, which are explained in more detail in the next chapter (4.2).

An important aspect that plays a role in the interpretation of microbial activity with regard to the influence of the gaps is the time of sampling. Microbial communities have been found to vary seasonally as well as annually (Lewandowski et al., 2015). Thus, results of investigations of microbial activity are clearly influenced by the time of sampling (Purahong et al., 2015). For example, Perreault et al. (2020) found a higher microbial community abundance in August than in May. A possible explanation for this result was, that microbial communities benefit from the buffering impact of herbaceous vegetation that grows on forest gaps during warm months (Perreault et al., 2020). Since herbaceous vegetation was also observed on the FOX gaps in August, even higher microbial activity can be expected during summer. Moreover, since decayed deadwood is a provider of C_{org} and nutrients (Vilhelmsson, 2013) and therefore has an impact on microbial activity (Perreault et al., 2020), results from soil samplings of different seasons over a longer period of time are necessary to understand the long-term impact of different forest treatments.

Finally, it will be discussed how forest gaps affected tea bag decomposition indices and what role the water content plays in this process. For mineral soils, the determined tea bag indices with a mean stabilisation factor *S* of 0.26 and a decomposition rate *k* of 0.02 are comparable to TBI values on Central European temperate deciduous forest sites from Keuskamp et al. (2013). Thus, the indices detected by this study are appropriate for this type of ecosystem. Although there are slight differences between the treatments in terms of stabilisation and degradation rates (Figure 27, Figure 29), simple linear models and ANCOVA did not show a significant effect of the different treatments when water content (Table 8) and microbial biomass (Table 9) were included in the models. In Figure 29 it is noticeable that there was a higher *k* in the soil on the GD treatments compared to the C plots, but this difference seems to be too weak for linear models to identify the influence of the treatments as significant. In general, increased decomposition would be expected as a result of an altered microclimate due to the gaps, as humid and warm environments lead to higher *k* (Keuskamp et al., 2013). However, investigations of litter decomposition rates on forest gap sites have not always shown consistent results as it is mentioned by Muscolo et al. (2014). It is argued that besides microbial activity, also higher solar radiation in gaps would promote photodegradation of plant litter and mass loss (Austin et al., 2015). Lin et al. (2015) have shown that although forest gaps did not have an immediate effect on decomposition after they are created, they did have positive effects on litter quality and the decomposition of fine-roots and leaf litter after 23 years. This shows again that the temporal aspect matters when assessing the impact of forest gaps on decomposition. Moreover, there are also controversial results regarding the influence of different sizes of gaps. While litter mass loss is significantly higher in small gaps (Ritter, 2005) and deciduous litter decomposition is slower at large gaps and clear cut sites (Jerabkova et al., 2011), there are also reports of particularly high degradation rates on large gaps (Zhu et al., 2003). Although different forest management practices can have a particular impact on litter quality and decomposition (Purahong et al., 2015), there is also evidence that different treatments have no significant effect

on decomposition. A litter bag study by Denslow et al. (1998) in a tropical wet forest in Costa Rica, for example, found no significant effect of canopy gaps on decomposition rates. Even after 8 years, Bauhus et al. (2004) found no correlation between the influence of gaps (30 m diameter) and litter degradation rates, which was related to insufficient changes of the temperature in gaps. Wang et al. (2019) found only minor effects of different environmental treatments on the mass loss of tea substrate as well. The controversial results of the mentioned studies underline that the influence of gaps on decomposition cannot always be clearly determined. In this study, the results regarding the identification of treatment effects on decomposition are not as demonstrative as expected. Since decomposition measures are dependent on various factors and environmental influences (Keuskamp et al., 2013; Prescott, 2010), more variables must be considered when investigating forest gap effects.

Nevertheless, although no treatment effect was found, differences in degradation behaviour were found in relation to the type of tea, the depth, and the water content. These results will be highlighted here. In agreement with various studies (Djukic et al., 2018; Keuskamp et al., 2013; Mori et al., 2021; Ochoa-Hueso et al., 2020; Wang et al., 2019), it was found that green tea was decomposed to a much greater degree than rooibos tea as shown in Figure 24. This can be attributed to the fact that green tea contains a greater proportion of labile and soluble compounds, while rooibos tea is composed of rather recalcitrant substrate (Keuskamp et al., 2013; Ochoa-Hueso et al., 2020).

Additionally, differences in the degradation behaviour with regard to depth position of the installed tea bags were found, which addresses the second research objective (ii) in further detail. In mineral soil there is a significant and, in the litter a marginally significant relationship between S and k (Figure 31). This relationship was also noted by Ochoa-Hueso et al. (2020), who interpreted that there is rapid decomposition in the early stage, with a disproportionately higher stabilisation or accumulation of recalcitrant and biochemically transformed plant material in the later stage of decomposition. A potential reason for this discovery was that parts of the microbial community may be specialised in easily degradable substances and thus outcompete microbial players that would rather degrade recalcitrant material at the site. It is also possible that some present microbes were unable to break down the tea substrate for various reasons, so that the plant residues are eventually biochemically stabilised (Ochoa-Hueso et al., 2020). In this study these processes could have played an important role in the mineral soil, where the S factor tends to be slightly higher than in the litter (Figure 27, Table 7). Additionally, decomposition rate k is clearly higher in litter than in mineral soil (Figure 29, Table 7). There are several possible explanations for this observation. Various studies report that microbial activity is higher in the litter than in the mineral soil, which would thus lead to a higher decomposition rate in leaf litter (Andersson et al., 2004; Liu et al., 2020; Schinner and Sonnleitner, 1996). This relationship cannot be proven in this study, as the microbial activity for the litter layer was not analysed. But since Herold et al. (2014a) found decreased potential and specific enzyme activities in deeper soil horizons in the Hainich biodiversity exploratory,

it can also be assumed for the results of this study that enzymatic activity and thus decomposition rates decline with increasing depth. Srinivasarao et al. (2017) found higher enzyme activities in the litter than in the mineral soil as well. The interaction of microbial biomass and enzyme activity on decomposition indices is discussed in the next chapter (4.2). A further possibility to explain the increased *k* in litter is that tea bags installed in litter were especially exposed to environmental fluctuations such as temperature, light conditions and moisture (Chapin et al., 2011). As these environmental factors are rather stable in the mineral soil compared to the litter layer (Chapin et al., 2011; Keuskamp et al., 2013), decomposition is therefore slower in the soil. This is particularly evident in Figure 25, where water contents vary strongly in the litter layer, but not in the mineral soil.

In this context, it is of great interest to investigate the influence of the water content determined in the tea bags at the time of their re-collection and how the moisture varies between the different treatments in litter and soil. Figure 25 presents that in the litter layer tea bags had a lower moisture content on the gaps than under closed canopy. At the same time, especially the G treatment shows slowest decomposition rates (Figure 29) and lowest mass loss of both tea types (Figure 24). The reason why the water content is lowest on the G treatment could be that the tea bags buried in the litter layer dry out more quickly due to the strong exposure to light and temperature, which inhibits the decomposition of the tea substrate. Wang et al. (2019) also observed slow decomposition rates in the litter layer under forest gaps and attributed it to dry and warm weather and increased light conditions during the summer months.

In the mineral soil, the gaps were wetter than C and D plots, as the soil samples taken in May showed as well. To repeat, this is because soils under forest gaps are exposed to higher precipitation and keep the humid conditions more stable (Keuskamp et al., 2013; Zhu et al., 2003). However, especially in the litter layer water contents should be interpreted with caution, as they only provide temporary information.

The relationship of mass loss and water contents is presented in Figure 31, showing significant positive interactions in the litter for both tea types. That decomposition increases with increasing water content is an important result of this study and in good agreement with general assumptions of Chapin et al. (2011) and studies by Mori et al. (2021) and Prescott (2010). While *S* in this study, as with Elumeeva et al. (2018), decreased with higher water content, the *k* increased. This was also observed by Mori et al. (2021), who conducted an incubation experiment testing the effect of water content on the TBI. Besides litter quality and microbial activity, the physical environment is a crucial factor controlling decomposition (Chapin et al., 2011). The origin of the significant influence of water content on decomposition may be the leaching effect. In this context, leaching is a physical process in which water dissolves organic components, nutrients and mineral ions from decomposing litter (Berg and McClaugherty, 2008; Chapin et al., 2011). These compounds are transported out of the detritus material (Berg and McClaugherty, 2008; Chapin et al., 2011), which causes the loss of mass. Especially during the first stages of decomposition, a high leaching rate can contribute strongly to mass loss of litter (Berg and McClaugherty, 2008; Djukic et al., 2018; Elumeeva et al., 2018). Since Wang

et al. (2019), attributed the mass loss of tea substrate partly to physical leaching in their investigation, it can be assumed that leaching also contributed to mass loss in this study. Furthermore, leaching is supported by high temperatures (Mori et al., 2021), which possibly occurred in forest gaps (GD treatment). For this reason, and due to the higher humidity in gaps (Zhu et al., 2003), the decomposition in gaps should be higher than under closed canopy as it was also found by Jerabkova et al. (2011). In the field experiment, the tea bags were often found uncovered in the litter layer, as gaps are generally known to have less leaf litter compared to closed stands (Lin et al., 2015; Zhu et al., 2003). This assumption was confirmed by field observations. The tea bags in the leaf litter are therefore particularly susceptible to leaching.

However, neither the average decomposition indices for the treatments nor linear models found a significantly higher decomposition on forest gaps. One aspect that should be considered is the above ground vegetation on the gaps. As a result of favourable light, temperature and moisture conditions, herbaceous vegetation has developed on the gaps (*Impatiens noli-tangere, Urtica dioica*). This vegetation could also have indirectly inhibited decomposition on the gaps, which also affects nutrient cycling and Carbon storage through the competition of plants for nutrients (Horwath, 2017; Long et al., 2016). It was observed that especially on forest gaps herbaceous cover can decrease solar radiation reaching the ground even more than under closed canopies (Perreault et al., 2020). Wang et al. (2019) reported inhibited mass loss of tea bag substrate due to plant cover as well.
It should be noted that only tall trees were felled as part of the FOX experiment. Although Ammer et al. (2020) state in their project description that all trees on the gaps were felled, areas with densely grown young beech trees up to 3 m high were observed on e.g. HEW05-G or HEW48-G. The decomposition in this case is subject to the effect of vegetation cover, even though these are gap areas. Thus, the experiment may be biased. High spatial variability is also evident on Figure 28 and Figure 30, where no clear trends in decomposition behaviour are visible. Since decomposition is influenced by many different factors (Keuskamp et al., 2013) and microbial organisms vary largely in their degradation behaviour and reaction to different soil conditions, there is a spatial and temporal heterogeneity (Chapin et al., 2011; Frey, 2007; Swift et al., 1979) and no uniform trends should be expected in general. The ANCOVA tables (Table 6, Table 8b, Table 9) demonstrate that variations of several parameters in the mineral soil were significantly explained by the location of the nine HEWs, which is indicated by the impact of the Plot ID. This may be due to the effect of different soil types (Luvisol, Stagnosol and presumably Leptosol on HEW32-GD and -D) and their inherent soil and vegetation characteristics, e.g., the high C_{org} content on HEW06-G or irregular vegetation cover on certain treatments. These observed examples can cause high spatial heterogeneity of soil properties and decomposition indices, that considerably complicate the statistical detection of significant effects of forest gaps on soil properties, microbial activity and decomposition in models.

So far, the results of this study show that a sole analysis of the forest gaps is not sufficient to explain considerable differences in soil properties, microbial activity and degradation. There

are significant interactions between the three biogeochemical parameter groups, which will be examined in more detail in the following chapter.

4.2 Interaction of soil properties, microbial activity and tea bag decomposition indices

The results of this study have shown that microbial parameters such as C_{mic} and enzyme activity were significantly related to abiotic soil properties (Table 6). This observation is in good agreement with a number of studies (Andersson et al., 2004; Herold et al., 2014b; Liu et al., 2020; Perreault et al., 2020; Solly et al., 2014). These relationships exist because the activity of microorganisms is controlled by numerous factors in the immediate environment that surrounds them (Baldrian, 2014; Loeppmann et al., 2020). Therefore, microbial activity is affected by, e.g. temperature, precipitation, litter quality, dominant vegetation and the presence of plant roots (Baldrian, 2014), nutrient and Carbon sources and also the topography shaping the microclimate (Berg and McClaugherty, 2008). Among others, Horwath (2017) describes soil factors such as mineralogy, parent material, particle size and soil pH as important measures impacting microbial biomass. The composition and structures of microbial communities and populations are controlled by general soil properties as well, especially N concentrations in litter and humus (Berg and McClaugherty, 2008). As soil enzymes are mainly of microbial origin (Beck and Beck, 1996), there are also significant relationships of C_{mic} and enzyme activities. This is proven by this study (App. Table IV, App. Figure II). Increasing enzyme activities in mineral soils with higher C_{mic} contents point to the fact that enzyme activities are related to active microorganisms that produce enzymes (Andersson et al., 2004). Moreover, it was found, that 65 % of the variance of C_{mic} is explained by C_{org} contents (Figure 23). The high correlation of C_{mic} and C_{org} is generally known (Andersson et al., 2004; Srinivasarao et al., 2017). This is because organic Carbon compounds are a major food source for microbes in the soil organic matter, that is processed and transformed into secondary Carbon sources and nutrients (Loeppmann et al., 2020). Thus microbial activity and mineralisation increases with higher resource availability (Herold et al., 2014b; Horwath, 2017). This is why enzyme activities were not only related to C_{mic} but also significantly corresponded to C_{org} (Beck and Beck, 1996), as shown by Table 6. This positive relationship is in line with Herold et al. (2014b), Sinsabaugh et al. (2008) and Meena and Rao (2021). Especially Štursová and Baldrian (2011) and Srinivasarao et al. (2017) highlight the importance of C_{org} as the main factor regulating the microbial and enzymatic activity in soils.

Further there was a significant negative interaction of C_{mic} and C/N ratio, indicating N availability and activity of microorganisms (App. Figure II). As expected, soils with a high C/N-ratio showed lower microbial activity (Bartsch and Röhrig, 2016). Enzyme activities also increased with increasing N and S contents.

The pH value is often mentioned as one of the most important factors influencing soil enzymes (Baldrian, 2014; Štursová and Baldrian, 2011). As with Sinsabaugh et al. (2008), all potential enzyme activities in this study were significantly influenced by the pH value (Table 6). The

more acidic the pH value, the lower the enzyme activity, because low soil pH is found to limit the mobility of enzymes (Štursová and Baldrian, 2011) as soil microbes react sensitively to soil chemical properties (Baldrian, 2014). In addition to a direct influence of pH on the microbial community, pH can also have an indirect effect by shaping nutrient availability and C_{org} content in the soil (Rousk et al., 2010; Zheng et al., 2019). As an exception for the interaction of pH and enzymatic activity, sPhos showed declining activity with increasing pH. This is due to the fact that the acid phosphatases have their optimum in the acidic range of 4-5 (Turner, 2010). Comparable results to this significant negative relationship were also obtained by Acosta-Martínez and Tabatabai (2000) and Herold et al. (2014b). A significant relationship of pH with water and estimated clay content, C_{org}, N and S content is also observed in this study and displayed in correlation matrix in App. Figure II. The significant positive relationships between enzyme activities and estimated clay and water content can be attributed to the fact that soil properties such as grain size and texture regulate the air, water and energy balance in soils and thus impact the living conditions of soil microorganisms (Baldrian, 2014; Beck and Beck, 1996). Clay minerals and their interaction with organic matter and the formation of clay-humus complexes essentially determine the stabilisation of enzymes (Burns, 1982). In line with the results of this study Herold et al. (2014a) found a highly positive relationship between clay content and enzyme activities.

Next, it will be considered to what extent decomposition is related to abiotic parameters in the mineral soil. Regarding the impact of the pH value, it was found that the decomposition of green tea is higher in the more acidic milieu than at higher pH values. This observation is contrary to numerous other studies using litter bags. Tóth et al. (2018), for example, report a positive effect of the pH value on decomposition, as the alkaline milieu promotes a better environment for soil microorganisms to decompose soil organic matter. So although decomposition should increase with higher pH (Djukic et al., 2010), the trend is reversed in this study. Stabilisation (*S*) also increased (weakly) with increasing pH, while the relationship was negative at Elumeeva et al. (2018). However, they also attributed the mass loss to leaching. The effect of this process was already described in the previous chapter (4.1). A possible explanation for the unexpected relationship between pH value and decomposition is that certain microbial players, especially fungi, may have their optimum in the acidic milieu (Walse et al., 1998), resulting in a higher abundance of microorganisms and thus decomposition degree. This relationship could be confirmed by examining the microbial community composition, as this is closely related to the pH value (Elumeeva et al., 2018).
In line with assumptions of Berg and McClaugherty (2008), high N and S contents promoted microbial activity and led to a higher decomposition rates (App. Figure II). Although the correlations of decomposition rate (*k*) and N and S contents as shown in App. Figure VII and App. Figure VIII are not very strong, the results underline the importance of nutrients for the microbial breakdown of cellulose, hemicelluloses and other solubles (Berg and McClaugherty, 2008). Purahong et al. (2016) also found a positive effect of macronutrients on communities of

fungi and bacteria.

In this study, however, no correlations between the C/N-ratio and decomposition indices were found and also enzyme activities showed no or only a marginally significant influence (BG) on the decomposition (App. Figure II). This suggests that other processes such as leaching (Elumeeva et al., 2018) or even spatial differences, have contributed more to decomposition as it is shown in Table 8. Djukic et al. (2010) also describe important variables such as MAT, MAP, and litter quality, which were not or not sufficiently investigated in this study but are found to significantly impact decomposition measures. In addition, when comparing the soil data with tea bag indices, it is important to note that in this study the data on abiotic soil parameters, microbial biomass and enzyme activity were obtained from soil samples taken in spring (May). The tea bag indices, though, reflect a period of 90 days during summer months (May-August). The fact that no relationship was found between the indicators of soil health (C/N-ratio, C_{mic} and enzyme activity) studied in the laboratory and the decomposition indices studied in the field is in good agreement with the comparative study by Middleton et al. (2021). They observed no or only negative correlations of laboratory measurements and field TBI decomposition data, too.

However, the Table 9 shows that there is a significant influence of C_{mic} on the decomposition of green tea and a marginal impact on stabilisation factor (*S*), when considering treatment and site-information (Plot ID) as covariables in linear models. Contrary to general assumptions, the decomposition did not increase with increasing microbial biomass, but decreased. The relationship was therefore negative ($R^2 = 0.10$) (App. Figure IX). This surprising relationship could be because in the tea bag experiment at sites with a high microbial presence, there was not only a mass loss of the tea substrate but also a mass increase by microorganisms and therefore the decomposition appeared lower. To be more precise, especially fungi may have caused a mass increase as they are the first invaders of fresh substrate through their filamentous growth (Berg and McClaugherty, 2008; Paul, 2015). In forest soils fungi usually prevail the microbial community (Loeppmann et al., 2020), while bacteria are less mobile and colonise new substrate at later decomposition stages (Berg and McClaugherty, 2008). Nevertheless, both groups of organisms should be taken into account, as they have different decomposition behaviours according to the type of habitat, e.g. deadwood (Tóth et al., 2018). An increase in mass can have various causes, which are also related to each other. Firstly, an ingrowth of microbial biomass is possible and also the transport and accumulation of microbial side products and nutrients by microorganisms can cause an increase in mass (Berg and McClaugherty, 2008; Ochoa-Hueso et al., 2020). For example, in a litter bag experiment conducted by Purahong et al. (2016) an accumulation of N by nitrogen-fixing bacteria (diazotrophs) was observed. Moreover, there is also a potential of soil entering the tea bags and fine roots (Keuskamp et al., 2013) which were also found in this study but were removed as far as possible. Even soil macrofauna (earthworm) was found inside of one tea bag buried in the mineral soil (App. Figure IV). Tóth et al. (2018) have used the TBI approach as well and found

a negative relationship between soil organic matter content and *k*. They attributed this to the observation that mineralisation has been less intensive than humification, which has led to an accumulation of soil organic matter (Tóth et al., 2018). For the results of this study, it can be assumed that at sites with a high microbial biomass (e.g. HEW32-GD, -D) the loss of mass through decomposition is influenced by an increase in mass through soil organisms and fine roots. However, temporal as well as spatial aspects of colonisation by fungi and decomposition have to be taken into account. In this study, the different soil types (Luvisol, Stagnosol and presumably Leptosol) and their soil properties may have influenced the decomposition results. An impact of local environmental fluctuations of temperature and precipitation during the summer months is possible. Leaching as a physical effect must be considered as an influence on weight loss (Chapin et al., 2011) and that external and indirect environmental factors can have had just as much of an impact as internal microbial ones (Andersson et al., 2004; Wang et al., 2019). The quality of the litter and the soil are particularly crucial for decomposition (Chapin et al., 2011; Tóth et al., 2018). In this study these possibly influencing factors make decomposition a highly heterogeneous process.

The identified relationships of this chapter highlight the complexity of the interaction of abiotic soil properties, microbial activity, and decomposition. If forest management measures such as gap formation or deadwood enrichment are to be assessed in terms of their effect on biodiversity and structural richness, the interactions between abiotic and microbial parameters and, if possible, more influencing variables and processes should be considered.

4.3 Limitations of the study and suggestions for improvement

As already pointed out in the previous chapters, there are some limitations of this study. One limiting factor is the variability of soil properties. This is indicated by the significant impact of the HEW locations (Plot ID) for different soil properties, microbial activities, and decomposition indices. Apparently, the soil characteristics are depending on the HEW, although the nine replicates should be well comparable to enable the identification of treatment effects. Since there are three different soil types with different soil characteristics, these site-specific differences complicate the determination of the impact of forest gaps and deadwood addition in the forest gap experiment. Differences in site conditions are particularly evident on HEW06-G and HEW32-GD, where soil properties have not been fully identified. While the experimental or control plots (C) in the Hainich-Dün Biodiversity Exploratory are well monitored in terms of their soil properties, important data such as the soil type and soil texture but also the litter quality is lacking for the treatments. Information on these measures should be obtained for future research and comparisons of treatment and control plots.
In this study, the number of soil samples was reduced from originally 117 to 36 by preparing composite samples to improve the feasibility of the analyses. Thereby, important information about spatial differences may have been lost. For a greater statistical power and better understanding of small-scale spatial variability of soil data, it is advisable to examine more

observations by analysing samples of the N-E-S-W sub-plots on the treatments and the control plots, instead of working with composite samples.

Four extracellular enzymes were investigated in this study but analysing further enzymes with diverse functions may provide new insights into microbial activity. In addition, data on the community composition of bacteria and fungi would be helpful to analyse colonisation patterns and the impact of deadwood.

With regard to the influence of the timing of soil sampling it should be noted that the microbial parameters captured in this study reflect the microbial biomass and potential enzyme activities at the time of sampling in May 2021. As there are indications of higher microbial activity, e.g. of cellulase and chitinase in July (Andersson et al., 2004), re-sampling during summer time should be considered for subsequent studies in this field. This is also recommended for improving the comparability of soil data with tea bag indices, which cover a period of 90 days. Another limitation is that the tea bag indices in the litter layer cannot be linked to the soil data in this study. It is thus advisable to examine the microbial activity not only in the mineral soil but also in the litter and to investigate carbon stocks in the organic layer, which may be more sensitive to changes in forest management.

A methodical weakness of the tea bag experiment is that the indices do not provide information on the contribution of leaching of organic matter and microbial decomposition to the mass loss of tea substrate. As mentioned above, the mass loss, which presents the basis for calculating the decomposition indices, can also be biased by an increase in mass, e.g., due to fine roots or fungal spores. Despite many replicates, there was a high sample loss of tea bags locally on some plots, especially in the litter layer. There the sample loss was more than five times higher than in the mineral soil. Several alternative field methods using inexpensive household items for analysing biological activity are currently being discussed (Middleton et al., 2021). Some of these methods, could provide results with less sample loss. According to Middleton et al. (2021) the application of bleached cotton underwear can be useful in detecting significant treatment effects. However, in this study, the TBI method emerged as a suitable and efficient approach to characterise differences in decomposition behaviour in litter and soil, detect the influence of water content and describe decomposition as a spatial heterogeneous process.

A regular measurement and monitoring of temperature and humidity, that affect various biological and physico-chemical parameters, would improve the knowledge of the impact of these environmental variables, especially in forest gaps. Further, the observation period of only one year after forest gap formation in this study might not be long enough to detect treatment effects. Besides a detailed and regular assessment of abiotic properties and vegetation patterns on the FOX plots, decomposition studies should be continued over a longer period of time. The investigation of soil parameters and environmental factors in the beginning of an ecological experiment is particularly important in order to have a basis for comparison in later studies. This fundamental initial record on the effect of forest gaps is provided by the results of this thesis.

5 Conclusion

The main objective of this study was to investigate the effect of forest gaps on soil properties, microbial biomass, enzymatic activity, and decomposition in mineral soils under different treatments (i). It was initially hypothesised, that forest gaps would promote microbial activity and decomposition. The results of this study do not provide full evidence of a positive effect of forest gaps.

Although gap sites had on average a higher water content and slightly higher microbial biomass and enzymatic activity, linear models revealed that when soil properties (C_{org} and pH) were considered as covariables, the treatment had no significant effect on microbial biomass and the activity of NAG, Sulf and Phos enzyme activity. However, forest gaps had a significant positive impact on the activity of BG, an enzyme involved in the breakdown of cellulose. Furthermore, it was found that physico-chemical soil properties explained a significant proportion of variation in microbial biomass and enzyme activities. All enzyme activities were positively related to pH (except for Phos), element concentration of C_{org}, N and S, clay and water content and C_{mic} (objective iii). These results confirm the assumption that microbial parameters are significantly related to soil properties.

Addressing the second objective of this study (ii), differences in the TBI decomposition behaviour were found with respect to tea type and the depth the tea bags were buried in (leaf litter, mineral soil). The decomposition rate *k* was higher in the leaf litter than in mineral soil. The results also showed that the decomposition of tea substrate in litter and soil was better explained by water content than by treatment with gaps and deadwood. The water content significantly promoted the mass loss of the tea material, especially in the litter layer. Although no correlation between enzymatic activity and decomposition indices could be detected, the decomposition of the green tea decreased with increasing C_{mic}. The assumption that high microbial activity is reflected in high decomposition cannot be confirmed in this study, because processes such as leaching of tea substrate and a fungal colonisation have likely influenced the mass loss of tea substrate.

Further, this study found spatial variability of soil related properties, due to specific abiotic soil and site conditions. Since the location of the investigated study sites (Plot ID) significantly explained variations in microbial parameters and decomposition indices, the spatial heterogeneity represents an uncertainty of this study that made it difficult to detect significant effects of forest management treatments. Although it was not possible to draw clear conclusions about the influence of forest gaps in this study after one year of gap formation, the FOX promises to provide great potential for further insights into functional relationships in a longer investigation period. Several studies report the positive effect of deadwood in forest gaps, which promotes microbial structure and activity with the ongoing decay of deadwood. According to the current state of research especially small gaps seem to have a positive influence on microbial activity and biodiversity, which illustrates the value of the forest gap experiment.

To answer the question of how the forests may benefit from canopy openings in terms of structural diversity and biodiversity at the microbial level, additional long-term research on soil microclimate and different drivers of microbial activity and decomposition is necessary. A single experimental study cannot fully describe the created spatial and temporal diversity of ecological conditions around forest gaps. Therefor more robust data on biological and functional aspects in forest ecosystems are needed.

All in all, the results of this study highlight the importance of the interaction of abiotic soil properties, microbial activity and decomposition indices that is crucial for the understanding of biogeochemical cycles. This thesis provides an initial basis for monitoring and further research on the influence of forest gaps on abiotic, microbial and decomposition parameters in the context of the forest gap experiment and functional biodiversity research in the Hainich-Dün Biodiversity Exploratory. Thereby, this work contributes to a better understanding of the complexity of forest ecosystems and thus supports environmental science in its goal of developing methods that promote biodiversity and implement a sustainable management of forests to counteract climate change.

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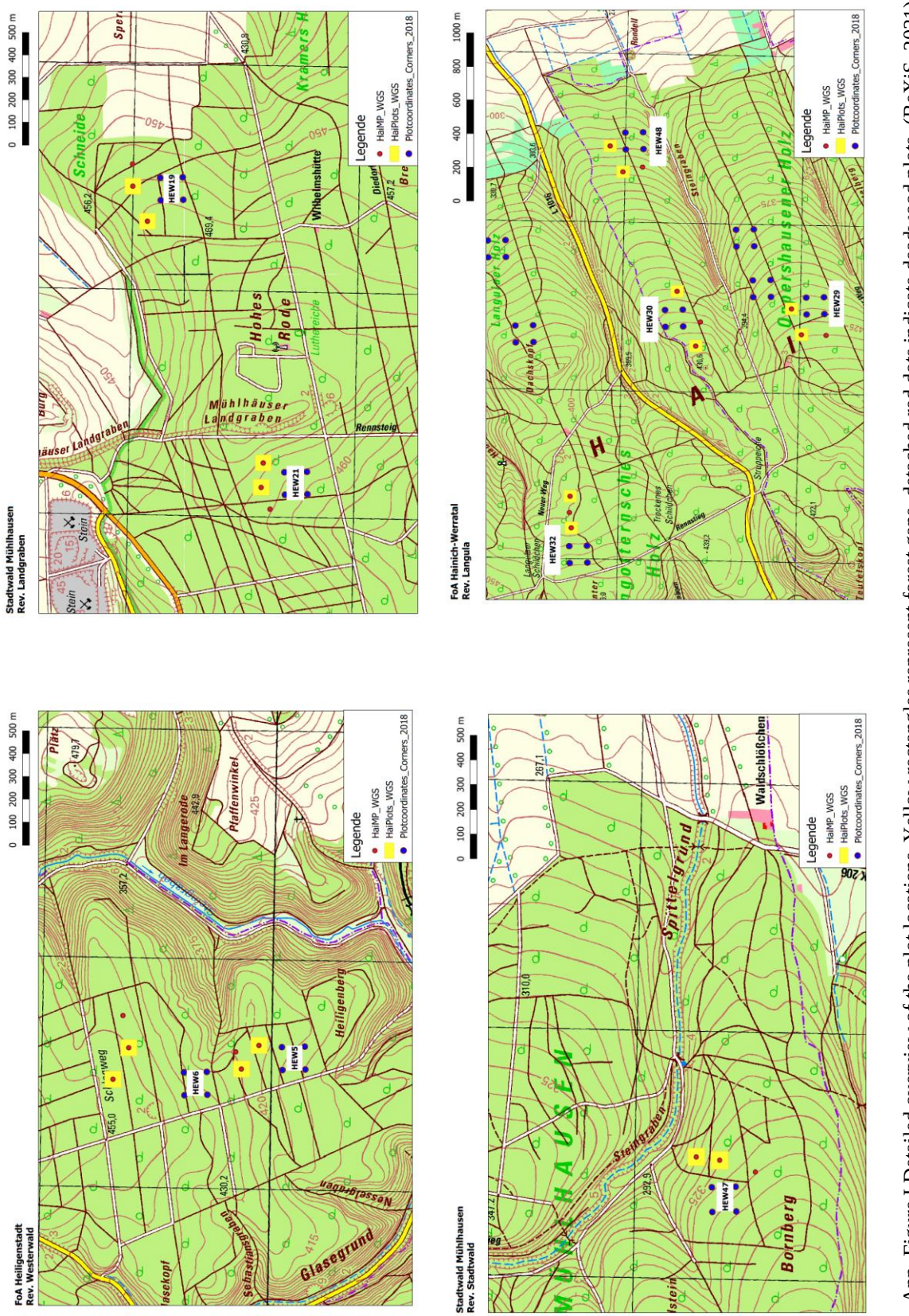
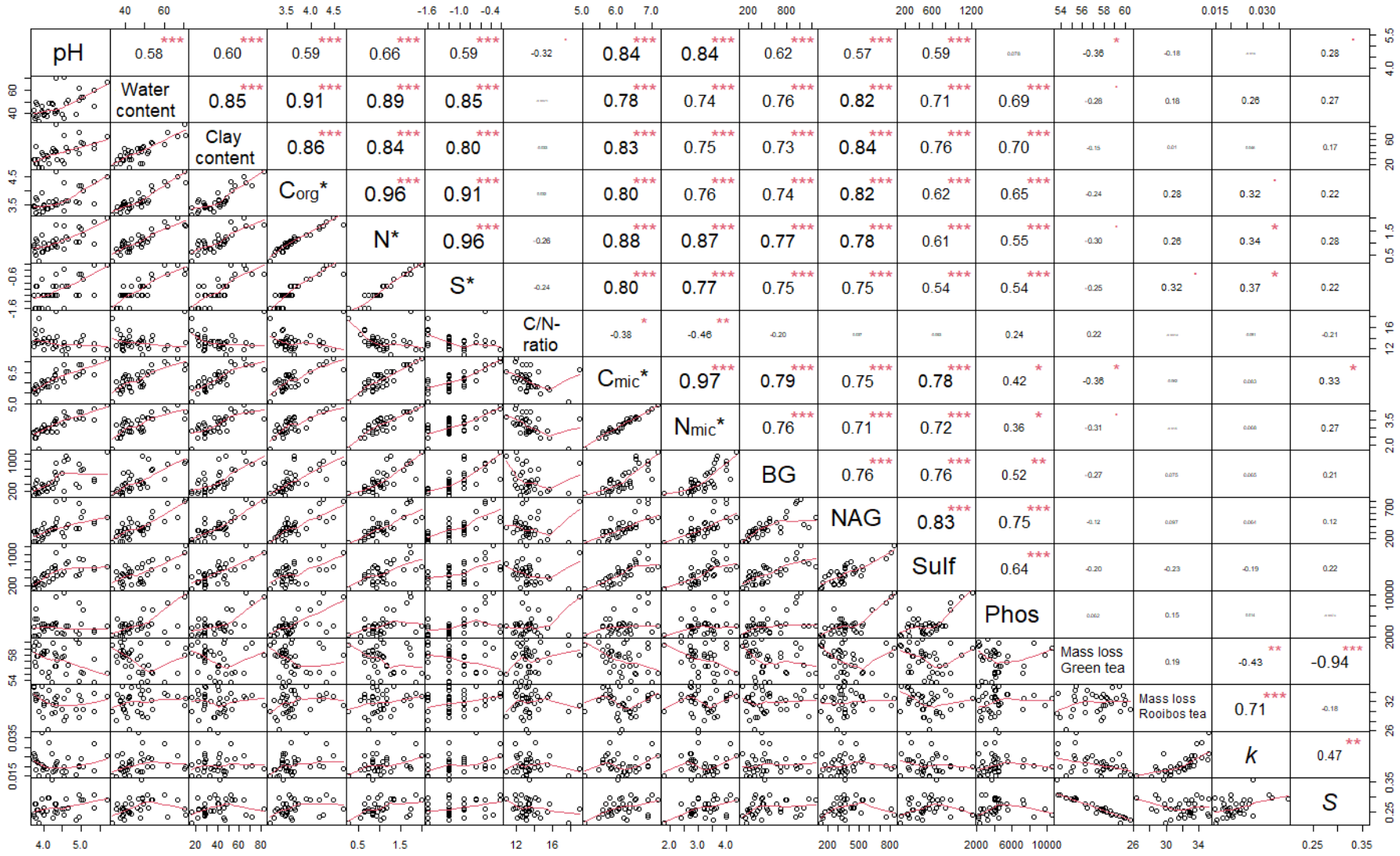
Appendix A



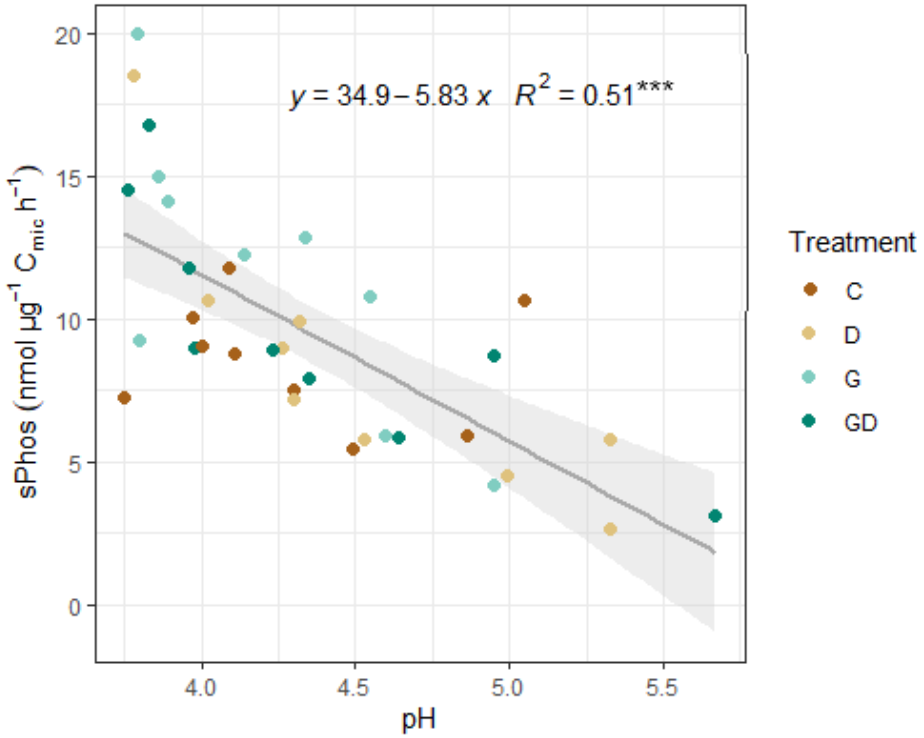
App. Figure I Detailed overview of the plot locations. Yellow rectangles represent forest gaps, detached red dots indicate deadwood plots. (BeXiS, 2021).



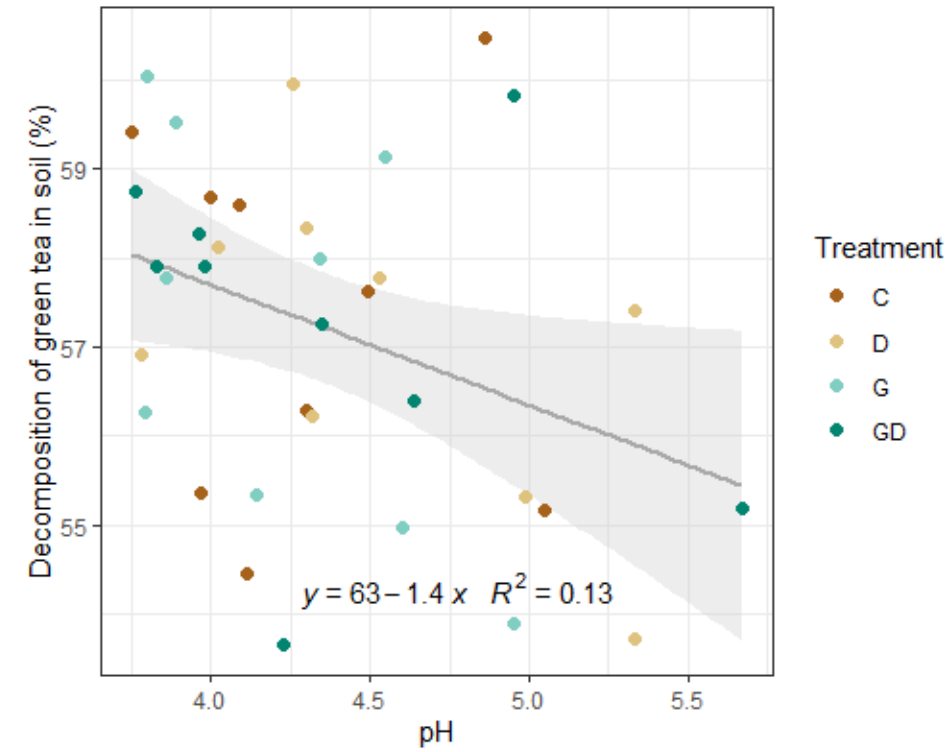
App. Figure II Correlation matrix for abiotic and microbial parameters and decomposition indices (Pearson). Black asterisks indicate log-transformation of parameters. Red asterisks indicate significance levels: *p<0,05; **p<0,01; ***p<0,001.

App. Figure III Shallow soil (presumably Leptosol) with high gravel content on HEW32-GD (Enyedi, 2021).

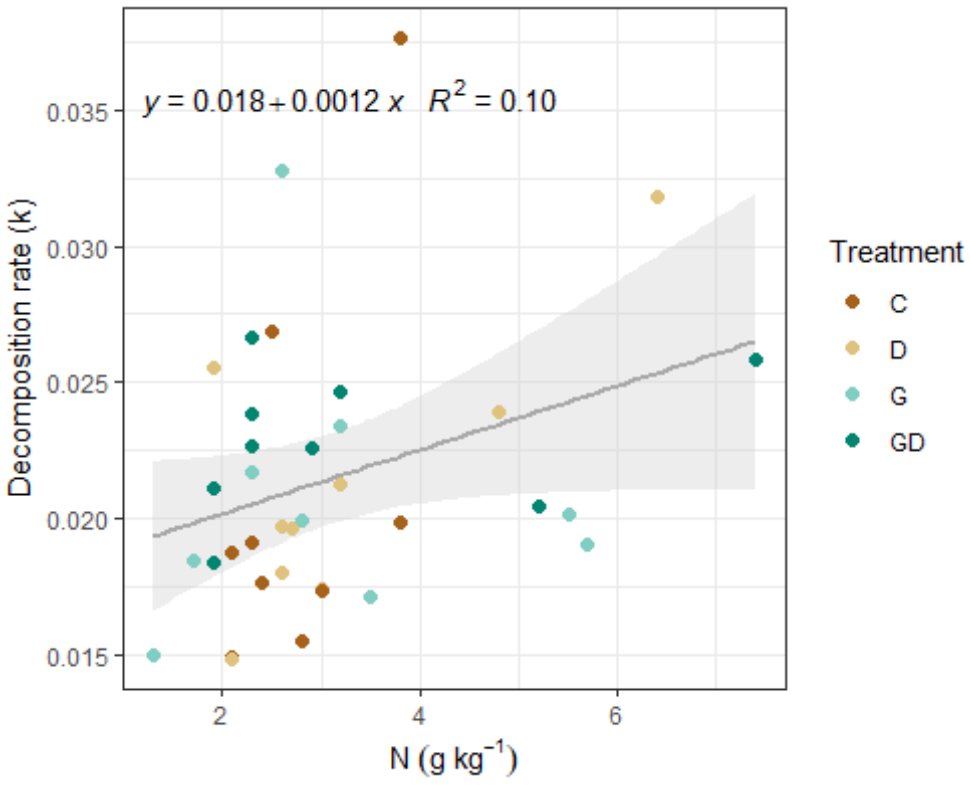
App. Figure IV Tea bag with earthworm inside taken from mineral soil on HEW29-G.



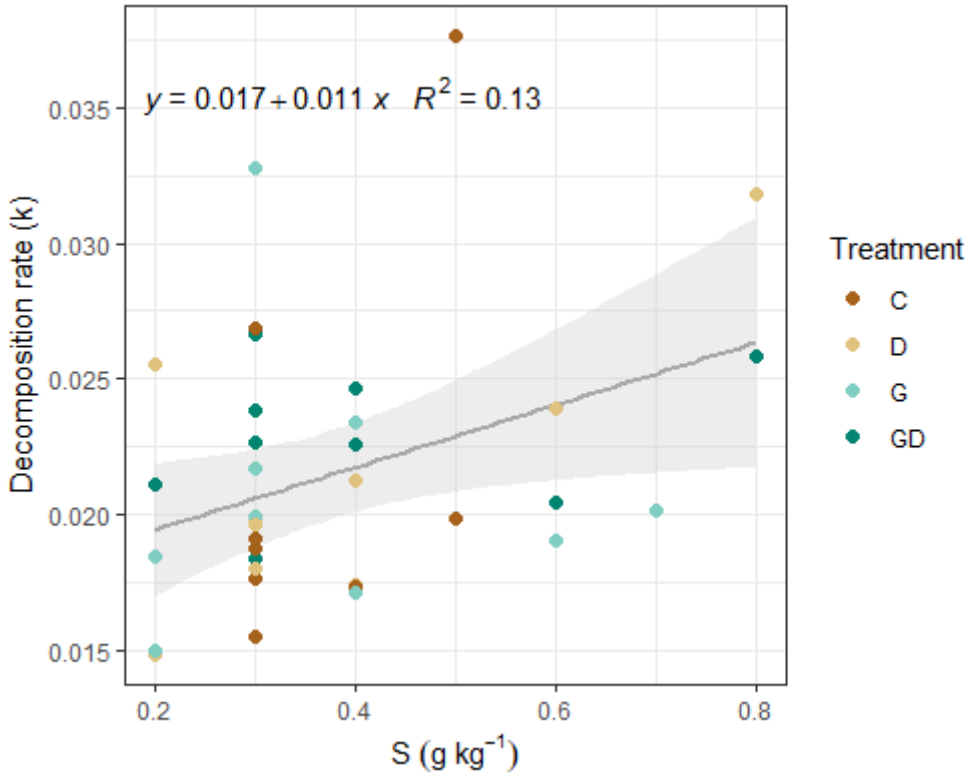
App. Figure V Linear model of pH and specific Phos enzyme activity with 36 observations and the regression equation. Significance is assessed by + <0,1; *p<0,05; **p<0,01; *** p<0,001.



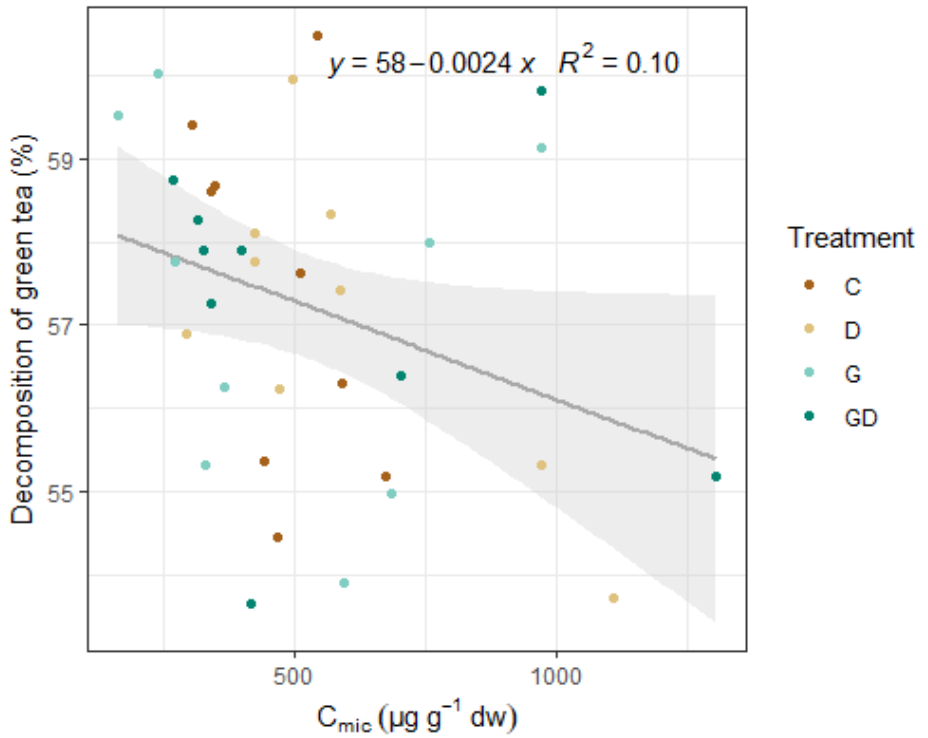
App. Figure VI Linear model of pH and decomposition of green tea in soils (%) with 36 observations and the regression equation.



App. Figure VII Linear model of N content and decomposition rate (k) with 36 observations and the regression equation.



App. Figure VIII Linear model of S content and decomposition rate (k) with 36 observations and the regression equation.



App. Figure IX Linear model of microbial biomass C_{mic} and decomposition of green tea (%) with 36 observations and the regression equation.

App. Table I Overview of abiotic and microbial parameters for each mineral soil sample (n = 36).

Plot ID	Treat-ment	pH	Residual water content	Water content	Dry matter content	Estimated clay content	C_{org}	C_{inorg}	C	N	S	C/N-ratio	C_{mic}	N_{mic}	BG	NAG	Sulf	Phos	sBG	sNAG	sSulf	sPhos
			(%)			(g kg^{-1})	(g kg^{-1})						(µg g^{-1})		(nmol g^{-1} dw h^{-1})				(nmol µg^{-1} C_{mic} h^{-1})			
HEW05	C	5,1	2,82	53,93	63,13	52,92	54,5	0,5	55,0	3,8	0,5	14,4	675	34	804,72	627,91	872,24	7178,79	1,19	0,93	1,29	10,64
HEW05	G	4,6	3,86	70,81	56,28	66,65	71,8	0,4	72,2	5,5	0,7	13,1	972	54	1012,81	837,48	1213,42	10481,69	1,04	0,86	1,25	10,78
HEW05	GD	5,0	4,23	61,82	59,19	71,54	65,1	0,3	65,4	5,2	0,6	12,6	970	54	936,31	761,07	879,80	8445,14	0,97	0,78	0,91	8,71
HEW05	D	5,0	3,03	53,60	63,13	55,7	65,0	0,3	65,2	4,8	0,6	13,6	970	54	781,45	539,32	597,52	4390,04	0,81	0,56	0,62	4,53
HEW06	C	4,3	1,85	40,01	70,10	40,12	32,4	0,0	32,4	2,4	0,3	13,4	590	29	432,24	363,82	713,14	4443,72	0,73	0,62	1,21	7,53
HEW06	G	4,3	5,12	70,11	55,78	83,28	107,6	0,3	107,9	5,7	0,6	18,9	757	35	959,67	805,87	1045,69	9718,56	1,27	1,06	1,38	12,84
HEW06	GD	4,0	1,54	41,06	69,80	36,03	25,5	0,0	25,5	1,9	0,3	13,4	399	17	338,91	215,02	466,00	3599,08	0,85	0,54	1,17	9,02
HEW06	D	4,3	2,31	47,80	66,10	46,19	36,2	0,1	36,3	3,0	0,4	12,1	494	26	279,25	336,39	375,97	4428,23	0,57	0,68	0,76	8,96
HEW19	C	4,5	0,87	38,90	71,37	27,18	35,0	0,0	35,0	3,0	0,4	11,6	511	28	695,59	302,84	465,14	2786,79	1,36	0,59	0,91	5,45
HEW19	G	4,6	2,40	48,91	65,54	47,38	38,2	0,2	38,4	3,5	0,4	10,9	685	40	1151,67	381,62	748,68	4066,56	1,68	0,56	1,09	5,94
HEW19	GD	4,6	1,89	51,63	64,71	40,65	38,3	0,2	38,5	3,2	0,4	12,1	702	38	1065,53	424,03	799,66	4137,71	1,52	0,60	1,14	5,89
HEW19	D	4,3	1,98	41,82	69,12	41,84	32,5	0,1	32,6	2,6	0,3	12,6	570	31	705,01	370,00	629,44	4095,27	1,24	0,65	1,10	7,18
HEW21	C	4,1	1,32	42,26	69,36	33,12	28,0	0,2	28,2	2,1	0,2	13,1	466	20	283,64	285,29	561,95	4110,98	0,61	0,61	1,21	8,82
HEW21	G	5,0	1,64	51,59	64,89	37,35	34,6	0,2	34,8	2,8	0,3	12,6	593	36	308,73	357,81	481,29	2494,16	0,52	0,60	0,81	4,21
HEW21	GD	4,2	1,96	48,41	66,06	41,57	30,5	0,2	30,7	2,3	0,3	13,4	416	16	441,77	360,60	663,91	3713,61	1,06	0,87	1,60	8,93
HEW21	D	5,3	2,27	46,12	66,88	45,66	34,0	0,1	34,1	2,6	0,3	13,0	587	32	475,88	447,94	666,22	3399,92	0,81	0,76	1,13	5,79
HEW29	C	4,0	0,93	39,25	71,15	27,98	32,5	0,0	32,5	2,3	0,3	14,0	347	18	255,43	263,71	233,33	3151,86	0,74	0,76	0,67	9,08
HEW29	G	4,1	0,47	48,71	66,93	21,9	36,3	0,1	36,5	2,6	0,3	14,1	327	20	412,73	327,24	292,40	4017,11	1,26	1,00	0,89	12,28
HEW29	GD	3,8	0,48	47,70	67,38	22,04	39,3	0,2	39,5	2,9	0,4	13,6	326	18	493,44	332,87	309,20	5465,43	1,51	1,02	0,95	16,77
HEW29	D	4,0	0,93	38,66	71,45	27,98	31,7	0,1	31,9	2,7	0,3	11,9	424	25	368,24	444,59	501,56	4513,35	0,87	1,05	1,18	10,64
HEW30	C	3,8	0,97	38,33	71,59	28,5	31,5	0,3	31,7	2,5	0,3	12,4	305	15	99,08	146,83	137,81	2218,64	0,32	0,48	0,45	7,27
HEW30	G	3,9	0,95	38,59	71,47	28,24	29,6	0,2	29,8	2,3	0,3	13,1	269	14	180,72	241,71	306,85	4039,50	0,67	0,90	1,14	15,02
HEW30	GD	3,8	0,98	43,13	69,19	28,64	30,1	0,2	30,3	2,3	0,3	13,1	266	16	301,39	306,37	227,73	3867,38	1,13	1,15	0,86	14,54
HEW30	D	4,3	1,93	43,16	68,50	41,18	39,3	0,2	39,5	3,2	0,4	12,4	472	26	633,40	461,50	551,89	4684,95	1,34	0,98	1,17	9,93
HEW32	C	4,0	1,85	46,53	66,98	40,12	49,8	0,2	50,0	3,8	0,5	13,3	443	20	401,54	355,14	235,71	4444,60	0,91	0,80	0,53	10,03
HEW32	G	3,8	2,48	49,31	65,32	48,44	42,0	0,2	42,2	3,2	0,4	13,3	365	17	421,79	261,53	251,51	7280,94	1,16	0,72	0,69	19,95
HEW32	GD	5,7	3,67	66,85	57,74	64,14	88,5	0,5	89,0	7,4	0,8	12,0	1306	78	1251,26	532,61	684,69	4068,80	0,96	0,41	0,52	3,12
HEW32	D	5,3	2,80	60,07	60,72	52,66	75,3	0,4	75,7	6,4	0,8	11,8	1111	62	532,51	469,36	595,30	2984,41	0,48	0,42	0,54	2,69
HEW47	C	4,9	2,33	39,71	69,91	46,46	36,5	0,0	36,5	2,8	0,3	13,1	542	34	396,88	364,85	447,95	3208,09	0,73	0,67	0,83	5,92
HEW47	G	3,8	0,47	37,29	72,50	21,9	25,0	0,0	25,0	1,7	0,2	14,6	237	11	149,30	151,29	262,10	2199,68	0,63	0,64	1,11	9,28
HEW47	GD	4,4	0,95	41,95	69,78	28,24	32,9	0,2	33,1	2,3	0,3	14,3	341	16	529,36	524,32	452,73	2702,18	1,55	1,54	1,33	7,92
HEW47	D	4,5	0,49	35,50	73,44	22,17	26,8	0,0	26,8	2,1	0,2	12,7	424	25	204,44	225,82	559,79	2458,98	0,48	0,53	1,32	5,80
HEW48	C	4,1	1,39	37,41	71,76	34,05	28,4	0,2	28,5	2,1	0,3	13,3	340	16	214,12	317,92	442,91	4017,49	0,63	0,94	1,30	11,82
HEW48	G	3,9	0,00	33,61	74,85	15,7	23,2	0,0	23,2	1,3	0,2	17,7	161	6	160,87	137,44	114,30	2277,17	1,00	0,85	0,71	14,14
HEW48	GD	4,0	0,00	42,14	70,35	15,7	29,8	0,0	29,8	1,9	0,2	15,4	316	17	199,27	175,80	437,16	3728,03	0,63	0,56	1,38	11,80
HEW48	D	3,8	0,95	36,15	72,76	28,24	30,3	0,0	30,3	1,9	0,2	15,7	294	11	256,49	312,95	384,05	5434,22	0,87	1,06	1,31	18,48

App. Table II Detailed overview of decomposition indices (stabilisation factor S, decomposition rate k) and tea bag water content in litter and mineral soil.

					Green tea		Rooibos								Green tea		Rooibos	
Plot ID	Treat-ment	Depth	*S*	*k*	water content (%)	decom-position (%)	water content (%)	decom-position (%)	Plot ID	Treat-ment	Depth	*S*	*k*	water content (%)	decom-position (%)	water content (%)	decom-position (%)
HEW05	C	Litter	0,236	0,021	211,49	59,56	173,22	34,12	HEW05	C	Mineral soil	0,293	0,019	168,45	55,17	140,46	30,47
HEW05	D	Litter	0,270	0,025	149,15	56,94	202,21	33,89	HEW05	D	Mineral soil	0,292	0,023	170,51	55,31	137,92	32,41
HEW05	G	Litter	0,187	0,034	217,01	62,87	205,66	39,95	HEW05	G	Mineral soil	0,242	0,020	259,86	59,15	197,58	32,81
HEW05	GD	Litter	0,279	0,028	168,64	56,54	173,31	34,49	HEW05	GD	Mineral soil	0,234	0,020	199,41	59,83	192,60	33,32
HEW06	C	Litter	0,265	0,023	224,23	55,96	199,20	34,13	HEW06	C	Mineral soil	0,279	0,017	178,62	56,30	115,98	29,75
HEW06	D	Litter	0,268	0,032	290,29	55,95	181,87	35,73	HEW06	D	Mineral soil	0,231	0,017	199,84	59,96	183,90	31,47
HEW06	G	Litter	0,256	0,023	158,37	58,90	153,41	33,41	HEW06	G	Mineral soil	0,264	0,019	271,11	57,99	239,63	31,19
HEW06	GD	Litter	0,229	0,032	137,75	60,24	211,82	37,96	HEW06	GD	Mineral soil	0,257	0,018	234,51	57,91	184,51	28,82
HEW19	C	Litter	0,259	0,030	170,85	57,77	177,89	36,54	HEW19	C	Mineral soil	0,263	0,017	181,40	57,63	164,16	30,48
HEW19	D	Litter	0,193	n.d.	253,34	63,03	213,73	42,35	HEW19	D	Mineral soil	0,254	0,019	184,01	58,33	161,39	32,65
HEW19	G	Litter	0,304	0,017	34,84	55,43	91,14	29,88	HEW19	G	Mineral soil	0,296	0,017	172,43	54,97	153,85	28,99
HEW19	GD	Litter	0,230	0,025	220,58	59,97	235,64	36,11	HEW19	GD	Mineral soil	0,266	0,024	211,16	56,40	200,16	34,38
HEW21	C	Litter	0,245	0,024	190,74	59,00	185,90	32,40	HEW21	C	Mineral soil	0,299	0,014	192,14	54,45	148,95	26,31
HEW21	D	Litter	0,188	0,023	280,72	62,90	229,99	36,61	HEW21	D	Mineral soil	0,265	0,018	189,58	57,41	134,77	29,97
HEW21	G	Litter	0,314	0,025	90,98	53,70	175,15	32,03	HEW21	G	Mineral soil	0,310	0,019	237,66	53,90	191,13	28,71
HEW21	GD	Litter	0,255	0,021	194,75	58,25	158,83	32,74	HEW21	GD	Mineral soil	0,362	0,026	222,19	53,65	177,31	29,71
HEW29	C	Litter	0,247	0,032	109,70	57,18	141,62	36,78	HEW29	C	Mineral soil	0,244	0,019	175,97	58,68	137,81	31,76
HEW29	D	Litter	0,258	0,024	81,46	57,95	80,56	33,68	HEW29	D	Mineral soil	0,266	0,019	188,43	58,12	140,64	31,35
HEW29	G	Litter	0,260	0,025	84,32	57,24	153,64	34,31	HEW29	G	Mineral soil	0,302	0,031	215,59	55,33	178,23	34,39
HEW29	GD	Litter	0,278	0,025	47,91	56,50	111,90	33,86	HEW29	GD	Mineral soil	0,259	0,022	203,90	57,91	148,27	33,52
HEW30	C	Litter	0,268	0,030	44,36	57,18	100,67	35,47	HEW30	C	Mineral soil	0,239	0,026	209,61	59,41	160,07	35,24
HEW30	D	Litter	0,268	0,024	64,81	57,24	91,39	33,62	HEW30	D	Mineral soil	0,281	0,021	156,32	56,23	108,45	31,69
HEW30	G	Litter	0,275	0,018	49,63	56,64	66,49	30,17	HEW30	G	Mineral soil	0,261	0,021	163,33	57,77	135,15	33,03
HEW30	GD	Litter	0,235	0,024	70,37	59,62	154,88	35,20	HEW30	GD	Mineral soil	0,246	0,023	240,62	58,75	181,39	35,08
HEW32	C	Litter	0,278	0,025	219,51	56,92	272,52	34,13	HEW32	C	Mineral soil	0,297	0,036	224,98	55,36	191,21	35,19
HEW32	D	Litter	0,292	0,026	186,54	55,46	175,40	33,96	HEW32	D	Mineral soil	0,312	0,031	206,27	53,73	167,19	33,69
HEW32	G	Litter	0,302	0,019	93,03	54,55	146,56	31,04	HEW32	G	Mineral soil	0,281	0,023	263,98	56,26	191,55	32,76
HEW32	GD	Litter	0,307	0,034	70,13	52,79	62,90	38,50	HEW32	GD	Mineral soil	0,294	0,025	188,12	55,18	155,98	33,08
HEW47	C	Litter	0,231	0,028	140,18	60,17	91,71	36,09	HEW47	C	Mineral soil	0,226	0,015	171,43	60,48	156,53	30,50
HEW47	D	Litter	0,245	0,025	118,42	58,89	165,56	35,26	HEW47	D	Mineral soil	0,262	0,014	174,58	57,77	150,08	28,21
HEW47	G	Litter	0,307	0,025	106,08	54,06	126,16	32,58	HEW47	G	Mineral soil	0,232	0,018	247,50	60,03	193,37	32,21
HEW47	GD	Litter	0,196	n.d.	172,00	62,18	n.d.	n.d.	HEW47	GD	Mineral soil	0,250	0,013	219,76	57,26	188,88	33,03
HEW48	C	Litter	0,195	0,019	223,47	61,74	167,71	35,12	HEW48	C	Mineral soil	0,255	0,018	156,09	58,61	136,02	32,07
HEW48	D	Litter	0,292	0,029	71,53	55,15	123,93	34,30	HEW48	D	Mineral soil	0,281	0,025	159,74	56,91	130,43	33,51
HEW48	G	Litter	0,180	0,020	228,47	62,41	185,67	35,15	HEW48	G	Mineral soil	0,215	0,015	219,61	59,53	150,37	29,95
HEW48	GD	Litter	0,251	0,031	132,12	58,40	122,43	36,58	HEW48	GD	Mineral soil	0,254	0,021	217,58	58,27	163,23	32,90

App. Table III Multiple linear model for potential enzyme activities multiple. Explanatory variables are C_{org} (log-transformed), pH.

	Dependent variable:			
	BG	NAG	Sulf	Phos
C_{org}	473,61***	344,38***	273,74**	4,906,35***
	(115,39)	(56,45)	(107,11)	(762,03)
pH	176,21**	43,98	173,58**	-1,822,63***
	(84,31)	(41,24)	(78,26)	(556,80)
Constant	-1,989,75***	-1,064,18***	-1,236,71***	-5,549,38**
	(360,01)	(176,11)	(334,17)	(2,377,51)
Observations	36	36	36	36
R^2	0,59	0,68	0,46	0,56
Adjusted R^2	**0,57**	**0,66**	**0,43**	**0,53**
Residual Std. Error (df = 33)	203,77	99,68	189,15	1,345,73
F Statistic (df = 2; 33)	24,22***	35,55***	14,03***	20,96***
Note:				*p<0,1; **p<0,05; ***p<0,01

App. Table IV Simple linear model for potential enzyme activities with Cmic (log-transformed) as explanatory variable.

	Dependent variable:			
	BG	NAG	Sulf	Phos
C_{mic}	522,49***	274,12***	415,40***	1,748,24**
	(69,23)	(41,38)	(56,62)	(651,54)
Constant	-2,713,28***	-1,305,30***	-2,040,77***	-6,368,25
	(427,49)	(255,50)	(349,63)	(4,022,99)
Observations	36	36	36	36
R^2	0,63	0,56	0,61	0,17
Adjusted R^2	**0,62**	**0,55**	**0,60**	**0,15**
Residual Std. Error (df = 34)	192,83	115,25	157,71	1,814,67
F Statistic (df = 1; 34)	56,95***	43,89***	53,82***	7,20**
Note:				*p<0,1; **p<0,05; ***p<0,01

App. Table V ANCOVA for microbial biomass C_{mic}. pH, Treatment and Plot ID are explanatory covariables and given in rows in the order of entering the analysis. Degrees of freedom (df), mean squares (MS) and F-values (F) are presented in the table. Significance is assessed by *p<0,05; **p<0,01; ***p<0,001.

		Microbial biomass C_{mic}	
	df	MS	F
pH	1	1741105	136.337 ***
Treatment	3	6442	0.504
Plot ID	8	50396	3.946 **
Residuals	23	12771	

Declaration of authorship

I hereby declare that I am the sole author of this master's thesis and that I have not used any sources other than those listed in the bibliography and identified as references. I further declare that I have not submitted this thesis at any other institution in order to obtain a degree.

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Place, Date Signature