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Collembola communities across forest ecosystems in Germany: Long-term dynamics and interrelationships with fungal food resources

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(*Charlie Mackesy, "The Boy, the Mole, the Fox and the Horse", 2019*)

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Summary

Collembola, one of the most abundant animals in soils worldwide, are important drivers of soil ecosystem services such as carbon sequestration and the cycling of organic matter. By feeding on fungal hyphae and spores, they are shaping fungal community structure and dispersal by transporting spores through the gut passage and body surfaces. Trophic niches and thereby feeding preferences are known to depend on Collembola life form and phylogenetic identity. Thus, the community composition of Collembola is an important driver of ecosystem services. Even though it is known that Collembola prefer saprotrophic over ectomycorrhizal fungi, more specific information on Collembola fungivory in natural systems is still lacking. Therefore, the identification of Collembola community composition and abundance and knowledge on Collembola diets is crucial for understanding soil ecosystem functioning.

We established a comprehensively tested and standardized method for the decontamination of Collembola body surfaces for the use in DNA-based analysis of trophic interactions in soil microarthropods (Chapter 2). In this study, we identified chlorine bleach (Sodium hypochloride, NaClO; 5 % for Acari; 1.5 % for Collembola) to be most efficient in decreasing fungal surface contaminants by at least 80 % without harming the detection of ingested prey material.

Further, we analyzed the gut content and possible gut symbionts of six Collembola species, *Ceratophysella denticulata*, *Isotomiella minor*, *Lepidocyrtus lanuginosus*, *Folsomia quadrioculata*, *Paristoma notabilis* and *Protaphorura armata*, of beech and spruce forests (Chapter 3). Targeted amplicon sequencing of the ITS2 gene fragment was used to identify a broad spectrum of ingested fungal genera. No indications for fungal gut symbionts were detected in starved Collembola. Unexpectedly, the composition of the fungal genera identified in the gut of Collembola did not differ significantly between spruce and beech forests. Collembola preferred saprotrophic or pathotrophic fungal genera, such as *Cladosporium*, *Ramularia*, *Mycena* and *Encoelia* over ectomycorrhizal fungi indicating that the latter are of minor importance for Collembola nutrition. Unfortunately, we only obtained a limited number of OTUs assigned to ingested fungal genera, which presumably was caused by the amplification bias of the used primers towards Collembola DNA. In addition, we found evidence of a cuticle infection with the entomopathogenic genus *Scopulariopsis* in all analyzed Collembola.

In a long-term soil fauna monitoring experiment conducted in the framework of the Biodiversity Exploratories, we identified factors affecting Collembola abundance and species composition in a forest management intensity gradient in three regions across Germany (Chapter 4). Samples were taken during spring every three years between 2008 and 2020. Collembola abundances and species richness did not decline with time. However, abundances and community compositions fluctuated with time depending on regional and climatic factors. Variations in Collembola abundances were high in sand-rich soils in the Schorfheide and shallow soils in the Swabian Alb. These soils have lower water holding capacity compared to clay-rich soils in the Hainich, and thus Collembola seem to be strongly affected by drought periods in the former regions. Further, Collembola community composition was affected by the identity of the dominant tree species (coniferous and deciduous), but no significant effect of forest management intensity on Collembola community structure or abundance was detected. Precipitation and microbial biomass were identified as the strongest factor affecting litter dwelling life forms (epedaphic and hemiedaphic) of Collembola, which mainly feed on microbial material. Surprisingly, euedaphic Collembola were not significantly affected by changing climatic conditions or regional specific factors across the study period and sampling sites. However, highest proportions of parthenogenetically reproducing species, among them many euedaphic species, were at a maximum in the Schorfheide indicating that parthenogenetic species are able to recover quickly after harsh environmental disturbances, such as drought. Overall, the results highlight that detrimental effects of weather extremes are less pronounced in regions with clay-rich soils, which facilitate higher soil hydration and nutrition.

In conclusion, the results of this thesis suggest that the soil system in temperate forests is well buffered against climatic changes and responds much slower to global climate change than aboveground arthropods. Therefore, more intensive monitoring of a wider range of forest types is needed to investigate long-term changes. Collembola in general seem to be resilient to environmental changes presumably related to their flexibility in using a wide range of food resources and recovering quickly after population declines due to harsh environmental conditions. However, the influence of climate change factors on feeding strategies and nutrition of Collembola and its implications for ecosystem services needs more detailed attention.

1 General Introduction

1.1 The forest ecosystem

Forests represent structural and species rich ecosystems providing essential ecosystem services such as carbon sequestration, climatic regulation, biomass production, water purification and habitat for a wide range of organisms (Decocq et al., 2016; Mori et al., 2017; Thompson et al., 2011). Even though most forest biomass and productivity exists aboveground, the belowground system, which is connected to the aboveground through plant roots, has major implications for forest ecosystems (Bardgett & van der Putten, 2014). Mainly abiotic soil properties determine ecosystem functions for the above- and belowground community, such as the physical structure of soils, soil hydration and acidity.

1.1.1 Soil structure and abiotic compartments

"The soil is the living, breathing skin of our planet" (Orgiazzi et al., 2016). With soils being key to environmental, social and economic services and consist of minerals, organic matter, living organisms, water and air.

Soil is characterized by its texture, which is due to differences in the size and type of mineral particles. According to the diameter of mineral particles the texture varies between gravel (> 2 mm), sand (2.0 – 0.063 mm), silt (0.63 – 0.02 mm) and clay (< 0.002 mm) (Orgiazzi et al., 2016). Usually, particles are intermixed with various forms of organic materials and living organisms. The soil structure describes the arrangements of soil particles into larger aggregates of different sizes and the formation of water or air filled pore spaces between them (Tisdall & Oades, 1982). These soil pores are habitat to a diversity of biological life forms and have large influence on the soil water content and organic matter turnover (Elliott & Coleman, 1988; Foster, 1988; Tisdall, 1994). The texture and pore sizes of soils strongly affect the soil water content and the matric potential of soil water (Passioura, 1980; Whalley et al., 2013). Large pores drain first, through low hygroscopic forces within large spaces. Therefore, sandy soils have lower water-holding capabilities compared to clay-rich soils. Compared to aboveground, the soil is not as exposed to extreme fluctuations of climatic conditions. However, climatic factors such as temperature and precipitation affect soil carbon sequestration, soil organism diversity and abundances, and plant carbon and nutrient inputs via roots and leaf litter (Drenovsky et al., 2010; Eisenhauer et al., 2012; Killham et al., 1993; Luo et al., 2021; Seneviratne et al., 2010; Smith, 2012). The soil pH is one of the main drivers of biogeochemical processes, such as mineralization of organic matter, nitrification and denitrification, and on the

other hand is influenced by leaf litter/plant identity, precipitation of pollutants (heavy metals) (Neina, 2019). The community and diversity of soil biota is strongly related to soil acidity (Bardgett, 2005; Birkhofer et al., 2012).

1.1.2 Links of aboveground and belowground systems and climate change

The dominant tree species has a large influence on the soil community composition of fungi and invertebrates (Aupic-Samain et al., 2019; Cesarz et al., 2013; Chen et al., 2019; Eissfeller et al., 2013; Ferlian et al., 2021; Henneron et al., 2017; Tedersoo et al., 2015). Thereby, the structure and quality of leaf litter and root exudation is of crucial importance for decomposition process (Albers et al., 2006; Berg & McClaugherty, 2014; Pollierer et al., 2007). Since humans have been manipulating forest tree species composition to use forests economically, most forests in temperate regions are more or less monocultures and of the same stand age. These tree monocultures are vulnerable to climate change, with severe temperature rises and drought events leading to high risks of bark beetle infestations in spruce plantations (Christiansen & Bakke, 1988; Marini et al., 2017) and storms and other weather extremes causing damages as well (Felton et al., 2010). The less obvious effect of forest management and climate change on the soil biodiversity and function is not yet resolved. Changes in land use and increasing intensity of management together with climate change affect abiotic and biotic factors, as stressed repeatedly (Birkhofer et al., 2012; Seibold et al., 2019). However, less is known how biotic factors, such as trophic interactions and community structure of soil animals alter ecosystem functions and services in forests. Within the last ~15 years the aboveground arthropod biodiversity declined rapidly (Hallmann et al., 2017; Raven & Wagner, 2021; Seibold et al., 2019). However, it is not yet known if this decline is also mirrored in the belowground biodiversity.

1.1.3 Soil biota

The soil is one of the most diverse habitats on earth, but organisms within are hardly visible due to their mostly small size and the opaque character of the habitat. Soil biota mainly consist of archaea, bacteria, protists, rotifers, tardigrades, nematodes, mites, springtails, earthworms, macroarthropods and burrowing mammals as well as plant roots, fungi and lichens (Bardgett, 2005; Orgiazzi et al., 2016). These organisms interact through direct feeding interactions (Scheu, 2002) or indirect by structuring the soil e.g., by burrowing activities of earthworms. One of the most abundant animals not solely in forest soils are Collembola.

The structure of the soil matrix influences trophic interactions of soil organisms (Erktan et al., 2020). Microarthropods cannot access water filled pores, which may include possible food or prey. Therefore, drought events can be advantageous for soil arthropods. In soil most communication and orientation happens through the detection of chemical cues (volatiles) released by roots, bacteria, fungi and soil animals (Insam & Seewald, 2010; Wenke et al., 2009). A consumer can detect its diet over some distance as shown in Collembola and their detection of different fungal species (Bengtsson et al., 1988). The transport of volatiles is ten-fold faster through air (gas) filled space than through water (Moldrup et al., 2000). Therefore, hydration of the soil does not only influence the accessibility, but also the detection of food. Soil biota also alter the soils physical and chemical composition, by organic matter turnover and nutrient cycling (Bardgett, 2005; Lehmann et al., 2017).

Fungi make up a large proportion of overall soil biomass and are most diverse in forest soils (Orgiazzi et al., 2016). This kingdom comprises around 150,000 described species of estimated millions of species present. Fungi can be assigned to functional groups according to their nutrient assimilation strategy, which consists of saprotrophic, symbiotic and pathotrophic fungi. Saprotrophic fungi are decomposing organic material in soil, leaf litter and dead wood (Boddy & Hiscox, 2016; Rousk & Bååth, 2011). Fungi, which form a mutualistic relationship with plants and are connected via roots are termed symbiotic or mycorrhizal fungi (Antunes & Koyama, 2017). In temperate forests ectomycorrhizal fungal species predominate. However, fungal presence is not always beneficial for plant growth. Pathotrophic fungi are responsible for diseases and can cause lethal damage to plants, other fungi and invertebrates (Singh, 1982). Moreover, fungi are also strongly involved in the formation of soil aggregates (Rillig & Mummey, 2006).

The biomass of saprotrophic and ectomycorrhizal fungi varies with forest tree composition (Awad et al., 2019; Nacke et al., 2016; Urbanová et al., 2015). Main drivers for differences in fungal abundance and community composition are soil acidity, organic matter input and soil structure. Ectomycorrhizal fungi are closely coupled to trees (Nacke et al., 2016) and are strongly influenced by soil conditions such as pH, moisture and temperature (Awad et al., 2019). In contrast, saprotrophic fungi are strongly influenced by forest related factors such as carbon-to-nitrogen (C:N) ratio and general quality of leaf litter (Purahong et al., 2016). Seasonality drives variations in fungal and bacterial community composition in deciduous forests (Nacke et al., 2016). Fungal communities in coniferous forests have higher proportions of saprotrophic fungi compared to monoculture beech forests (Likulunga et al., 2021). Even

between differently managed beech forests, no difference of fungal community composition was detected (Wubet et al., 2012). Indicating that fungal community structure is closely linked towards forest tree identity and rather independent of forest management practices.

1.2 The role of Collembola in ecosystems and trophic ecology

Collembola display high numbers with estimated c. 2 x 10^{18} individuals worldwide which corresponds to an estimated total biomass of 27.5 megatons carbon (Potapov et al., 2023). These enormous numbers not only reflect that Collembola are extremely abundant, but also that these animals contribute largely to ecosystem functioning. By feeding on different organic resources, they are able to recycle nutrients, facilitate carbon sequestration, regulate and disperse microbes and thereby enhance plant growth and soil quality (Rusek, 1998). Their main food sources are decaying plant material, living plant material, algae, lichens, bacteria, fungi and also nematodes (Ngosong et al., 2011; Heidemann et al., 2014; Pollierer & Scheu, 2021; Potapov et al., 2021). Feeding startegies and trophic position depends on the phylogenetic identity and life form of Collembola (Chahartaghi et al., 2005; Ferlian et al., 2015; Potapov et al., 2016). The trophic positions seem to be independent of macrohabitat, but to rely more on the structure of the microhabitat (Ferlian et al., 2015). Even though Collembola are known to feed on various resources, their diet typically comprise to a large extent fungal material. Collembola prefer saprotrophic over mycorrhizal fungi as indicated by stable isotope and amino acid analysis (Pollierer & Scheu, 2021; Potapov & Tiunov, 2016), presumably due to the thick cell walls and toxic secondary metabolites in living hyphae of mycorrhiza (Kaneda & Kaneko, 2004). Collembola have been shown to preferentially consume dark melanized fungi (dematiaceous fungi) in food choice experiments (Scheu & Simmerling, 2004). Dematiaceous fungi can be found already on living leaves and within the leaf litter, where these fungi act as primary saprotrophs (Klironomos et al., 1992). Typical dematiaceous fungi are *Alternaria* and *Cladosporium*, which are preferred by Collembola over typical soil fungi, such as *Penicillium* and *Trichoderma*, which act as secondary saprotrophs (Klironomos et al., 1992). However, Collembola are known to feed on a mixture of fungal species, which may help in improving the nutritional value of the diet and possibly dilutes toxins (Chauvat et al., 2014; Jørgensen et al., 2003, 2005; Scheu & Folger, 2004). If and how Collembola species and functional groups selectively search for and utilize fungal diets in the soil system is still unclear.

Around 6,500 Collembola species are described globally with approximately 600 species present in Germany (Orgiazzi et al., 2016). Soil communities can differ in their species richness depending on large scale regional and ecosystem structures with highest species richness in

tropical forests with on average 36.6 species/site and lowest temperate agricultural systems with on average 19.5 species/site (Potapov et al., 2013). The community composition of Collembola can vary between different regions or forest types, even if species richness is stable (Pollierer & Scheu, 2017).

Collembola morphology displays several adjustments towards the habitat/soil layer they colonize. Body forms vary from globular to slender and have a soft wax like cuticle, sometimes covered in setae or scales (in epedaphic species) or pseudocelli (in euedaphic species). The name giving body structure is a collophore, or ventral tube, an organ important for fluid and electrolyte exchange, and also used for adhesion to smooth surfaces (Hopkin, 1997). The common name for Collembola is springtails, which is based on the jumping organ, the furca, that evolved from a paired appendage and is used to escape from predators. Length of antennae (usually four segments), legs and furca correlate with vertical stratification of the Collembola species in soil (Salmon et al., 2014). The coloration and presence of ocelli is also decreasing with soil depth. Soil inhabiting Collembola are usually covered with various sensory organs, which presumably help them to sense food sources through smell and taste, atmospheric pressure, humidity, temperature and different concentrations of oxygen and carbon dioxide (Bengtsson et al., 1991; Hedlund et al., 1995; Zettel, 1984). Especially blind Collembola rely on the ability to sense food resources through volatiles (Bengtsson et al., 1988). Due to their soft body structures and life-long molting Collembola are sensitive towards desiccation and retreat to moist microsites at dry conditions (Verdeny-Vilalta & Moya-Laraño, 2014). Within the soil structure they tend to prefer soil pores of > 100 µm but are able to find refuge in smaller pores if predators are present (Vreeken-Buijs et al., 1998).

Collembola community composition and diversity may differ between forest types and may be influenced by management intensity, potentially affecting trophic interactions and functions. Additionally, Collembola functional groups (= life forms) vary in abundance in differently managed forest systems and regions (Henneron et al., 2017; Hishi et al., 2022; Martins da Silva et al., 2016; Pollierer & Scheu, 2017; Vandewalle et al., 2010).

1.2.1 Functional groups – Life forms

Collembola can be grouped into four life forms according to their morphology and vertical stratification in soil (after Gisin, 1943; modified by Potapov et al., 2016, Fig. 1.1).

Atmobiotic life form:

Collembola species that inhabit the surfaces of plants and the uppermost surfaces of leaf litter, including the largest Collembola species with various coloration and ocelli. Their appendages are well developed and long and they are therefore able to quickly escape from predators. Their preferred food sources are algae, lichens and fresh plant materials.

Epedaphic life form:

Collembola species that inhabit the upper leaf litter layer and sometimes the bark of dead wood. The have relatively large body sizes with coloration and are often covered by setae (hairs) or scales. They possess up to eight ocelli per eye spot and have well developed appendages including furca. This life form can be divided into two feeding guilds. One guild is mainly feeding on plant material and microorganisms. These species mainly affect the first stages of litter decomposition and are considered more selective. The second guild is mainly feeding on microorganisms present in the leaf litter layer, such as saprotrophic fungi, and on nematodes.

Hemiedaphic life form:

Collembola species that inhabit the lower leaf litter and upper soil layer and are able to move between the two layers. They are smaller than the former two groups and less colored. The number of ocelli can be reduced and the appendages and furca are shortened but still present. Their main food resource consists of microorganisms that are present in strongly decaying leaf litter and detritus itself. By feeding on detritus they are forming the physical structure of litter and therefore shaping the microbial community composition.

Euedaphic life form:

Collembola species that are permanently inhabiting the soil. Their bodies are mostly slender and white with very short appendages and often completely reduced furca. These species are blind, but possess several sensory organs all over their bodies. The main food source are microbes present in the rhizosphere. They feed on bacteria, saprotrophic and mycorrhizal fungi, thereby playing an important role in the decomposition of soil organic matter.

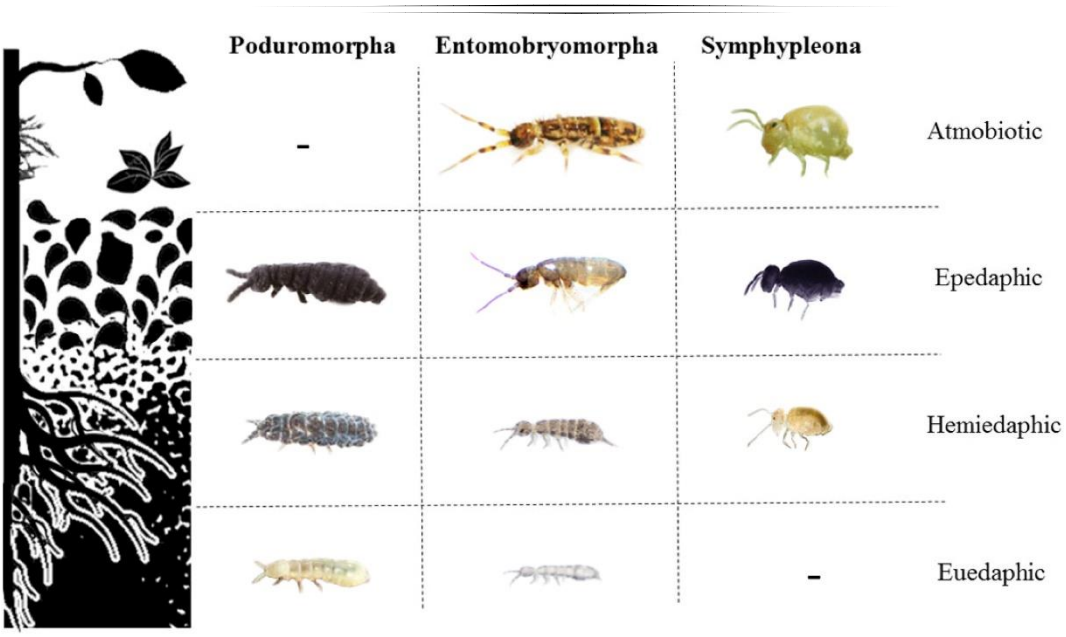

Fig. 1.1: Overview of Collembola life forms (horizontal) with their respective taxonomic order (vertical) (from Potapov et al., 2016)

1.2.2 Reproductive mode

In general, the bisexual reproductive mode is prevalent among Collembola (Chahartaghi et al., 2006). However, the deeper soil habitat is inhabited by a larger fraction of species with parthenogenetic reproduction. Sexual species, which have the largest share on the surface and within the litter layer, presumably outcompete parthenogenetic species due to larger genetic variability (Chahartaghi et al., 2009). Some species even display complex mating rituals as seen in Sminthuridae. In Collembola, the proportion of parthenogenetic species increases with soil depth as most belong to the euedaphic group (Petersen, 1980). Parthenogenetic species seem to take advantage of the more stable habitat in deeper soil layers even though the resources may be of lower nutritional value compared to the litter layer (Scheu & Drossel, 2007). They are fast colonizers and can quickly occupy new resources. Parthenogenesis can sometimes be an adaptation to geographic and seasonal patterns (Song et al., 2011; Vrijenhoek & Parker, 2009).

Collembola abundance and community composition, including the relative abundance of parthenogenetic vs. sexual species, vary with time, but variations are presumably driven by abiotic environmental factors and may not show a constantly decreasing trend over the long term.

Due to the high abundances in nearly all ecosystems worldwide and their role in crucial ecosystem processes, Collembola can serve as a model group to understand the effects of land use intensity and abiotic factors on trophic interactions and temporal dynamics of soil mesofauna.

1.3 Study region and methodology

1.3.1 Biodiversity Exploratories

The long-term and large-scale project "Biodiversity Exploratories" is aiming at linking biodiversity and ecosystem functioning research (www.biodiversity-exploratories.de; Fischer et al., 2010). The three regions are located in a north-south gradient across Germany, with the Schorfheide-Chorin (Schorfheide) in the north, Hainich-Dün (Hainich) in the middle and Swabian Alb in the south of Germany. These regions feature replicated sets of forest and grassland experimental grid plots but vary due to regional specific environmental factors such as soil structure, pH and climatic conditions.

The Schorfheide exploratory is characterized by a young glacial landscape with mainly dystric Cambisol soils, often with a texture from sandy loam to pure sand located at an altitude of 3-140 m a.s.l. The soils in the Schorfheide have a pH of 3.3 ± 0.19, which is the most acidic among the three regions (Pollierer & Scheu, 2017). Due to its location, it is influenced by a continental climate with larger extremes between seasons. In general it is the warmest and driest region with an annual mean temperature of 8.0-8.5 °C and an annual mean precipitation of 500-600 mm. Since soils in this region are of sandy texture, the water holding capacity is extremely low. The Hainich exploratory is located in central Germany at an altitude of 285-550 m a.s.l. and characterized by calcareous bedrock with mainly eutric Cambisol, Luvisol and sometimes Stagnosol soils (with clayey and loamy texture). These soils are nutrient rich with a high water-holding capacity. The pH value is 4.59 ± 0.67, which is the highest among the three regions. The mean annual temperature is 6.5-8.0 °C and mean annual precipitation is 500-800 mm. The Swabian Alb exploratory is located in the southwest of Germany at an altitude of 460-860 m a.s.l. and is characterized by calcareous bedrock with karst phenomena with mainly clay rich eutric Cambisol and Leptosol soils. These soils are shallow structured and rocks (karst) appears within the upper 20 cm of soil. The soils pH value is 4.51 ± 0.72. It is the coldest and wettest of the three exploratories with an annual mean temperature of 6.0-7.0 °C and an annual mean precipitation of 700-1000 mm. Each region/exploratory consists of 50 grassland and 50 forest experimental grid plots of 100 m x 100 m.

The studies of this dissertation were part of the Biodiversity Exploratories contributing project 'LitterLinks', aiming at identifying changes within the soil food web structure with land use intensity in forest systems. Therefore, here we describe only the forest experimental plots. In each exploratory, four forest types with four replicates were sampled. Forest types consisted of coniferous forests, young even-aged beech stands (*Fagus sylvatica*, age ~30 years; young managed beech forest), mature even-aged beech stands (age ~70 years; old managed beech forest) and beech stands that were left unmanaged for at least 60 years (age ~150 years; unmanaged beech forest). Forest types are characterized by different management intensities, decreasing from coniferous to young managed beech to old managed beech to unmanaged beech forests. Coniferous forests included pine stands (*Pinus sylvatica*, age ~50 years) in Schorfheide and spruce stands (*Picea abies*, age ~60 years) in Hainich and Swabian Alb. Soil samples of different diameter (5 cm for soil mesofauna, 20 cm for soil macrofauna) and samples for microbial biomass measurement were taken with soil corers, including the litter layer and soil to a depth of 5 cm. Earthworms were extracted by a mustard-water solution poured twice every 15 min (2 x 5 l) and incubated for a maximum of 30 min over the bare soil of ¼ m² area. Sampling took place during spring (May-June) every three years between 2008 and 2020. At each grid plot an environmental monitoring unit was installed and climatic factors, such as air temperature, precipitation and soil moisture were recorded.

The wide array of abiotic and biotic data collected for each site within the Biodiversity Exploratories allows for a more detailed understanding of the drivers of biodiversity and the concomitant effects on ecosystem functioning as affected by management practices. Research within the Biodiversity Exploratories is still ongoing and new experiments are set up to gain a more functional understanding, e.g. on the effects of forest gap formation on the ecosystem.

1.3.2 Molecular Gut Content Analysis of Collembola

A precise tool to analyze species –specific feeding interactions in natural settings is the molecular analysis through polymerase chain reaction of animals gut contents (Symondson, 2002; Nielsen et al., 2018). By using general primers, targeting a gene region shared with a broad range of possible food materials and within high species-specific sequence variability, the detection of various food species is possible within one consumer (King et al., 2008). Especially in cryptic environments such as the soil, which is inhabited by rather small invertebrate organisms, direct observation of feeding interactions is nearly impossible. This

method is extremely sensitive and even short and degraded DNA fragments can be detected through amplification via PCR (Symondson, 2002). Databases including sequence information of all kingdoms are growing due to the improvement and accessibility of different sequencing techniques and thereby allow for a detection of a broad range of fungal species as well (Pompanon et al., 2012; Tedersoo & Smith, 2013).

1.3.3 Analysis of community structure of Collembola – taxonomic determination

Even though the century of molecular barcoding of species through high throughput sequencing techniques has long begun (Valentini et al., 2009; Ye et al., 2019), the classical taxonomic approach of species determination is more needed than ever (Mace, 2004; Padial et al., 2010).

Especially the identification of Collembola can be challenging. Collembola have soft tissue and are relatively small with body sizes ranging for example from 0.4 mm (*Megalothorax minimus*) to 6.2 mm (*Pogonognathellus longicornis*) in temperate European regions (Hopkin, 2007). In addition, the sizes can vary immensely within one species due to lifelong molting of Collembola. Collembola are sometimes identified through coloration. However, a study on different species of *Lepidocyrtus*, a very common genus in forests and grassland in Europe, has identified that these species (*L. cyaneus* and *L. lanuginosus*) are polyphyletic suggesting that coloration is a weak descriptor of species identity (Zhang et al., 2019). There is a strong need for the combination of classical and molecular approaches for species determination, since phylogenetic identity is a strong indicator for functional relationships and biodiversity research.

We used classical taxonomy in the determination of Collembola in a long term monitoring study within the Biodiversity Exploratories. For the determination keys by Gisin (1943), Fjellberg (1998, 2007) and Hopkin (2001) were used.

1.4 Scope of the Dissertation

The overall aim of this dissertation was to identify the trophic links between Collembola and fungi as well as to investigate the effect of forest type (forest management intensity) and environmental factors on Collembola abundance and community composition in a long term species monitoring experiment.

We investigated the following main hypotheses:

(1) The use and preference of fungal resources depends on the life form of Collembola and forest type. The proportion for ectomycorrhizal fungal material present in the gut of Collembola increases with soil stratification, i.e. euedaphic Collembola rely more on

mycorrhizal fungi. Epedaphic Collembola, feed solely on saprotrophic fungal species, whereas hemiedaphic Collembola, feed on both, saprotrophic and ectomycorrhizal fungal species.

(2) Collembola abundances and species richness decreases with time in the last decade due to climatic changes.

(3) Proportions of Collembola species vary with effects of region, forest type and climatic conditions depending on their life form and reproductive mode.

1.4.1 Chapter overview

In **chapter 2** we evaluated the effectiveness of ten possible body surface decontamination methods for the use on soil microarthropods. For the molecular analysis of gut contents of soil microarthropods, whole body DNA extractions are used. Any contamination with environmental DNA attached to the animals' body surface has to be eliminated to draw conclusions towards their diet. Therefore, we did not only test for the effective removal of surface contaminants, but also for the reliable detection of gut content material via polymerase chain reaction. We fed individuals of *Steganacarus magnus* (Acari, Oribatida) with an oversupply of nematodes of the genus *Plectus* for several days. Subsequently mites were frozen and rolled over fungal mycelia of *Chaetomium globosum* for a controlled contamination of known fungi. In the main experimental part, 20 individuals per method were sterilized according to the methods' protocol. We used PCR with species and genus specific primer for the detection of *C. globosum* and for the detection of *Plectus* sp. as a gut content control. Most sufficient results were detected after a bleach (5 %) and formaldehyde (37 %) treatment of mites with effective removal of fungal contaminants by 81 % and 88 % respectively. The most efficient method, in lower bleach concentrations, was tested for the use on soft cuticle microarthropods, such as Collembola. A treatment with 1.5 % bleach was most efficient, without harming the specimens.

In **chapter 3** we investigated the fungal diet of six Collembola species compared between beech and spruce forests. In addition to the analysis of possible fungal diet species, we let a sub group of all species starve, which we used to identify possible gut symbionts via metabarcoding. To remove possible environmental contaminants we used a treatment with bleach 1.5 %, which was the most efficient surface decontamination method identified in **chapter 2**. The six Collembola species could be assigned to different life forms including epedaphic (*Ceratophysella denticulata*, *Lepidocyrtus lanuginosus*), hemiedaphic (*Folsomia quadrioculata*, *Parisotoma notabilis*) and euedaphic (*Isotomiella minor*, *Protaphorura*

armata). We used targeted amplicon sequencing of the ITS 2 gene region, typically used in fungal metabarcoding, on an Illumina MiSeq (2x 150 bp) system. Around 100 OTUs were assigned to fungal genera. However, the read proportion per sample varied. Collembola did not differ in their use of fungal resources between forest types, suggesting that Collembola were less influenced in their use of fungal resources by macrosites, such as distinct forest types. Surprisingly, the dominant fungal genera associated with Collembola was the entomopathenogenetic fungi *Scopulariopsis* as well as the ubiquitous sporulating *Penicillium* and *Aspergillus*. The fungus *Scopuliaropsis* sp. seems to have infested the cuticles of Collembola, however, without causing any visible damage. *Penicillium* sp. and *Aspergillus* sp. are not likely to be ingested specifically and more by chance. However, fungal genera which are more likely to be intentionally ingested such as the saprotrophic and pathotrophic fungal genera *Cladosporium*, *Ramularia*, *Mycena* and *Encoelia* were present in gut contents. Ectomycorrhizal fungi seem not to be a main resource for Collembola, since a limited number of reads were assigned to mycorrhizal fungi. Unfortunately, this study had limitations such as the amplification bias towards Collembola DNA, which limited the detection of fungal DNA in our samples. Despite or especially through these limitations this study is giving the first insights of metarbarcoding analysis in a challenging consumer – diet system with some ideas for improvement.

In **chapter 4** we analyzed the effect of time, region, forest type and climatic factors on the abundance and community composition of Collembola. We used data compiled within the project Biodiversity Exploratories sampled during spring every three years between 2008 and 2020. Contrary to our expectations, Collembola abundances and species richness did not decline with time. However, temporal fluctuations of abundances were detected, caused by climatic fluctuations specifically of precipitation during winter and spring. Moreover, region and forest type identity affected Collembola abundance and community composition. Indicating that region specific factors, such as the soil type and structure mainly drive Collembola community structure effects. Soils with higher soil water content, such as the soils in the Hainich, display more stable abundances across forest types in comparison to the Schorfheide, which has sand rich soils, where Collembola abundances varied most. In addition, the Schorfheide had highest proportion of parthenogenetic Collembola species, which indicates that the ability of fast colonization in an environment with weather extremes and poor nutrient quality in leaf litter is advantageous. Litter inhabiting Collembola were mainly affected by precipitation and microbial biomass, presumably due to the decline of microbial food sources in litter and due to morphological traits that inhibit species' ability to retreat towards moist microsites present in

soil. Even though Collembola abundances did not decline with time, the negative effect of climate change was obvious in spruce forests in the Hainich. Spruce forest stands were cleared after bark beetle infestations and left the soil system more exposed to climatic extremes. Collembola are contributing to ecosystem services and are affected by a reduction in precipitation and change in habitat structure, therefore, long-term monitoring is needed to evaluate their response to ongoing climate change.

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2 Methods for removing body surface contaminants of soil dwelling invertebrates (Oribatida) using detection PCRs

Steganacarus magnus "chased" by lumbricidae ©by Sarah Bluhm

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Abstract

Molecular gut content analysis via detection PCR or high-throughput sequencing (metabarcoding) of consumers allows unravelling of feeding interactions in a wide range of animals. This is of particular advantage for analyzing the diet of small invertebrates living in opaque habitats such as the soil. Due to their small body size, which precludes dissection whole-body DNA extraction is necessary for molecular gut content analysis. This poses the problem that potential body surface contaminants, such as fungal spores may be incorrectly identified as ingested food particles. To minimize amplification of fungal body surface contaminants, a standardized and well tested surface decontamination method is required. We investigated the efficiency of ten methods for body surface decontamination in oribatid mites using *Steganacarus magnus* as model species. Furthermore, we tested side effects of the decontamination techniques on the detection of gut prey organisms. Prior to decontamination, mites were fed with nematodes (*Plectus* sp.) *ad libitum* and postmortem contaminated with fungal spores (*Chaetomium globosum*). We used detection PCR with primers specific for *C. globosum* and *Plectus* sp. to detect contaminants and prey, respectively. The results suggest that chlorine bleach (Sodium hypochloride, NaClO, 5 %) is most efficient in removing fungal surface contamination without significantly affecting the detection of prey DNA in the gut. Based on these results, we provide a standard protocol for efficient body surface decontamination allowing to trace the prey spectrum of microarthropods using molecular gut content analysis.

2.1 Introduction

Ecosystems comprise a dense network of direct and indirect interactions between organisms and their biotic and abiotic environment, with feeding interactions forming the basis of food webs. Identifying those trophic interactions through direct observations in the field are however difficult, especially in opaque habitats– such as in soil – or includes animals that are very small

– such as in many invertebrate taxa. Under these circumstances feeding interactions can be identified using DNA-based analysis of regurgitates, faeces and the gut content (King et al., 2008; Nielsen et al., 2018; Symondson, 2002). As even low amounts of ingested food DNA are detectable by amplification through polymerase chain reaction (PCR) and will subsequently be identified by high-throughput sequencing, these molecular methods are highly sensitive and specific. Whole-body DNA extraction of small invertebrates, such as mites and springtails, is necessary to obtain the gut content material. Thus, the DNA extract consists of DNA of various origin, i.e. the DNA of the consumer, environmental DNA from the consumers body surface, the consumers symbiotic gut microbiome and DNA of ingested food. This mixture of DNA will lead to false assignments in the analysis of the consumers diet. Further, digestion processes and the short duration of the gut passage pose additional challenges for prey detection, but if overcome may allow unprecedented insight into trophic networks (Agustí et al., 2003; Eitzinger et al., 2019; Read et al., 2006).

Soils are an example of an opaque habitat colonized predominantly by small invertebrates typically reaching high density and diversity, such as springtails and mites. Most of these microarthropod species live as generalist feeders consuming a wide range of diets in particular fungi and bacteria (Nielsen, 2019; Scheu & Setälä, 2002). Microbial feeding species may even regulate the activity and thereby the functions and services of microorganisms, including litter decomposition, carbon sequestration and nutrient cycling (Bardgett, 2005; Nielsen, 2019). Molecular gut content analysis is a promising approach allowing to track trophic interactions of microbial feeders (Gong et al., 2018; Jørgensen et al., 2005). However, for disentangling trophic relationships between soil microarthropods and microorganisms it is necessary to establish methods allowing to exclude body surface contaminants from bacteria and fungi in the gut of the consumers. This is especially challenging since living and crawling through the soil microarthropods are contaminated with bacteria and fungi, in particular fungal spores, and

in fact may contribute to their dispersal in soil (Anslan et al., 2016; Renker et al., 2005). Due to the high sensitivity of molecular gut content analysis, it may lead to the detection of microorganisms attached to the body surface, thereby compromising the analysis of prey species in the gut of microarthropods. These false positive results are likely to occur in molecular gut content analyses of small sized invertebrates requiring whole animal DNA extraction.

Chlorine bleach (Sodium hypochloride, NaClO) is commonly used as a sterilizing agent in molecular laboratories and has been successfully used to clean surfaces of insects of different life stages (Davidson et al., 1994; Linville & Wells, 2002) as well as bulk samples (Greenstone et al., 2012; Hausmann et al., 2021), and arachnids (Miller-ter Kuile et al., 2021) including oribatid mites (Remén et al., 2010). Many of these studies also focused on the detectability of prey DNA in the gut (Greenstone et al., 2012; Linville & Wells, 2002; Miller-ter Kuile et al., 2021; Remén et al., 2010), but also on the detection of endosymbionts (Meyer & Hoy, 2008). Doing that these studies also aimed at working towards a standard method for surface decontamination of invertebrates for metabarcoding. However, the numbers of replicates and tested decontamination protocols were limited. We tested 10 decontamination procedures and used 20 replicates in each of them with up to three PCR replicates to evaluate technical and intra specific variations. Our experimental set up consisted of the oribatid mite *Steganacarus magnus* as model microarthropod species, which was fed with nematodes of the genus *Plectus* and was postmortem contaminated in the mycelium of the fungus *Chaetomium globosum*. Both *S. magnus* and *Plectus* sp. are abundant in forests in Europe including beech forests in the vicinity of Göttingen (Lower Saxony, Germany) and *S. magnus* is known to feed on nematodes, especially *Plectus* sp. (Heidemann et al., 2011, 2014). The study represents the first comprehensive test of surface decontamination methods for establishing a standardized procedure for molecular gut content analysis in soil microarthropods.

2.2 Materials & Methods

2.2.1 Sampling of animals

Soil and litter dwelling animals were extracted over night from litter of a beech forest near the city of Göttingen using heat extractors (Kempson et al., 1963). Living animals were collected in containers covered with wet tissue to prevent desiccation of the animals. Individuals of *Steganacarus magnus* (Nicolet, 1855) were identified under a stereo microscope (Stemi 508, Zeiss, Jena, Germany) and starved for five days at 16 °C. Starved animals were fed with bacterial feeding nematodes [*Plectus minimus* (Cobb, 1893) or *P. velox* (Bastian, 1865), from nematode cultures] *ad libitum* for three days. Single individuals of *S. magnus* were then transferred into 1.5 ml Eppendorf tubes and frozen at -80 °C. The body surface of dead specimens was contaminated by rolling them over a colony of the fungus *Chaetomium globosum* (Kunze, 1817) cultivated on potato dextrose agar (PDA; Carl Roth, Karlsruhe, Germany). This was done by pushing the mites with a pair of tweezers over a distance of at least 2 cm over the fungal colony, so that the animals' surface was visibly contaminated. The contamination as well as the cleaning of animals was done under sterile conditions in a laminar flow hood.

2.2.2 Cleaning of the body surface

Cleaning of the body surface from fungal propagules (spores) was done by "washing" specimens using ten different methods (Table 1). At least 20 individuals were tested with each method. For every washing step individual specimens were transferred into a new sterile tube and shortly vortexed after the next cleaning solution was added (500 µl). To transfer specimens to a new tube spring steel tweezers were used, which were subsequently washed in ethanol and flamed to avoid contamination. High grade RNase free water and absolute ethanol (EtOH) was used in the additional steps before and after the main decontamination procedure. A droplet of

Tween 20 was added to water and decontamination liquids to break surface tension. After the last step of decontamination, each animal was transferred to a new sterile tube and either directly used for DNA extraction or stored at -20 °C until further usage.

Table 1. The ten methods used for decontaminating the body surface of *Steganacarus magnus*. All washing steps (except flame treatment) were done in 1.5 ml sterile tubes and vortexed. 'Wash 1' included washing steps with H_2O (2 min), followed by EtOH (5 min) and H_2O (2 min). 'Wash 2' included two H_2O (2 min) washing steps. "-" indicates that this washing step was omitted.

Method	Wash 1	Decontamination	Wash 2
Acetone + Tween 20 0.1 %	X	Incubate in Acetone; 5 min	X
Bleach 5% + Tween 20 0.1%	X	Incubate in Bleach; 5 min	X
Flame	-	Pass through flame; 1 sec	-
Formaldehyde 37 %	X	Incubate in Formaldehyde; 5 min	X
H_2O_2 (Hydrogen peroxide) 30 %	X	Incubate in H_2O_2; 5 min	X
Peracetic acid 2 %	X	Incubate in Peracetic acid; 5 min	X
SDS (Sodium dodecyl sulfate) 0.1 %	X	Incubate in SDS; 5 min	X
Sterillium®	X	Incubate in Sterillium®; 5 min	X
Ultrasound	X	Ultrasound bath; 10-30 sec	X
UV Light	X	Treat with UV light; 30 min	X

2.2.3 DNA extraction

Whole body DNA extraction was done using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturers protocol. Body tissue was mechanically crushed using steel pestles and tissue lysis was conducted on a heating and mixing platform (ThermoMixer®, Eppendorf SE, Hamburg, Germany) at 56 °C for 2 h. The final elution of DNA from the silica membrane was performed twice with 16 µl elution buffer "AE" (provided in the Kit) per specimen, i.e. DNA extracts were based on single specimens.

2.2.4 DNA amplification

DNA amplification using PCR targeted three fragments. The first PCR targeted a 320 bp fragment of the D3 region, part of the 28S rRNA gene, as a general invertebrate marker. This was carried out using the primer pair D3A – 5'- GACCCGTCTTGAAACACGGA-3' and D3B – 5'- TCGGAAGGAACCAGCTACTA -3' (Litvaitis et al., 1994; Maraun et al., 2003). For detecting contamination by *C. globosum*, the primer pair specific for *C. globosum* CHA F5 – 5'- GAGGTCACCAAACTCTTGATAATTT -3' and CHA R6 – 5'- CCTACTACGCTCGGTGTGACAG -3' targeting a 313 bp fragment was used. This primer pair was developed and optimized using a number of sensitivity and specificity tests before conducting this experiment (M. Jüds and K. Heidemann, unpubl. data). To test if the decontamination procedure also affects the gut content of the animals, a primer pair targeting a 156 bp fragment of the 18S rDNA gene specific for the nematode genus *Plectus* sp. was used (Heidemann et al., 2014). For each sample and primer pair two PCR reactions were carried out for the decontamination and gut content test. PCR conditions included 34 cycles with denaturation of DNA double strands at 95 °C for 30 s, annealing of primer at 58 °C (D3), 59 °C (Chae), 62 °C (Plec) for 45 s, elongation of strands at 72 °C for 30 s. PCR started with heat activation of the Taq polymerase at 95 °C for 15 min and ended with a final strand elongation at 72 °C for 10 min.

For all PCR reactions the SuperHotStar-Taq Master Mix was used (Genaxxon bioscience GmbH, Ulm, Germany). PCR for the D3 fragment was carried out using 1 µL of each primer (100 pmol μL^{-1}; Eurofins Genomics, Ebersberg, Germany), 1 µL 25 mM $MgCl_2$, 1 µL of BSA (3%), 12.5 µL of 2 x SuperHotStar-Taq Master Mix containing the polymerase and 2.5 µL DNA. PCR for *C. globosum* was carried out with 1 µL (100 pmol μL^{-1}, Eurofins Genomics, Ebersberg, Germany) of each primer and 12.5 µL of 2 x SuperHotStar-Taq Master Mix (Genaxxon) containing the polymerase and 2.5 µL DNA. PCR for amplifying a 156 bp fragment

of *Plectus* sp. was carried out using 2 µL (100 pmol μL^{-1}, Eurofins Genomics, Ebersberg, Germany) of each primer, 2 µL 25 mM $MgCl_2$, 2 µL of BSA (3%), 12.5 µL of HotStarTaq Master Mix Kit containing the polymerase and 2.5 µL DNA. In all reactions, RNase free water was used to fill up to 25 µL total reaction volume per sample.

PCR products were visualized and checked on a capillary electrophoresis system QIAxcel using AL320 as analyzing method (Qiagen, Hilden, Germany). In case of inconsistent results of a sample, a third PCR was executed. A subset of purified PCR products were Sanger sequenced for further validation of decontamination success or confirmation of positive detection of nematode and fungal DNA.

2.2.5 Statistical analysis

Variations in the efficiency of the decontamination procedures were inspected using analysis of variance (ANOVA). Differences between means were inspected using Tukey's honestly significant differences (HSD) test with the Holm correction method. For graphical presentation of the frequency of detection of *C. globosum* percentages were used illustrating the effectiveness of the different decontamination methods. All statistically analyses were done using R (R Core Team (2021), Version 4.2.1) and RStudio (RStudio 2022.07.0).

2.3 Results

2.3.1 Decontamination efficiency

First, to detect if the DNA extraction was successful and amplified by PCR, the DNA concentration was measured, and the D3 region was amplified and visualized. All tested DNA extracts were positive for the D3 region and thus used for detection of fungal and nematode DNA.

Before testing for successful decontamination of the animal body surface a contamination reference was tested. For the control group, 20 individuals of *S. magnus* were treated in the same way as the individuals used for the decontamination test but without cleaning. Generally, the detection of *C. globosum* varied significantly with decontamination methods ($F_{10,436}$ = 19.45, $P < 0.001$). In all non-decontaminated (= control) *S. magnus* we detected *C. globosum* (Fig. 1). In eight of the ten decontamination methods the percentage of oribatids with detectable traces of *C. globosum* were significantly lower than the control, which were Acetone 67 % *Chaetomium* sp. detection, hydrogen peroxide 55 %, UV-light 50 %, SDS 48 %, peracetic acid 35 %, ultrasound 35 %, chlorine bleach 19 % and formaldehyde 12 %. In four decontamination methods, the detection of *C. globosum* dropped to < 75 % compared to the control (formaldehyde, chlorine bleach, peracetic acid and ultrasound). Detection of *C. globosum* was lowest in bleach and formaldehyde treated individuals with a detection rate of 19 % and 12 %, respectively.

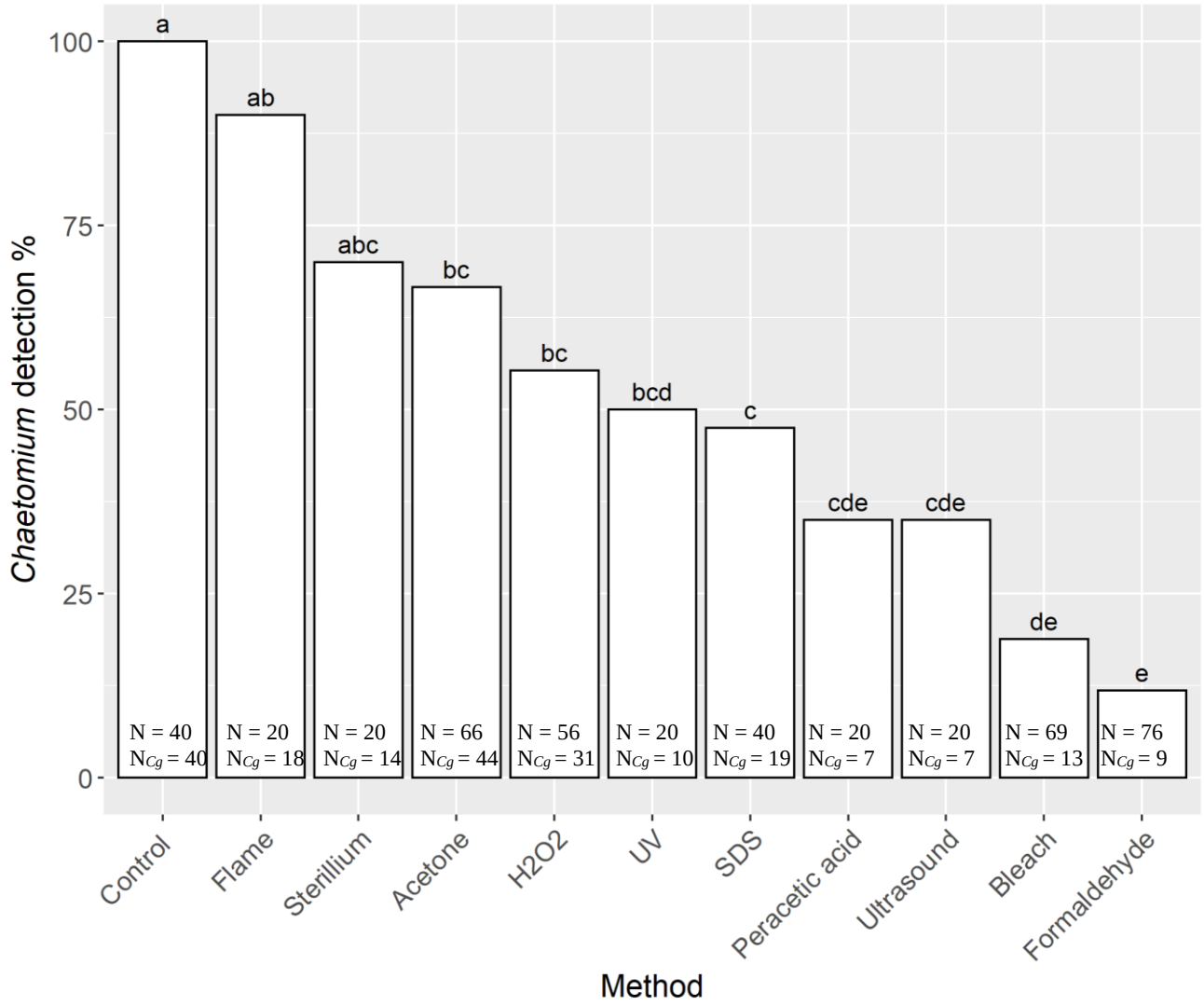

Figure 1: Effects of the method of decontamination on the detection frequency (%) of the fungus *Chaetomium globosum* in *Steganacarus magnus*. Bars sharing the same letter do not differ significantly ($p < 0.05$; Tukey's HSD test). Total numbers of tested individuals (N) and total numbers of detected *C. globosum* (N_{Cg}) are given at the bottom of the respective bars. For details of the decontamination methods see Table 1 and Methods.

2.3.2 Decontamination and gut content detection

Detection of nematode prey *Plectus* sp. in the gut of *S. magnus* varied significantly with decontamination methods ($F_{10,436} = 7.98$, $P < 0.001$). Generally, maximum detection of *Plectus* sp. was about 50 % with highest detection rates in non-decontaminated (53 %) and flame treated *S. magnus* (55 %; Fig. 2). Overall, detection frequency of *Plectus* sp. was not significantly reduced after decontamination with flame (55 %), bleach (49 %), SDS (48 %) and formaldehyde

(36 %). The other decontamination methods reduced the detection of *Plectus* sp. to below 20 %. In two decontamination treatments (peracetic acid and UV) *Plectus* sp. was not detected.

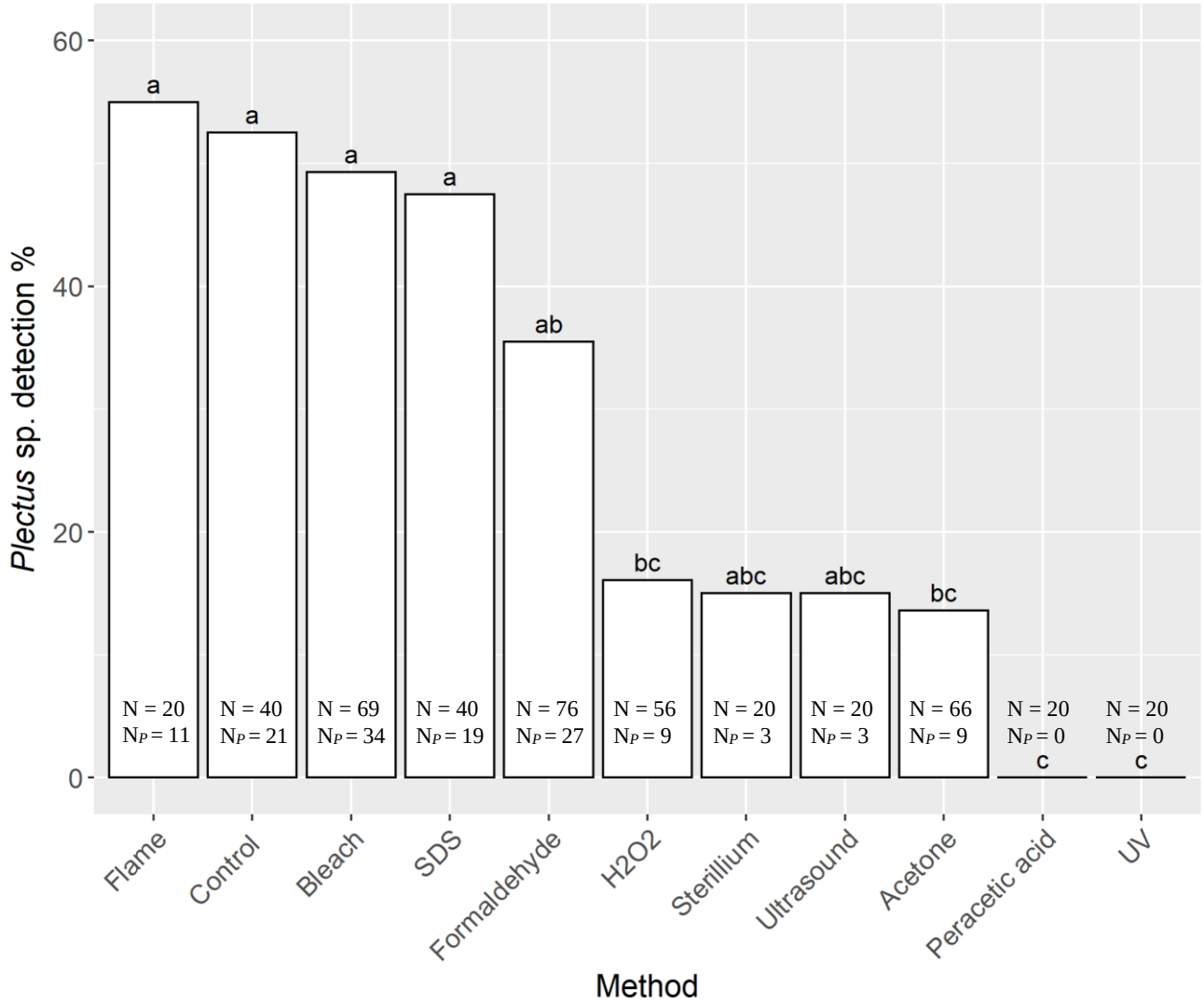

Figure 2: Effects of the method of decontamination on the detection frequency (%) of *Plectus* sp. in the gut of *Steganacarus magnus*. Bars sharing the same letter do not differ significantly ($p < 0.05$; Tukey's HSD test). Total numbers of tested individuals (N) and detected *Plectus* sp. (N_P) are given at the bottom of the respective bars. For details of the decontamination methods see Table 1 and Methods.

2.4 Discussion

We tested ten methods for their surface decontamination efficiency of a soil dwelling oribatid mite species, *S. magnus*, in a controlled laboratory experiment. Treatments with 5 % bleach and 37 % formaldehyde reduced contamination with fungal DNA significantly. Even though six other methods also reduced the detection of fungal DNA in contaminated *S. magnus*

individuals, the reduction rate varied and overall was not satisfying. Additionally, we tested if the decontamination methods affect the detection of prey DNA in the gut of *S. magnus* via PCR. Our results indicate that the detection of nematode DNA (*Plectus* sp.) is affected by decontamination methods. The detection of prey DNA was not significantly affected in specimens treated with bleach, whereas PCR amplification was reduced in specimens treated with formaldehyde. This suggests that even short formaldehyde exposure of oribatid mites for surface decontamination interfers with gut content material. Therefore, the treatment with bleach represents the best decontamination procedure for soil arthropods to be used for molecular gut content analysis. Other methods that did not affect the detection of gut DNA, such as flame and SDS, did not remove surface contamination or did not reduce sufficiently fungal contamination.

We chose the experimental set up with *S. magnus* feeding on *Plectus* sp. as nematode prey as it has been used previously in laboratory feeding experiments as well as in field studies investigating the gut content of soil microarthropods (Heidemann et al., 2014). Also, *S. magnus* can be collected in high numbers to allow solid replication of decontamination methods and to establish a reliable experimental basis. The primers used for the detection of the fungal contaminant *C. globosum* were sensitive to lowest amounts of DNA as they had been tested in serial dilution. Mites of the genus *Steganacarus* are characterized by a strongly sclerotized globular and ptychoid body shape and thus easy to handle and not to break easily during handling. Additionally, the notogaster of *S. magnus* is covered with several long setae on which microbial propagules are likely to stick. If handling soil animals from other taxa, e.g. with softer body cuticle or smaller body size, such as Collembola and Nematoda, the protocol for decontamination may need further refinement. The experimental set up and results of the present study may be taken as starting point to evaluate a wider range of body surface

contaminants as well as primers targeting to amplify the gut content of decomposer soil arthropods.

For analyzing the fungal diet of oribatid mites Remén et al. (2010) used a decontamination protocol containing 3.7 % bleach, but only obtained sufficient ingested fungal DNA material after pooling dissected guts. The dissection of guts is time-consuming and challenging for screenings of large numbers of soil microarthropod species for their gut content, since time between sampling and freezing of organisms is an important factor in the quality of gut DNA extracts (Alberdi et al., 2019). Additionally, as the authors also stated, dissecting the gut is not feasible for small or delicate microarthropods. Therefore, we tested the decontamination methods in combination with whole body DNA extraction and detection PCR. Greenstone et al. (2011) suggested to hand collect animals via a brush if possible to prevent cross contaminations in collection vessels containing a number of individuals for metabarcoding studies. While this might be possible for larger invertebrates living above the ground, it is not an option for animals living in opaque habitats such as the soil. Thus, Greenstone et al. (2012) also tested different bleach concentrations for their decontamination success and found that 2.5 % bleach and 40 min rotation provide the best results. However, since their study focused on insects living above the ground, we anticipated the procedure to be too harsh for small soil animals. Similarly, Miller-ter Kuile et al. (2021) suggested context specific decontamination treatments for DNA metabarcoding of invertebrates. However, they also argued that decontamination generally does not play a major role in metabarcoding of invertebrates of open terrestrial systems. Nonetheless, the authors stressed the need for a standardized and well tested method for surface decontamination of invertebrates. We shared this aim and tested a wide range of methods in a comprehensive and replicated manner aiming at establishing such standardized method.

Considering the enormous diversity of arthropod morphology and physiology establishing standard procedures for surface decontamination is challenging. This applies in particular for studies aiming at analyzing the gut content of microbe- and fungivorous animals in metabarcoding studies. We anticipated that incubation time of bleach or other decontamination reagents as well as handling techniques need to be adjusted accordingly. In ongoing experiments, we investigated in more detail the applicability of the best performing decontamination methods of this study for investigating the gut content of Collembola using metabarcoding (M. Jüds, unpublished data). Similar to oribatid mites, Collembola are mainly feeding on fungi, but have more delicate body cuticle. Due to their varying and small body sizes it is not possible to dissect the gut to exclude contamination from the body surface, similar to oribatid mites. In addition to the methods investigated in this study we also tested decontamination with a lower concentration of bleach (1.5 %) and shorter incubation time with formaldehyde (37 %, 3 min) and used cut pipette tips instead of tweezers for handling the animals. With the tested methods we were able to reduce fungal body surface contamination by over 95 %. Therefore, we suggest to use 1.5 % bleach as decontamination solution for arthropods with delicate cuticles such as Collembola.

Our results on the decontamination efficiency of ten methods suggest that the best practice for a reliable surface sterilization with no effect on the quality of the gut content in mites is a treatment with bleach. The treatment with formaldehyde (37 %) had sufficient decontamination results but might harm the gut content DNA. The use of other decontamination reagents, such as SDS and acetone, or treatments with UV radiation, did not result in valid decontamination of the body surface. We advise a standardized protocol consisting of three essential steps: “Wash 1”- rinsing and washing with water and ethanol to rinse off easy to remove contaminants, “Decontamination”- incubation in bleach (5 %) for 5 min, “Wash 2”- rinsing with water to remove residual bleach and contaminants. The concentration of bleach may be reduced to

1.5 % for delicate microarthropods such as Collembola and additionally the incubation time can be shortened as well. Only sterile tubes and tools should be used in a clean working environment, e.g. a laminar flow hood. How animals are transferred or how vigorous animals are mixed in the solution by vortexing or gently inverted by hand should be considered depending on the animals' surface structure and morphology. Despite further refinement and adjustment may be necessary for decontamination of other more delicate microarthropods, method proposed above provides a standardized protocol for the decontamination of a wide range of microarthropod taxa.

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3 Variations in the fungal diet of Collembola species with forest type as indicated by molecular gut content analysis

Ceratophysella denticulata in aquarelle by Svenja Meyer (*2023*)

Article in preparation

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Abstract

Forest soils are inhabited by an immense variety of microbes and animals. Fungi and bacteria typically live as saprotrophs and are of crucial importance for ecosystem processes such as leaf litter decomposition, carbon sequestration and nutrient cycling. However, they also function as food resource for soil dwelling invertebrates including microbivores and detritivores such as Collembola. Collembola are assumed to mainly feed on fungi, but detailed information on their fungal diet in natural systems is still missing. Amplicon metabarcoding of gut contents allows identification of fungi ingested by Collembola at the level of species. Here we analyzed the fungal diet of six Collembola species, *Ceratophysella denticulata*, *Isotomiella minor*, *Lepidocyrtus lanuginosus*, *Folsomia quadrioculata*, *Paristoma notabilis* and *Protaphorura armata*, in a beech and spruce forest in central Germany. We hypothesized that the fungal OTU composition in Collembola varies with the diversity of fungal genera in bulk soil of beech and spruce forests. Further, we hypothesized that the fungal diet at the level of genera as well as guilds varies among Collembola species. Contrasting these hypotheses, we neither found significant differences in fungal genera nor in fungal guilds associated with Collembola between forest types and Collembola species. Unexpectedly, across Collembola species and forest types the dominant fungal genus associated with Collembola was the entomopathogenic genus *Scopulariopsis* as well as ubiquitous sporulating fungal genera such as *Penicillium* and *Aspergillus*. The latter two taxa are likely to be ingested accidentally rather than selected as dietary taxa. As likely intentionally selected fungal taxa we identified mostly saprotrophic and pathotrophic fungal genera such as *Cladosporium*, *Ramularia*, *Mycena* and *Encoelia*, but the detection of these taxa also varied little among Collembola species and forest types. Overall, however, we only obtained a limited number of reads from our samples presumably due to amplification bias due to large amounts of Collembola DNA in our samples. Future studies on the molecular gut content of Collembola need to improve conditions for amplification of fungal DNA in bulk tissue samples of Collembola.

3.1 Introduction

Soils harbor an immense density and diversity of biota, which are linked by often hidden and cryptic interactions. In particular uncovering interactions between microbivore species and their microbial prey is challenging as most microbivore taxa feed on a wide range of microorganisms including bacteria and fungi (Hunt et al., 1987; Wall & Moore, 1999). However, knowledge on these interactions is crucial as they are likely to affect a wide range of soil processes, such as the decomposition of organic matter, nutrient cycling and soil structure formation (Bardgett, 2005).

The most important driver of soil processes are microbes, in particular fungi and bacteria, and soil living invertebrates, such as earthworms, oribatid mites and collembolans. Fungi can be categorized into functional groups (‘guilds’) by their nutrient assimilation strategies, including saprotrophic, symbiotrophic (mycorrhizal) and pathotrophic fungi. Fungal community composition differs between forest types and is coupled to the dominant tree species (Bahnmann et al., 2018). Saprotrophic fungi show higher abundances in coniferous forests than in monoculture beech stands. However, Ascomycota, the largest group of fungi including most saprotrophic fungi, also dominate in deciduous forests over Basidiomycota (Goldmann et al., 2015). Soil abiotic factors, such as pore size, pH and nutrient availability, but also biotic factors, such as species identity and architecture of plant roots, play an important role in determining the composition of fungal communities, and thereby alter the dietary spectrum of fungal feeding soil invertebrates. Collembola, one of the most abundant microarthropods in soils worldwide, may regulate microbial communities by feeding and dispersing fungi, i.e. transporting spores and hyphae within the gut system or attached to the body surface. Since soil microarthropods live in close vicinity of roots, dispersal may be particularly important for mycorrhizal fungi as well as potentially plant pathogens (Anslan et al., 2018). In general, by feeding on dead organic matter, bacteria, fungi as well as small animals such as nematodes, Collembola play an important role in litter decomposition and nutrient cycling, but also serve as important food source for predatory arthropods (Hopkin, 1997; Oelbermann et al., 2008). Generally, Collembola are assumed to predominantly feed on fungi, with preferring saprotrophic over ectomycorrhizal fungi in forest ecosystems (Pollierer & Scheu, 2021; Potapov & Tiunov, 2016). Food choice experiments have shown that Collembola preferentially feed on dark melanized fungi (Scheu & Simmerling, 2004). These dematiaceous fungi, such as *Alternaria* and *Cladosporium*, typically live as primary saprotrophs, which already colonize living leaves and are preferred by Collembola over typical soil fungi, such as *Penicillium* and *Trichoderma*,

acting as secondary saprotrophs (Klironomos et al., 1992). Direct feeding of Collembola on mycorrhizal fungi is considered scarce, but Kaneda & Kaneko (2004) found *F. candida* to preferentially feed on dead over living ecto-mycorrhizal fungal hyphae, possibly due to toxic secondary metabolites in living hyphae. However, experiments also indicated that Collembola feed on a mixture of fungal species with their preferences varying with the nutritional value of fungi. Mixed diets may help in improving the nutritional value of food, but also dilute toxins of individual species in the diet (Chauvat et al., 2014; Jørgensen et al., 2003, 2005; Scheu & Folger, 2004). Collembola not only directly use fungi as food, but may also benefit from fungal (and bacterial) exo-enzymes digesting complex organic compounds, thereby acting as an 'external rumen' (Swift et al., 1979). How Collembola select specific fungal functional groups and fungal species in the complex matrix of soil, however, remains little understood.

The use of basal resources and the trophic position of an organism can be reconstructed by measuring the body tissue composition of fatty acids and stable isotopes, respectively. Using fatty acid analysis the composition food resources can be traced to the level of phyla, such as plants, bacteria and fungi (Ruess & Chamberlain, 2010). In addition, the analysis of stable isotopes ratios of ^{13}C in fatty acids may allow to further trace the origin of the diet (Haubert et al., 2009; Ngosong et al., 2011). Stable isotope analysis adds another dimension to the diet of consumers by allowing to trace their trophic position using $^{15}N/^{14}N$ ratios (Potapov et al., 2019; Wada et al., 1991). The two methods provide crucial information on the position of a consumer within food webs, but are limited in the ability to identify species-specific links between consumers and their prey organisms. However, such information is necessary to shed light on the preferences of consumers for certain prey and, e.g. in Collembola to trace their link to saprotrophic and mycorrhizal fungal species. In contrast to fatty acid and stable isotope analysis, molecular gut content analysis using targeted amplicon sequencing (metabarcoding) allows species level analysis of food sources (King et al., 2008). Through the improvement and development of sequencing techniques, the parallel sequencing of millions of DNA fragments and the growing databases allow detecting of broad range fungal species (Pompanon et al., 2012; Tedersoo et al., 2022). Typically, general primers amplifying a barcoding region with ideally similar efficiency across taxa are used to detect a broad spectrum of food items in a consumer (Deagle et al., 2014; Taberlet et al., 2018).

Feeding preferences and trophic niches vary among species and ecological groups of Collembola, such as epedaphic, hemiedaphic and euedaphic Collembola (Ferlian et al., 2015; Potapov et al., 2021). Euedaphic Collembola, inhabiting soil layers, predominantly feed on

plant (root) resources and mycorrhizal fungi as indicated by stable isotope analysis. By contrast, hemiedaphic Collembola predominantly feed on microorganisms and heavily decomposed organic matter in the lower litter and upper soil layers, affecting the physical structure and mineralization of litter. The upper litter layer is colonized by epedaphic Collembola, are assumed to predominantly feed on saprotrophic fungi and non-vascular plant material, thereby affecting the decomposition of litter at early decomposition stages (Potapov et al., 2016).

We investigated the microbiome of Collembola in forests dominated by European beech and Norway spruce in central Germany (Hainich) using targeted amplicon sequencing of the most abundant Collembola species in both of these forests, i.e. *Lepidocyrtus lanuginosus* (Gmelin, 1788), *Ceratophysella denticulata* (Bagnall, 1941), *Parisotoma notabilis* (Schäffer, 1896), *Folsomia quadrioculata* (Tullberg, 1871), *Isotomiella minor* (Schäffer, 1896), *Protaphorura armata* (Tullberg, 1869). The six Collembola species represent three life forms, epedaphic (*L. lanuginosus, C. denticulata*), hemiedaphic (*F. quadrioculata, P. notabilis*) and euedaphic (*I. minor, P. armata).* In addition to Collembola freshly sampled in the field with filled guts, we also analyzed starved individuals to allow differentiation between the gut microbiome comprising ingested fungi on the one side and potential endosymbiotic fungi on the other. The study aimed at testing the following hypotheses:

(1) Fungal OTU richness of starved Collembola is less diverse than the OTU richness of non-starved Collembola and comprises mainly pathotrophic and symbiotrophic fungi, whereas the OTU richness of non-starved Collembola comprises mainly OTUs of saprotrophic fungi.
(2) The composition of gut OTUs in Collembola differs between forest types reflecting that the microbiome of beech and spruce forests differs.
(3) Euedaphic Collembola rely more on soil saprotrophic and root symbiotrophic fungal taxa including ectomycorrhizal species. Hemiedaphic Collembola feed on both soil fungi and fungi of the litter layer comprising mainly saprotrophic species. Epedaphic Collembola predominantly feed on saprotrophic fungi of the litter layer.

3.2 Materials & Methods

3.2.1 Study site

This study formed part of the "Biodiversity Exploratories", a large-scale, long-term functional biodiversity research project based in Germany (Fischer et al., 2010). We sampled Collembola specimens from two forest sites located in the Hainich-Dün region in central Germany (beech forest: 10.3592°E, 51.3371°N; spruce forest: 10.3236°E, 51.1853°N). One forest site was dominated by mature even aged European beech trees (*Fagus silvatica*; age ~70-120 years) stocking on Luvisol soil of a pH of 4.58-4.69. The forest was located at 432 m a.s.l., has a mean annual temperature of 7.6 °C and a mean annual precipitation of 619 mm. The second forest was dominated by Norway spruce (*Picea abies*; age ~60 years) stocking on Stagnosol soil of a pH of 5.41-6.79. The spruce forest was located at 427 m a.s.l., has a mean annual temperature of 7.8 °C and a mean annual precipitation of 622 mm.

3.2.2 Sampling of Collembola

Collembolans were extracted from litter and soil in November and December 2018. Soil animals were extracted by heat (40 °C) for a maximum of 3 h (Kempson et al., 1963) and collected alive in containers with wet tissue to avoid desiccation. Species were identified immediately under a stereo microscope and single specimens were transferred to sterile tubes and frozen at -80 °C. To ensure minimum changes in the gut content, we froze the specimens within a maximum time-span of 10 h after sampling from the field. A second set of the same species were placed into small containers lined with moist tissue and kept at 16 °C for five days for emptying their gut. After starvation, Collembola were transferred individually to sterile tubes and frozen at -80 °C.

3.2.3 Body surface decontamination

To avoid the detection of fungi attached to the body surface of Collembola, we sterilized each specimen by washing with bleach (1.5 %) for 3 min following the decontamination protocol of Jüds et al. (*in prep*). We washed animals under sterile conditions under a laminar flow hood and cooled samples on cooling blocks to avoid degradation of gut DNA during the decontamination procedure. The protocol consisted of three steps: (1) *wash 1*: rinsing of specimens in RNase free water and incubation in EtOH (98 %) for 5 min, (2) *decontamination*: incubation of specimens in bleach 1.5 % for 3 min, and (3) *wash 2*: rinsing in RNase free water

twice. At each step, animals were transferred with cut pipette tips to new sterile tubes, to which the next washing solution was added; then, the tubes were shaken carefully by hand.

3.2.4 DNA extraction, PCR and library preparation

Based on previous tests, we pooled eight specimens of the same species from the same forest type as one replicate to gain sufficient DNA for the analysis. DNA of pooled samples was extracted using the Agencourt DNAdvance Genomic DNA Isolation Kit (Magnetic Beads) (Beckman Coulter, Brea, USA) following the manufacturers protocol. Lysis was done in 100 µl lysis buffer and 10 µl proteinase K (20 mg/ml) at 55 °C on a mixing platform (ThermoMixer®, Eppendorf SE, Hamburg, Germany) over-night (18-20 h). Steps between lysis and elution were done following the manufacturers protocol. Final elution was done by adding 50 µl RNAse free water, resuspended magnetic beads and incubated at room temperature for 15 min. Then, the tubes were put on a magnet and incubated until the solution was completely clear, ~50 µl supernatant, containing the eluted DNA, was transferred to a new sterile tube and stored at -80 °C until further use. We added negative controls, i.e. RNase free water, to follow potential contaminations.

Amplification of the fungal ITS2 region was done using the primers including MiSeq adapters ITS3_KYO2 (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GATGAAGAACGYAGYRAA** -3') and ITS4 (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCCTCCGCTTATTGATATGC** -3') (primer region written in bold) (Toju et al., 2012). PCR was done using Phusion High-Fidelity DNA Polymerase master mix (Thermo Fisher Scientific, Waltham, USA). The total reaction volume was 25 µl per sample and consisted of 5 µl 5 x Phusion GC buffer, 0.5 µl $MgCl_2$, 1.25 µl 5% DMSO, 0.5 µl dNTPs (0.2 mM for each of the four nucleotides), 0.5 µl of each primer (0.2 µM of each primer), 20 ng of DNA, 1 unit Phusion High-Fidelity Polymerase. The master mix was filled up to a volume of 25 µl with RNase free water. The PCR program was set with following steps after an initial double strand denaturation at 98 °C for 1 min, followed by in total 25 repeating cycles of double strand denaturation at 98 °C for 45 s, primer annealing at 47 °C for 45 s and strand elongation at 72 °C for 30 s. The PCR program ended with one final strand elongation step at 72 °C for 5 min. The PCR was repeated three times, to gain three independent PCR replicates. The quality of PCR products was visualized and checked on a capillary electrophoresis system QIAxcel using the AL320 analyzing method (Qiagen, Hilden, Germany). PCR products were cleaned using the Beckman

Coulter™ Agencourt AMPure XP Kit (Beckman Coulter, Brea, USA) and DNA concentrations were measured using the QuantifFluor® dsDNA System (Promega, Madison, USA). PCR products of single technical triplicates were equimolar pooled to one replicate. A second PCR including the individual sample indexes and flow cell adapter primer was done using Nextera XT Index Kit following the manufacturers protocol (Illumina Inc, San Diego, USA). Tagged and pooled samples were sequenced on an Illumina MiSeq sequencing system producing paired end reads of 300 bp each. Sequencing was performed at the Göttingen Genomics Laboratory (G2L, Göttingen, Germany).

3.2.5 Bioinformatics

Raw paired-end reads were filtered for their quality using fastp (Chen et al., 2018, Version 0.23.2). Paired-end reads were merged using the software PEAR (Zhang et al., 2014, Version 0.9.11) and primers and adapters were cut off reads using cutadapt (Martin et al., 2011, Version 3.2). Additional read filtering was done using ITSx to exclude consumer ITS sequences (Version 1.1.3). VSEARCH OTU clustering, amplicon sequence variant ASV UCHIME 3 reference was performed subsequently (Rognes et al., 2016). Abundance tables of ASV/OTU were established with VSEARCH. OTUs were assigned to taxonomic level using BLASTn against the UNITE and NCBI nt database. Sequence identity scores/thresholds were set at > 93 %. Fungal functional groups assignments were done using FunGuild (Nguyen et al., 2016).

3.2.6 Statistics

Total read abundance data of fungal genera and fungal guilds were separated and analyzed independently to identify the effect of specific fungal genera and the effect of fungal functional groups on the gut content of Collembola. All analysis were performed using R (R Core Team (2021), Version 4.2.1) and RStudio. Two Collembola species needed to be excluded from the data analysis because both occurred only in one experimental treatment or forest type. Data from these species are given in Appendix Fig. 3. As the center log ratio data transformation requires a data set without zeros, we used the method count zero multiplicative (CZM) of the package ‘zCompositions’. This method transforms all zeros in a compositional dataset by a small proportion and in the same way modifies the non-zero values with a multiplicative way (Martín-Fernández et al., 2015). We then transformed total read numbers using the center log ratio transformation method (CLR) of the package ‘compositions’ in R. To identify differences in the OTU composition we used non-parametric multivariate analysis of variance

(PERMANOVA) based on euclidean distance measures with 9999 permutations. Graphics of barplots and ordinations were plotted using the 'GGPLOT2' package.

3.3 Results

Most of the original sequencing read output (8125 OTUs, 6,413,990 reads) contained reads of arthropod (Collembola) origin or could not be assigned to any fungal genus or guild and were excluded from the analysis. After removing all non-fungal sequences from the dataset, a total of 82 OTUs consisting of 1782 reads remained, which were assigned to the fungal kingdom. Most of these reads belonged to an epidermal pathogenic fungus of the genus *Scopulariopsis* and the sporulating fungal genus *Penicillium*. These fungal genera were equally distributed among Collembola species and forest types, and made up c. 50 % and 25 % of all reads, respectively (Appendix Fig 1).

The fungal functional group of "pathotroph-saprotroph-symbiotroph", which includes the fungal genus *Scopulariopsis*, made up > 50 % of the mean read abundance in the 'Guild dataset', followed by the "saprotroph" fungal functional group with > 30 % of the mean read abundance. The functional group of symbiotrophs was only found with < 4 % of the mean read abundance.

As the pathogenic *Scopulariopsis* is unlikely to form part of the diet of Collembola and the sporulating *Penicillium* likely represents accidentally ingested spores also not contributing to the diet of Collembola, we excluded both genera from the following analyses. Excluding these genera a total of 80 OTUs remained consisting of 660 reads.

Reads from the two Collembola species which occurred only in one experimental treatment or forest type, i.e. *C. denticulata* and *I. minor* are shown in Appendix Fig. 3. Both species were not included in the statistical analysis since they could only be tested for one forest type or one experiment. Generally, however, the fungal OTU compositions of both species was similar to the other four species analyzed.

We split the remaining dataset into starved and non-starved Collembola and analyzed these two datasets separately. In the non-starved Collembola dataset, we were able to assign 80 OTUs to fungal genera out of a total of 541 reads. The dataset of starved Collembola comprised only 19 OTUs and 119 reads. Most fungal OTUs of the non-starved Collembola dataset were assigned to the fungal functional group "pathotroph-saprotroph" with 56 reads (Fig. 1B) and comprised fungal genera such as *Aspergillus* and *Cladosporium*, i.e. typical widespread sporulating fungi (Fig. 1A). In addition, these non-starved Collembola contained saprotroph fungal genera, such

as *Encoelia*, *Lycoperdon* and *Preussia*, as well as some ectomycorrhizal fungi, although they were rare (Appendix table 1). We did not find indications for further symbiotrophic fungi in the starved Collembola dataset in addition to the (excluded) genus *Scopulariopsis.*

The reads of the non-starved Collembola dataset comprised 22.1 % pathotrophs, 22 % pathotroph-saprotrophs, 16.5 % saprotrophs, 14 % saprotroph-symbiotrophs, 10 % pathotroph-symbiotrophs, 9.3 % symbiotrophs and 6.2 % pathotroph-saprotroph-symbiotrophs (Fig. 1B).

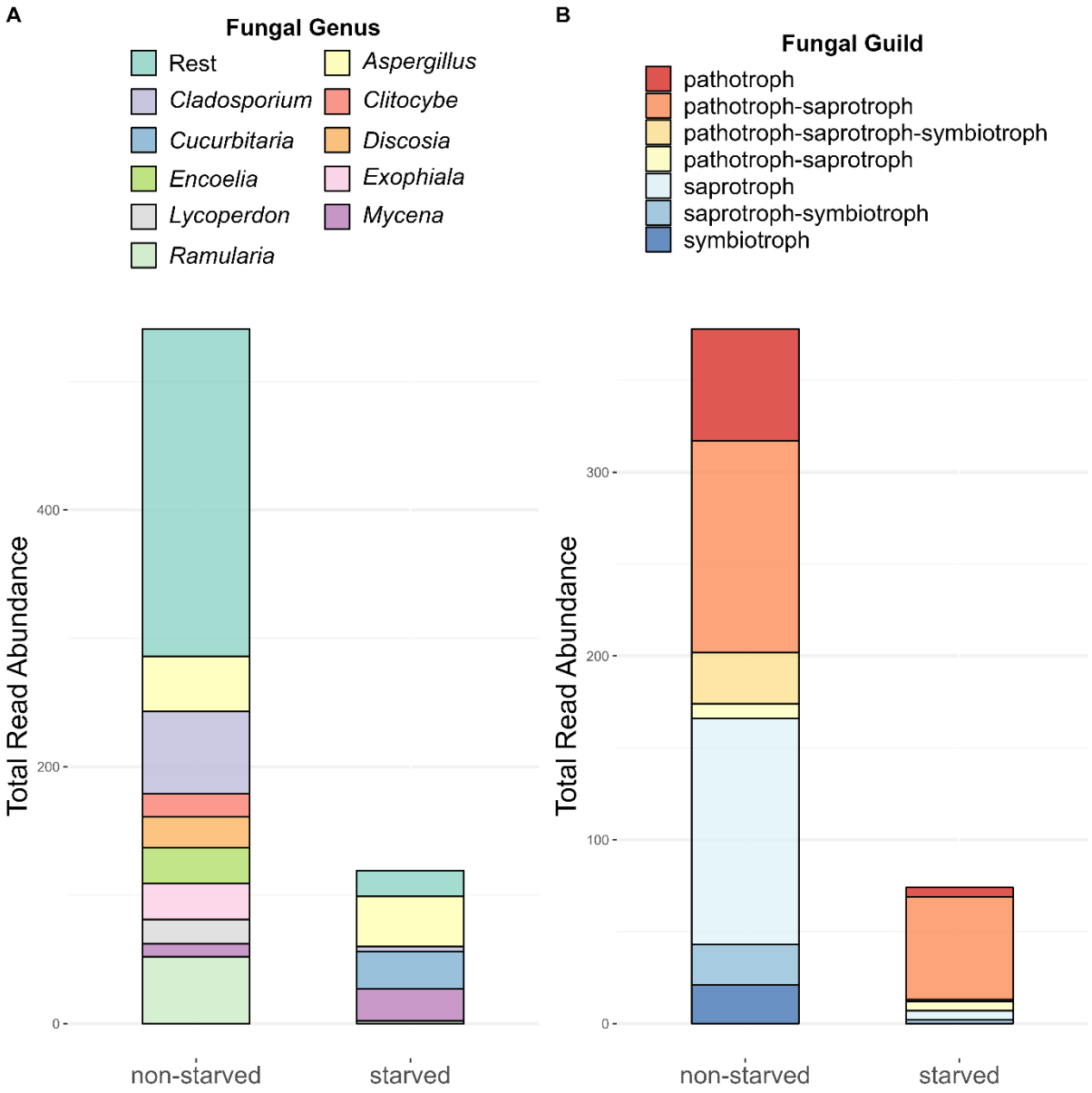

Figure 1: Total read abundance of fungi amplified from non-starved (n=58) and starved (n=50) Collembola assigned to fungal genera (A) and functional groups (B).

PERMANOVA did not identify significant differences between fungal functional group composition in non-starved Collembola, neither among species ($F_{(3,31)}$ = 1.1; P = 0.33) nor between forest types ($F_{(1,31)}$ = 1.2; P = 0.30), and the same was true for fungal OTU composition (Collembola species: $F_{(3,38)}$ = 0.46; P = 0.87); Forest type: $F_{(1,38)}$ = 0.42; P = 0.71). The lack of differences among Collembola species presumably was due to the fact that one species, *F. quadrioculata*, was responsible for the majority of read counts and contributed 389 reads with 68 assigned OTUs from the overall 541 reads. Of those reads 376 came from *F. quadrioculata* of the spruce forest (Fig. 2A; Appendix Table 2). The ten most abundant reads could be assigned to the genera *Ramularia* (52 reads), *Cladosporium* (46 reads), *Discosia* (24 reads), *Encoelia* (20 reads), *Clitocybe* (18 reads), *Lycoperdon* (18 reads), *Preussia* (14 reads), *Rhexocercosporidium* (11 reads), *Typhula* (11 reads) and *Psathyrella* (10 reads). These genera comprised mainly two fungal functional groups, pathotroph-saprotrophs and saprotrophs (Fig. 2B), which resembled the fungal functional group composition of the total non-starved Collembola dataset (Fig. 1B). Ectomycorrhizal fungi, such as *Tomentella* and *Dermocybe*, were rare with five and two reads, respectively, and this was also true for the total non-starved dataset with 5 and 2 reads (Sebacina: 6 reads, Russula: 4 reads; Inocybe: 2 reads). *Aspergillus* was not detected in the spruce forest samples of *F. quadrioculata*, although it was abundant in the other Collembola gut content samples with 43 reads in total.

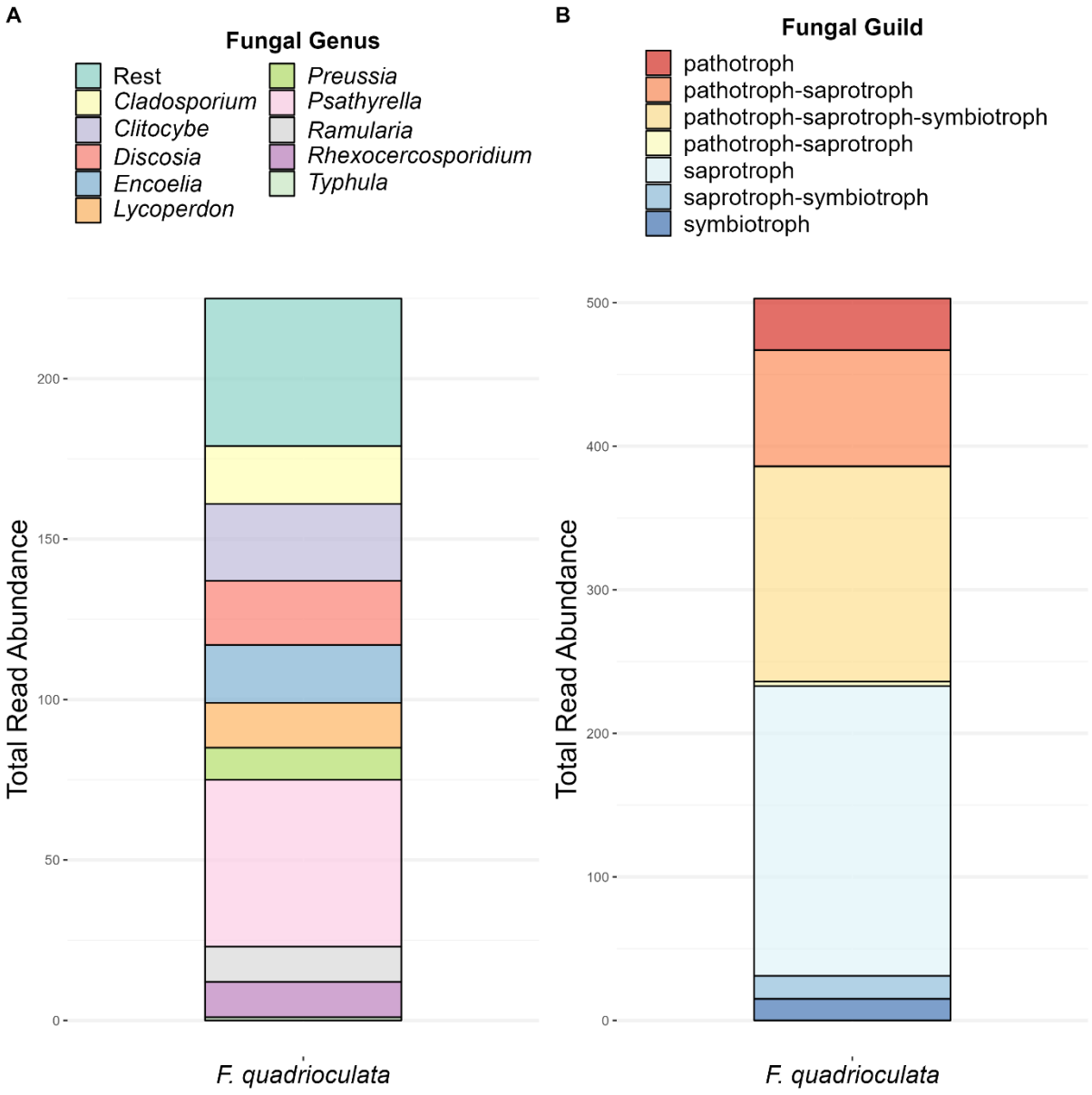

Figure 2: Total read abundance of fungi amplified from non-starved *Folsomia quadrioculata* assigned to fungal genera (A) and functional groups (B).

3.4 Discussion

We aimed at identifying variations in the fungal diet of Collembola with Collembola species and forest type. In the following, we will first discuss the findings in relation to our research questions and then discuss limitations of our study and possible improvements for future experiments.

3.4.1 Fungi associated with starved and non-starved Collembola

To discriminate between fungi in the diet of Collembola from possible fungal symbionts present in the digestive tract, we amplified fungal DNA from non-starved and starved Collembola species. Conform to our first hypothesis, starvation strongly reduced the number of reads compared to non-starved Collembola species by about 80% indicating that starvation effectively reduced amplification of fungi from the gut of Collembola. Consequently, the results suggest that fungi amplified from non-starved Collembola in large reflect fungi in the gut and therefore fungi which formed part of the diet of Collembola. This is supported by the fact that the OTUs amplified from non-starved Collembola predominantly comprised saprotrophic fungal genera, such as *Encoelia*, *Lycoperdon* and *Preussia*, but also some ectomycorrhizal fungi, although they were rare even in non-starved Collembola. Conversely, OTUs of ectomycorrhizal fungi were absent in starved animals. In both, starved and non-starved Collembola, however, the majority of the reads originated from cuticle pathogens (*Scopulariopsis*) and omnipresent spore forming fungi such as *Penicillium*, which are unlikely to form part of the diet of Collembola.

Contrasting our second hypothesis that differences in fungal communities between beech and spruce forests are reflected in the fungal diet of Collembola, the OTU composition between Collembola of spruce and beech forests did not differ significantly. Fungal community composition is known to differ strongly between coniferous and deciduous forests (Goldmann et al., 2015; Pollierer et al., 2015). Therefore, the results suggest that Collembola predominantly feed on fungal taxa present in both forest types pointing towards the selection of certain fungal taxa as food. Earlier studies indicated that Collembola indeed preferentially select certain fungal taxa (Chauvat et al., 2014; Jørgensen et al., 2003, 2005). Supporting our results, studies based on stable isotope and fatty acid analysis of the same Collembola species as investigated in this study also did not find significant differences between coniferous and beech forests indicating that their diet varies little with forest type (Ferlian et al., 2015). Overall, the results suggest that the composition of the diet of Collembola is rather independent of forest tree identity.

3.4.2 Variations with Collembola species and functional groups

We hypothesized that fungal diet of Collembola differs between Collembola species, due to their distinct vertical stratification in litter and soil in forests. In contrast to this hypothesis, the fungal OTU composition did not differ significantly between Collembola species regardless of forest type. Even though the Collembola species studied have been shown previously to feed

on different resources and occupy distinct trophic niches in forests (Chahartaghi et al., 2005; Pollierer et al., 2015), this was not reflected in the fungal diet as analyzed by molecular gut content analysis. Similar results were found in an earlier study on fungal feeding epedaphic Collembola, in which the fungal diet only varied with season but not between Collembola species (Anslan et al., 2018).

In non-starved Collembola, fungi of each of the functional groups considered (pathotroph, saprotroph, symbiotroph) were detected in each of the species studied. However, the assignment of a fungal genus to a certain functional group is not always straightforward as ecological roles of species within genera may vary. The most abundant functional groups identified across Collembola species were pathotrophs and pathotroph-saprotrophs with 22.1 % and 22.0 % mean read abundance, respectively. Pure pathotrophs were rare and did not form part of the 10 most abundant fungal genera across Collembola species, but were present in many genera with 5.5 reads on average. One of the (plant) pathotrophs was the genus *Ramularia*, which, however, may also be assigned to saprotrophs. Saprotrophs and saprotoph-symbiotrophs were detected with 16.5 % and 14.0 % mean read abundance across Collembola species. Preferential feeding of Collembola on saprotrophic fungi has been reported repeatedly (Anslan et al., 2018; Pollierer & Scheu, 2021; Potapov & Tiunov, 2016). The proportion of pure symbiotrophic fungal genera was generally low with 9.3 % mean read abundance across Collembola species suggesting that ecto-mycorrhizal fungi play only a minor role in the diet of Collembola, which is conform to earlier studies (Potapov & Tiunov 2016; Pollierer & Scheu, 2021). At least in part this may be explained by mycorrhizal hyphae repelling fungal feeders due to possessing thick cell walls and releasing toxins (Klironomos et al., 1992). Kaneda and Kaneko (2004) reported that *F. candida* preferentially feeds on dead rather than living ectomycorrhizal hyphae supporting that defense mechanisms of living hyphae contribute to low consumption by fungivores.

In contrast to our study, a similar study on the diet of Oribatida species using molecular gut content analysis found species-specific differences in the fungal food, with the fungi in the gut being closely related to the trophic niche of the mites (Gong et al., 2018). The lack of differences in fungi in the gut of Collembola species in our study points to a less intense selection of fungi in Collembola compared to Oribatida. However, the similar fungal taxa amplified from non-starved Collembola across the Collembola species studied may also have been due to amplification bias caused by large amounts of animal DNA in the samples.

In *F. quadrioculata* from the studied spruce forest, which contributed most to the reads of the Collembola species studied, most of the reads belonged to ascomycetes and could be assigned

to *Ramularia*, a plant pathogen, *Cladosporium*, a dematiaceaus hyphomycete genus, *Discosia*, a plant pathogen and *Encoelia*, a saprotroph. Only two genera, both saprotrophs, *Clitocybe* and *Lycoperdon*, within the ten most abundant fungi amplified belonged to Basidiomycota. This supports earlier findings that the diet of Collembola predominantly comprises Ascomycota rather than Basidiomycota (Anslan et al., 2016). A number of studies reported the preference of Collembola for dematiaceous Ascomycota, such as *Cladosporium*, indicating high nutritional quality of these fungi, although the reason for the preference of Collembola for dematacious hyphomycetes remains unclear (Scheu & Simmerling, 2004). Overall, the results support that Collembola selectively consume plant pathogenic and saprotrophic fungal taxa with mycorrhizal taxa being less important.

3.4.3 Fungal pathogens and ubiquists in Collembola

To the best of our knowledge, this is the first report of the fungal genus *Scopulariopsis* in Collembola. *Scopulariopsis* is a common saprotrophic facultative entomopathogenic fungal genus and was isolated from soils, air, decaying plant material and humid indoor environments (Domsch et al., 2007). Fungi of this genus were found to be associated with a number of arthropods (Bałazy et al., 1987; Mbuthia et al., 2012) and their role as entomopathogenic fungi is well documented in ticks, where infections by *Scopulariopsis* may be lethal to the host (Bonnet et al., 2021; Suleiman et al., 2016). However, as documented by Yoder et al. (2008), infection of the American dog tick *Dermacentor variabilis* by *Scopulariopsis brevicaulis* may also increase host resistance against infection by another fungal species, *Metharizium anisole*, which is responsible for lethal desiccation of the host, thereby *S. brevicaulis* functions as mutualist. Generally, however, the consequences of infection of insects and other arthropods by *Scopulariopsis* are little studied. In the Collembola species investigated in our study, the fungal infection was not detected visually from the outside and animals appeared healthy, despite all of the Collembola species studied were infected by *Scopulariopsis*.

Beside *Scopulariopsis* the second most frequently amplified fungi were of the ubiquitous genus *Penicillium*. Further, fungi of the ubiquitous genus *Aspergillus*, were frequently amplified. Food choice experiments have shown that soil microarthropods typically avoid chitinolytic fungi, such as *Penicillium* and *Trichoderma*, as food, potentially because these fungi have the ability to digest microarthropods (Maraun et al., 2003). Further, fungi of the genera *Penicillium* and *Aspergillus* contain toxins, and therefore typically are little fed by fungivore soil microarthropods (Scheu & Simmerling, 2004; Staaden et al., 2010). However, since these fungi

produce ample amounts of spores and therefore are ubiquitous in soil, they are likely to be accidentally ingested associated with other resources by soil detritivores.

3.4.4 Limitations and outlook

There are a number of limitations of our study, most of them related to the challenges of working with small soil organisms. We collected and sequenced up to 100 individuals per species and forest type in both the starved and non-starved treatments, and pooled eight individuals for amplification. Nevertheless, we only obtained a limited number of reads. The low number of reads may have been due to low amount of fungal tissue in the gut of Collembola. Although we minimized the handling time of Collembola before DNA extraction, handling may have resulted in reduced load of fungi in the gut. Further, it is known that Collembola empty their gut before molting (Hopkin, 1997) and this likely resulted in including Collembola specimens with empty guts in the analyses. An early study analyzing Collembola gut contents by microscopy found 40 - 50 % of the analyzed species to have empty guts (Anderson & Healey, 1972). Further, the primer used for amplifying the fungal ITS2 region also amplifies eukaryotic ITS2 sequences and amplification of Collembola ITS2 in our bulk samples may have hampered amplification of fungal ITS2. Due to their small body sizes and delicate cuticle, dissection of Collembola and using only gut material for amplification is not feasible. Rather, whole animal DNA extracts need to be used to amplify fungal DNA in the gut. Further, to allow focusing on gut DNA possible body surface contamination by microorganisms needs to be eliminated by body surface sterilization. Here, we used a washing protocol containing 1.5 % bleach (NaClO). Although we tested this washing protocol intensively (Jüds et al., submitted), it may have also have resulted in reduced detection of fungal DNA in the gut. Future studies therefore may employ blocking primers inhibiting the amplification of consumer DNA to improve the detection of fungal DNA from the gut of Collembola (Vestheim & Jarman, 2008), but the use of blocking primers also is not without problems (Piñol et al., 2015). Additionally, amplification bias towards certain taxa may have resulted in overrepresentation of these taxa (Elbrecht & Leese, 2015; Piñol et al., 2019). Further, different amounts of Collembola DNA due to variations in Collembola body size may have affected amplification of fungal DNA. Finally, even if a fungal genus was detected in the gut of Collembola, the genus may not necessarily be digested during the gut passage and thereby contribute to the diet of the Collembola species studied. In particular the latter problem may be overcome by combining molecular gut content analysis with stable isotope, fatty acid or compound specific stable isotope analysis of amino acids of consumers (Larsen et al., 2013; Pollierer & Scheu, 2021). Combining these methods

ultimately may allow understanding trophic relationships in fungivore food chains and resolve the structure of cryptic soil food webs.

Despite of the above limitations, we were able to identify a number of fungal genera contributing to the diet of Collembola, in particular considering the data from *F. quadrioculata*. The results suggest that the diet of Collembola of different ecological groups comprises mainly saprotrophic and pathotrophic taxa of Ascomycota. This underlines the role of Collembola as modulators of fungal-mediated decomposition processes as well as infections of plants by pathogenic fungi. Further, we found evidence for the ubiquitous infection of Collembola by the entomopathenogenic fungal genus *Scopulariopsis*. Uncovering the role of these fungi for Collembola populations and their functioning warrants further investigation.

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Appendix

Soil and Litter controls

We sampled bulk litter and top soil material (0-5 cm depth) of three randomly chosen sites from each of the two forest sites, which served as eDNA reference and positive control for the analyses.

We extracted DNA of bulk litter and top soil of both forests using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturers protocol. A total of 250 mg of each triplicate soil and litter sample was added to a PowerBead Pro Tube with lysis solution. Samples were vortexed on a FastPrep Tissue Homogenizer (Thermo Fisher Scientific, Waltham, USA) in two steps at 5.5 speed for 30 s. For the following steps of DNA extraction we followed the manufacturers protocol. DNA was subsequently stored at -80 °C until further use. DNA concentrations of each sample and control were measured using the Qubit dsDNA HS- and BR-Assay-Kits (Thermo Fisher Scientific, Waltham, USA).

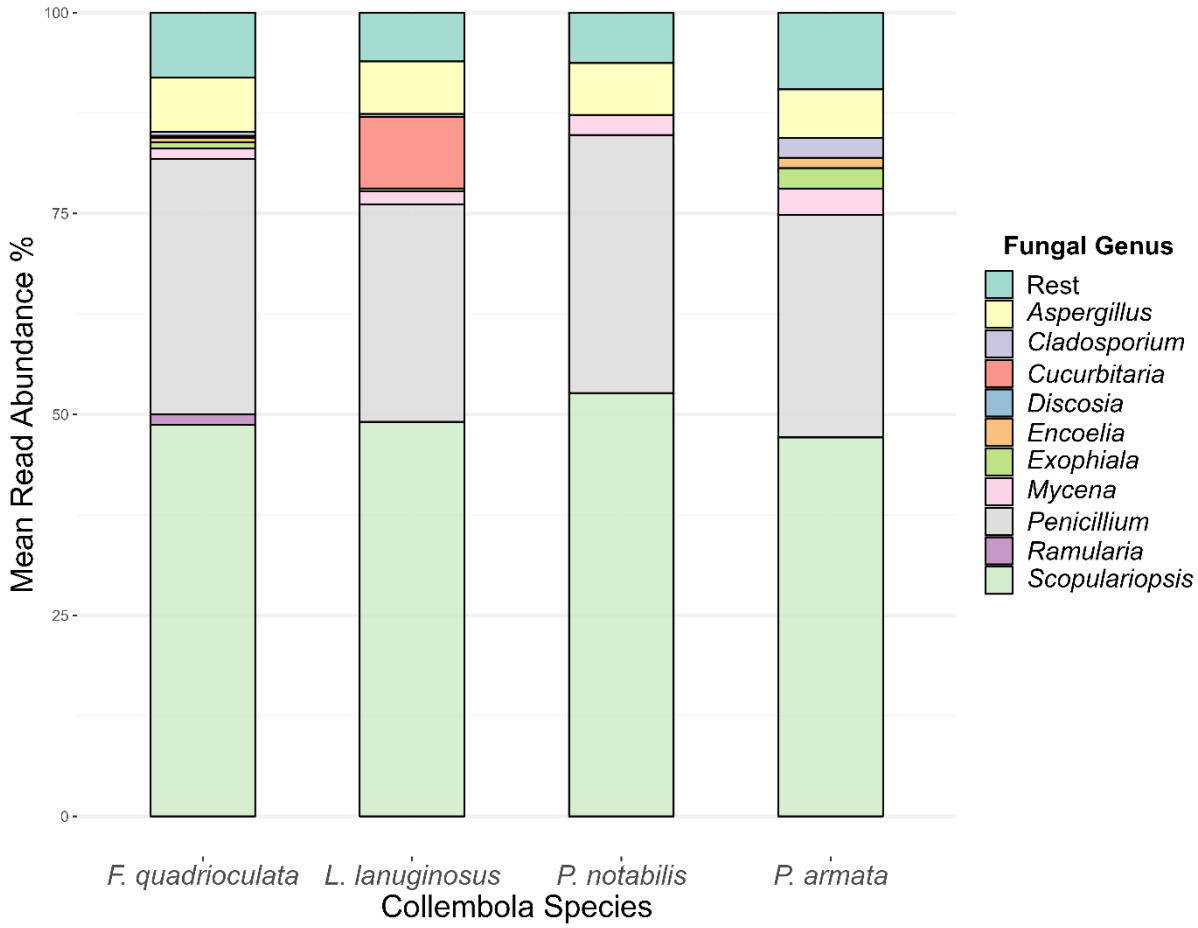
Appendix Table 1: List of the most abundant fungal taxa amplified from non-starved Collembola species *Folsomia quadrioculata*, *Lepidocyrtus lanuginosus*, *Parisotoma notabilis* and *Protaphorura armata* excluding the fungal genera *Scopulariopsis* and *Penicillium* from the studied spruce and beech forest with phylogentic identity and assigned fungal functional groups

total reads	fungal genus	phylum	order	family	guild
64	*Cladosporium*	Ascomycota	Capnodiales	Cladosporiaceae	NA
52	*Ramularia*	Ascomycota	Capnodiales	Mycosphaerellaceae	Pathotroph-Saprotroph
43	*Aspergillus*	Ascomycota	Eurotiales	Aspergillaceae	NA
28	*Encoelia*	Ascomycota	Helotiales	Cenangiaceae	Saprotroph
28	*Exophiala*	Ascomycota	Chaetothyriales	Herpotrichiellaceae	Pathotroph-Saprotroph
24	*Discosia*	Ascomycota	Xylariales	Sporocadaceae	NA
19	*Lycoperdon*	Basidiomycota	Agaricales	Lycoperdaceae	Saprotroph
18	*Clitocybe*	Basidiomycota	Agaricales	Tricholomataceae	Pathotroph-Saprotroph
15	*Preussia*	Ascomycota	Pleosporales	Sporormiaceae	Saprotroph
14	*Mortierella*	Mucoromycota	Mortierellales	Mortierellaceae	Saprotroph-Symbiotroph
12	*Lophodermium*	Ascomycota	Rhytismatales	Rhytismataceae	Pathotroph
12	*Rhizosphaera*	Ascomycota	Dothideales	Dothideaceae	Pathotroph
11	*Rhexocercosporidium*	Ascomycota	Helotiales	NA	Pathotroph
11	*Typhula*	Basidiomycota	Thelephorales	Typhulaceae	Pathotroph
10	*Mycena*	Basidiomycota	Agaricales	Mycenaceae	Pathotroph-Saprotroph
10	*Psathyrella*	Basidiomycota	Agaricales	Psathyrellaceae	Saprotroph
9	*Chloridium*	Ascomycota	Chaetosphaeriales	Chaetosphaeriaceae	Pathotroph-Saprotroph-Symbiotroph
8	*Cristinia*	Basidiomycota	Atheliales	Atheliaceae	Saprotroph
8	*Thelebolus*	Ascomycota	Thelebolales	Thelebolaceae	Saprotroph-Symbiotroph
7	*Brunnipila*	Ascomycota	Helotiales	Hyaloscyphaceae	Saprotroph
7	*Buckleyzyma*	Basidiomycota	NA	NA	Saprotroph
7	*Hymenoscyphus*	Ascomycota	Helotiales	Helotiaceae	Pathotroph-Saprotroph-Symbiotroph
7	*Trichoderma*	Ascomycota	Hypocreales	Hypocreaceae	Pathotroph-Saprotroph-Symbiotroph
6	*Fuscostagonospora*	Ascomycota	Pleosporales	NA	NA
6	*Oidiodendron*	Ascomycota	NA	Myxotrichaceae	Pathotroph-Symbiotroph
6	*Sebacina*	Basidiomycota	Sebacinales	Sebacinaceae	Symbiotroph
5	*Tomentella*	Basidiomycota	Thelephorales	Thelephoraceae	Symbiotroph
5	*Vishniacozyma*	Basidiomycota	Tremellales	Bulleribasidiaceae	NA
4	*Angustimassarina*	Ascomycota	Pleosporales	Amorosiaceae	NA
4	*Russula*	Basidiomycota	Russulales	Russulaceae	Symbiotroph
3	*Apiotrichum*	Basidiomycota	Trichosporonales	Trichosporonaceae	Saprotroph
3	*Chaetocladium*	Mucoromycota	Mucorales	Chaetocladiaceae	Saprotroph
3	*Coprinellus*	Basidiomycota	Agaricales	Psathyrellaceae	Saprotroph
3	*Cordana*	Ascomycota	NA	NA	Pathotroph-Saprotroph
3	*Hypholoma*	Basidiomycota	Agaricales	Strophariaceae	Saprotroph
3	*Hypomyces*	Ascomycota	Hypocreales	Hypocreaceae	Pathotroph
3	*Neonectria*	Ascomycota	Hypocreales	Nectriaceae	Pathotroph
2	*Chaetomium*	Ascomycota	Sordariales	Chaetomiaceae	NA

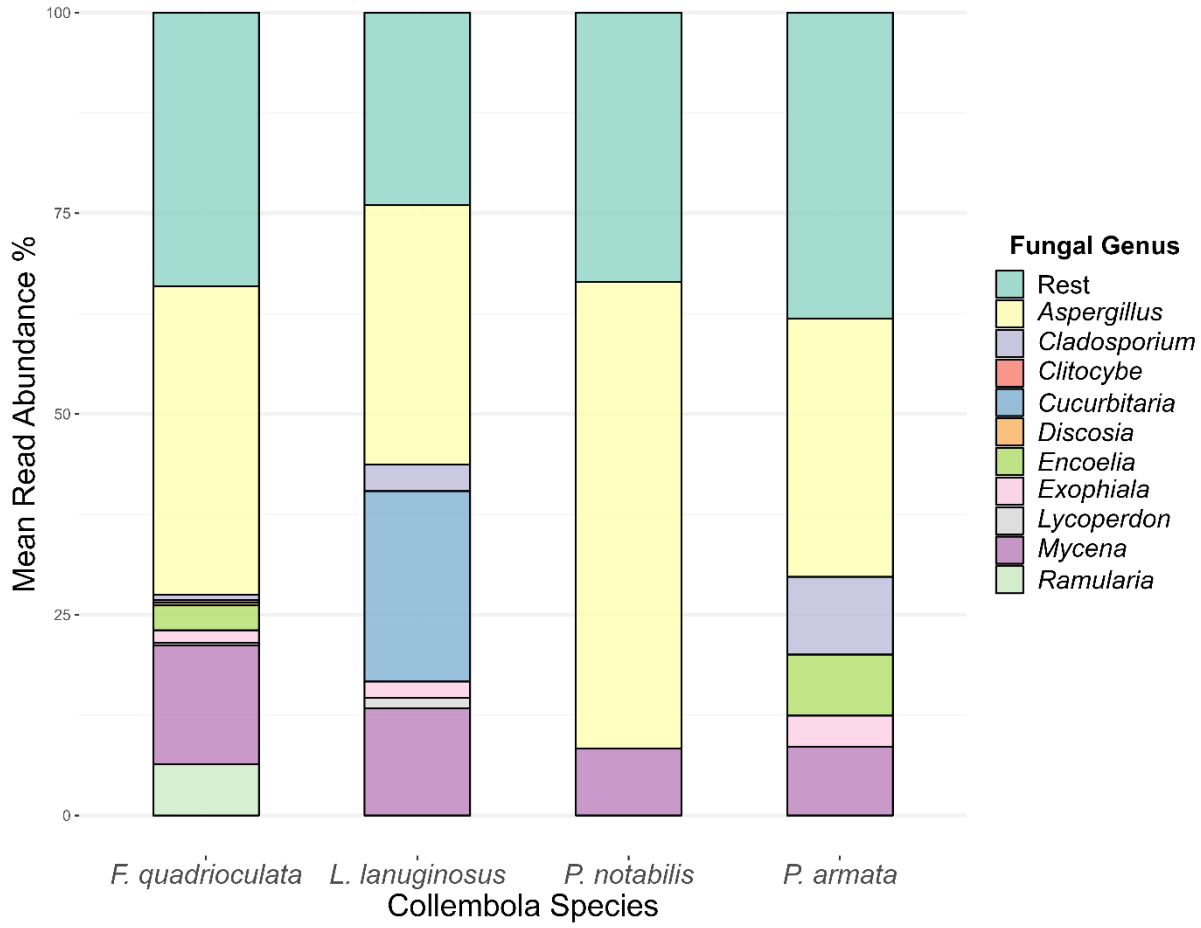
2	*Dendrophoma*	Ascomycota	Chaetosphaeriales	NA	Pathotroph
2	*Dermocybe*	Basidiomycota	Agaricales	Cortinariaceae	Symbiotroph
2	*Erythrobasidium*	Basidiomycota	Erythrobasidiales	Erythrobasidiaceae	NA
2	*Erythrobasidium*	Basidiomycota	Erythrobasidiales	Erythrobasidiaceae	NA
2	*Exobasidium*	Basidiomycota	Exobasidiales	Exobasidiaceae	Pathotroph
2	*Galerina*	Basidiomycota	Agaricales	Strophariaceae	Pathotroph-Saprotroph
2	*Glonium*	Ascomycota	NA	Gloniaceae	NA
2	*Gorgomyces*	Ascomycota	Helotiales	Leotiaceae	NA
2	*Inocybe*	Basidiomycota	Agaricales	Inocybaceae	Symbiotroph
2	*Lasiosphaeria*	Ascomycota	Sordariales	Lasiosphaeriaceae	Saprotroph
2	*Lecanicillium*	Ascomycota	Hypocreales	Cordycipitaceae	Pathotroph
2	*Lecanora*	Ascomycota	Lecanorales	Lecanoraceae	Symbiotroph
2	*Marasmius*	Basidiomycota	Agaricales	Marasmiaceae	Pathotroph-Saprotroph-Symbiotroph
2	*Neohelicomyces*	Ascomycota	Tubeufiales	Tubeufiaceae	Pathotroph-Saprotroph
2	*Striatibotrys*	Ascomycota	Hypocreales	Stachybotryaceae	NA
2	*Tubaria*	Basidiomycota	Agaricales	Strophariaceae	Saprotroph
1	*Allophylaria*	Ascomycota	Helotiales	Helotiaceae	Saprotroph
1	*Alternaria*	Ascomycota	Pleosporales	Pleosporaceae	Pathotroph-Saprotroph-Symbiotroph
1	*Beauveria*	Ascomycota	Hypocreales	Cordycipitaceae	Pathotroph
1	*Camposporium*	Ascomycota	NA	NA	NA
1	*Colacogloea*	Basidiomycota	NA	NA	Pathotroph
1	*Cordyceps*	Ascomycota	Hypocreales	Cordycipitaceae	NA
1	*Cystolepiota*	Basidiomycota	Agaricales	Agaricaceae	Saprotroph
1	*Dioszegia*	Basidiomycota	Tremellales	Bulleribasidiaceae	NA
1	*Entoloma*	Basidiomycota	Agaricales	Entolomataceae	Pathotroph-Saprotroph-Symbiotroph
1	*Gelasinospora*	Ascomycota	Sordariales	Sordariaceae	Saprotroph
1	*Geomyces*	Ascomycota	NA	Pseudeurotiaceae	Saprotroph
1	*Gliomastix*	Ascomycota	Hypocreales	Bionectriaceae	Saprotroph
1	*Humicola*	Ascomycota	Sordariales	Chaetomiaceae	NA
1	*Macrotyphula*	Basidiomycota	Agaricales	Clavariaceae	Saprotroph
1	*Microcyclospora*	Ascomycota	Capnodiales	NA	NA
1	*Microsphaeropsis*	Ascomycota	Pleosporales	NA	Pathotroph-Symbiotroph
1	*Neosetophoma*	Ascomycota	Pleosporales	Phaeosphaeriaceae	Saprotroph
1	*Ophiobolus*	Ascomycota	Pleosporales	Leptosphaeriaceae	Saprotroph
1	*Phlebiella*	Basidiomycota	Corticiales	Corticiaceae	Saprotroph
1	*Phragmocephala*	Ascomycota	Pleosporales	Melanommataceae	Saprotroph
1	*Phyllactinia*	Ascomycota	Erysiphales	Erysiphaceae	Pathotroph
1	*Plectosphaerella*	Ascomycota	Glomerellales	Plectosphaerellaceae	Pathotroph-Symbiotroph
1	*Pseudodictyosporium*	Ascomycota	Pleosporales	Dictyosporiaceae	Saprotroph
1	*Pseudogymnoascus*	Ascomycota	NA	Pseudeurotiaceae	Pathotroph-Saprotroph-Symbiotroph
1	*Rhodocollybia*	Basidiomycota	Agaricales	Tricholomataceae	Saprotroph
1	*Tetracladium*	Ascomycota	NA	NA	Saprotroph

Appendix Table 2: List of the most abundant fungal taxa amplified from non-starved *Folsomia quadrioculata* from the studied spruce forest with phylogentic identity and assigned fungal functional groups

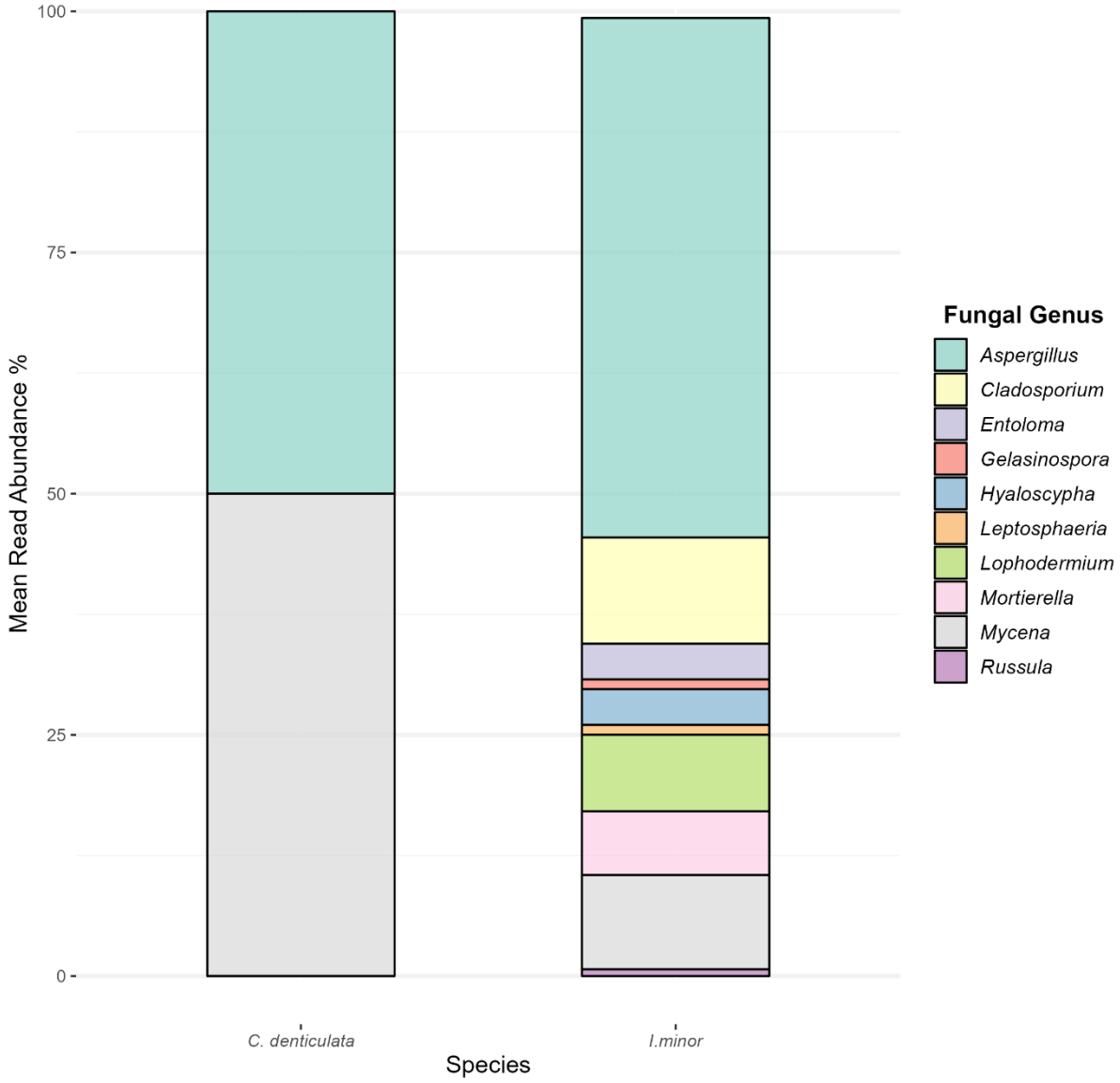
total reads	fungal genus	phylum	order	family	guild
52	*Ramularia*	Ascomycota	Capnodiales	Mycosphaerellaceae	Pathotroph-Saprotroph
46	*Cladosporium*	Ascomycota	Capnodiales	Cladosporiaceae	NA
24	*Discosia*	Ascomycota	Xylariales	Sporocadaceae	NA
20	*Encoelia*	Ascomycota	Helotiales	Cenangiaceae	Saprotroph
18	*Clitocybe*	Basidiomycota	Agaricales	Tricholomataceae	Pathotroph-Saprotroph
18	*Lycoperdon*	Basidiomycota	Agaricales	Lycoperdaceae	Saprotroph
14	*Preussia*	Ascomycota	Pleosporales	Sporormiaceae	Saprotroph
11	*Rhexocercosporidium*	Ascomycota	Helotiales	NA	Pathotroph
11	*Typhula*	Basidiomycota	Thelephorales	Typhulaceae	Pathotroph
10	*Psathyrella*	Basidiomycota	Agaricales	Psathyrellaceae	Saprotroph



Appendix Fig. 1: Mean relative read abundances of fungal genera amplified from the non-starved Collembola species *Folsomia quadrioculata*, *Lepidocyrtus lanuginosus*, *Parisotoma notabilis* and *Protaphorura armata* including the fungal genera *Scopulariopsis* and *Penicillium.*

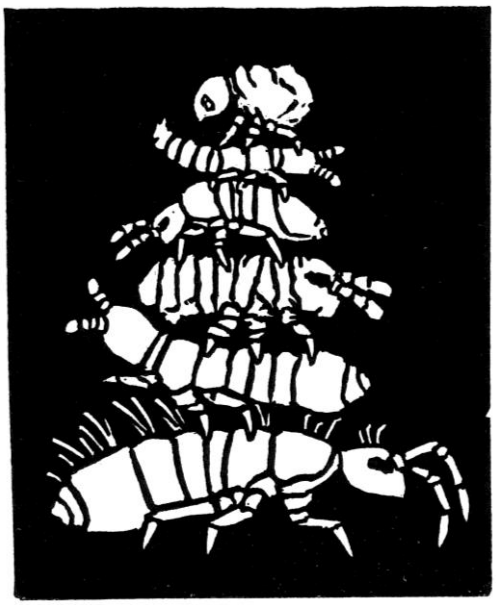


Appendix Fig. 2: Mean relative read abundances of fungal genera amplified from the non-starved Collembola species *Folsomia quadrioculata*, *Lepidocyrtus lanuginosus*, *Parisotoma notabilis* and *Protaphorura armata* without the fungal genera *Scopulariopsis* and *Penicillium.*



Appendix Fig. 3: Mean relative read abundances of fungal genera amplified from the non-starved Collembola species *Ceratophysella denticulata* and *Isotomiella minor* without the fungal genera *Scopulariopsis* and *Penicillium.*

4 Long term changes in Collembola community composition and abundance: the role of forest type and precipitation



"Stapled Collembola" linol cut print by Svenja Meyer (2023)

Article in preparation:

Jüds M, Bluhm S, Junggebauer A, Pollierer M and Scheu S. Long term changes in Collembola community composition and abundance: the role of forest type and precipitation. *in prep*. (2023).

Abstract

Analyzing biodiversity loss and its effect on ecosystem functioning is one of the main challenging tasks in face of global changes. We analyzed Collembola abundances, species richness and community structure at five sampling dates between 2008 and 2020. Samples were taken in four forest types consisting of coniferous forests of either pine or spruce, and young managed beech, mature managed beech and unmanaged beech forests in three regions in Germany, i.e. the Schorfheide in northern Germany, the Hainich in central Germany and the Swabian Alp in southern Germany. Generally, neither Collembola abundance nor species richness declined with time. However, depending on region Collembola abundance and community composition fluctuated. These fluctuations were linked to regional specific climatic conditions and soil characteristics. Precipitation was the main driver of fluctuations in Collembola abundance in the Schorfheide region, characterized by the harshest climatic conditions and by soils of sandy texture of low water holding capacity. Forest management intensity generally did not affect soil Collembola communities. Rather, the identity of the dominant tree species (deciduous or coniferous) was the main driver of Collembola community composition. Interestingly, the frequency of parthenogenetically reproducing Collembola species was at a maximum in the Schorfheide region, indicating that parthenogenetic reproduction facilitates recovery from harsh environmental conditions. Less pronounced response to climatic fluctuations in the Swabian Alp and especially the Hainich indicates that higher water holding capability buffers climate extremes. Overall, the results suggest that Collembola form an ideal model group for studying regional and habitat specific responses of soil animal communities to climate change.

4.1 Introduction

Soils form the basis of terrestrial food webs and provide essential services to humans. Soils harbor high biomass and diversity of a wide range of taxa including bacteria, fungi and animals. One of the main challenges of today is the loss of biodiversity documented for a wide range of ecosystems including temperate forests (Seibold et al., 2019). Therefore, monitoring of biodiversity and identifying consequences of the loss of species on the functioning of forests and other ecosystems is crucial (Storch et al., 2023). Doing that is the goal of the long-term, large-scale project “Biodiversity Exploratories” in Germany (Fischer et al., 2010). In the framework of this project, we investigated variations in Collembola communities with forest types and land-use intensity. Collembola form a crucial part in the functioning of ecosystems

in the soil as they feed on various resources, such as dead organic matter and microbes and thereby shape the litter structure and break up nutrients (Hopkin, 1997). Thus within the soil system a complex network of interactions takes place and provides essential ecosystem services, as carbon sequestration, decomposition of organic structures and nutrient cycling (Bardgett, 2005). To study interactions of soil microarthopods in this cryptic environment, feeding networks are reconstructed with diverse methods (Digel et al., 2014; Larsen et al., 2013; Pollierer & Scheu, 2021; Potapov, Tiunov, et al., 2019; Scheu & Setälä, 2002; Wada et al., 1991).

Collembola are one of the most abundant arthropods in soil worldwide (Potapov et al., 2023). They occupy an important node in soil food webs functioning as grazers of fungi but also as important prey for predators (Agustí et al., 2003; Eitzinger et al., 2013; Mundy et al., 2000; Potapov, Scheu, et al., 2019; Potapov, Tiunov, et al., 2019; Potapov & Tiunov, 2016). Until today, approximately 9400 Collembola species have been described (Bellinger, Christiansen & Janssens, 1996-2023), but this likely only represents a small fraction of the species existing worldwide (Orgiazzi et al., 2016). Trophic relationships of Collembola in soil foods received considerable attention, with recent studies based on stable isotope and fatty acid analysis stressing the wide range of trophic niches of Collembola varying among species and life forms (Chahartaghi et al., 2005; Pollierer et al., 2015; Potapov & Tiunov, 2016).

Starting with Gisin (1943) and later modified by Rusek (1989), Collembola have been assigned to functional groups (= life forms) and this grouping has been proven useful for analyzing the response of Collembola to environmental changes as well as their functioning in soil systems (Chahartaghi et al., 2005; Ferlian et al., 2015; Potapov et al., 2016). Atmobiotic Collembola inhabit the litter surface as well as the surface of plants. They are characterized by large body size, long appendages, pigmented body and coverage by setae. They are assumed to mainly feed on algae, lichens, plants and fungi (Potapov et al., 2016; Rusek, 1998). Epedaphic Collembola inhabit the upper litter layer or the surface of bark and dead wood. They also are characterized by large body size, with well developed appendages and visual organs. These species feed on a wide spectrum of diets including leaf litter, algae and fungi, but also nematodes (Chahartaghi et al., 2005). Atmobiotic and epedaphic Collembola typically reproduce sexually with sometimes complex courtship behavior (Sminthuridae) (Bretfeld, 1970; Chahartaghi et al., 2006). Hemiedaphic species typically are smaller than atmobiotic and epedaphic species and characterized by shorter appendages and less pronounced coloration with moderately developed visual organs. They move between litter and soil layers and mainly feed

on decomposed litter material and fungi (Potapov et al., 2016). Hemiedaphic Collembola either reproduce sexually or via parthenogenesis (Chahartaghi et al., 2006). Euedaphic species typically live in the mineral soil and are characterized by a lack of pigmentation, elongated body with short appendages and often reduced furca and ocelli (Potapov et al., 2016; Rusek, 1998). They mainly feed on microorganisms in the rhizosphere which also includes mycorrhizal fungi (Innocenti & Sabatini, 2018; Maraun et al., 2003). The majority of parthenogenetically reproducing Collembola species are of this life form (Chahartaghi et al., 2006; Chernova et al., 2010).

Collembola reach high densities and species richness in temperate forest ecosystems with abundance typically varying in the range of 40,000 – 60,000 ind/m² with species numbers in the range of 30 - 60 (Hopkin, 1997; Petersen & Luxton, 1982; Pollierer & Scheu, 2017; Potapov et al., 2023). By feeding on fungi, including pathotrophic species, and by stimulating nutrient mobilization they may beneficially affect plant growth (Innocenti & Sabatini, 2018; Sabatini & Innocenti, 2001). In addition, Collembola also serve as food for predatory arthropods, both below and above the ground (Eitzinger et al., 2013; Toju & Baba, 2018). In forests, the dominant tree species has large influence on belowground soil biota through resource input via leaf litter and root exudates (Aupic-Samain et al., 2019; Cesarz et al., 2013; Chen et al., 2019; Eissfeller et al., 2013; Ferlian et al., 2021; Henneron et al., 2017; Tedersoo et al., 2015). Thus, the quality and structure of leaf litter and root exudation shapes belowground decomposition processes (Albers et al., 2006; Berg & McClaugherty, 2014; Pollierer et al., 2007). Spruce leaf litter had the most distinct fungal community structure in a study examining the litter decomposition ability of saprotrophic fungi in temperate forest (Kubartová et al., 2009). In addition abiotic factors such as soil type and structure, acidity and hydration have large impacts on the soil community structure (Birkhofer et al., 2012; Brockett et al., 2012; Erktan et al., 2020). Since Collembola essentially rely on microbial food resources, the type of forest and thus litter quality and structure might also impact their abundances and community composition.

Within the last 15 years aboveground arthropod diversity on temperate grassland and forest systems declined rapidly and was closely associated to land-use practices (Seibold et al., 2019). How and if this decline is mirrored in the belowground arthropod system is not known.

We studied the temporal variations in Collembola abundances and community composition in three regions across Germany with coniferous and deciduous forests representing a management intensity gradient including the effect of environmental factors. Pollierer & Scheu

(2017) compared the temporal dynamics of two sampling dates of the same study system and found mainly differences in community composition caused by region and not by forest type. However, if Collembola species respond differently in distinct forest types of management intensity under changing climatic conditions is still unclear. The need for long term monitoring of soil animals has been stressed repeatedly (Guerra et al., 2021).

(1) The overall abundances and species richness of Collembola decrease with time
(2) Collembola abundance and community composition depends on forest type, with the abundance decreasing with forest management intensity and community composition differing most between coniferous and young beech forests.
(3) Collembola life forms and reproductive modes are affected differently by regional factors, forest type and precipitation throughout the years

4.2 Materials & Methods

4.2.1 Study sites

The study was conducted in the framework of the Biodiversity Exploratories, a large scientific platform aiming at linking biodiversity and ecosystem functioning research (www.biodiversity-exploratories.de; Fischer et al. 2010). Field sites of the Biodiversity Exploratories are located in three regions across Germany, Schorfheide-Chorin (Schorfheide), Hainich-Dün (Hainich) and Swabian Alb. The Schorfheide exploratory is located in the northeast of Germany at an altitude of 3-140 m a.s.l. and is characterized by a young glacial landscape with mainly Dystric Cambisol soils often displaying a texture from sandy loam to pure sand. It is the warmest and driest exploratory in this study with an annual mean temperature of 8.0-8.5 °C and an annual mean precipitation of 500-600 mm. The Hainich exploratory is located in central Germany at an altitude of 285-550 m a.s.l. and characterized by calcareous bedrock with mainly Eutric Cambisol, Luvisol and sometimes Stagnosol soils (with clayey and loamy texture). The annual mean temperature is 6.5-8.0 °C and annual mean precipitation is 500-800 mm. The Swabian Alb exploratory is located in the southwest of Germany at an altitude of 460-860 m a.s.l. and is characterized by calcareous bedrock with karst phenomena with mainly clay rich Eutric Cambisol and Leptosol soils. It is the coldest and wettest of the three exploratories with an annual mean temperature of 6.0-7.0 °C and an annual mean precipitation of 700-1000 mm. Soils

differ in acidity between regions with pH 3.3 ± 0.19 in the Schorfheide to 4.51 ± 0.72 in the Swabian Alb and 4.59 ± 0.67 in the Hainich (Pollierer & Scheu, 2017).

In each exploratory, four forest types with four replicates each were sampled. Forest types consisted of coniferous forests, young even-aged beech stands (*Fagus sylvatica*, age ~30 years; young managed beech forest), mature even-aged beech stands (age ~70 years; old managed beech forest) and beech stands that were left unmanaged for at least 60 years (age ~150 years; unmanaged beech forest). Forest types are characterized by different management intensities, decreasing from coniferous to young managed beech to old managed beech to unmanaged beech forests. Coniferous forests included pine stands (*Pinus sylvatica*, age ~50 years) in the Schorfheide and spruce stands (*Picea abies*, age ~60 years) in the Hainich and Swabian Alb. Experimental plots were located in 100 m x 100 m grid plots with a minimum distance of 200 m to another plot as well as a minimum of 100 m distance to the forest edge.

4.2.2 Sampling, extraction and determination of Collembola

Sampling took place in a standardized manner every three years from spring 2008 to spring 2020, with five sampling campaigns at three-year intervals. Sampling took place in 3 m x 3 m subplots located within the 100 m x 100 m grid plot representing the general vegetation structure of the forest. One soil core was taken on each plot for the extraction of soil microarthropods. The sample was divided into litter layer and the upper 5 cm of soil and in each exploratory a total of 16 soil cores were taken with forest type replicated four times. For the extraction of Collembola soil cores of a diameter of 5 cm were used. For the analysis of microbial biomass, three soil cores (5 cm) were taken and divided into litter and upper 5 cm of soil. Individual litter and soil samples were mixed and analyzed by measuring the maximum initial respiratory response (for further details see Klarner et al. (2014).

Soil animals were extracted using a heat gradient for approximately 10 days (Macfadyen, 1961). The extraction started at room temperature (22.5 °C) for 2 d, increased by 2.5 °C during days 3-5, then by 5 °C during days 6-7 and stayed at 40 °C during days 8-10. A solution of glycol and water (1:1) was used for collecting the animals. After the extraction was completed, animals were transferred to 70 % ethanol until further usage.

Collembola species were identified using a light microscope equipped with phase contrast using keys of Gisin (1960), Fjellberg (1998, 2007) and Hopkin (2001). Specimens were brightened with a solution of lactic acid and glycerol (2:1) or by hydrogen peroxide if necessary (Pollierer & Scheu, 2017).

4.2.3 Statistical analysis

We used R (R Core Team, 2021, v.4.2.1) and R Studio (v.2022.07.0) for all statistical analysis. Climatic parameters and the Silvicultural Management Intensity (SMI) index of the studied experimental plots were obtained from the "Biodiversity Exploratories Information System" (BExIS, www.bexis.uni-jena.de).

For the analyses we pooled data from the two layers (litter and soil) of one sampling time and plot. Abundance count data was log transformed before analysis. Data from the experimental grid plots HEW2 and HEW51 (Hainich, coniferous forest), were excluded from the analysis, since, due to climate induced decline of spruce forests in Germany, they could only be sampled for three and two sampling times, respectively. We performed a generalized linear mixed-effect model using the function 'glmer' of the 'lme4' package with a negative binomial data distribution to identify changes in Collembola abundances between years, regions and forest types. Plot identity (Plot ID) was set as a random factor to prevent plot specific effects to influence the analysis, even though the explained variance by 'Plot ID' was very low. We set 'year' as factor in the analysis and added environmental factors including mean precipitation during winter and spring, and SMI and microbial biomass in litter to the model. All environmental factors were standardized before analysis. Preliminary analyses indicated that mean temperature in winter and spring did not improve the model and correlated strongly with precipitation.

To analyze changes in Collembola species composition between sampling years, regions and forest types, we used non-metric multidimensional scaling (NMDS) and extracted species and site scores of the first two axis. We then used permutational multivariate analysis of variance (PERMANOVA) with 999 permutations and Euclidean distance matrix to identify if Collembola community composition varies significantly with the analyzed factors.

We used canonical correspondence analysis (CCA) to identify and visualize differences in Collembola community composition as affected by environmental variables. Additionally we analyzed Collembola community structure using the 'gllvm' package with a reduced species matrix, filtered for Collembola species with abundances > 5 per soil core and species that occurred in several sampling years. We grouped Collembola per Plot ID and used this grouping as a random factor. Data distribution family was negative binomial. We inspected the effect of mean precipitation in winter and spring and SMI using the function 'gllvm'. Effect size plots were calculated using the function 'coefplot.gllvm'.

In addition to the species level community analysis we also assigned the analyzed Collembola species to functional groups (life forms) including atmobiotic, epedaphic, hemiedaphic and euedaphic. Further, we divided the dataset into sexual or parthenogenetic reproducing species. Information on functional groups assignment and reproductive was compiled by Pollierer & Scheu, 2017 and through further literature research (Chahartaghi et al., 2006; Chernova et al., 2010; see Appendix table 1). Both trait sets were analyzed using the community weighted mean function (CWM), which transforms abundance data of Collembola species to proportional data of traits per sampling ID/Plot ID. Variations in Collembola life forms and reproductive mode with environmental factors were identified using generalized linear mixed models with a "beta" distribution family, typical of proportional data, and ANOVA. Numbers of the different Collembola functional groups and frequency of reproductive modes were compared between years, region and forest types using Tukey's HSD test.

4.3 Results

4.3.1 Abundances

Across the five sampling campaigns from 2008 – 2020 we collected 18,612 Collembola individuals consisting of comprising > 100 species. Temporal dynamics of Collembola species diversity, richness and evenness are displayed comprising all regions and forest types in Appendix Fig. 1. Collembola abundance was significantly affected by sampling year (χ^2 = 21.02, P = 0.0003), forest type (χ^2 = 8.62, P = 0.035) and the interaction between region and sampling year (χ^2 = 18.78, P = 0.016) and region and mean precipitation in spring season in the respective sampling year (χ^2 = 8.18, P = 0.017) as identified by the generalized linear mixed effect model. Collembola abundances in the Hainich were stable across the sampling dates, but fluctuated in the Swabian Alb and most pronounced in the Schorfheide (Fig. 1A). Precipitation in winter and spring before sampling fluctuated in a similar way between sampling years (Fig. 1B & C). Total Collembola abundance was similar between regions with on average 39,867 ± 26,998 ind./m² in the Swabian Alb, 40,084 ± 29,270 ind./m² in the Hainich-Dün and 38,468 ± 32,940 ind./m² in the Schorfheide-Chorin. By contrast, total Collembola abundance was significantly higher in coniferous forests (51,290 ± 36,430 ind./m²) compared to the 30, 70 and 150 year old beech forests (32,975 ± 27,488, 37,895 ± 26,187 and 35,732 ± 24,656 ind./m², respectively). Figures representing the microbial biomasses of litter and soil and SMI between years, region and forest types are shown in the appendix (App. Figs. 2 A, B; 3).

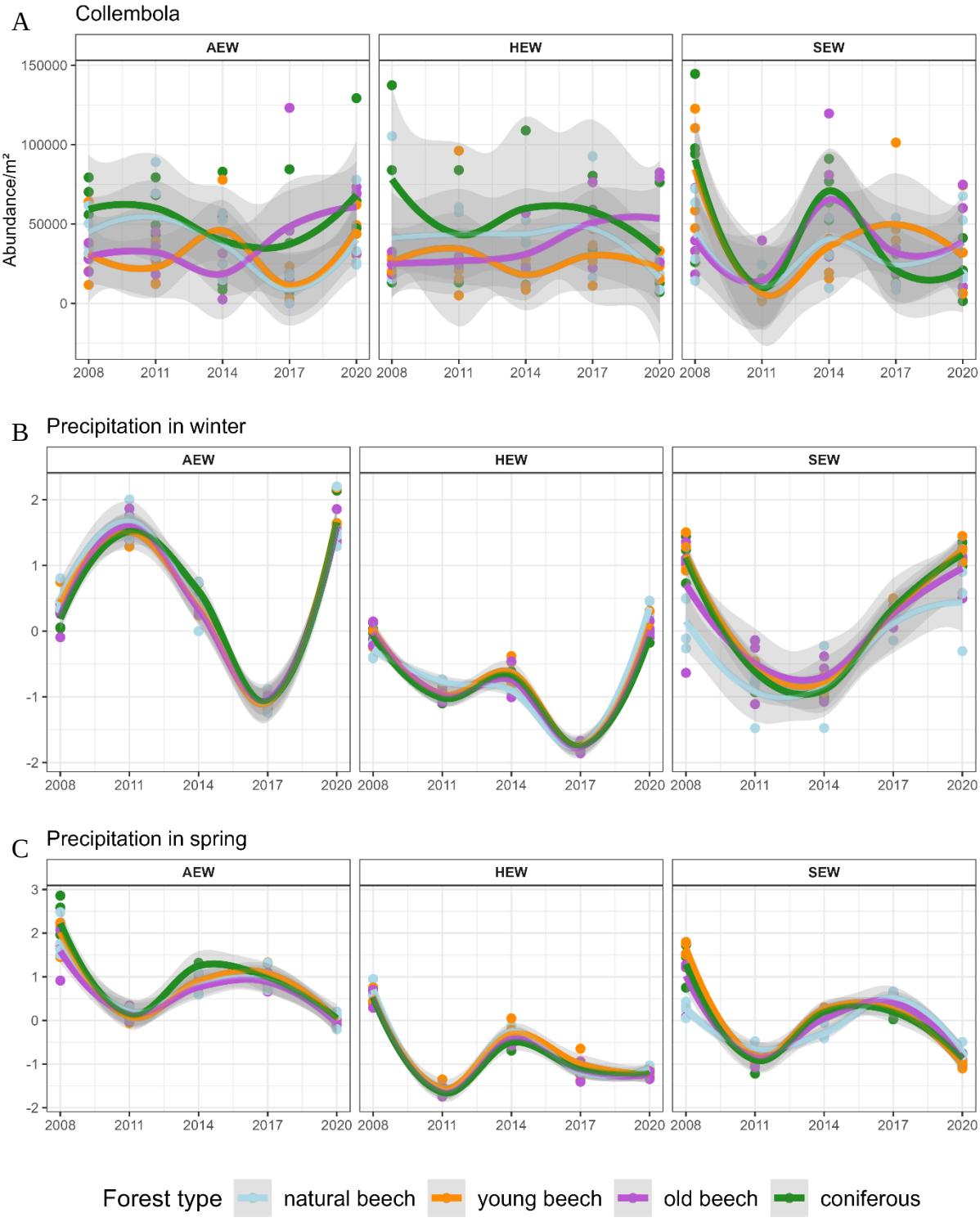


Figure 1: Temporal variation in the abundance per m² of Collembola (A), precipitation in winter (B) and spring before sampling (C) in the Swabian Alb (AEW), Hainich (HEW) and Schorfheide (SEW). Forest types in trend lines and plots (dots; mean per sampling year) are indicated by color. Precipitation is standardized as used in the statistical analysis.

4.3.2 Community Composition

Collembola species composition differed significantly between sampling year ($F_{4,168}$ = 8.08, P = 0.001), region ($F_{2,168}$ = 10.04, P = 0.001) and forest type ($F_{3,168}$ = 3.31, P = 0.001) (Fig. 2 A-C). In addition, the community composition was also affected by the mean temperature in spring of the respective sampling year ($F_{1,168}$ = 2.25, P = 0.019), and the silvicultural management intensity as represented by the SMI ($F_{1,168}$ = 2.21, P = 0.020). The interaction between region and forest type ($F_{6,168}$ = 1.61; P = 0.005) as well as between region and sampling year ($F_{8,168}$ = 1.95, P = 0.001) also significantly affected Collembola community composition. Environmental variables were plotted using canonical correlation analysis (CCA) (Fig. 3).

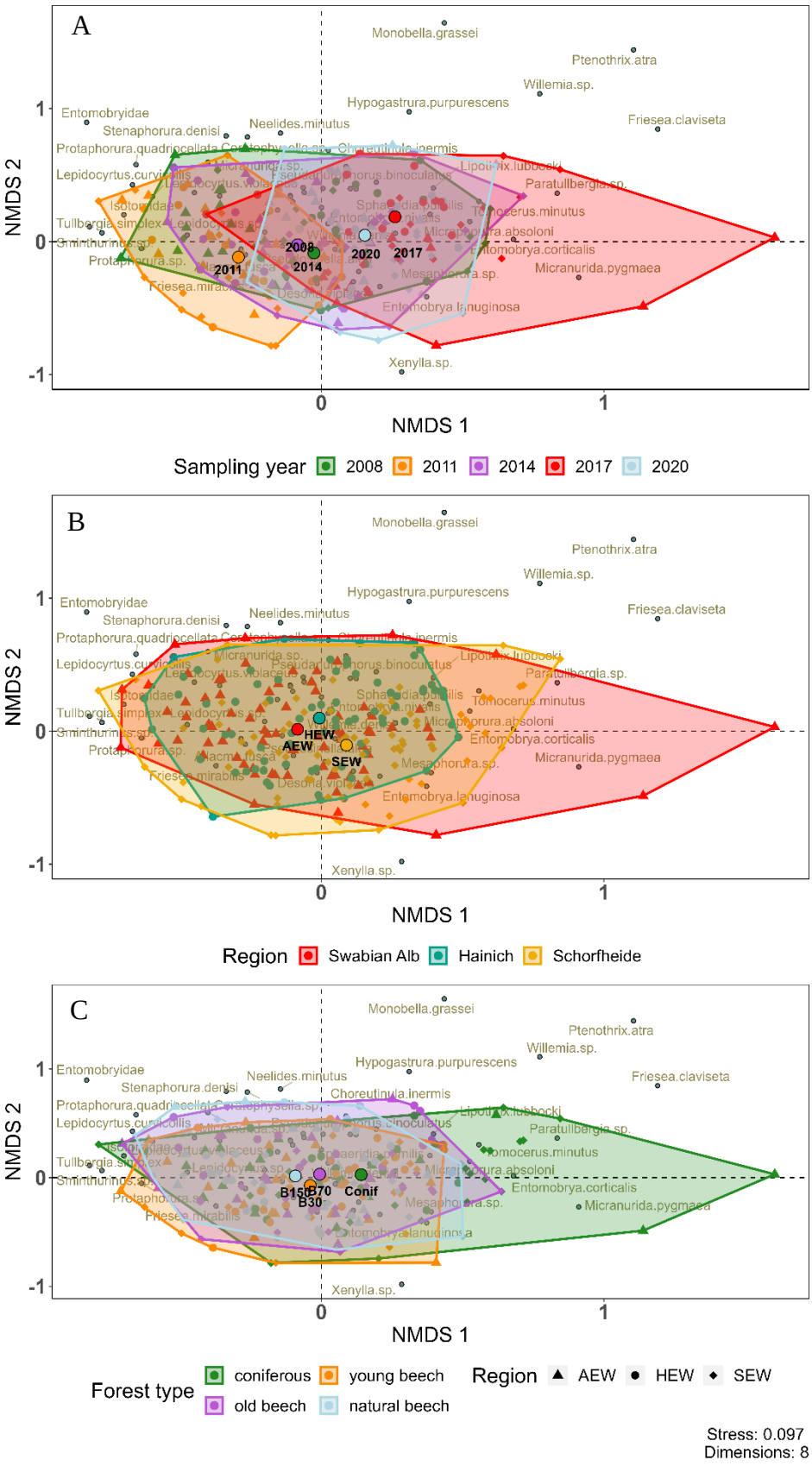


Figure 2: Variations in Collembola community composition between sampling years (A), regions (B) and forest types (C) based on the first two axes of non-metric multi-dimensional scaling.

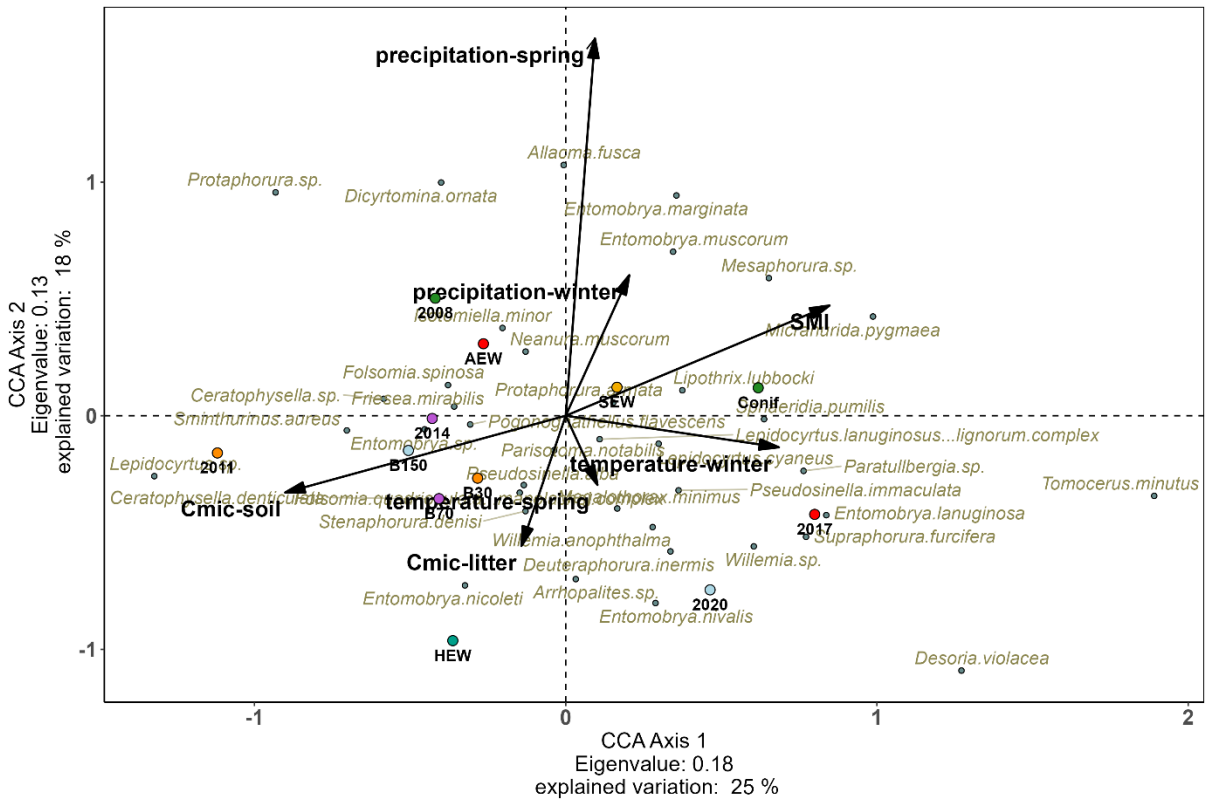
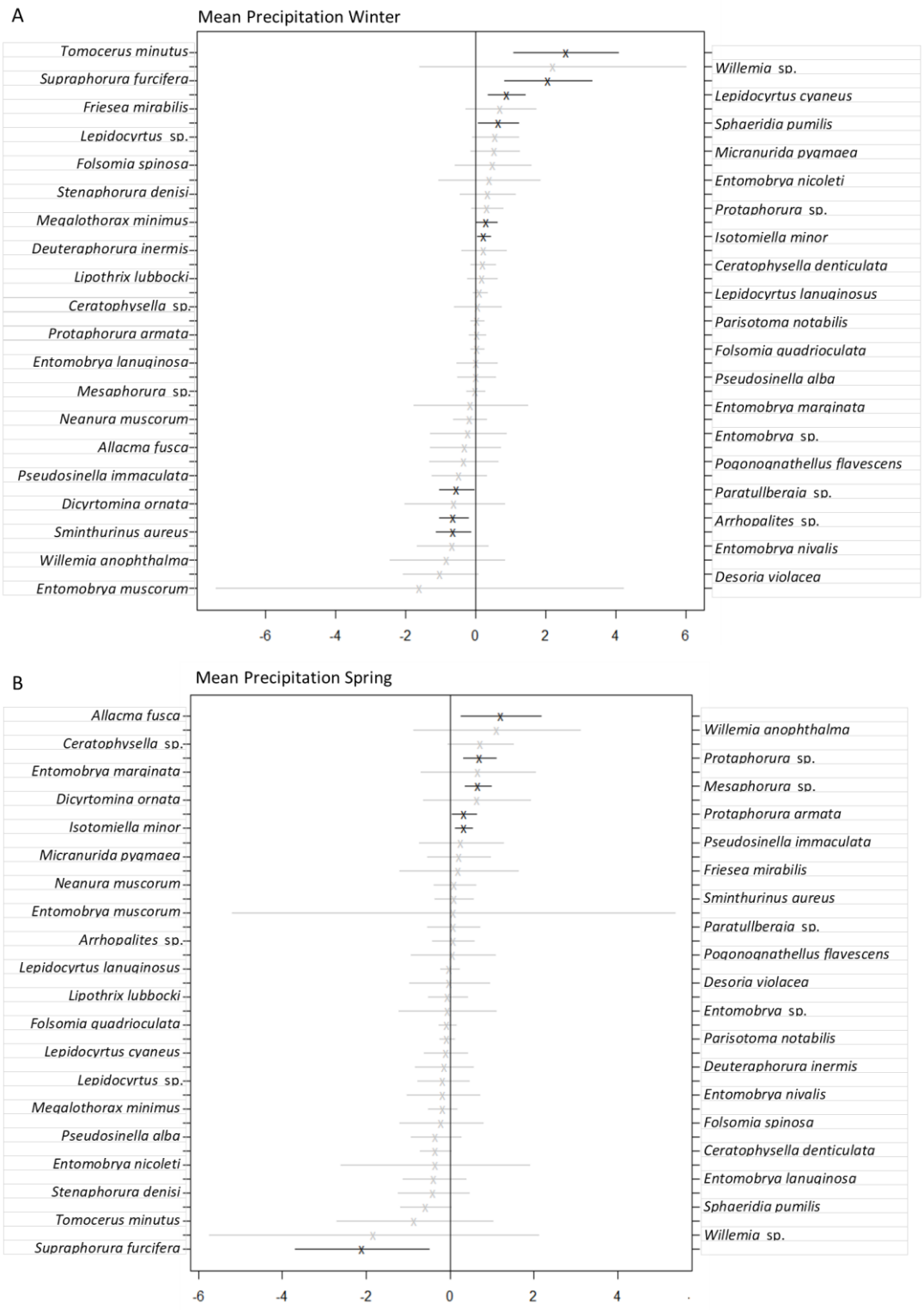


Figure 3: Canonical correspondence analysis (CCA) on the influence of environmental factors on the Collembola community composition. Sampling year (2008, 2011, 2014, 2017, 2020), region (Swabian Alb (AEW), Hainich (HEW), Schorfheide (SEW) and forest types (young beech (B30), old beech (B70), natural beech (150), coniferous (conif)) are plotted as centroids. Explained variation and eigenvalues are displayed at the corresponding axis.

We tested further how climatic conditions and forest management affected Collembola community structure, by using generalized linear latent variable models (gllvm). Through this method the influence of precipitation in winter and spring on the following Collembola species/genera was identified: *Allacma fusca*, *Arrhopalites* sp., *Isotomiella minor*, *Lepidocyrtus cyaneus*, *Megalothrorax minimus*, *Mesaphorura* sp., *Paratullbergia* sp., *Protaphorura armata*, *Sminthurinus aureus*, *Sphaeridia pumilis* and *Supraphorura furcifera* (Fig. 4 A, B). However, some species /genera including *Ceratophysella* sp., *L. cyaneus*, *Mesaphorura* sp., *Pogonognathellus flavescens*, *Pseudosinella alba*, *S. aureus* and *S. pumilis* were also affected by forest management intensity as indicated by the SMI (Fig. 4 C).


A
Mean Precipitation Winter
Tomocerus minutus
Supraphorura furcifera
Friesea mirabilis
Lepidocyrtus sp.
Folsomia spinosa
Stenaphorura denisi
Megalothorax minimus
Deuteraphorura inermis
Lipothrix lubbocki
Ceratophysella sp.
Protaphorura armata
Entomobrya lanuginosa
Mesaphorura sp.
Neanura muscorum
Allacma fusca
Pseudosinella immaculata
Dicyrtomina ornata
Sminthurinus aureus
Willemia anophthalma
Entomobrya muscorum
Willemia sp.
Lepidocyrtus cyaneus
Sphaeridia pumilis
Micranurida pygmaea
Entomobrya nicoleti
Protaphorura sp.
Isotomiella minor
Ceratophysella denticulata
Lepidocyrtus lanuginosus
Parisotoma notabilis
Folsomia quadrioculata
Pseudosinella alba
Entomobrya marginata
Entomobrya sp.
Pogonognathellus flavescens
Paratullbergia sp.
Arrhopalites sp.
Entomobrya nivalis
Desoria violacea
-6
-4
-2
0
2
4
6
B
Mean Precipitation Spring
Allacma fusca
Ceratophysella sp.
Entomobrya marginata
Dicyrtomina ornata
Isotomiella minor
Micranurida pygmaea
Neanura muscorum
Entomobrya muscorum
Arrhopalites sp.
Lepidocyrtus lanuginosus
Lipothrix lubbocki
Folsomia quadrioculata
Lepidocyrtus cyaneus
Lepidocyrtus sp.
Megalothorax minimus
Pseudosinella alba
Entomobrya nicoleti
Stenaphorura denisi
Tomocerus minutus
Supraphorura furcifera
Willemia anophthalma
Protaphorura sp.
Mesaphorura sp.
Protaphorura armata
Pseudosinella immaculata
Friesea mirabilis
Sminthurinus aureus
Paratullbergia sp.
Pogonognathellus flavescens
Desoria violacea
Entomobrya sp.
Parisotoma notabilis
Deuteraphorura inermis
Entomobrya nivalis
Folsomia spinosa
Ceratophysella denticulata
Entomobrya lanuginosa
Sphaeridia pumilis
Willemia sp.
-6
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4

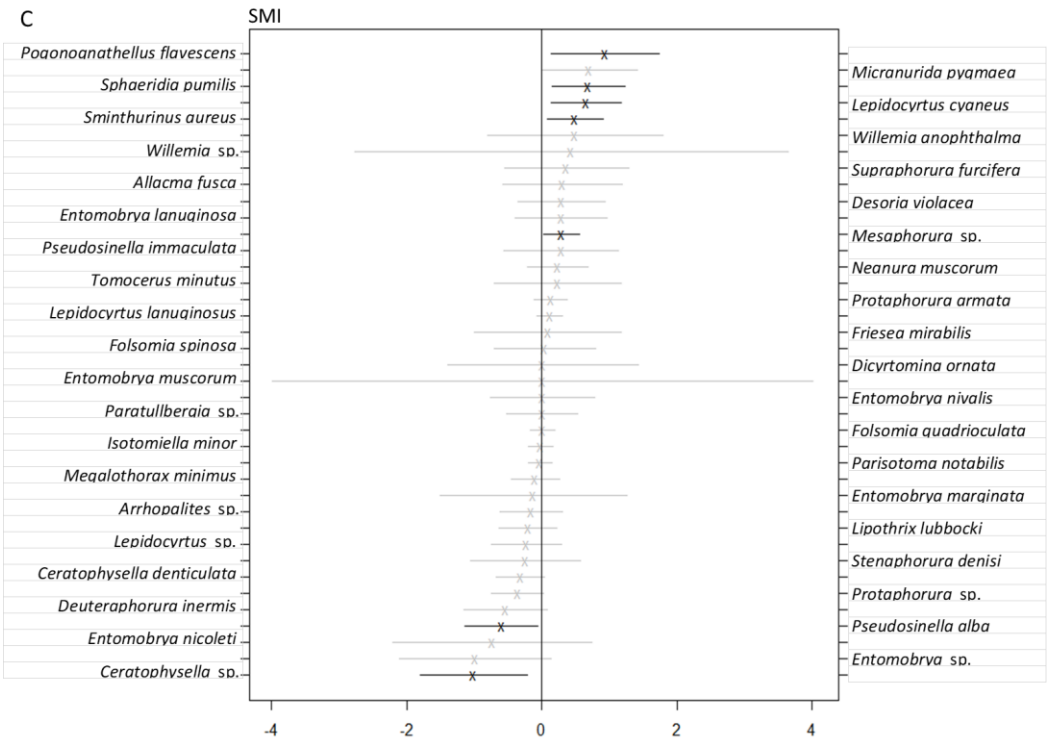

Figure 4: Effect size plots of the covariate coefficients and confidence intervals of the Collembola community with their response to precipitation in winter (A) and spring before sampling (B) and management intensity displayed in the silvicultural management index (SMI). Significant effects are highlighted in black. The length of the line indicates the confidence interval. Values of the environmental factors were standardized before analysis.

4.3.3 Collembola functional groups and reproductive mode

To analyze the effect of region, forest type and environmental factors on the functional group (life form) and reproductive mode of Collembola, we transformed the species matrix based on count data to a new matrix with (abundance) weighted proportions of traits. The proportions of each 'trait' were then analyzed by generalized linear mixed effect models.

For the 'trait' Collembola life form, the proportion of epedaphic Collembola differed significantly between region ($\chi^2 = 16.26$, $P = 0.003$) and was affected by microbial biomass in litter ($\chi^2 = 5.81$, $P = 0.016$). Multiple comparisons of means indicated that the Schorfheide was the main driver of differences between proportions of epedaphic Collembola (Tukey's HSD; Fig. 5 A). The proportion of hemiedaphic Collembola differed significantly between sampling years ($\chi^2 = 13.14$, $P = 0.011$), mean precipitation in spring ($\chi^2 = 5.13$, $P = 0.024$) and microbial biomass in litter ($\chi^2 = 4.48$, $P = 0.034$). Differences in the proportions of hemiedaphic Collembola were most pronounced between years 2008 and 2017 (Tukey's HSD; Fig. 5 B).

Euedaphic Collembola proportions varied marginal between years ($\chi^2 = 7.78$, P = 0.1) but were not affected by any other factor in the model.

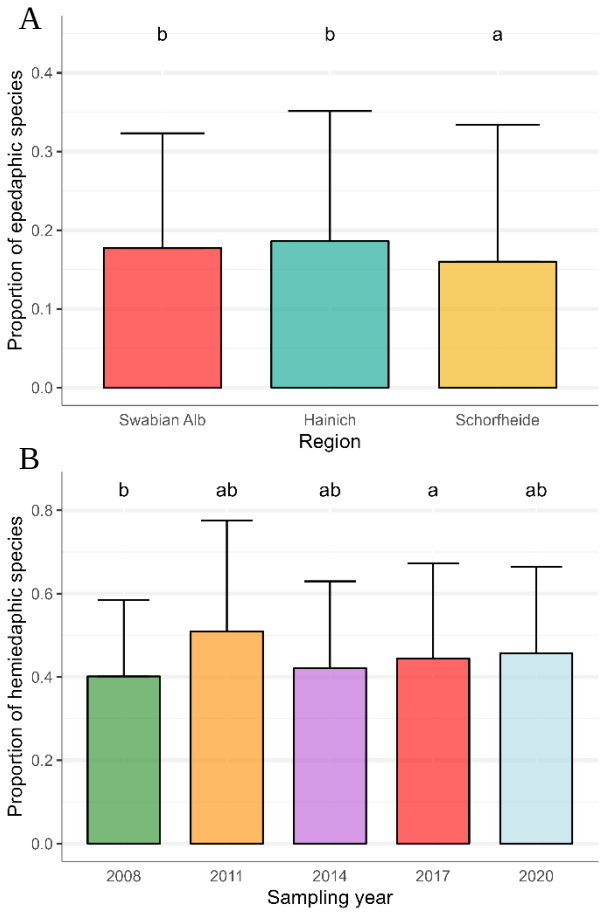

Figure 5: Proportion of epedaphic Collembola in the three regions studied (A) and of hemiedaphic Collembola in the five sampling years (B). Means with standard deviations. Bars sharing the same letter do not differ significantly ($p < 0.05$; Tukey's HSD test).

For the 'trait' reproductive mode, forest type ($\chi^2 = 11.77$, P = 0.008) and region ($\chi^2 = 8.33$, P = 0.016) significantly affected the frequency of sexually reproducing Collembola, with mean values differing significantly between the Schorfheide and Hainich, and coniferous forests and young and mature beech forests (Tukey's HSD; Fig. 6 A, B). Differences in the proportion of parthenogentically reproducing Collembola varied significantly between regions ($\chi^2 = 6.23$, P = 0.044), sampling year ($\chi^2 = 12.99$, P = 0.011) and the mean precipitation in winter ($\chi^2 = 5.94$, P = 0.015) and spring ($\chi^2 = 6.37$, P = 0.011). Again, differences were most pronounced between Schorfheide and Hainich region, between the sampling years 2008 and 2020, and 2014 and 2020 (Tukey's HSD, Fig. 6 C, D).

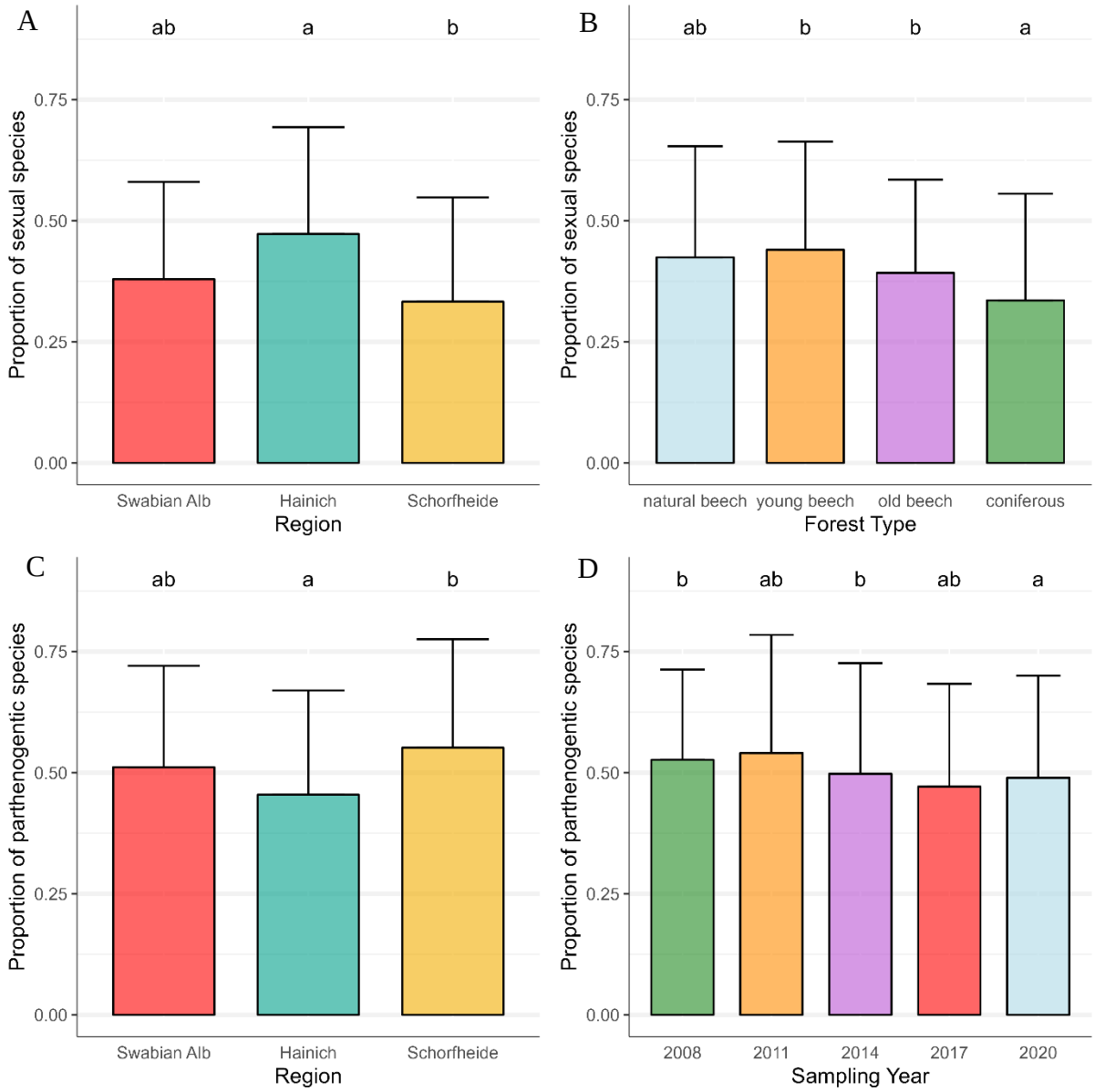

Figure 6: Proportion of sexually reproducing species in the three regions studied (A) and the four forest types (B) and proportion of parthenogenetically reproducing species in the three regions (C) and sampling years (D). Means with standard deviations. Bars sharing the same letter do not differ significantly ($p < 0.05$; Tukey's HSD test).

4.4 Discussion

4.4.1 Collembola abundances over time

Collembola abundances varied significantly over time, but in contrast to our first hypothesis did not decline with time. Rather, we identified that the fluctuations in Collembola abundances depended on regional factors and climatic conditions, in particular precipitation. Abundances varied most pronounced in the Schorfheide, where abundances decreased intensely from 2008

to 2011 (mean 65,216 ind/m² in 2008; 10,275 ind/m² in 2011) but recovered quickly in 2014 (mean 53,159 ind/m²). Collembola in the Swabian Alb also varied in abundances with time but less pronounced. By contrast, Collembola abundances in the Hainich fluctuated little with time. Abundances of Collembola were highest in 2008 (overall mean 50,115 ind/m²) and lowest in 2011 (overall mean 29,914 ind/m²), but after this decline they appeared to have recovered in 2014 Collembola (overall mean 43,233 ind/m²) and then stayed at a similar level in 2017 (overall mean 34,368 ind/m²) and 2020 (overall mean 39,733 ind/m²).

The pronounced fluctuations of Collembola abundance in the Schorfheide may be related to the more continental climate compared to the other two regions, which is characterized by large differences in temperature and precipitations between summer and winter as concluded previously (Pollierer & Scheu, 2017). Interestingly, precipitation in spring was identified as main factor driving Collembola abundance in the Schorfheide. Similarly, for Collembola in the Swabian Alb precipitation in winter was identified as main factor driving Collembola abundance. Presumably, moist conditions during winter and spring allow Collembola to recover from population decline during summer. Precipitation affected Collembola abundance in the two regions with most opposite climatic conditions, i.e. the Schorfheide in the north with the warmest and driest conditions and the Swabian Alb in the south with coldest and wettest conditions. However, an important component for the effect of climatic factors on Collembola abundance likely are soil conditions. Soils in the Schorfheide are of sandy texture with poor water holding capacity. Therefore, Collembola in the Schorfheide are likely to suffer most from low precipitation and high temperature in summer, typical for the continental climate in this region. Soils in the Swabian Alb comprise Cambisols which are rich in clay able to store more water, however, the soils typically are shallow also may dry during summer. Due to the shallow soils, Collembola in the Swabian Alb likely heavily depend on precipitation during winter rehydrating soils. In contrast to the Schorfheide and the Swabian Alb, fluctuations in precipitation did not affect Collembola abundances in the Hainich. Soils in the Hainich are characterized by Cambisols, Luvisols and Stagnosols with the calcareous bedrock typically overlain by loess sediments resulting in deeper soils with high water holding capacity and high activity of burrowing soil animals such as earthworms. Earthworms, in particular deep burrowing species such as *Lumbricus terrestris*, are less abundant in the Schorfheide and Swabian Alb due to low pH and shallow soils, respectively (Ponge et al., 2014; S. Scheu, pers. comm.).

Earthworms affect Collembola and other soil mesofauna in various ways (Eisenhauer, 2010). They incorporate litter material into the soil making it available for soil other soil detritivores such as Collembola (Ferlian et al., 2022). Further, due to their burrowing activity the increase soil pore space facilitating movement of other soil invertebrates in particular mesofauna. Among Collembola only the euedaphic Onychiuridae are known to actively burrow to some extent (Rusek, 1985). Other Collembola rely on the availability to soil pores > 50 µm for movement and accessing food resources as well as for hiding from predators (Erktan et al., 2020). Further, soil hydration via soil pores is important since most of the prey taxa of Collembola including microorganisms but also nematodes rely on water films. However, if also larger pores are filled with water, movement and feeding of Collembola and other microarthropods is hampered. Therefore, soil structure and climate are likely to interact in affecting Collembola abundance and community composition and this is consistent with our findings. Interestingly, in the Schorfheide, the region with the most adverse environmental conditions and strongest climatic variations parthenogentically reproducing Collembola reached highest dominance indicating that parthenogenetic reproduction helps in recovering from low population density due to environmental harshness as shown before (Pollierer & Scheu, 2017).

Analyzing changes in Collembola community composition in more detail indicated that effects of precipitation were most pronounced in sexually reproducing epedaphic species, such as *Tomocerus minutus* and *Allacma fusca*. However, precipitation in winter and spring also correlated with the density of parthenogenetic euedaphic species, such as *Megalothorax minimus*, *Isotomiella minor* and *Mesaphorura* sp. Even though the silvicultural management index did not correlate with total Collembola abundance or community composition, a number of species responded to management intensity. The sexually reproducing species *Pogonognathellus flavescens*, *Sphaeridia pumilis* and *Lepidocyrtus cyaneus* as well as the parthenogenetic, euedaphic genus *Mesaphorura* appeared to have benefitted from forest management intensity. However, this may also be due to benefitting from coniferous trees, which may apply in particular to *Mesaphorura* sp.

4.4.2 Effect of forest type on Collembola abundance and community composition

Collembola abundances were significantly affected by forest type and the communities changed with forest type over time. However, Collembola abundances and community composition virtually exclusively differed between coniferous and beech forests, regardless of the age and management intensity of beech forests. Generally, Collembola abundances in coniferous forests

exceeded those in beech forests irrespective of region. Higher abundances of soil microarthropods in coniferous than deciduous forests have been reported repeatedly (Erdmann et al., 2012; Potapov et al., 2023). Interestingly, however, differences between coniferous and beech forests were most pronounced in 2008, 2011 and 2014, whereas in 2017 and 2020 abundances in managed beech forest (young and mature) exceeded those in coniferous forests with the exception of the Swabian Alb in 2020. Presumably, extreme droughts and high temperatures in summer and autumn in 2016 and 2017 caused large scale dieback of spruce in Germany, and the following drought induced bark beetle infestations (Marini et al., 2017) also affected soil animals including Collembola. Among our study sites in particular spruce forests in the Hainich suffered heavily from bark beetle infestation. In two out of the four coniferous sites in the Hainich all spruce trees died. In 2017 Collembola density in coniferous forests in the Hainich was 51,155 ind/m² and declined to 41,484 ind/m² in 2020. In parallel, also microbial biomass in the litter layer declined. Presumably, reduced input of both aboveground litter and root-based resources combined with more pronounced exposure to sunlight and associated higher temperatures detrimentally affected soil biota including Collembola.

In beech forests, Collembola abundance and species composition was relatively stable suggesting that Collembola are little affected by forest management practices. Since the main factors influencing Collembola communities are the identity of the dominant tree species, precipitation and microbial biomass, indicating a resilient response towards management disturbance. Unexpectedly, the abundance or species richness of Collembola did not increase in natural beech forests, although this forest type is rich in deadwood. Interestingly, Oribatida abundances were also detected to be low in natural beech forests (Erdmann et al., 2012). Further, fungal community structure was also not affected by different management of beech forests indicating that soil physio-chemical parameters, such as pH and sand content, as well as parameters related to herbaceous plants and litter cover impact fungal communities (Wubet et al., 2012).

The observed differences in Collembola abundance and community composition between beech and coniferous forests is likely related to a number of factors. The litter of coniferous forests typically is of low quality, due to high proportion of lignin, however, due to the thick leaf litter layer a stable habitat with fungal rich resources is created. Beech litter has higher C:N ratio, but the soils in deciduous forests are more exposed to seasonality effects. Collembola inhabit distinct trophic niches depending on their life form and phylogenetic identity (Ferlian et al., 2015; Potapov et al., 2016). However, Collembola seem to utilize similar resources

irrespective of forest type indicating that their trophic niche is relatively stable (Ferlian et al., 2015; Jüds et al., *in prep*). Differences in Collembola abundances and community composition between forest types presumably were caused by differences in the litter layer, understory plant composition and soil pH related to the dominant tree species.

4.4.3 Variations in Collembola functional groups and reproductive mode

4.4.3.1 Life Forms

Among the four functional groups (life forms) of Collembola mainly the relative abundance of epedaphic and hemiedaphic species varied among years. Generally, the relative abundance of epedaphic Collembola was significantly lower in the Schorfheide than in the Hainich and the Swabian Alb. The Schorfheide region is the warmest and driest of the three studied regions and drought and heat in particular affect soil invertebrates in the litter layer including epedaphic Collembola. Further, as discussed above, the effects of drought are likely to be most pronounced in the sandy soils of the Schorfheide. Even though epedaphic Collembola are generally considered relatively drought resistant and move up into the vegetation (Hopkin, 1997), still longer periods of drought may be detrimental.

The relative abundance of hemiedaphic Collembola varied in particular between 2008 and 2017. Interestingly, in 2008 when the relative abundance of hemiedaphic species was lowest, total abundance of Collembola was highest. Hemiedaphic species abundances were significantly affected by precipitation during spring as well as through microbial biomass in litter, underlining their dependence on high bacterial and fungal densities. However, neither region nor forest type affected hemiedaphic Collembola, which suggests that hemiedaphic species rely on resources little affected by environmental fluctuations. In comparison to epedaphic species, hemiedaphic Collembola have shorter appendages, smaller bodies with less pronounced setae, and are able to migrate between the soil and litter. In general, epedaphic and hemiedaphic species are considered to be well adapted to climatic fluctuations, since their main habitat is exposed to environmental fluctuations (van Dooremalen et al., 2013). Nonetheless, these species seem to depend on moist environments, which could also affect biomass of their preferred food and conditions for molting and reproduction.

Euedaphic Collembola neither were affected by regional nor forest type related factors nor climatic conditions such as precipitation. This contrasts results of Pollierer & Scheu (2017), who detected significant effects of region and forest type, suggesting that euedaphic Collembola are negatively affected by warmer climate and lower soil hydration in the Schorfheide. In fact,

the density of euedaphic Collembola was highest in 2008 and lowest in 2011, but in our long-term dataset correlations with environmental factors were not significant.

4.4.3.2 Reproductive Mode

We analyzed the effect of sampling year, region, forest type and environmental factors on Collembola with either sexual or parthenogenetic reproductive mode. The relative abundance of sexually reproducing Collembola was significantly lower in the Schorfheide than the Hainich. This pattern resembled epedaphic Collembola, which mainly reproduce sexually. In addition, the relative abundance of sexually reproducing Collembola was significantly lower in coniferous than in managed beech forests. Parthenogenetic Collembola showed the opposite pattern regarding the study regions, but their relative abundance generally peaked in 2011 when total Collembola abundance was lowest which was associated with lowest precipitation in spring. This is likely to be related to the faster reproduction of parthenogenetic compared to sexual species and thereby faster recovery from population declines as identified previously in Collembola from the Schorfheide (Pollierer & Scheu, 2017). Generally higher relative abundance of parthenogenetic than sexual species in the Schorfheide than the Hainich therefore likely reflects that they better cope with the more pronounced fluctuating climatic conditions in the Schorfheide.

4.4.4 Conclusion

We investigated the effects of time, region and forest type (also reflecting different management intensity) on the abundances and community composition of Collembola. The results documented that Collembola abundances vary between years, with the fluctuations being related to variations in climatic conditions in particular precipitation in winter and spring. Collembola were most affected by environmental factors in regions with harsh climatic conditions and sandy soils of low water holding capacity (Schorfheide) or shallow soils on limestone parent rock (Swabian Alb). Forest management neither significantly affected abundances nor community composition of Collembola irrespective of year and region. By contrast, both Collembola abundances and community structure differed between deciduous and coniferous forests. Coniferous forests, characterized by poor litter quality and thick litter layers, provide ample habitat for soil mesofauna such as Collembola. Low precipitation in spring and summer and associated drought periods in the last years resulted in the dieback of spruce resulting in reduced density of Collembola in particular in the Hainich. Epedaphic and hemiedaphic Collembola species were significantly affected by precipitation, indicating that

these life forms depend on moist microsites in the litter layer, where their main food sources are located. Most epedaphic species reproduce sexually and reached lowest relative abundance in the Schorfheide. The Schorfheide displays the harshest climatic conditions with low soil hydration, with parthenogenetically reproducing species being well adapted to these conditions due to their fast colonization abilities after population declines.

Generally, compared to aboveground insects investigated at the same study sites (Seibold et al. 2019), Collembola abundances and community structure fluctuated little between years and did not show trends of decline indicating that environmental extremes are buffered in soil and that Collembola are able to recover quickly from population decline during summer drought if precipitation resumes in winter and spring. Overall, effects of climate change on soil invertebrates in the temperate zone may generally be less pronounced than on aboveground invertebrates due to their ability to counteract population declines during summer by being active in winter when soil moisture conditions become favorable again allowing them to reproduce, thereby compensating the effects of adverse environmental conditions in summer.

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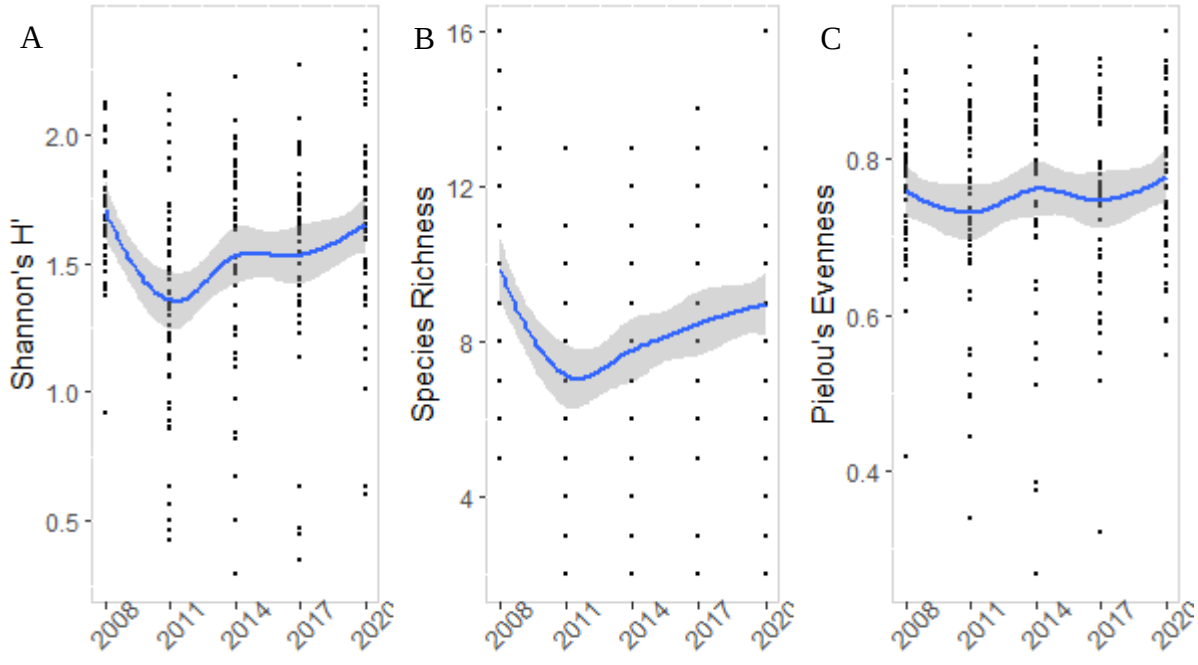
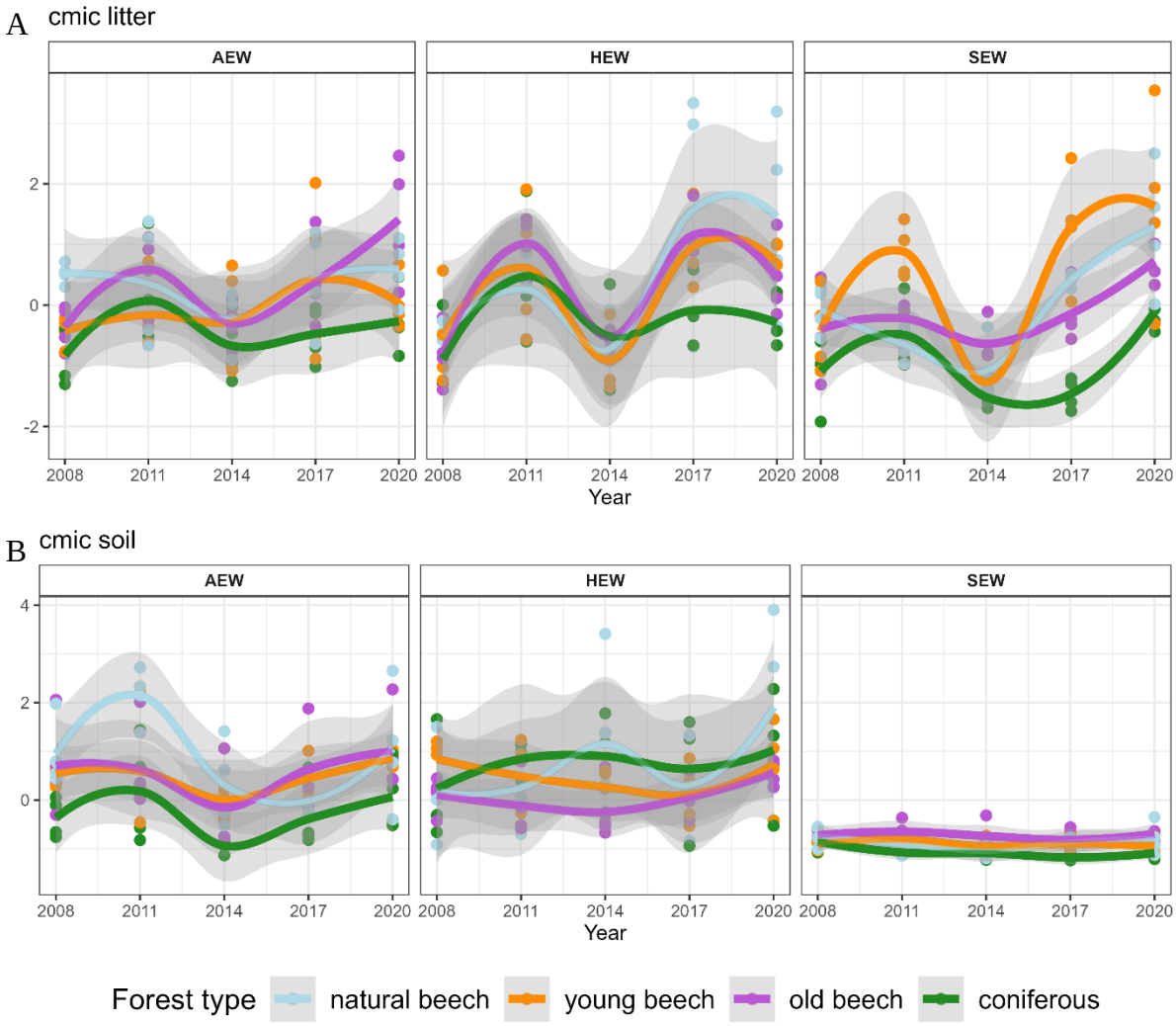
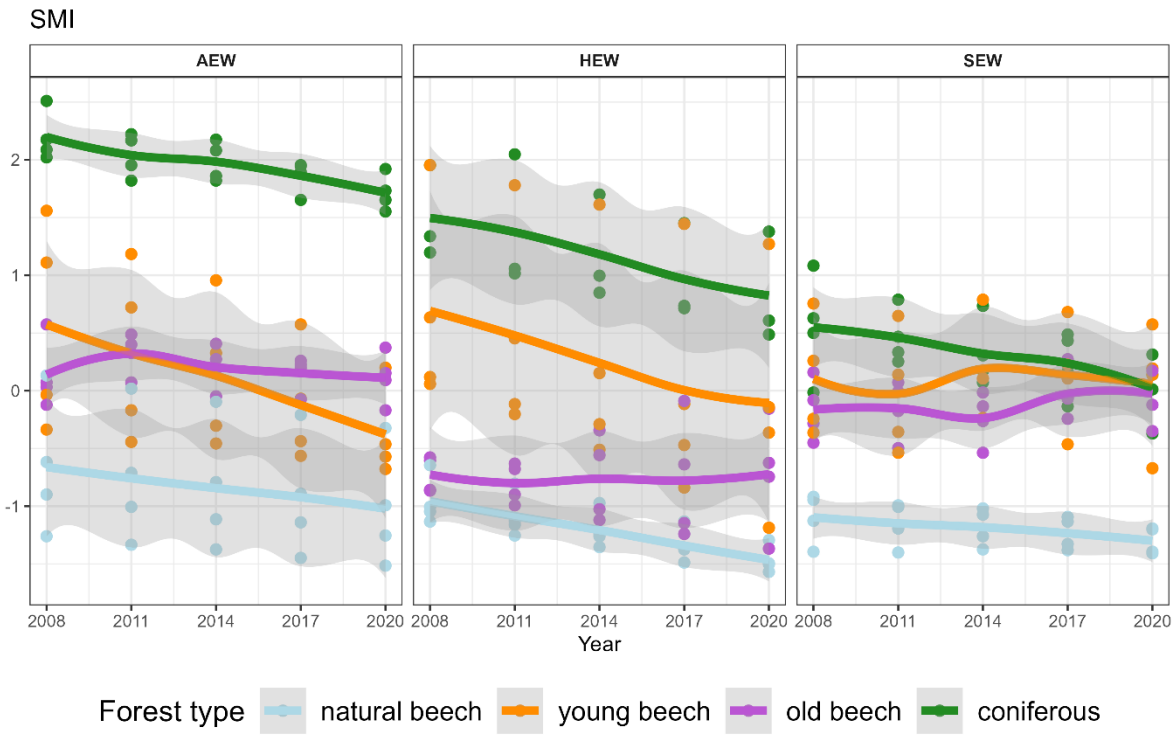
Appendix



Appendix Fig. 1: Temporal dynamics of Collembola species diversity (A), species richness (B) and species evenness (C).



Appendix Fig. 2: Temporal dynamics of standardized microbial biomass (means) in litter (A) and soil (B) in the Swabian Alb (AEW), Hainich (HEW) and Schorfheide (SEW). Colors indicate the forest type.



Appendix Fig. 3: Temporal dynamics of the forest management intensity (means) as indicated by the silvicultural management intensity index (SMI) for the Swabian Alb (AEW), Hainich (HEW) and Schorfheide (SEW). Colors represent forest types.

5 General Discussion

5.1 The role of Collembola in future ecological studies

Due to their high abundances and species numbers worldwide, Collembola are a promising model organism to study the effect of climate change and human caused ecosystem disturbance on the biodiversity and ecosystem functioning of soils (Potapov et al., 2020, 2023). By feeding on dead organic matter and microbes they shape soil structure and functioning (Rusek, 1998). Collembola occupy distinct trophic niches, which are related to their life form and phylogenetic identity (Chahartaghi et al., 2005; Ferlian et al., 2015; Potapov et al., 2019). Thereby, analyzing the response of Collembola life forms, community composition and feeding activity to different environmental drivers, allows to identify climatic and environmental drivers that are important for the functioning of soil systems. Especially in face of climate change and biodiversity loss, monitoring associated effects on the soil system, with potentially detrimental influence on carbon sequestration and nutrient cycling, is crucial. Collembola, due to their physiology and use of a wide range of resources, sensitively react to drought (Peguero et al., 2019; Petersen, 2011; Pflug & Wolters, 2001). Although the diet of Collembola has been investigated intensively, species-specific analysis of their feeding behavior in natural environments is still poor. In the study presented in Chapter 3 first insights into the feeding on fungal resources at the level of genera by different Collembola species were obtained via DNA based analysis. Even though the use of metabarcoding of gut contents in Collembola is challenging, it is a promising tool to study the diet of soil microarthropods more detailed. The combination of molecular analyses of trophic interactions and the analysis of habitat structure and climatic conditions may help to identify drivers of shifts in ecosystem functioning.

Collembola, even though tiny and rather delicate invertebrates, display resilient and flexible responses towards changes of their environment (Berg & Bengtsson, 2007; Filser et al., 2002; Pollierer & Scheu, 2017). The analysis of feeding interactions in opaque habitats, such as the soil, is still challenging even in face of novel techniques such as molecular methods. Since Collembola live in close vicinity of their food sources, material such as spores or hyphae could potentially attach to the body surface of animals. Thus, after whole body DNA extraction a DNA mixture of consumer tissue, gut contents and environmental material may be obtained and thereby lead to false positive results by detection of non-ingested material in those samples. To gain reliable and trustworthy results in the molecular analysis of Collembola gut contents, we tested ten surface decontamination methods in **Chapter 2**. A washing treatment with bleach

(5 % for mites, 1.5 % for Collembola) and formaldehyde 37 % was most efficient in removing surface contamination, succeeding in at least 80 % of tested animals. In addition, the detection of DNA of prey previously consumed by the tested mites, was highest among bleach treated animals with 50 % (similar to the control group). Unexpectedly, five treatments did not remove surface contaminants in more than 50 % of the tested animals; these least efficient treatments were flaming of body surface, hand sanitizer solution, acetone and hydrogen peroxide. Therefore, based on our results, we suggest to use a treatment with bleach for removing surface contaminations of soil microarthropods for the use in molecular analyses of trophic interactions. This study was the first to comprehensively test methods for the decontamination of microarthropod body surfaces aiming at establishing a standardized method for the use in metabarcoding studies.

After establishing an effective washing protocol as presented in Chapter 2, in **Chapter 3** we used molecular techniques for the analysis of fungal diets of Collembola from spruce and beech forests. Collembola were divided into two groups, one was analyzed for their gut contents and the other was starved and analyzed for possible gut symbionts. No fungal gut symbionts were detected in starved Collembola, indicating that fungal genera detected in the gut content analysis were actually ingested. Surprisingly, large proportions of an entomopathogenic fungal genus, *Scopulariopsis*, was present in all Collembola samples. *Scopulariopsis* is well known for infecting the cuticles of ticks where it can cause lethal damages (Bonnet et al., 2021; Suleiman et al., 2016). However, Yoder et al. (2008) found mutualistic effects in the American dog tick against the infection by another fungus. The effect of this fungus on Collembola is unclear, and the studied specimens appeared healthy. Further, we found that Collembola fed preferentially on saprotrophic or pathotrophic fungal genera, such as *Ramularia*, *Cladosporium*, *Discosia* and *Encoelia,* which are all Ascomycota. In general, the proportions of Ascomycota were larger than those of Basidiomycota in the studied Collembola gut contents, which is in line with the findings by Anslan et al. (2016). Ectomycorrhizal fungi were detected in a limited number of reads, suggesting that this fungal functional group is not forming a main part of Collembola diet as reported previously (Pollierer & Scheu, 2021; Potapov & Tiunov, 2016). Fungal diets of Collembola between spruce and beech forests did not differ significantly, although the fungal community in litter and soil varies distinctly between these two forest types (Goldmann et al., 2015). Our results suggest that Collembola browse more selectively on fungal genera in soil, which was also suggested earlier (Chauvat et al., 2014; Jørgensen et al., 2003, 2005). Further, Collembola trophic interactions, abundances and community structures seem to be shaped by environmental factors of microsites (= soil pores) within the larger macrosites (=

forest stands), as discussed in Chapter 4. Due to the high amplification rate for Collembola DNA, information on fungal diets was relatively limited. Thus, it was difficult to detect differences between fungal diets in different species and life forms of Collembola. Nevertheless, the results of this study indicate that the studied species fed on similar fungal resources.

In **Chapter 4** we aimed at identifying the main drivers of Collembola abundance and community structure in temperate forests ecosystems by investigating long-term population dynamics. Neither Collembola abundances nor species richness declined uniformly along the five sampling dates between 2008 and 2020. Changes in abundances were rather affected by precipitation during winter and spring before the sampling. In contrast to the Schorfheide and the Swabian Alb, Collembola abundances in the Hainich were relatively stable. Regional specific changes in abundance and community structure were presumably caused by differences in soil type and structure, such as sand rich soils in the Schorfheide and shallow soils in the Swabian Alb. In addition, Collembola seem to benefit from the large amount of leaf litter in coniferous forests, which likely buffers soil desiccation, resulting in high Collembola abundances overall, but especially in coniferous forests in the Schorfheide in 2008. However, in the Hainich, spruce forests were strongly affected by bark beetle infestations resulting in the dieback of trees in two experimental plots. This extreme disturbance presumably caused low Collembola abundances in the coniferous forests in the Hainich in 2020.

In contrast to density and diversity, Collembola community structure varied between years as well as between region and forest type, which indicates a turnover of species with changing environmental conditions. In contrast, different intensity of management practices in beech forests did not affect Collembola abundance and community structure indicating that tree species is the main driver of community structure in forests, as has been previously shown for Collembola and fungi (Ferlian et al., 2015; Goldmann et al., 2015). The functional groups of Collembola responded differently to changes in climatic conditions and between regions and forest types. Epedaphic Collembola differed strongly between regions, with lowest proportions in the Schorfheide compared to the Hainich and Swabian Alb. In general, both litter dwelling life forms (epedaphic and hemiedaphic) were affected by changes in microbial biomass in litter, underlining that these species mainly rely on microbial resources in the litter layer (Potapov et al., 2016). Parthenogenetic reproducing Collembola were most abundant in the Schorfheide with harshest climatic conditions and soils with lowest hydration capability, suggesting that their ability for fast colonization after weather extremes is advantageous compared to sexual

reproducing species. Similarly, the frequency of parthenogenetic reproduction increased with soil depth, emphasizing the soil as a stable habitat with sufficient food sources for Collembola buffering varying climatic conditions. The results of the study presented in Chapter 4 indicates that Collembola abundance and species richness is resilient against climatic variations and management practices. However, Collembola community structure is affected by regional, forest type and climatic factors, indicating a turnover in species composition with varying environment and climate.

5.2 Optimization of molecular techniques for the analysis of fungal feeding in Collembola

In our study on the molecular analysis of gut contents in Collembola presented in Chapter 2 we experienced several limitations and challenges due to our study system which are listed below with some ideas for optimization.

First, Collembola are relatively small and whole body DNA extraction had to be done. To gain sufficient DNA concentrations, necessary for the sequencing system, we had to pool eight individuals of the same species as one sample. We used at least five replicates of each species per forest type per experiment, therefore a large number of Collembola invividuals was needed. Unfortunately, the most abundant Collembola species and species inhabiting the lower litter and soil layer are relatively small. Specimens were sterilized with bleach (1.5 %), since Collembola could be surface contaminated by environmental DNA including the target group of fungi. Furthermore, the small size and lack of coloration of some species made this procedure challenging as well. This issue is unlikely to be solved by using larger atmobiotic and epedaphic species, since the whole trophic ecology of Collembola would only be partly displayed. However, more individuals of species and larger replicates could improve the study and compensate for losses of specimens during handling.

Second, the quantity and quality of fungal DNA, consisting of different species mixtures in varying digestive stages, is relatively low compared to the vast amount of Collembola DNA material of high quality. We used targeted amplicon sequencing for amplifying DNA in gut material. Even though the primers used in this study have been frequently utilized for metabarcoding of fungi, the primers also amplify the ITS2 region of arthropods due to their shared eukaryotic basis. We propose to use blocking primers which inhibit the amplification of a consumer and thereby improve the detection of gut content DNA (Vestheim & Jarman, 2008).

However, these primers may also cause problems and may exhibit amplification bias towards certain taxa (Elbrecht & Leese, 2015; Piñol et al., 2015, 2019).

Third, some Collembola may have had empty digestive tracts, due to molting or simply not feeding. Collembola are known to empty their guts before molting (Hopkin, 1997) and during sampling it was not possible to identify Collembola with empty guts. However, we sampled up to 1800 individuals of Collembola to limit the effect of possible empty guts and to replicate each species at least five times per forest type and experiment. Nonetheless, larger replicate numbers are preferred.

Even though we observed several challenges as listed above, we still were able to gain some insight into Collembola fungal feeding habits and unexpectedly detected evidence for infection with an entomopathenogenic fungus of all Collembola species studied.

5.3 What are the factors influencing Collembola feeding strategies?

Collembola inhabit various trophic niches depending on their ecomorphological life form and phylogenetic identity (Chahartaghi et al., 2005; Ferlian et al., 2015; Potapov et al., 2016). Collembola seem to feed on similar resources and are not influenced by habitat structure between macrostructures such as forest type or microbial community composition (Ferlian et al., 2015). Similarly, we did not detect differences in feeding on fungal material between spruce and beech forests in **Chapter 3**. Even though these results indicate that Collembola specifically search for certain fungal resources, the general feeding pattern seems to be opportunistic and general. Feeding on diverse resources is advantageous, since the mixing of diets provides a broad nutritional value (Scheu & Folger, 2004). The regions studied in **Chapter 4** are characterized by differences in soil type and structure as well as differences in climatic conditions. Soils compromise mixtures of minerals, organic materials and air or water filled pores (Orgiazzi et al., 2016). The size and structure of pores and their connections (corridors) affect trophic positions of soil microbiota and animals (Erktan et al., 2020). The main reason is the physical accessibility of food resources, since organic carbon can only be processed by soil mircoarthropods and microbiota if they have access to it (similarly to root derived carbon). In general, pore size influences the accessibility of soil resources to soil biota, with bacteria inhabiting water filled micropores (> 1.2 µm; Hassink et al., 1993), fungi growing in air filled pores > 10 µm but prefering pore sizes > 100 µm (Effmert et al., 2012; Soufan et al., 2018), and Collembola and other soil microarthropods colonizing air filled pores > 50 µm (Joschko et al., 1990). Soil inhabitants are influenced by pore size and hydration (water and air filled pores).

Since most bacteria and nematodes are aquatic and many fungi and microarthropods are depending on (moist) air filled pores (Hopkin, 1997; Schimel, 2018). The accessibility of possible food or prey is higher in coarse structured soil compared to fine structured soil. However, as in sandy soils, even if the accessibility increases, the hydration potential of the soil decreases and species are forced to move to moist microsites, where organisms are at risk of being consumed (Erktan et al., 2020). Collembola depend on several factors in their resource use, such as the general availability of food resources, the structure of the habitat and climatic conditions influencing the preferred resource and residency.

5.4 Impact of climate change on forest ecosystems and implications for Collembola

The Biodiversity Exploratories aim at monitoring the effects of forest (and grassland) management on the ecosystem and its species, and thereby on ecosystem functioning. On the basis of scientific data, recommendations for policy makers on management practices in forest and grassland are made. In the forests studied, we did not detect significant effects of management practices on Collembola abundance and community structure, indicating that the soil system is well buffered against disturbances due to forest management and is little affected by tree stand age. However, we found effects of tree species identity and precipitation during winter and spring on the abundance and community structure of Collembola. The effects of global warming are visible also in temperate forests (Allen et al., 2010, 2015). A large bark beetle outbreak damaged spruce stands all over Germany with extreme infestations in the Harz region. In face of climate change, scientists and forestry people are searching for new strategies to restore biodiversity and economic value of forests (Ammer, 2019; Ammer et al., 2018). One idea is to increase the proportion of mixed stands or to introduce non-native Douglas fir as spruce replacement species, since it is resilient against warmer temperatures and drought periods (Pötzelsberger et al., 2020; Schmid et al., 2014). As shown recently, the establishment of non-native Douglas fir plantations may affect microbial community composition especially in nutrient poor soils (Lu & Scheu, 2021). Despite no general effect on soil microarthropod species diversity was found, the abundance of euedaphic Collembola was reduced in pure Douglas fir stands compared to beech forests (Lu, 2021), underlining the influence of tree species identity. Overall, however, microarthropods in soil seem to be resilient towards climatic changes. Nevertheless, monitoring and studying of soil animal community composition and their responses towards environmental disturbances and climate change is required to obtain insight into potential long-term changes.

5.5 Concluding remarks

Within the scope of this dissertation, we established a standardized procedure to decontaminate body surfaces of microarthropods for the use in DNA based analysis of trophic interactions (**Chapter 2**). We used the established body surface decontamination procedure from Chapter 2 in field sampled Collembola of the study presented in **Chapter 3** for the analysis of molecular gut contents via high-throughput sequencing. Here, we identified several fungal genera, which were fed on by Collembola independent of forest type and life form. Collembola clearly preferred saprotrophic and pathotrophic fungi over ectomycorrhizal species. Surprisingly, a fungal genus known to infect cuticles of arthropods was detected in all Collembola samples, but the role of this fungus in Collembola is unclear. In **Chapter 4** we identified environmental parameters affecting Collembola abundance and community composition in a long-term monitoring experiment along a forest type management gradient. Forest type and precipitation were important drivers of community structure and abundance of Collembola, but differences were modulated by soil type and structure. Trait-based analysis are promising for identifying the effects of environmental factors and climate change on animal communities. Collembola functional groups react distinctly towards changes in climate and abiotic factors, even though trophic niches are relatively stable within life forms. In addition, the molecular analysis of Collembola gut contents, related to their life forms, may help to disentangle environmental effects on Collembola life forms in a wide range of habitats.

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List of Publications

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Jüds M, Heidemann K, Eitzinger B and Scheu S. Methods for removing body surface contaminants of soil dwelling invertebrates (Oribatida) using detection PCRs. *in prep*. (2023).

Jüds M, Schneider D and Scheu S. Variations in the fungal diet of Collembola species with forest type as indicated by molecular gut content analysis. *in prep*. (2023).

Jüds M, Bluhm S, Junggebauer A, Pollierer M and Scheu S. Long term changes in Collembola community composition and abundance: the role of forest type and precipitation. *in prep*. (2023).

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Thesis Declaration

Declaration of the author's own contribution to manuscripts with multiple authors

Chapter 2: Methods for removing body surface contaminants of soil dwelling invertebrates (Oribatida) using detection PCRs

Authors: Melissa Jüds, Kerstin Heidemann, Bernhard Eitzinger, Stefan Scheu

I am the first author of this paper. I was involved in the conduction of experiments, collection of the data, analyzing of the data and writing the manuscript. Kerstin Heidemann, Bernhard Eitzinger and Stefan Scheu were involved in the conception of the experiments. Kerstin Heidemann, Bernhard Eitzinger and Stefan Scheu contributed in finalizing the manuscript.

Chapter 3: Variations in the fungal diet of Collembola species with forest type as indicated by molecular gut content analysis

Authors: Melissa Jüds, Dominik Schneider, Stefan Scheu

I am the first author of the paper. I was involved in the conceptualization, set up of the experiment, collected and analyzed the data and wrote the manuscript. Stefan Scheu developed the study conceptualization. Dominik Schneider contributed to the data analysis. Dominik Schneider and Stefan Scheu were involved in finalizing the manuscript.

Chapter 4: Long term changes in Collembola community composition and abundance: the role of forest type and precipitation

Authors: Melissa Jüds, Sarah Bluhm, André Junggebauer, Melanie Pollierer, Stefan Scheu

I am the first author of this paper. I was involved in the data collection, data analysis and wrote the manuscript. Stefan Scheu was involved in the conceptualization of the experiment. Sarah Bluhm and Melanie Pollierer were involved in the data collection. André Junggebauer and Melanie Pollierer were involved in the data analysis. All co-authors were involved in finalizing the manuscript.

Plagiarism declaration

I declare that I have written this doctoral dissertation independently. All persons contributing to the manuscripts have been named so. All sentences or passages quoted from other people's work have been specifically acknowledged by clear cross-referencing. I have not submitted this thesis in any form for another degree at any university or institution. I confirm that the printed and the digital version of my doctoral dissertation are identical.

Melissa Jüds
Göttingen, April 2023