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Changes in Microbial Resources and Trophic Niches of Microarthropods in Forest Ecosystems with Elevation

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“The tiny creatures of the undergrowth were the first animals of any kind to colonize the land. They established the foundations of the land’s ecosystems. […] If we and the rest of the backboned animals were to disappear overnight, the rest of the world would get on pretty well. But if THEY were to disappear, the land’s ecosystems would collapse. […] These small creatures are within a few inches of our feet wherever we go on land, but often they’re disregarded. We would do very well to remember them.”

– Sir David Attenborough

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Summary

Mountain forests play a major role for biodiversity conservation, carbon storage and hydrological cycling. Therefore, understanding different components of mountain forest ecosystems is essential to predict potential responses to global climate change. To study such responses, the investigation of elevational gradients is an important tool as they comprise a wide range of climatic variables on a small spatial scale. While changes in aboveground biodiversity along elevational gradients have been studied intensively, information on belowground systems is scarce. Besides studies focusing on litter decomposition, there is few comprehensive data available on how food web functioning changes across elevations. The soil food web is a major component of nutrient cycling within forest systems and thereby is linked to mineralization rates and tree growth. In this thesis I investigated changes in microbial resources, trophic changes in microarthropods in general and trophic changes within the functional Collembola communities along an elevational gradient (800 – 1700 m asl) of primary forests on Changbai Mountain, China. We established seven sites spaced by elevational steps of 150 m along an elevational gradient at the northern slope of Changbai Mountain. The northern slope is characterized by primary forests dominated by Korean pine with decreasing admixtures of deciduous tree species (<1100 m asl) and dark-bark spruce (>1100 m asl). The sites were sampled in early September 2019, before litter fall began. The general scope of this thesis is to understand how trophic interactions among different components of the soil food web change with elevation.

In Chapter 2 we focused on the characterization of microbial community composition and functioning across elevations as microbes are the major agents of decomposition in forest systems and form an important food resource for animal decomposers. Microbial communities in litter and soil were investigated using phospholipid fatty acid (PLFA) profiles, and microbial biomass and activity were measured using substrate-induced respiration. Our results suggest that microbial communities on Changbai Mountain are heavily influenced by elevation. Microbial communities showed strong turnover in the litter layer, most pronounced in the transition zone between the two major forest types (1100 m – 1250 m asl). While only temperature seasonality significantly structured PLFA profiles in the litter layer, temperature and precipitation seasonality, resource availability, soil moisture, Ca concentrations and pH structured microbial communities in soil. Indicators of microbial stress and microbial biomass were higher in the litter layer at higher elevations, both correlated negatively with Ca concentrations indicating increased nutritional stress in high microbial biomass communities at sites with low Ca availability.

In Chapter 3 we report trophic positions of Collembola and Oribatida at group level, measured by bulk and amino acid stable isotopes, to increase with increasing elevation. Further, trophic positions of Collembola and Oribatida correlated with microbial stress ratios and microbial biomass. The energy channels, measured by ^{13}C values of amino acids and neutral lipid fatty acids (NLFA), on the other hand, did not change across elevations suggesting fungi to be the major resources of both Collembola and Oribatida along the whole gradient.

In Chapter 4 we targeted trophic changes of Collembola communities across elevations at species level. Collembola species contributing 80% to the Collembola abundance per elevation were categorized into life forms and their individual body lengths and bulk stable isotopes were measured. Abundance weighted mean and minimum Δ^{15}N values as well as Δ^{13}C values increased with elevation, while the range of Δ^{15}N values decreased. Isotopic uniqueness, a measure of isolated trophic niches, increased with elevation. Δ^{15}N values of Collembola species occurring across all elevations increased with elevation, with this being most pronounced in the cosmopolitan species *Folsomia octoculata*. Shifts in mean Δ^{15}N with elevation depended on the life form of Collembola and were most pronounced in litter-dwelling, hemiedaphic species. We further found changes in Δ^{15}N with body size to depend on Collembola life form, with Δ^{15}N increasing with body size in hemiedaphic and euedaphic species, but not in epedaphic species.

The results reported in Chapter 3 indicate trophic shifts of Collembola and Oribatida communities towards functioning more as secondary decomposers with increasing elevation. The results further indicate that these shifts are likely determined by increasing microbial stress ratios and microbial biomass with elevation, which represent the availability of microbial resources. In Chapter 4 we indeed found species functioning as primary decomposers to decrease with increasing elevation as indicated by increasing minimum Δ^{15}N values and decreasing range of Δ^{15}N values with elevation. These shifts in (especially) Collembola trophic niches with increasing elevation are likely linked to increasing accessibility of microbial resources with decreasing quality of litter, as reported in Chapter 2. Collembola traits, represented by Collembola life forms and body size, likely determine the capability of Collembola to access these alternative microbial resources along the elevational gradient as suggested by the results presented in Chapter 4. This thesis is the first to report detailed data on trophic changes in microarthropods along elevational gradients and therefore contributes significantly to the understanding of food web functioning in mountain forest ecosystems.

Like in mountainous ecosystems across the world, annual mean temperatures on Changbai Mountain increased and annual precipitation decreased over the last decades. According to the strong dependence on local litter factors, the trophic positions of microarthropods at higher elevations may decrease in the future as tree species from lower elevations may expand to higher elevations. There is an urgent need for comparable studies along other elevational gradients including different seasons to verify implications of our study and to come up with more general statements. This thesis represents a starting point of such studies.

Chapter 1: General Introduction

Tianchi Lake at Changbai Mountain, China

1.1 On Mountain Forests

The protection of mountain ecosystems is one of the major tasks in modern societies as mountains are directly linked to human welfare. Mountain systems are among the greatest water suppliers for humanity, especially in semiarid and arid regions, where they usually contribute to more than 50% of the total water discharge (Messerli et al., 2004). Therefore, mountains play a critical role in food supply also for the lowland area, this is particularly true for many developing counties (Messerli et al., 2004), indicating upcoming challenges in the face of global warming. For example, glacial water supports many big Andean cities, while glaciers vastly retreated all over the Andes in the last decades (Coudrain et al., 2005). Additionally, mountain areas (especially forests) harbour a major fraction of the world's biodiversity. Tropical mountains for example harbour over 70% of the worlds bird diversity (Quintero & Jetz, 2018). Further, the plant diversity in alpine habitats is higher than expected from their covered area (Körner, 2004). One of the reasons for high biodiversity in mountains likely is the rapid change of climatic factors over a small spatial scale (Körner, 2004). Additionally, mountain habitats often comprise many species with small-ranged distributions, which makes them major centres of endemism (Rahbek et al., 2019). In any case, mountain habitats have an intrinsic value given their natural history and biodiversity.

Even though it is hard to come up with generalised characterization of mountains areas, researchers agreed on a concept including both elevation and slope (Blyth et al., 2002). Higher Mountains are usually divided into three elevational belts with each belt sustaining a different fauna and flora. The montane belt is often considered the lowest belt, it usually comprises the area below the treeline. It is followed by the alpine belt (mostly tundra) and the nival belt where snowfall occurs all year around (Körner, 2004). These belts may be further subdivided due to climatic properties (Körner, 2011). Due to its large plant biomass (trees) and area (43% of the worldwide mountain area outside of Antarctica), the montane belt can be considered the most important in regard of carbon storage (Körner, 2011; Price et al. 2015). Since mountain forests make up 23% of the worldwide forest cover, this even holds true from a global point of view (Price et al., 2015). Besides their value as carbon sinks, mountain forests further play a critical role in maintaining hydrological cycles, erosion prevention and also timber production, to only name few functions (Price et al. 2015).

Like other mountain ecosystems, mountain forests are strongly affected by climate change. Matskovsky et al. (2021) predicted climate change to negatively affect tree growth of two endemic Chilean mountain tree species. Dakhil et al (2021) predicted a decrease in the area of occupancy for eight montane coniferous tree species endemic to southwestern China, of which four species were predicted to lose over 90% of their area of occupancy. Further, Kim et al. (2018) predicted a contraction of coniferous forests by 33 % in the North American Blue Mountains under climate change. Mountain forests are especially vulnerable to climate change as dominant tree species in the montane belt often are endemic coniferous species with a small spatial distribution. Accordingly, Albrich et al. (2020) predicted a climate induced transition of coniferous forests towards more broadleaf forests in the European Alps.

Such changes in tree species composition will likely affect the composition and functioning of animal, plant and microbial communities. Leidinger et al. (2021) found differences in species numbers in a variety of taxa between beech forests with different admixtures of other tree species, with the type of the response depending on the respective taxon. Vehviläinen et al. (2008) found varying responses of predatory arthropod abundances to different types of forest stands. The investigation of elevational gradients in undisturbed forests may help to uncover the response of forest dwelling taxa to climate driven forest conversion. Such a gradient, including the natural transition zone between broadleaf and coniferous forests, is present on the northern slope of Changbai Mountain, China (Tang et al. 2011).

1.2 Changbai Mountain

The Changbai Mountain area in Northeast China stretches along the borders of the provinces Jilin and Liaoning with North Korea (42°8’25.4004”N, 128°7’36.2352”E). Changbai Mountain itself is the highest mountain in the area (2750 m asl). It was formed and shaped through volcanic activity, besides the formation of a basalt plateau between 20 and 3 mya, the actual Changbai Mountain shield and its cone were formed between 2.8 and 0.01 mya (Li et al. 2021). Given the volcanic history, geological groups at the northern slope comprise mainly stomatal and laminated basalt, alkali pumice, trachyte and tuff, covering the bedrock consisting mainly of dolomitic marble (Yan et al. 2018). Changbai Mountains climate is characterized as temperate continental mountain climate influenced by monsoons (Wang et al. 2020), including warm and wet summers and long winters. The northern slope of Changbai Mountain is part of the “Changbaishan National Nature Reserve”, founded in 1960 to preserve the unique fauna and flora of the existing primary forests. The primary forests are characterized by Korean pine (*Pinus koraiensis* Siebold & Zucc.) intermixed with deciduous trees which dominate forests between 800 and 1100 m asl. Above 1100 m the abundance of dark-bark spruce (*Picea jezoensis* var. *komarovii* Siebold & Zucc.) increases, making it the dominant tree species up to 1700 m, further followed by birch forests (*Betula ermanii* Cham.) and tundra (Liu 1997).

Several environmental factors change alongside with elevation and forest types across this elevational gradient. The most obvious being climatic variables which in part define tree species distribution boundaries. In general, annual mean temperature and temperature seasonality decrease and precipitation increases alongside with elevation. Whereas precipitation seasonality is highest at low, but lowest at intermediate elevations (Fig. 1a). These factors indicate that, even though the higher elevations are colder and have more rain and snow in general, the lower elevations show stronger fluctuations in both throughout the year. Litter related factors also change with elevation (Fig. 1b,c), which is likely linked to both climate and forest type. There are strong variations in C/N ratio and litter water content which both peak at 1250 m. The depth of the litter and fermentation layer is slightly higher at higher elevations (1250 - 1700 m), while the humus layer does not change, which already indicates alterations in carbon input and/or decomposition processes (Fig. 1b). Chemical properties of leaf litter can tremendously vary across forest types. On Changbai Mountain calcium concentrations are high in lower elevation litter as also reflected in the pH of litter. The strong fluctuations in nutrient content in general indicate differences in litter stoichiometry along the elevational gradient (Fig. 1c). This is in line with Ricklefs and Matthew (1982), who found large variations in e.g., calcium, lignin and N content between 34 temperate deciduous tree species, even on a small spatial scale (24 km gradient). Perez-Harguindeguy et al. (2000), reported variations in litter C/N ratio and physical litter toughness between tree functional groups such as deciduous and evergreen tree species.

1.3 Implications for Soil Food Web Functioning

Differences in local climate and the structural differences in litter across elevations likely influence the soil food web, due to its close ties to primary producers. Forest soil food webs are driven by the input of dead organic matter through primary production. In terrestrial ecosystems 10-30 % of net primary production (carbon) is released as litter and the same holds true for organic carbon provided by root exudates (Chapin et al. 2011). It must be mentioned that root derived resources can be of high importance for soil animals (Pollierer et al. 2007). Litter, however, typically is considered the most important resource especially for decomposers. In a greenhouse experiment Eisenhauer and Reich (2012) found the identity of above ground plant litter input to influence Oribatida abundance and decomposer performance (carbon use efficiency of microbes) stronger than root litter identity.

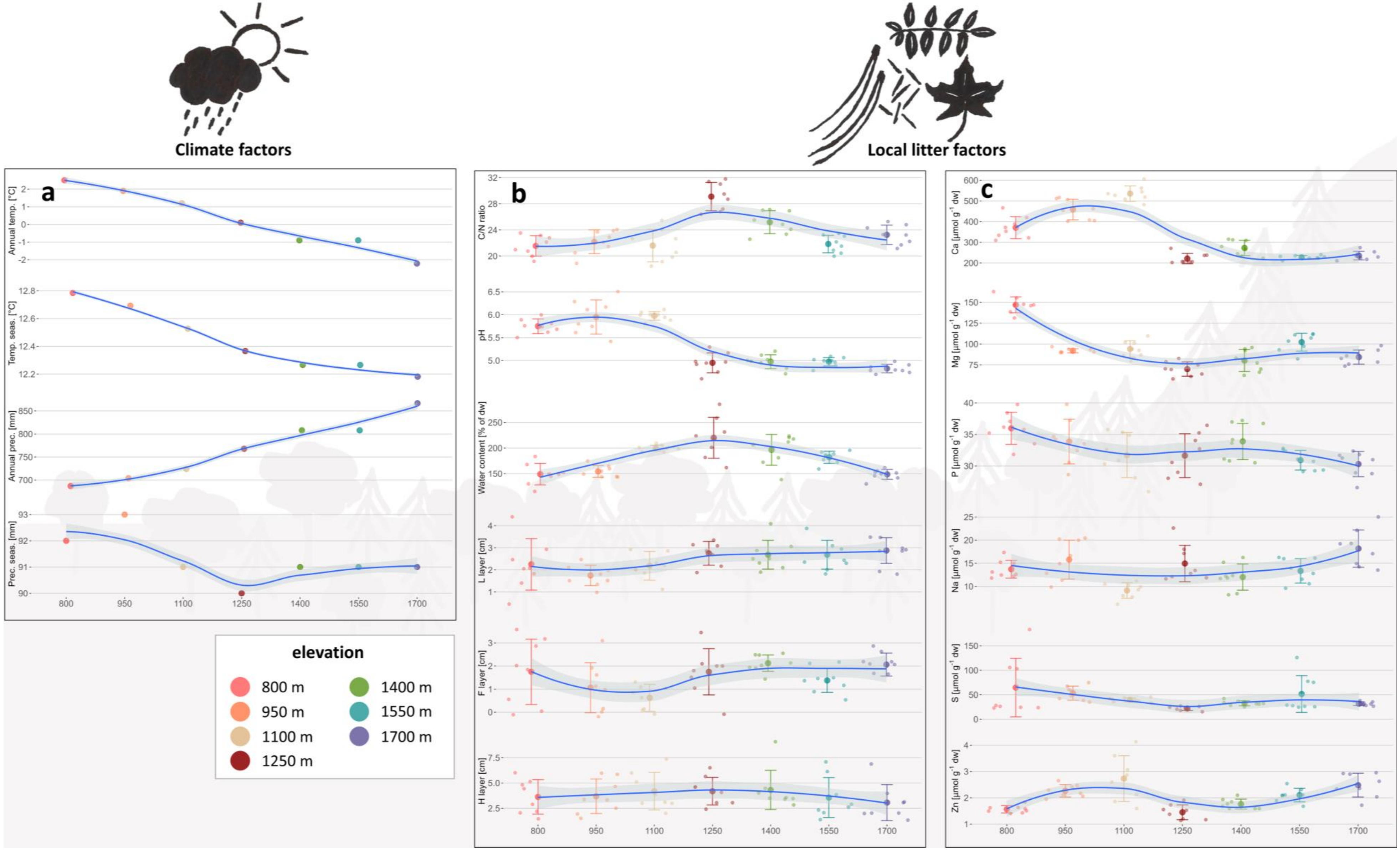

Figure 1: Characterization of climate (a) and local litter factors (b, c) at the northern slope of Changbai Mountain. (a) annual mean temperature [°C], temperature seasonality (standard deviation of mean monthly temperature) [°C], annual mean precipitation [mm] and precipitation seasonality (standard deviation of mean monthly precipitation) [mm] were retrieved from “Worldclim2” at 30 s resolution (Fick & Hijmans, 2017; Hijmans, 2021). (b) represents common local litter characteristics including the depth of the intact litter (L), fermentation (F) and humus (H) layer, while (c) displays litter nutrient content [µmol g^{-1} dw] measured from intact litter; The measurements of respective factors are described in Chapter 2.

Consequently, differences in physical and chemical properties of litter may directly influence litter decomposition rates. For example, the contribution of soil mesofauna to decomposition is higher in nutrient rich compared to more recalcitrant litter (Fujii et al. 2018) and leaf toughness and C/N ratio can negatively influence litter weight loss through decomposition (Perez-Harguindeguy et al. 2000). In forests litter decomposition is mainly driven by microorganisms, especially bacteria and fungi (Bani et al. 2018). Within soil food webs, decomposers are among the most crucial components of forest growth as they recycle nutrients and counteract nutrient leaching. Besides microbes, microarthropods also contribute to litter decomposition and recycling of nutrients (Seastedt, 1984; Kampichler & Bruckner, 2009). Collembola and Oribatida are the most abundant microarthropod taxa in forests and therefore influence nutrient cycling (Hättenschwiler et al. 2005; Bani et al. 2018). Both taxa span a wide range of trophic niches (Scheu and Falca 2000; Schneider et al. 2004; Chahartaghi et al. 2005), with the majority in forests being primary and secondary decomposers: Chahartaghi et al (2005) categorized five and eight of the twenty investigated Collembola taxa from three deciduous forests as primary and secondary decomposers, respectively. Similarly, Schneider et al (2004) categorized ten and ten out of 26 assigned oribatid taxa from four forests as primary and secondary decomposers, respectively.

Primary and secondary decomposers are not distinct, rather, they form a continuum with both feeding on litter at different microbial colonization stages (Scheu and Falca 2000; Maraun et al. 2023). Even though Collembola and Oribatida species may vary considerably in their trophic niches (Scheu and Falca 2000; Pollierer et al. 2009), in Collembola habitat-dependent intraspecific variation in trophic ranges may be almost as high as the interspecific variation (Korotkevich et al. 2018). Consequently, changes in resource availability due to altered decomposition processes and litter quality along elevational gradients will likely cause shifts in trophic position of microarthropod communities and species and thus food web functioning.

Even though responses of soil food web’s trophic structure along elevational gradients are likely, they have never been investigated in detail. Most studies on soil food webs along elevational gradients focus on community composition, including microbes (Shen et al. 2013; Liu et al. 2019), Collembola (Xie et al. 2022) and Oribatida (Pan et al. 2023). There is urgent need to identify trophic responses of different soil food web compartments to elevation to predict potential impacts of climate and forest change. In fact, to the best of our knowledge there has only been one study reporting changes in trophic positions of soil microarthropods (Oribatida) across an elevational gradient, and the main focus of that study was not trophic changes with elevation (Fischer et al. 2014).

1.4 Methodologies

This thesis forms part of the “Changbai Project”, a joint project between the Key Laboratory of Wetland Ecology and Environment of the Chinese Academy of Sciences, Changchun, China and the Working Group of Animal Ecology of the University of Göttingen, Germany. The Project is supported by both, the National Natural Science Foundation of China (NSFC; No. 31861133006-42071059) and the German Science Foundation (DFG; SCHE/376/42-1) in the framework of the Sino-German agreement between the DFG and the NSFC.

In this framework we established seven sites with elevational steps of 150 m along an elevational gradient at the northern slope of Changbai Mountain, China, covering the primary forest area between 800 and 1700 m asl, as characterized before. At each site we established eight plots, serving as replicates for the respective measurements. Samples were taken in early September 2019, before litter fall began (see chapters for sampling methods).

To understand interactions between microbes and microarthropods it is important to characterize the microbial community and functioning. A well-established approach for identifying changes in microbial communities is phospholipid fatty acid (PLFA) analysis. PLFAs form the major component of cell membranes and, by varying among microbial groups, provide insight into microbial community structure (Bossio and Scow 1997; Moore-Kucera and Dick 2008; Frostegård et al. 2011). Certain PLFA ratios on the other hand can serve as indicators of environmental stress and substrate availability (Bossio and Scow 1997; Moore-Kucera and Dick 2008; Frostegård et al. 2011). Further, microbial basal respiration and substrate-induced respiration provide insight into gross characteristics of microbial communities such as microbial biomass and the microbial carbon use efficiency (Anderson and Domsch 1978; Scheu 1992; Anderson and Domsch 1993). This information is crucial in terms of soil food web functioning as it provides insight into decomposition processes, but also quantifies availability of microorganisms as potential resource for the soil animal food web.

Investigating trophic relationships in soil food webs is a difficult task, impeded by several factors. The habitat itself does not allow direct observations and the body size and feeding mode of focal organisms complicates many established methods such as visual gut content inspection. An alternative method used is the identification of gut enzymes (Potapov et al. 2021), however, both methods do only allow a temporal snapshot of the diet, a problem revisited under the more modern approach of molecular gut content analysis. Bulk stable isotopes of consumers (usually ^{15}N and ^{13}C) on the other hand can give long-term diet information (Nielsen et al. 2018). The method of bulk stable isotopes was adopted for soil food web analyses in 2000 (Ponsard and Arditi 2000; Scheu and Falca 2000), and became one of the best established methods for the analyses of the trophic structure of soil animals (Potapov et al. 2019a; Maraun et al. 2023). However, the information about a consumer’s basal resource gained from bulk stable isotopes is of low resolution and only allows broad differentiation of e.g., plant, algae and lichen feeders (Maraun et al. 2023). Compound-specific ^{13}C analysis of amino acids (CSSIA-AA; Larsen et al. 2013, 2016; Pollierer et al. 2019) or biomarker neutral lipids (NLFA) in consumers (‘dietary routing’; Ruess and Chamberlain 2010) can increase the resolution as they allow to differentiate more resources (e.g., fungal types and functional bacterial groups). Additionally, both methods are highly complementary: CSSIA-AA reflects basal resources used for protein synthesis and growth, whereas NLFA analysis reflects basal resources used for storage lipids, i.e. energy metabolism.

Bulk stable isotopes (^{15}N) are further used to differentiate trophic positions like primary and secondary decomposers or predators, however, to estimate trophic positions the correct estimation of the baseline, the basal resource of the food chain in most cases, is crucial (Gannes et al. 1997; Martínez Del Rio et al. 2009; Potapov et al. 2019b). ^{15}N analysis of amino acids allows baseline independent estimates of the trophic position of consumers (Chikaraishi et al. 2007, 2010, 2014) and is therefore well suited to increase reliability of trophic position estimates.

Consequently, using a combination of different complementary methods for trophic analyses could be a promising approach to counteract methodical weaknesses and evaluate common methods.

1.5 Scope of the Thesis

Microbial decomposition and soil microarthropod community functioning may change strongly with changing environmental factors and this thesis for the first time tackles the question: ”How do trophic interactions among different components of the soil food web change with elevation?”. In this work we access changes in microbial communities, biomass and functioning and their major driving factors (**Chapter 2**), following up we identify how the overall trophic functions of Collembola and Oribatida change across elevations and how this is linked to microbial and litter resources (**Chapter 3**). Further we address the trophic composition of the Collembola community with a focus on their role as primary decomposers (**Chapter 4**) and comprehensively discuss the relation of these results (**Chapter 5**).

1.6 Chapter Overview

In the following chapters we investigated three main hypotheses:

1. Microbial community functioning is influenced by elevation, with increasing microbial biomass and carbon use efficiency with increasing elevation.

2. The role of Collembola and Oribatida as primary decomposers diminishes at higher elevations, whereas their role as secondary decomposers is strengthened.

3. The trophic range of the Collembola community shrinks towards higher elevations, caused by a decrease/shift in primary decomposers and an increase in secondary decomposers, but this varies with Collembola life form and body size.

In Chapter 2 we investigated substrate-induced respiration and phospholipid fatty acids in litter and two soil layers across the studied elevational gradient. Microbial biomass and indicators of microbial stress were higher in the litter layer of higher elevations, both correlated negatively with Ca concentrations indicating increased nutritional stress in high microbial biomass communities at sites with low Ca availability. PLFA profiles in the litter layer separated low and high elevation sites, but this was less pronounced in soil. Therefore, the litter layer likely buffers the impact of climate factors on soil microbial communities. Annual variations in temperature influenced PLFA profiles in all three layers, while annual variations in precipitation influenced upper soil microbes only. Furthermore, the availability of resources, soil moisture, Ca concentrations and pH structured microbial communities. Pronounced changes in microbial biomass between litter of pine dominated (800 - 1100 m) and spruce dominated (1250 - 1700 m) forests indicated a shift in the structure and functioning of microbial communities between forest types along the elevational gradient. Microbial community structure and functioning strongly depends on elevation, but driving factors vary between soil layers. Our results suggest that, besides annual variations in temperature and precipitation, carbon accumulation and nitrogen acquisition shape changes in microbial communities with elevation on Changbai Mountain.

The study presented in **Chapter 3** focused on trophic variations in Collembola and Oribatida along the studied elevational gradient. We applied three state-of-the-art methods to elucidate trophic positions and basal resource use of both taxa at community level: Bulk stable isotope analysis of nitrogen and carbon (SI_{bulk}), compound-specific stable isotope analysis of amino acids (CSSIA-AA) and dietary routing through neutral lipid fatty acids (NLFA). $\Delta^{15}N_{bulk}$ and trophic position calculated using CSSIA-AA (TP_{CSSIA}) both increased with elevation in both taxa and correlated with each other. Stable isotope mixing models using $\delta^{13}C$ of essential amino acids indicated fungi as most important resource at most elevations for both taxa. Proportions of marker NLFAs changed little across elevations in both taxa, indicating strong variations in trophic positions with elevation but conserved energy channels in both taxa. Collembola showed higher relative amounts of bacterial markers than Oribatida. $\Delta^{13}C_{bulk}$ did not respond linearly to the elevational gradient and the response depended on taxon. A strong linear correlation between $\delta^{15}N$ of phenylalanine in consumers and $\delta^{15}N$ of litter indicated litter as basal resource of their energy channel. Our results indicate changes in food web functioning with changing forest types along elevational gradients, where microarthropods might switch from feeding at the base of the food web to feeding at higher trophic levels. This may potentially compromise their role in litter decomposition and nutrient cycling.

In **Chapter 4**, based on the implications from Chapter 3, we examined the effects of elevation on trophic variations of Collembola at the species level. Collembola species were categorized into life forms and individual body lengths were measured. Abundance weighted mean and minimum $\Delta^{15}N$ values as well as abundance weighted $\Delta^{13}C$ values increased with increasing elevation, while the range of $\Delta^{15}N$ values decreased. Maximum and minimum values of $\Delta^{13}C$ differed between elevations but showed no linear response. Isotopic uniqueness linearly increased with elevation. $\Delta^{15}N$ values of Collembola species occurring across all elevations increased with elevation, this shift was strongest in *Folsomia octoculata*. Elevational changes in mean $\Delta^{15}N$ values also depended on Collembola life form and were most pronounced in hemiedaphic species. Further, $\Delta^{15}N$ values increased with decreasing body size but only in hemiedaphic and euedaphic species. Linear changes in $\Delta^{13}C$ values with elevation depended on Collembola life form, with euedaphic species showing the strongest shift. Overall, the results suggest that Collembola species functioning as primary decomposers shift towards using more microbial diets and functioning as secondary decomposers or even living as predators or scavengers at higher elevations. The results further indicate that the ability to access alternative food resources depends on Collembola life form and body size, but that this relationship varies between ecosystems. Climate driven forest conversion may affect the trophic structure of Collembola communities as Collembola functioning as secondary decomposers in coniferous forests may shift towards functioning as primary decomposers if species boundaries of broad-leaf tree species at lower elevations expand towards higher elevations.

1.7 References

Albrich K, Rammer W, Seidl R (2020) Climate change causes critical transitions and irreversible alterations of mountain forests. Glob Change Biol 26:4013–4027. https://doi.org/10.1111/gcb.15118

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 10:215–221. https://doi.org/10.1016/0038-0717(78)90099-8

Anderson TH, Domsch KH (1993) The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol Biochem 25:393–395. https://doi.org/10.1016/0038-0717(93)90140-7

Bani A, Pioli S, Ventura M, et al (2018) The role of microbial community in the decomposition of leaf litter and deadwood. Appl Soil Ecol 126:75–84. https://doi.org/10.1016/j.apsoil.2018.02.017

Bossio DA, Scow KM (1997) Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb Ecol 35:265–278. https://doi.org/10.1007/s002489900082

Chahartaghi M, Langel R, Scheu S, Ruess L (2005) Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biol Biochem 37:1718–1725. https://doi.org/10.1016/j.soilbio.2005.02.006

Chapin FS, Matson PA, Vitousek PM (2011) Principles of terrestrial ecosystem ecology. Springer New York, New York, United States

Chikaraishi Y, Kashiyama Y, Ogawa NO, et al (2007) Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: Implications for aquatic food web studies. Mar Ecol Prog Ser 342:85–90. https://doi.org/10.3354/meps342085

Chikaraishi Y, Ogawa NO, Ohkouchi N (2010) Further evaluation of the trophic level estimation based on nitrogen isotopic composition of amino acids. In: Earth, life, and isotopes. Kyoto University Press, Kyoto

Chikaraishi Y, Steffan SA, Ogawa NO, et al (2014) High-resolution food webs based on nitrogen isotopic composition of amino acids. Ecol Evol 4:2423–2449. https://doi.org/10.1002/ece3.1103

Döll K, Chatterjee S, Scheu S, et al (2013) Fungal metabolic plasticity and sexual development mediate induced resistance to arthropod fungivory. Proc R Soc B Biol Sci 280:20131219. https://doi.org/10.1098/rspb.2013.1219

Eisenhauer N, Reich PB (2012) Above- and below-ground plant inputs both fuel soil food webs. Soil Biol Biochem 45:156–160. https://doi.org/10.1016/j.soilbio.2011.10.019

Fischer BM, Meyer E, Maraun M (2014) Positive correlation of trophic level and proportion of sexual taxa of oribatid mites (Acari: Oribatida) in alpine soil systems. Exp Appl Acarol 63:465–479. https://doi.org/10.1007/s10493-014-9801-3

Frostegård Å, Tunlid A, Bååth E (2011) Use and misuse of PLFA measurements in soils. Soil Biol Biochem 43:1621–1625. https://doi.org/10.1016/j.soilbio.2010.11.021

Fujii S, Cornelissen JHC, Berg MP, Mori AS (2018) Tree leaf and root traits mediate soil faunal contribution to litter decomposition across an elevational gradient. Funct Ecol 32:840–852. https://doi.org/10.1111/1365-2435.13027

Gannes LZ, O'brien DM, Martínez del Rio C (1997) Stable isotopes in animal ecology: Assumptions, caveats and a call for more laboratory experiments. Ecology 78:1271–1276. https://doi.org/10.1890/0012-9658(1997)078[1271:siiaea]2.0.co;2

Guckert JB, Hood MA, White DC (1986) Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: Increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. Appl Environ Microbiol 52:794–801. https://doi.org/10.1128/aem.52.4.794-801.1986

Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annu Rev Ecol Evol Syst 36:191–218. https://doi.org/10.1146/annurev.ecolsys.36.112904.151932

Kim JB, Kerns BK, Drapek RJ, et al (2018) Simulating vegetation response to climate change in the Blue Mountains with MC2 dynamic global vegetation model. Clim Serv 10:20–32. https://doi.org/10.1016/j.cliser.2018.04.001

Knivett VA, Cullen J (1965) Some factors affecting cyclopropane acid formation in *Escherichia coli*. Biochem J 96:771–776. https://doi.org/10.1042/bj0960771

Korotkevich AY, Potapov AM, Tiunov AV, Kuznetsova NA (2018) Collapse of trophic-niche structure in belowground communities under anthropogenic disturbance. Ecosphere 9: e02528. https://doi.org/10.1002/ecs2.2528

Krajewska B (2018) Urease-aided calcium carbonate mineralization for engineering applications: A review. J Adv Res 13:59–67. https://doi.org/10.1016/j.jare.2017.10.009

Larsen T, Ventura M, Andersen N, et al (2013) Tracing carbon sources through aquatic and terrestrial food webs using amino acid stable isotope fingerprinting. PLoS ONE 8:e73441. https://doi.org/10.1371/journal.pone.0073441

Larsen T, Pollierer MM, Holmstrup M, et al (2016) Substantial nutritional contribution of bacterial amino acids to earthworms and enchytraeids: A case study from organic grasslands. Soil Biol Biochem 99:21–27. https://doi.org/10.1016/j.soilbio.2016.03.018

Li MM, Xu ZT, Ventura G, et al (2021) Geochronology and petrogenesis of early pleistocene dikes in the Changbai Mountain volcanic field (NE China) Based on geochemistry and Sr-Nd-Pb-Hf isotopic compositions. Front Earth Sci 9:729905. https://doi.org/10.3389/feart.2021.729905

Liu M, Sui X, Hu Y, Feng F (2019) Microbial community structure and the relationship with soil carbon and nitrogen in an original Korean pine forest of Changbai Mountain, China. BMC Microbiol 19:218. https://doi.org/10.1186/s12866-019-1584-6

Liu QJ (1997) Structure and dynamics of the subalpine coniferous forest on Changbai mountain, China. Plant Ecol 132:97–105. https://doi.org/10.1023/A:1009705319128

Maraun M, Thomas T, Fast E, et al (2023) New perspectives on soil animal trophic ecology through the lens of C and N stable isotope ratios of oribatid mites. Soil Biol Biochem 177:108890. https://doi.org/10.1016/j.soilbio.2022.108890

Martínez Del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111. https://doi.org/10.1111/j.1469-185X.2008.00064.x

Minocha R, Chamberlain B, Long S, et al (2015) Extraction and estimation of the quantity of calcium oxalate crystals in the foliage of conifer and hardwood trees. Tree Physiol 35:574–580. https://doi.org/10.1093/treephys/tpv031

Moore-Kucera J, Dick RP (2008) PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microb Ecol 55:500–511. https://doi.org/10.1007/s00248-007-9295-1

Nielsen JM, Clare EL, Hayden B, et al (2018) Diet tracing in ecology: Method comparison and selection. Methods Ecol Evol 9:278–291. https://doi.org/10.1111/2041-210X.12869

Pan X, Xie Z, Sun X, et al (2023) Changes in oribatid mite community structure along two altitudinal gradients in Asia and Europe as related to environmental factors. Appl Soil Ecol 189:104912. https://doi.org/10.1016/j.apsoil.2023.104912

Perez-Harguindeguy N, Dıaz S, Cornelissen JHC, et al (2000) Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. Plant Soil 218:21–30

Pollierer MM, Langel R, Körner C, et al (2007) The underestimated importance of belowground carbon input for forest soil animal food webs. Ecol Lett 10:729–736. https://doi.org/10.1111/j.1461-0248.2007.01064.x

Pollierer MM, Langel R, Scheu S, Maraun M (2009) Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios (^{15}N/^{14}N and ^{13}C/^{12}C). Soil Biol Biochem 41:1221–1226. https://doi.org/10.1016/j.soilbio.2009.03.002

Pollierer MM, Larsen T, Potapov AM, et al (2019) Compound-specific isotope analysis of amino acids as a new tool to uncover trophic chains in soil food webs. Ecol Monogr 89:e01384. https://doi.org/10.1002/ecm.1384

Ponsard S, Arditi R (2000) What can stable isotopes (^{15}N and ^{13}C) tell about the food web of soil macro-invertebrates? Ecology 81:852–864. https://doi.org/10.2307/177382

Potapov AM, Tiunov AV, Scheu S (2019a) Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biol Rev 94:37–59. https://doi.org/10.1111/brv.12434

Potapov AM, Tiunov AV, Scheu S, et al (2019b) Combining bulk and amino acid stable isotope analyses to quantify trophic level and basal resources of detritivores: A case study on earthworms. Oecologia 189:447–460. https://doi.org/10.1007/s00442-018-04335-3

Potapov AM, Pollierer MM, Salmon S, et al (2021) Multidimensional trophic niche revealed by complementary approaches: Gut content, digestive enzymes, fatty acids and stable isotopes in Collembola. J Anim Ecol 90:1919–1933. https://doi.org/10.1111/1365-2656.13511

Price MF, Gratzer G, Duguma LA, et al (2015) Mountain forests in a changing world - realizing values, addressing challenges. https://doi.org/10.13140/2.1.2386.5283

Ricklefs RE, Matthew KK (1982) Chemical characteristics of the foliage of some deciduous trees in Southeastern Ontario. Can J Bot 60:2037–2045. https://doi.org/10.1139/b82-251

Ruess L, Chamberlain PM (2010) The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biol Biochem 42:1898–1910. https://doi.org/10.1016/j.soilbio.2010.07.020

Rusek J (2007) A new classification of Collembola and Protura life forms. Contrib Soil Zool Cent Eur II:109–115

Scheu S (1992) Automated measurement of the respiratory response of soil microcompartments: Active microbial biomass in earthworm faeces. Soil Biol Biochem 24:1113–1118. https://doi.org/10.1016/0038-0717(92)90061-2

Scheu S, Falca M (2000) The soil food web of two beech forests (*Fagus sylvatica*) of contrasting humus type: stable isotope analysis of a macro- and mesofauna-dominated community. Oecologica 123:285–286. https://doi.org/10.1007/s004420051015

Schneider K, Migge S, Norton RA, et al (2004) Trophic niche differentiation in soil microarthropods (Oribatida, Acari): Evidence from stable isotope ratios ($^{15}N/^{14}N$). Soil Biol Biochem 36:1769–1774. https://doi.org/10.1016/j.soilbio.2004.04.033

Shen C, Xiong J, Zhang H, et al (2013) Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. Soil Biol Biochem 57:204–211. https://doi.org/10.1016/j.soilbio.2012.07.013

Tang L, Li A, Shao G (2011) Landscape-level forest ecosystem conservation on Changbai Mountain, China and North Korea (DPRK). Mt Res Dev 31:169–175. https://doi.org/10.1659/MRD-JOURNAL-D-10-00120.1

Volk GM, Lynch-Holm VJ, Kostman TA, et al (2002) The role of druse and raphide calcium oxalate crystals in tissue calcium regulation in *Pistia stratiotes* leaves. Plant Biol 4:34–45. https://doi.org/10.1055/s-2002-20434

Wang H, Xu J, Sheng L, et al (2020) Study on the characteristics of climate change in Changbai Mountain National Natural Reserve from 1958 to 2017. Arab J Geosci 13:777. https://doi.org/10.1007/s12517-020-05808-7

White PJ (2003) Calcium in plants. Ann Bot 92:487–511. https://doi.org/10.1093/aob/mcg164

Xie Z, Sun X, Lux J, et al (2022) Drivers of Collembola assemblages along an altitudinal gradient in Northeast China. Ecol Evol 12:e8559. https://doi.org/10.1002/ece3.8559

Yan B, Liang X, Xiao C (2018) Hydrogeochemical characteristics and genesis model of Jinjiang and Julong hot springs in Changbai Mountain, Northeast China. Geofluids 2018:1694567. https://doi.org/10.1155/2018/1694567

Chapter 2: Changes in Microbial Community Structure and Functioning with Elevation Are Linked to Local Soil Characteristics as Well as Climatic Variables

Oribatid mite larvae (*Damaeus* sp.) feeding on fungal hyphae

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2.1 Abstract

Mountain forests are important carbon stocks and biodiversity hotspots but are threatened by increased insect outbreaks and climate driven forest conversion. Soil microorganisms play an eminent role in nutrient cycling in forest habitats and form the basis of soil food webs. Uncovering the driving factors shaping microbial communities and functioning at mountainsides across the world is of eminent importance to better understand their dynamics at local and global scales. We investigated microbial communities and their climatic and local soil related drivers along an elevational gradient (800 - 1700 m asl) of primary forests at Changbai Mountain, China. We analysed substrate-induced respiration and phospholipid fatty acids (PLFA) in litter and two soil layers at seven sites. Microbial biomass (C_{mic}) peaked in the litter layer and increased towards higher elevations. In the litter layer the increase in C_{mic} as well as in stress indicator ratios were negatively correlated with Ca concentrations indicating increased nutritional stress in high microbial biomass communities at sites with lower Ca availability. PLFA profiles in the litter layer separated low and high elevations, but this was less pronounced in soil, suggesting that the litter layer functions as buffer for soil microbial communities. Annual variations in temperature correlated with PLFA profiles in all three layers, while annual variations in precipitation correlated with PLFA profiles in upper soil only. Furthermore, the availability of resources, soil moisture, Ca concentrations and pH structured the microbial communities. Pronounced changes in C_{mic} and stress indicator ratios in the litter layer between pine dominated (800 - 1100 m) and spruce dominated (1250 - 1700 m) forests indicated a shift in the structure and functioning of microbial communities between forest types along the elevational gradient. The study highlights strong changes in microbial community structure and functioning along elevational gradients, but also shows that these changes and their driving factors vary between soil layers. Besides annual variations in temperature and precipitation, carbon accumulation and nitrogen acquisition shape changes in microbial communities with elevation at Changbai Mountain.

2.2 Introduction

Forests store large amounts of carbon, fixed in standing plant (tree) biomass as well as soil organic matter. Mountain forests contribute significantly to these carbon stocks as 41% of the worldwide mountain area is covered by forests and mountain forests sum up to 23% of the worldwide forest cover (Price et al., 2015). Global warming is expected to strongly alter mountain forests. Albrich et al. (2020) projected changes of coniferous mountain forests towards broadleaf forests at lower elevations in the European Alps. These changes in vegetation are likely to affect the structure and functioning of microbial and animal communities. Although microbial communities along elevational gradients received more attention in the last years, there is still a lack of knowledge on the factors driving microbial community composition and functioning along such gradients (Looby & Martin, 2020). Studies addressing this lack of knowledge are best to be done in mountain areas little affected by humans allowing to uncover the response of natural communities to global change factors. The northern slope of Changbai Mountain in Northeast China represents such a natural forest gradient as these forests have never been logged (Tang et al., 2011). The forests comprise mainly primary forests with a transition between deciduous and mixed forests at lower elevations towards pure coniferous and birch forests at higher elevations (Liu, 1997; Tang et al., 2011).

Litter entering the belowground system is decomposed predominantly by microorganisms, mainly bacteria and fungi (Bani et al., 2018), and therefore, microorganisms play a critical role in the mineralisation of carbon and nitrogen (Hobara et al., 2014). Considering that microbial activity is intricately linked to temperature, mountains provide ideal settings to investigate the role of temperature and associated changes in forest type on the structure and functioning of microbial communities. Studying these changes is of particular relevance in face of global climate change. With decreasing temperature and shorter vegetation periods at higher elevation, microorganisms may have to concentrate their metabolic activities to the limited period of high temperature and microbial communities have to adapt to the short period they can be active. Conform to these assumptions, Massaccesi et al. (2020) found microbial biomass to increase with increasing elevation in coniferous forests in the European Apennine indicating higher resource availability at high elevations. Changbai Mountain forests at high elevations are dominated by spruce (Liu, 1997) and spruce is known to retard decomposition processes by high concentration of polyphenols in needles contributing to the accumulation of carbon at high elevation (Gallet & Lebreton, 1995). This is likely to be associated by distinct microbial communities.

Harsh environmental conditions including climatic as well as soil factors at high elevations also likely increase physiological stress of microorganisms. Both low temperature and pH are known to result in alterations in the structure of microbial membranes (Knivett & Cullen, 1965; Guckert et al., 1986; Russel, 2008), with major consequences for microbial community composition and functioning. Conform to these considerations, Shen et al (2013) identified pH as main driver of changes in microbial community composition with elevation at Changbai Mountain. Effects of low temperature and pH on soil microorganisms, however, are likely to vary with soil depth due to the buffering of adverse climatic conditions by the litter layer and typically higher pH in litter than in soil. Further, the decreases in organic matter with soil depth and associated decline in resource availability (Kramer & Gleixner, 2008; Hobley & Wilson, 2016) may aggravate microbial stress in soil. Therefore, driving factors of microbial community structure and activity are likely to differ between litter and soil. As both processes in litter and soil contribute to carbon and nutrient cycling, understanding the driving factors of microbial community composition and functioning in both litter and soil is of fundamental importance. However, studies investigating changes in microbial communities along elevational gradients often only focus on soils and neglect the litter layer (Chang et al., 2016; Liu et al., 2019).

To investigate changes in microbial communities along environmental gradients phospholipid fatty acids (PLFAs) are commonly used (Xu et al., 2014; Chang et al., 2016; Liu et al., 2019). PLFAs form the major component of cell membranes and, by varying among microbial groups, provide insight into microbial community structure (Bossio & Scow, 1997; Moore-Kucera & Dick, 2008; Frostegård et al., 2011). Further, PLFA ratios serve as indicators of environmental stress and substrate availability (Bossio & Scow, 1997; Moore-Kucera & Dick, 2008; Frostegård et al., 2011). Thereby, PLFAs provide insight into changes in the structure and functioning of microbial communities along altitudinal gradients (Liu et al., 2019; Klimek et al., 2020). Similarly, microbial basal respiration and substrate-induced respiration (SIR) provide insight into gross characteristics of microbial communities such as microbial biomass and activity as well as the efficiency in the use of carbon resources by microorganisms (Anderson & Domsch, 1978; Scheu, 1992; Anderson & Domsch, 1993).

We used PLFAs and SIR to follow changes in microbial community structure and functioning in litter and soil of forests along an altitudinal transect of Changbai Mountain, China. We hypothesised (i) microbial biomass and metabolic quotient to increase with increasing elevation, but to decrease with soil depth; (ii) microbial community composition, represented by PLFA profiles, to change with elevation and soil depth, with the changes being less pronounced in soil than in litter; (iii) elevation-related climatic variables and pH to be the major factors structuring microbial communities in litter, while in soil local soil characteristics to be most important; and (iv) physiological and nutritional stress indicators to increase with increasing elevation (due to increased environmental harshness) and soil depth (due to increased resource shortage).

2.3 Material and Methods

2.3.1 Study Site and Sampling

Changbai Mountain (42°8’25.4004”N, 128°7’36.2352”E) extends along the border between the Chinese provinces Jilin and Liaoning and North Korea, with the “Changbaishan” being the highest mountain (2750 m asl). Samples were taken along the northern slope of the mountain forming part of the “Changbaishan National Nature Reserve”. The alkaline geological groups in the sampling area comprise stomatal and laminated basalt, alkali pumice, trachyte and tuff, reflecting the volcanic history of the mountain (Yan et al., 2018). The area belongs to the temperate climate regime and is characterized by long winters and short and warm summers. Between 1959 and 1988 the annual mean temperature ranged from -7 to 3°C and precipitation ranged from 700 to 1400 mm (Chen et al., 2011). The mountain vegetation mainly comprises broadleaved and mixed forests with high abundance of Korean pine (*Pinus koraiensis* Siebold & Zucc.) at lower elevation (up to 1100 m) and spruce – fir coniferous forests at higher elevation (up to 1700 m) followed by birch forests and tundra (Tang et al., 2011; Yu et al., 2013). The current study focuses on the forest area between 800 and 1700 m asl, where seven plots of an elevational difference of 150 m, were sampled. Every plot was subdivided into four subplots with at least 50 m distance between them (Appendix Fig. 1). Samples were taken in early September 2019. Three soil cores of a diameter of 5.5 cm were randomly taken at each subplot, the cores were divided into litter layer, upper (0 - 5 cm) and lower (5 - 10 cm) soil layer. The three samples per layer were pooled and considered as one replicate, resulting in four replicates per elevation. Samples were transported in cooling boxes to the laboratory and frozen at -26°C. Prior to further analyses thawed litter samples were cut into pieces of ca. 2.5 cm × 2.5 cm by scissors and thawed soil samples were sieved through 2 mm mesh and thoroughly mixed.

2.3.2 Chemical and Microbial Analyses

Soil and litter pH was measured in 0.01 M $CaCl_2$ solution. For carbon and nitrogen analyses 2 g of soil and 1 g of litter were dried at 70°C for 24 h and milled. Aliquots of ca. 1.5 mg litter and ca. 10 mg of soil were transferred into tin capsules. Carbon and nitrogen content, and natural $^{13}C/^{12}C$ isotope ratios (Table 1) were measured using an isotopic mass spectrometer (Delta plus XP, Thermo Electron, Bremen, Germany) coupled via an interface (Conflo III, Thermo Eclectron, Bremen, Germany) to an elemental analyser (Flash 2000, Thermo Fisher Scientific, Cambridge, UK). The abundance of ^{13}C was expressed as δ values, calculated as $\delta\,(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 10^3$, with R_{sample} and $R_{standard}$ being the $^{13}C/^{12}C$ ratio in the sample and standard. Vienna Pee Dee belemnite was the primary standard for ^{13}C. Acetanilide was used as internal standard.

Table 1: Means ± se of natural $\delta^{13}C$ [‰] values across elevations and soil layers.

layer	800 m	950 m	1100 m	1250 m	1400 m	1550 m	1700 m
litter	-27.7 ± 0.38	-28.0 ± 0.39	-27.5 ± 0.36	-28.3 ± 0.77	-27.7 ± 0.43	-27.8 ± 0.61	-27.5 ± 0.60
0-5 cm	-26.5 ± 0.18	-26.6 ± 0.43	-26.4 ± 0.64	-26.5 ± 0.65	-25.9 ± 0.30	-25.7 ± 0.34	-25.8 ± 0.32
5-10 cm	-25.7 ± 0.27	-26.0 ± 0.24	-25.3 ± 0.41	-25.9 ± 0.18	-25.2 ± 0.05	-25.0 ± 0.22	-25.4 ± 0.20

A set of climatic variables retrieved from worldclim2 was ascribed to every elevational plot and extracted via the “raster” package at 30 s resolution (Fick & Hijmans, 2017; Hijmans, 2021). Precipitation and temperature seasonality were calculated as standard deviation of the yearly precipitation and temperature (mean of monthly means), respectively.

For measuring the concentrations of eleven elements in litter and soil (aluminium, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulphur, zinc), subsamples of the litter layer were dried (60°C, 48 h), milled and digested with 65 % HNO_3 at 195°C for 8 h. For soil layers the cation exchange capacity was measured from 2.5 g of fresh soil. Samples were saturated with 0.2 N $BaCl_2$ overnight, followed by a 4 h percolation phase in which the solved ions were exchanged with Ba^{2+}. Extracted ions were analysed by ICP-OES (inductively coupled plasma optical emission spectrometry, ICAP 7000 ICP-OES Analysator, ThermoFisher Scientific, USA).

For measuring microbial respiration and biomass, samples were placed at 4°C for 72 h for thawing prior to the analyses and then pre-incubated for seven days at room temperature. A total of 0.8 g of litter and 2 g of each soil layer were used for measuring basal respiration (BR) and substrate-induced respiration (SIR) following Anderson & Domsch (1978). O_2 consumption ($\mu l\ O_2\ g^{-1}$ soil dw h^{-1}) was measured every 0.5 h at 22.0°C using an automated respirometer based on electrolytic O_2 compensation (Scheu, 1992). For BR the mean of readings between 6-12 h after attachment of the vessels to the respirometer were used. For measuring SIR, a glucose solution was added with 80 mg g^{-1} dry weight added to litter and 8 mg g^{-1} dry weight to soil. The mean of the lowest three measurements of the glucose-amended samples was used as maximum initial respiratory response (MIRR; $\mu l\ O_2\ g^{-1}$ dry weight h^{-1}). Microbial biomass (C_{mic}) was calculated as MIRR × 38 × 0.7 (Beck et al., 1997). The specific respiration (qO_2; $\mu l\ O_2\ mg^{-1}\ C_{mic}\ h^{-1}$) was calculated as quotient between BR and C_{mic}. To facilitate comparisons between soil layers C_{mic} was expressed per gram organic carbon as mg $C_{mic}\ g^{-1}C$.

Phospholipids were extracted using a modified high throughput method based on Buyer & Sasser (2012). Lipids were separated through silica columns (0.5 g silicic acid, 3 ml; HF

BOND ELUT-SI, Varian Inc., Darmstadt, Germany). Twenty µl of internal standard (FAME CRM47885, C11 to C24; BAME 47080-U, C11 to C20; Sigma-Aldrich, Darmstadt, Germany) was added before the evaporation at the end of lipid extraction. Samples were evaporated for 40 min at 50°C and then at 37°C using a vacuum centrifuge. Then, 0.2 ml transesterification reagent was added and the vials incubated at 37°C for 15 min before adding 0.4 ml of acetic acid (0.075 M) and 0.4 ml chloroform. The lower phase containing the phospholipid methyl esters (FAMEs) was transferred into new vials and the separation step was repeated with another 0.4 ml of chloroform and evaporated at room temperatures. As dissolvent for FAMEs we used 75 µl isooctane. The resulting fatty acid methyl esters were analysed in a gas-chromatograph (GC-FID Clarus 500; PerkinElmer Corporation, Norwalk, USA) equipped with an Elite 5 column (30 m × 0.32 mm inner diameter, film thickness 0.25 µm). The abundance of the lipids was calculated as nmol per gram of dry material and then transformed to mole percentages (Pollierer et al., 2015).

To further characterise the microbial community, the ratio between fungal (18:2ω6,9) and bacterial PLFAs (i15:0, a15:0, i16:0, cy17:0, cy19:0) was calculated (fun/bac ratio) (Moore-Kucera & Dick, 2008). In addition, the ratio between the sum of cyclopropyl acids (cy17:0, cy19:0) and their monoenoic precursors (16:1ω7, 18:1ω7) was calculated (cyclo/pre ratio), and used as indicator for physiological stress e.g., caused by low pH and low nutrient supply (Knivett & Cullen, 1965; Guckert et al., 1986; Bossio & Scow, 1997). Furthermore, we calculated the ratio between saturated (14:0, 15:0, 16:0, 17:0, 18:0) and monounsaturated PLFAs (16:1ω7, 17:1, 18:1ω9, 18:1ω7), representing nutritional or substrate-related stress (Bossio & Scow, 1997; Moore-Kucera & Dick, 2008). Also, the ratio of branched-chain PLFAs (i15:0, a15:0, i16:0, i17:0), representing Gram$^+$ bacteria (except Actinobacteria), and cyclopropyl and vaccenic type PLFAs (cy17:0, cy19:0, 16:1ω7, 18:1ω7), representing Gram$^-$ bacteria were calculated (Ratledge & Wilkinson, 1988; Joergensen, 2022).

2.3.3 Statistical Analyses

Statistical analyses were performed in R v 4.0.4 (R Core Team, 2021). To analyse differences in microbial community composition among elevations and layers Bray-Curtis distance-based PERMANOVAs were performed using the “adonis” function. The input matrix included amounts of PLFAs as mole percentages as dependent variables. Elevation, soil layer and their interaction were included as independent factors. Non-metric multidimensional scaling (NMDS) was used to display differences in PLFA composition in 2-dimensional space. To identify the PLFAs responsible for most of the variation between elevations and soil layers, Bray-Curtis distance-based analysis of similarity percentages (“SIMPER”) was conducted (Oksanen et al., 2020).

To investigate environmental factors structuring the PLFA composition in litter and soil, redundancy analysis (RDA) was used. The response matrix was the same as for the

Bray-Curtis distance-based method described above. While the response matrix was left unscaled, the matrix containing the environmental factors, including local soil factors (including the eleven elements) as well as climatic factors, was scaled to values between 0 and 1 to secure comparability of effects. RDAs were calculated for all three layers and predictors were selected after correlation and co-linearity between each other; pH was included in all RDA models since it has been identified as the main structuring force for microbial communities at Changbai Mountain (Shen et al., 2013). With this pre-selected set of explanatory variables, a permutational, P-value-based forward-selection was ran via the "ordistep" function (Oksanen et al., 2020). The number of permutations was 1000. The significance of the variation explained by the selected model and its predictors was tested with the permutational-based "anova.cca" function and their explanatory impact was analysed via the adjusted R^2-values of the model (Oksanen et al., 2020). The RDA model was displayed as 2-dimensional biplot of "species"-scaled values to focus on the impact of the factors characterising community composition.

Variations in C_{mic} and qO_2 with elevation and soil layer were inspected using linear mixed effects models with plot-ID as random term (Bates et al., 2022); if necessary, data were $\log_{10}$ transformed to approximate Gaussian distribution. Independent variables were elevation, soil layer and their interaction. If the interaction between elevation and soil layer was significant each layer was analysed separately, using multiple linear models with the respective dependent variable as mentioned above and elevation as independent factor. Linear models met the assumption of homoscedasticity and independence. The independent factor elevation was ordered in all analyses. For visualisation of pairwise differences in figures, we computed Tukey's honestly significant difference (HSD) using the "emmeans" package (Lenth, 2022). Errors presented in text and figures represent the standard error of the mean (SEM). To gain a better understanding of the observed changes, we correlated factors varying with elevation (C_{mic}, qO_2, cyclo/pre and mono/sat ratios) with the environmental factors which were identified by forward-selection in the RDAs to significantly affect the PLFA patterns ($\delta^{13}C$, C/N, pH, Ca concentration, water content) using "Spearman rank correlation" to account for non-linear relationships revealed by visual inspection.

2.4 Results

2.4.1 Microbial Biomass across Elevations and Layers

To study the expected variations in microbial biomass (C_{mic}), we tested the influence of elevation and layer on C_{mic} and their interaction. Microbial biomass varied strongly among layers and generally declined from the litter layer (42.08 ± 1.84 mg C_{mic} g^{-1} C) to 0-5 and 5-10 cm soil by 75 % and 73 %, respectively, but the decline varied with elevation (significant layer × elevation interaction; $\chi^2 = 24.65$, $P = 0.017$). Separate analysis of each layer showed that C_{mic} only varied significantly with elevation in litter ($F_{6,21} = 2.67$, $P = 0.044$), where it first declined from 800 (38.82 ± 3.58 mg C_{mic} g^{-1} C) to 1100 m by 18 % and then increased from 1100 m (31.83 ± 3.93 mg C_{mic} g^{-1} C) up to 1700 m by 61 % (Fig. 1). By contrast, in 0-5 and 5-10 cm soil C_{mic} did not show a clear pattern, but was generally low at 1100 m and high at 1250 m. In contrast to C_{mic}, qO_2 varied significantly with elevation ($\chi^2 = 13.95$, $P = 0.03$), but not among soil layers; it was generally low at 950 m (overall mean across layers 5.49 ± 0.33 µl O_2 mg^{-1} C_{mic} h^{-1}) and highest at 1700 m (6.41 ± 0.30 µl O_2 mg^{-1} C_{mic} h^{-1}), but the variations were generally small (Appendix Fig. 2).

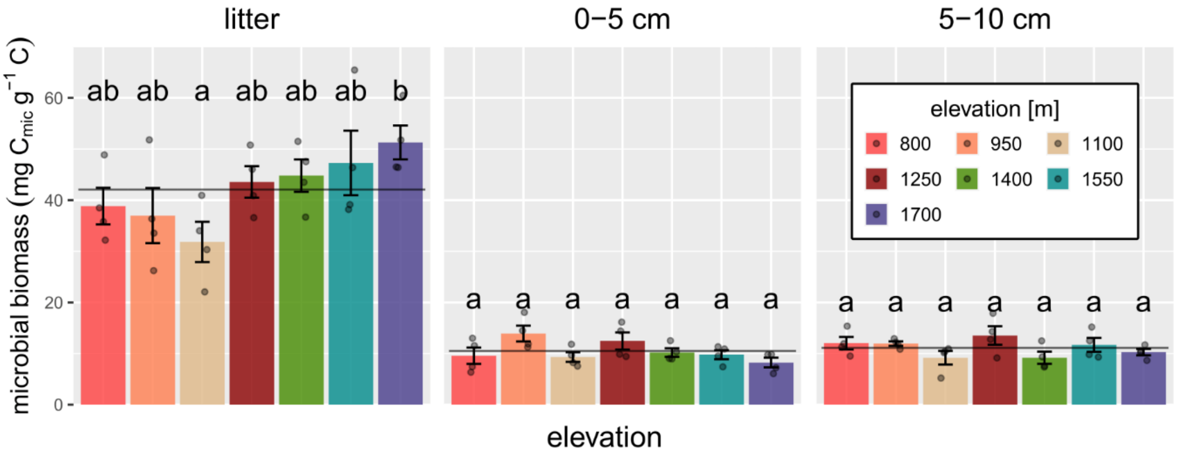

Figure 1: Changes in microbial biomass with elevation in litter, 0-5 and 5-10 cm soil depth. The solid line represents the mean across elevations, the dots represent data points, error bars represent the standard error of the mean and letters mark significant differences between means (Tukey's HSD test at $P < 0.05$). For results of linear models see text.

Correlation analysis between C_{mic} and qO_2 and environmental factors showed that in litter Ca concentration correlated negatively with C_{mic} and qO_2 ($\rho = -0.60$, $P < 0.001$ and $\rho = -0.54$, $P = 0.003$, respectively), while C/N ratio correlated positively with C_{mic} and qO_2 ($\rho = 0.38$, $P = 0.047$ and $\rho = 0.50$, $P = 0.006$, respectively). Also, pH correlated negatively with C_{mic} in the litter layer ($\rho = -0.61$, $P < 0.001$), reflecting that it was closely inter-correlated with Ca ($\rho = 0.63$, $P < 0.001$). Contrasting the litter, Ca concentrations and pH correlated positively with C_{mic} in 0-5 cm soil ($\rho = 0.38$, $P = 0.045$ and $\rho = 0.72$, $P < 0.001$, respectively). In

0-5 cm and 5-10 cm soil water content correlated negatively with C_{mic} (ρ = -0.39, P = 0.038 and ρ = -0.42, P = 0.026, respectively).

2.4.2 Microbial Community Composition across Elevations and Layers

PLFA profiles as proxy for microbial community composition varied significantly among layers ($F_{2,63}$ = 98.64, P = 0.001) as well as among elevations ($F_{6,63}$ = 5.05, P = 0.001), with both interacting significantly ($F_{12,63}$ = 2.21, P = 0.006). SIMPER analysis detected the fungal marker 18:2ω6,9 as the most important PLFA separating litter and the two soil layers (Appendix Table 1) decreasing from litter to 0-5 cm by 83 % and to 5-10 cm by 85 %. The Gram$^-$ bacterial marker 18:1ω7 was the PLFA accounting for most of the dissimilarity between 0-5 cm and 5-10 cm, but it decreased from 0-5 cm to 5-10 cm by only 3 % reflecting the overall similarity of the PLFA profiles of these two layers.

Due to the significant interaction between layer and elevation, we inspected the layers separately and displayed the PLFA profiles of individual layers (Fig. 2). In the litter layer PLFA profiles significantly changed with elevation ($F_{6,21}$ = 2.49, P = 0.007). The sites at 800, 950 and 1100 m separated from those at 1550 and 1700 m, while the PLFA profiles at 1250 and 1400 m largely overlapped with the other elevations (Fig. 2A). Although PLFA profiles in 0-5 cm ($F_{6,21}$ = 5.66, P < 0.001) and 5-10 cm ($F_{6,21}$ = 4.81, P < 0.001) also differed significantly with elevation, the differences were less pronounced compared to litter (Fig. 2B, C). However, as in litter, the sites at 800, 950 and 1100 m clustered close together, and this also applied to the sites at 1250, 1400, 1550 and 1700 m. In litter, the monounsaturated PLFAs 18:1ω7, 18:1ω9 and 16:1ω7 were more abundant at lower elevations, while the abundance of the saturated fatty acids 14:0, 16:0 and 17:0 peaked at 1550 and 1700 m (Fig. 2A). PLFA 16:0 accounted for most of the dissimilarity between 800 and 1700 m (4.35 %) and PLFA 18:1ω7 accounted for the second most dissimilarity (3.08 %; Appendix Table 2). In 0-5 cm depth, the PLFA pattern generally resembled that in litter, however, the PLFA accounting for most of the dissimilarity between 800 and 1700 m was 18:1ω7 (2.41 %; Appendix Table 2). In 5-10 cm depth, 800 m separated from the higher elevations, being most dissimilar to 1700 m (group-dissimilarity 10.56 %), with PLFA a15:0 accounting for most of the dissimilarity (2.34 %, Appendix Table 2). Branched-chain PLFAs a15:0, i16:0 and cy17:0 were associated with 800, 950 and 1100 m, while the unsaturated PLFAs 18:2ω6,9 and 18:1ω7 were most abundant at 1400, 1550 and 1700 m.

Table 2: F- and P-values for pH, Ca concentrations, C/N ratio, $\delta^{13}C$ values, water content, temperature seasonality and precipitation seasonality as predictors of PLFA patterns in litter, 0-5 and 5-10 cm soil as analysed by RDA and presented in Fig. 3. Environmental factors were chosen via P-value based forward selection per layer. All selected factors are displayed, "-" indicates that facors were not chosen for the respective layer. pH was included in all RDAs due to its importance for microbial community composition shown in a previous study at Changbai Mountain (Shen et al., 2013).

Predictor	Litter layer		0-5 cm		5-10 cm	
	F-value	P-value	F-value	P-value	F-value	P-value
pH	0.45	0.66	24.03	<0.001	0.64	0.468
Ca	-	-	3.56	0.027	-	-
C/N	-	-	50.38	<0.001	-	-
$\delta^{13}C$	-	-	6.44	0.003	-	-
Water content	-	-	-	-	8.21	0.007
Temperature seasonality	4.71	0.008	4.87	0.014	5.44	0.019
Precipitation seasonality	-	-	5.00	0.010	-	-

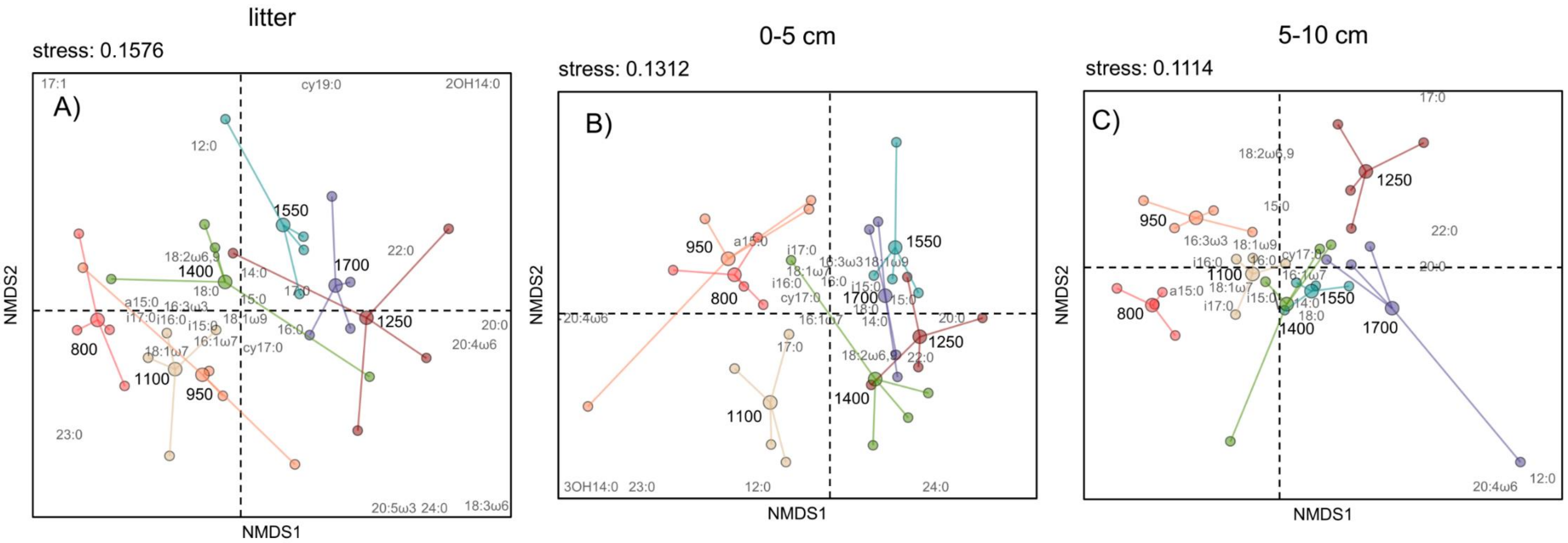

Figure 2: Biplots of two dimensional NMDS of phospholipid fatty acid (PLFA) profiles across elevations (● 800 m; ● 950 m; ● 1100 m; ● 1250 m; ● 1400 m; ● 1550 m; ● 1700 m) in (A) litter, (B) 0-5 cm soil and (C) 5-10 cm soil. Lines connect the elevation centroids to the subplots of the respective elevation.

As indicated by RDA, the environmental factors that correlated with certain PLFAs varied between layers (Table 2, Fig. 3) and this was also true for the variance in PLFA patterns explained by RDA axes 1 and 2 in litter, 0-5 and 5-10 cm depth (10.4 %, 69.2 % and 30.2 %, respectively). Temperature seasonality was the only significant environmental variable in the litter layer and was highest at 800 and 950 m. In the litter layer, the monounsaturated PLFAs 16:1ω7, 18:1ω7 and 18:1ω9 increased with temperature seasonality (Fig. 3A). In 0-5 cm depth, C/N ratio, pH, Ca concentrations, $\delta^{13}C$, precipitation seasonality and temperature seasonality significantly explained the PLFA distribution (Table 2, Fig. 3B). PLFA 16:0 increased with C/N ratio and reached a maximum at intermediate elevations (1250 and 1400 m). Soil pH and Ca concentrations increased towards lower elevations parallel to PLFA cy17:0. Temperature seasonality and precipitation seasonality increased parallel to PLFA a15:0 reaching a maximum at 800 m. $\delta^{13}C$ reached a maximum at 1550 and 1700 m. In 5-10 cm depth, soil water content and temperature seasonality significantly explained the variation in the PLFA distribution (Table 2, Fig. 3C). Temperature seasonality increased with the PLFAs cy17:0 and i15:0 and the soil water content increased with the $Gram^{+}$ marker PLFAs i16:0 and i17:0, and PLFA a15:0 being highest at 1250, 950 and 800 m, respectively.

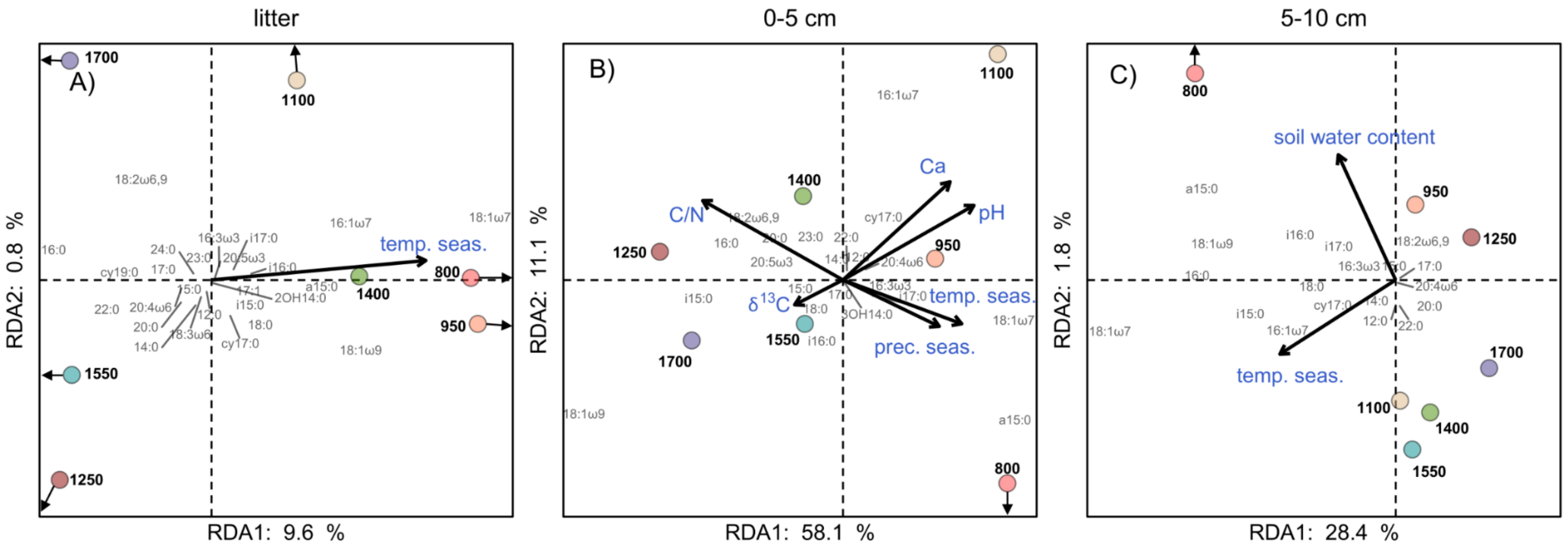

Figure 3: RDA biplots on the relationship between phospholipid fatty acids and significant environmental factors (pH, temperature seasonality, precipitation seasonality, $\delta^{13}C$ of the respective layer and soil water content of the respective layer; identified by P-value based forward selection) in (A) litter, (B) 0-5 cm and (C) 5-10 cm soil depth. Centroids of elevations are represented by colored dots (● 800 m; ● 950 m; ● 1100 m; ● 1250 m; ● 1400 m; ● 1550 m; ● 1700 m). The variation explained by the RDA axes is given as percentages of total. Elevations marked with arrows are positioned beyond the plotted borders of the RDA axes; arrows point in the direction of their position. All coordinates were species-scaled.

2.4.3 Indicators of Community Changes and Nutritional Stress

Of the four common PLFA indicator ratios, the fun/bac PLFA ratio significantly decreased from litter (overall mean 0.8 ± 0.18) to 0-5 and 5-10 cm depth by 89.2 % and 91.4 %, respectively ($\chi^2 = 278.58$, $P < 0.001$; Fig. 4A); it did not vary significantly with elevation. The cyclo/pre ratio, as a measure of physiological stress, significantly decreased from litter (overall mean 0.15 ± 0.01) to 0-5 and 5-10 cm by 14.9 % and 5.7 %, respectively ($\chi^2 = 10.25$, $P = 0.006$; Fig. 4B), however, the decline depended on elevation (significant elevation × layer interaction; $\chi^2 = 40.96$, $P < 0.001$). As indicated by separately analysing the three layers, the cyclo/pre ratio only varied significantly with elevation in litter ($F_{6,21} = 5.86$, $P = 0.001$), where it was high at elevations ≥1250 m and low at elevations ≤1100 m. Variations in the sat/mono ratio, a measure for substrate-induced stress, also depended on both soil layer and elevation (significant elevation × layer interaction; $\chi^2 = 25.92$, $P = 0.011$), and followed a very similar pattern to the cyclo/pre ratio (Fig. 4C). In contrast to the cyclo/pre ratio, however, the separate analysis of the three layers indicated that the sat/mono ratio changed significantly with elevation in each of the layers being high at elevations ≥1250 m and low at elevations ≤1100 m in litter ($F_{6,21} = 3.45$, $P = 0.016$), and at a maximum at 1250 m in 0-5 ($F_{6,21} = 2.67$, $P = 0.044$) and 5-10 cm depth ($F_{6,21} = 2.93$, $P = 0.031$). In contrast to the other three ratios, the Gram$^+$/Gram$^-$ ratio generally increased from litter to 0-5 and 5-10 cm depth by 23.7 % and 63.4 %, respectively ($\chi^2 = 73.83$, $P < 0.001$; Fig. 4D), however, again the effect of layer depended on elevation (significant elevation × layer interaction; $\chi^2 = 25.92$, $P = 0.011$). As indicated by separately analysing the three layers, changes with elevation where only significant in litter ($F_{6,21} = 4.23$, $P = 0.006$) and 5-10 cm depth ($F_{6,21} = 10.09$, $P < 0.001$), but in trend also in 0-5 cm depth ($F_{6,21} = 2.47$, $P < 0.058$). In litter the Gram$^+$/Gram$^-$ ratio was highest at 1550 m and lowest at 950 and 1250 m, whereas in 0-5 cm depth it was similarly high at 800, 1250 and 1700 m, and lowest at 1100 m, and in 5-10 cm depth it was highest at 800 m and again lowest at 1100 m. Subsequent correlation analysis indicated that in litter Ca concentrations correlated strongly negatively with the sat/mono and cyclo/pre ratios ($\rho = -0.70$, $P < 0.001$ and $\rho = -0.72$, $P < 0.001$, respectively). As being closely inter-correlated, pH also correlated positively with the sat/mono and cyclo/pre ratios in litter ($\rho = 0.49$, $P = 0.008$ and $\rho = -0.56$, $P = 0.002$, respectively). Further, the C/N ratio correlated positively with the cyclo/pre ratio in litter ($\rho = 0.49$, $P = 0.008$), but negatively with the Gram$^+$/Gram$^-$ ratio ($\rho = -0.56$, $P = 0.002$). As in litter, Ca concentrations in 0-5 cm soil depth correlated negatively with the sat/mono and cyclo/pre ratios ($\rho = -0.50$, $P = 0.007$ and $\rho = -0.54$, $P = 0.003$, respectively), and for the sat/mono ratio this was also true for pH ($\rho = -0.44$, $P = 0.021$). Further, the C/N ratio correlated positively with the sat/mono and cyclo/pre ratios ($\rho = 0.68$, $P = < 0.001$ and $\rho = 0.45$, $P = 0.015$, respectively), while pH correlated negatively with the Gram$^+$/Gram$^-$ ratio ($\rho = -0.57$, $P = 0.002$). In 5-10 cm soil depth, only Ca content correlated negatively with the cyclo/pre ratio ($\rho = -0.39$, $P = 0.042$).

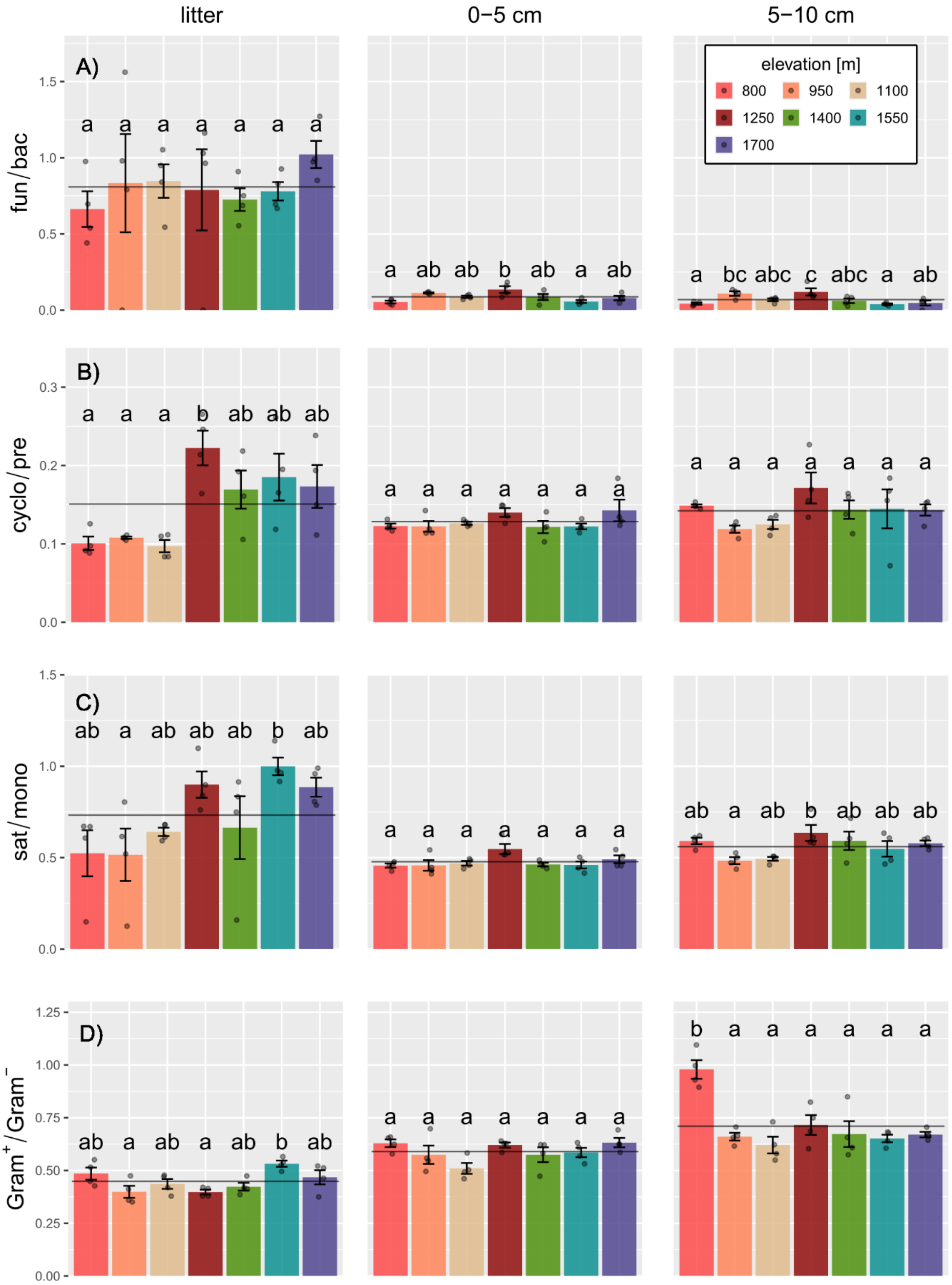

Figure 4: Changes in the (A) fungal/bacterial (fun/bac), (B) cyclopropyl/monoenoic (cyclo/pre), (C) saturated/monounsaturated (sat/mono) and (D) Gram$^+$/Gram$^-$ marker PLFA ratios (Gram$^+$/Gram$^-$) in litter, 0-5 and 5-10 cm soil depth with elevation. The solid line represents the overall mean, the dots the data points. Error bars represent the standard error of the mean and letters mark significant differences between means (Tukey's HSD test at $P < 0.05$). For results of linear models see text.

2.5 Discussion

Using a combination of respiration-based parameters and PLFA patterns, we identified variations in microbial community composition and functioning in litter and soil of natural forests across an elevational gradient at Changbai Mountain. The results showed strong variations in microbial communities between litter and soil along the studied elevational gradient. Further, the results indicate that the factors responsible for the changes in the structure and functioning of microbial communities also differed between layers. Only temperature seasonality affected the PLFA patterns in a uniform way across soil layers.

Supporting our first hypothesis, C_{mic} strongly decreased from litter to 0-5 and 5-10 cm soil depth, presumably reflecting the decrease in resource availability from litter to deeper soil layers. However, qO_2 did not differ significantly between soil layers suggesting that the efficiency in the use of carbon resources by microorganisms is similar across soil layers (Cao et al., 2019). Interestingly, C_{mic} responded differently to the elevational gradient in litter and soil. In litter C_{mic} increased with increasing elevation above 1100 m and correlated negatively with the concentration of Ca along the elvational gradient. Ca is involved in a number of bacterial processes, one of the most important being the recovery of nitrogen from urea via urease-reaction (Castanier et al., 1999; Krajewska, 2018). The role of Ca and the contribution of microorganisms to the cycling of nitrogen has been investigated in detail in arable soils (Klose & Tabatabai, 2000; Bowles et al., 2014), while its role in forest soils remains little studied. Klose and Tabatabai (1999) found urease activity to be mainly of microbial origin in a variety of soils, underlining the potential influence of Ca on the mobilisation of nitrogen and microbial nitrogen nutrition.

The increases in C_{mic} and in part of qO_2 towards higher elevations, and its (strongly) negative correlation with Ca (and pH) and (moderately) positive correlation with litter C/N ratio may reflect that nutritional shortage is more pronounced in communities of high C_{mic} and microbial activity. In fact, microbial activity can increase with stronger nitrogen limitation and decrease with the addition of nitrogen (Craine et al., 2007; Averill & Waring, 2018) following the “microbial nitrogen mining” hypothesis (Moorhead & Sinsabaugh, 2006). Wild et al. (2017) showed that a short-term input of carbon increases microbial growth as well as the microbial demand for nitrogen, but does not influence nitrogen mining. Therefore, high carbon availability at high elevations may also explain the positive correlation between PLFA stress indicators and litter C/N ratio due to increased nitrogen demand as well as increased C_{mic}. In addition, high Ca concentrations at lower elevations may facilitate microbial nitrogen acquisition and therefore result in lower microbial stress. Overall, our first hypothesis was only supported in part; in the litter layer C_{mic} responded as hypothesised, even though not linear, with the main drivers being variations in the availability of carbon and nitrogen but also Ca along the elevational gradient, while C_{mic} in the two soil layers was rather constant across elevations.

Supporting our second hypothesis PLFA profiles clearly separated the three layers, and this was mainly due to the decrease in fungal PLFA markers from the litter to the two soil layers as well as the decrease in Gram$^-$ bacterial markers from 0-5 to 5-10 cm. This is in line with results of the study of Šnajdr et al. (2008) who documented a rapid decrease in fungal biomass from the litter to the fermentation layer in forests. Fungi are known to be the major decomposers of recalcitrant carbon compounds and typically dominate in the litter layer, while bacteria play a larger role in the decomposition of root exudates thereby dominating in soil (de Boer et al., 2005). Further, the Gram$^+$/Gram$^-$ ratio increased with soil depth, since Gram$^-$ bacteria heavily depend on plant-derived carbon, such as litter, while Gram$^+$ bacteria preferentially use soil organic matter-derived carbon (Kramer & Gleixner, 2008).

PLFA profiles in the litter layer also varied with elevation, and temperature seasonality was the only environmental variable studied significantly affecting them, which is in line with our third hypothesis, even though we expected more climatic variables to influence PLFA profiles in litter. Temperature seasonality represents the variation in temperature during the year and litter is more heavily exposed to such fluctuations in temperature than deeper soil layers. Generally, increasing temperature accelerates the decomposition of litter (Kirschbaum, 1995) resulting in more shallow organic layers (Raich et al., 2006). Associated with higher temperature, decomposition rates of forest litter typically increase towards lower elevations (Salinas et al., 2011). However, in addition to the increase in temperature at lower elevations, temperature variation within the year also increases at lower elevations, and the vegetative period starts earlier and lasts longer compared to higher elevations. In spring decomposition rates of litter strongly increase (Kreyling et al., 2013), but at higher elevations this is less pronounced resulting in litter accumulation and reduced nutrient mobilisation. At Changbai Mountain coniferous stands of spruce and fir dominate at elevations between 1100 and 1700 m (Tang et al., 2011; Yu et al., 2013). Coniferous needles contain high amounts of lignin and polyphenols (Gallet & Lebreton, 1995; Taylor et al., 1989; Parkinson & Parsons, 1989), thereby typically decomposing more slowly than deciduous litter (Prescott, 2010).

Although litter decomposition is hampered during winter it does not stop and may benefit from snow cover preventing or reducing freezing of litter and soil (Schimel et al., 2004, 2007; Kreyling et al., 2013). Uchida et al. (2005) reported that 26% of the annual mass loss of litter to occur under snow. Notably, interception of snow by trees is higher and therefore snow cover is sparser in evergreen coniferous compared to deciduous forests (Vikhamar & Solberg, 2003; Noguchi & Nishizono, 2010). This reduced snow cover, which is related to low temperature seasonality, together with low quality needle litter may explain the accumulation of litter at high elevations, while the opposite may be true at low elevations, with these differences likely affecting microbial biomass and community structure. Neither pH nor Ca significantly explained variations in PLFA profiles in litter, but the negative correlations of PLFA stress ratios with litter pH and concentrations of Ca indicate that microbial communities and their functioning is in fact affected by the availability of base cations.

Changes in microbial parameters in 0-5 and 5-10 cm soil depth were similar and differed strongly from those in litter. Of the studied environmental factors, only temperature seasonality structured microbial community composition across layers. In 0-5 cm depth the C/N ratio of the soil explained a large fraction of the variations in PLFA profiles supporting our third hypothesis. This is in line with the findings of Liu et al. (2019) that carbon and nitrogen concentrations strongly affect PLFA patterns at Changbai Mountain, however, compared to the current study, they only investigated soil layers of a smaller elevational gradient comprising only pine forests. Notably, the C/N ratio in 0-5 cm depth was much lower than in the litter layer indicating increased microbial access to nitrogen. Across the elevational gradient the C/N ratio in 0-5 cm depth was highest at 1250 m and this was associated with an increase in PLFA 15:0 and an increased sat/mono ratio pointing to nutritional stress at this nitrogen poor site. Additionally, C_{mic} was high at 1250 m and, as in the litter layer, this may have aggravated nitrogen limitation (Dubinkina et al., 2019). Another soil related factor that correlated with microbial community structure in 0-5 cm depth were $\delta^{13}C$ values of soil organic matter. $\delta^{13}C$ increased towards higher elevations indicating an increasing state of decomposition of organic matter (Melillo et al. 1989; Potapov et al. 2019), related to high microbial biomass and activity in litter. Other soil factors driving the PLFA composition in 0-5 cm depth were Ca concentrations and pH, which increased towards lower elevations, indicating again that the effect of pH on microbial community composition at our study sites is not linked to physiological stress by acidity but the abundance of base cations. Variations in Ca concentrations rather than pH itself may be responsible for the widely reported correlation between pH and the structure of microbial communities (Högberg et al., 2007; Männistö et al., 2007; Zhou et al., 2017).

Besides these local soil related factors, temperature seasonality explained a large fraction of the variation in PLFA profiles in 0-5 and 5-10 cm depth, and in 0-5 cm also precipitation seasonality, contrasting the litter layer. Changbai Mountain has a rather constant warm climate during the relatively short vegetative period followed by harsh winters with mean monthly temperatures below -20°C in January (Yu et al., 2013). Temperature and precipitation seasonality increase towards lower elevations, reflecting longer and warmer summers as well as more pronounced seasonality at lower elevations. In particular marker PLFAs for $Gram^+$ bacteria increased towards lower elevations, especially in 5-10 cm depth, where the $Gram^+/Gram^-$ ratio was highest at 800 m. Due to their strong and interlinked peptidoglycan cell walls, $Gram^+$ bacteria are more resistant to temperature and moisture changes than $Gram^-$ bacteria (Schimel et al. 2007). Interestingly, the $Gram^+/Gram^-$ decreased from 800 to 1100 m and this was most pronounced in 5-10 cm depth. $Gram^-$ bacteria depend more heavily on labile carbon resources, while $Gram^+$ bacteria can access more recalcitrant carbon compounds (Kramer & Gleixner, 2008; Fanin et al., 2019). High microbial activity and biomass due to high temperatures during the vegetative period may hamper the leaching of labile carbon compounds into the soil, which is supported by the strong increase in the Gram$^+$/Gram$^-$ ratio from litter to 0-5 and 5-10 cm soil depth. Notably, we took our samples in September before the deciduous trees shed their leaves and the litter layer comprised predominantly leaf litter material of the previous year depleted in labile compounds, which may have contributed to low availability of labile carbon compounds in soil and therefore to the increase in Gram$^+$ bacteria in soil at 800 m.

The identified effects of temperature seasonality on the structure and functioning of microbial community in each of the layers is of special relevance for the response of decomposer systems and decomposition processes to global warming, which is expected to be associated with increased seasonal temperature fluctuations (Tian et al., 2015). Transplantation experiments along elevational gradients showed decomposition to increase in litter translocated to lower elevations (Salinas et al., 2011), where temperature and temperature seasonality are higher. Therefore, climate change may affect in particular the functioning of microbial communities at high elevations with potential detrimental consequences for carbon sequestration.

Contrasting our fourth hypothesis both stress indicator ratios were highest in the litter layer, but their response also depended on elevation. The moderate positive correlation of the litter C/N ratio with the cyclo/pre ratio in the litter indicates an increase in nutritional stress under high C/N ratios especially at 1250 m and 1400 m. In litter, temperature seasonality correlated positively with PLFAs 18:1ω7 and 16:1ω7 indicating lower microbial stress at lower elevations with higher temperature seasonality, which is further supported by the increase in the sat/mono and cyclo/pre ratio. Besides being an indicator of nutritional stress, the cyclo/pre ratio increased in *E. coli* with the acidity of the environment (Knivett & Cullen, 1965; Moore-Kucera & Dick, 2008). Conform to these findings, at our study sites the cyclo/pre ratio in the litter layer was negatively correlated with pH and Ca concentrations. Both pH and Ca concentrations changed with the transition from pine to spruce forests at 1100 m, potentially explaining the changes in stress indicator ratios likely due to reduced Ca concentrations as well as increased litter C/N ratio.

2.5.1 Conclusion

Our study aimed at uncovering variations in microbial community composition and functioning along a natural elevational gradient of forests and identifying the factors responsible for these variations. We identified temperature and precipitation seasonality as major climatic factors driving microbial communities in litter and soil, which is likely due to the pronounced difference between harsh winters and constant warm and wet summers at Changbai Mountain. Besides climatic factors, the availability of resources played a critical role in structuring microbial communities in litter and upper soil, as indicated by $\delta^{13}C$ values reflecting the stage of organic matter decomposition as well as C/N ratio reflecting the availability of nitrogen. The effect of Ca concentrations and pH on the microbial community in upper soil might be linked to nitrogen acquisition via urease reaction. This, however, needs to be proven in future studies measuring both urease activity and Ca concentrations. Pronounced changes in microbial biomass and stress indicator ratios in the litter layer between 1100 and 1250 m indicate a prominent shift in the structure and functioning of microbial communities between pine-dominated and spruce-dominated forests. Montane forests are increasingly threatened due to global warming and increased infections by herbivore pest species and therefore, there is the need to better understand their functioning and regulatory forces including feedbacks between the below- and aboveground system. The present study forms a starting point for such studies. Future studies need to include other soil food web components including soil invertebrates and their relationship with tree species and forest types.

2.6 Acknowledgments

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2.7 References

Albrich K, Rammer W, Seidl R (2020) Climate change causes critical transitions and irreversible alterations of mountain forests. Glob Change Biol 26:4013–4027. https://doi.org/10.1111/gcb.15118

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 10:215–221. https://doi.org/10.1016/0038-0717(78)90099-8

Anderson TH, Domsch KH (1993) The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol Biochem 25:393–395. https://doi.org/10.1016/0038-0717(93)90140-7

Averill C, Waring B (2018) Nitrogen limitation of decomposition and decay: How can it occur? Glob Change Biol 24:1417–1427. https://doi.org/10.1111/gcb.13980

Bani A, Pioli S, Ventura M, et al (2018) The role of microbial community in the decomposition of leaf litter and deadwood. Appl Soil Ecol 126:75–84. https://doi.org/10.1016/j.apsoil.2018.02.017

Bates D, Maechler M, Bolker B, et al (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Beck T, Joergensen RG, Kandeler E, et al (1997) An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biol Biochem 29:1023–1032. https://doi.org/10.1016/S0038-0717(97)00030-8

Bossio DA, Scow KM (1997) Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microb Ecol 35:265–278. https://doi.org/10.1007/s002489900082

Bowles TM, Acosta-Martínez V, Calderón F, Jackson LE (2014) Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biol Biochem 68:252–262. https://doi.org/10.1016/j.soilbio.2013.10.004

Buyer JS, Sasser M (2012) High throughput phospholipid fatty acid analysis of soils. Appl Soil Ecol 61:127–130. https://doi.org/10.1016/j.apsoil.2012.06.005

Cao Y, Xu L, Zhang Z, et al (2019) Soil microbial metabolic quotient in Inner Mongolian grasslands: patterns and influence factors. Chin Geogr Sci 29:1001–1010. https://doi.org/10.1007/s11769-019-1084-5

Castanier S, le Métayer-Levrel G, Perthuisot JP (1999) Ca-carbonates precipitation and limestone genesis - the microbiogeologist point of view. Sediment Geol 126:9–23. https://doi.org/10.1016/S0037-0738(99)00028-7

Chang EH, Chen TH, Tian G, Chiu CY (2016) The effect of altitudinal gradient on soil microbial community activity and structure in moso bamboo plantations. Appl Soil Ecol 98:213–220. https://doi.org/10.1016/j.apsoil.2015.10.018

Chen L, Wu S, Pan T (2011) Variability of climate-growth relationships along an elevation gradient in the Changbai Mountain, Northeastern China. Trees - Struct Funct 25:1133–1139. https://doi.org/10.1007/s00468-011-0588-0

Craine JM, Morrow C, Fierer N, et al (2007) Microbial nitrogen limitation increases decomposition. Ecology 88:2105–2113. https://doi.org/10.1890/06-1847.1

de Boer W, Folman L, Summerbell RC, Boddy L (2005) Living in a fungal world: Impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 29:795–811. https://doi.org/10.1016/j.femsre.2004.11.005

Dubinkina V, Fridman Y, Pandey PP, Maslov S (2019) Multistability and regime shifts in microbial communities explained by competition for essential nutrients. eLIFE 8: e49720. https://doi.org/10.7554/eLife.49720

Erhardt S, Hochreuther P, Schütz M, Berger M (2022) OpenTopoMap - Topographische Karten aus Openstreetmap. https://opentopomap.org

Fanin N, Kardol P, Farrell M, et al (2019) The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. Soil Biol Biochem 128:111–114. https://doi.org/10.1016/j.soilbio.2018.10.010

Fick SE, Hijmans RJ (2017) WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. Int J Climatol 37:4302–4315. https://doi.org/10.1002/joc.5086

Frostegård Å, Tunlid A, Bååth E (2011) Use and misuse of PLFA measurements in soils. Soil Biol Biochem 43:1621–1625. https://doi.org/10.1016/j.soilbio.2010.11.021

Gallet C, Lebreton P (1995) Evolution of phenolic patterns and associated litters and humus of a mountain forest ecosystem. Soil Biol Biochem 27:157–165. https://doi.org/10.1016/0038-0717(94)00167-Y

Guckert JB, Hood MA, White DC (1986) Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: Increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. Appl Environ Microbiol 52:794–801. https://doi.org/10.1128/aem.52.4.794-801.1986

Hijmans RJ (2021) Raster: Geographic data analysis and modeling. R package version 3.5-21

Hobara S, Osono T, Hirose D, et al (2014) The roles of microorganisms in litter decomposition and soil formation. Biogeochemistry 118:471–486. https://doi.org/10.1007/s10533-013-9912-7

Hobley EU, Wilson B (2016) The depth distribution of organic carbon in the soils of eastern Australia. Ecosphere 7:e01214. https://doi.org/10.1002/ecs2.1214

Högberg MN, Högberg P, Myrold DD (2007) Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150:590–601. https://doi.org/10.1007/s00442-006-0562-5

Joergensen RG (2022) Phospholipid fatty acids in soil—drawbacks and future prospects. Biol Fertil Soils 58:1–6. https://doi.org/10.1007/s00374-021-01613-w

Kirschbaum MUF (1995) The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biol Biochem 27:753–760. https://doi.org/10.1016/0038-0717(94)00242-S

Klimek B, Jaźwa M, Choczyński M, et al (2020) The drivers of soil microbial communities structure on forest stands along the altitudinal gradient in western Carpathians. Acta Oecologica 108:103643. https://doi.org/10.1016/j.actao.2020.103643

Klose S, Tabatabai MA (1999) Urease activity of microbial biomass in soils. Soil Biol Biochem 31:205–211. https://doi.org/10.1016/S0038-0717(98)00090-X

Klose S, Tabatabai MA (2000) Urease activity of microbial biomass in soils as affected by cropping systems. Biol Fertil Soils 31:191–199. https://doi.org/10.1007/s003740050645

Knivett VA, Cullen J (1965) Some factors affecting cyclopropane acid formation in *Escherichia coli*. Biochem J 96:771–776. https://doi.org/10.1042/bj0960771

Krajewska B (2018) Urease-aided calcium carbonate mineralization for engineering applications: A review. J Adv Res 13:59–67. https://doi.org/10.1016/j.jare.2017.10.009

Kramer C, Gleixner G (2008) Soil organic matter in soil depth profiles: Distinct carbon preferences of microbial groups during carbon transformation. Soil Biol Biochem 40:425–433. https://doi.org/10.1016/j.soilbio.2007.09.016

Kreyling J, Haei M, Laudon H (2013) Snow removal reduces annual cellulose decomposition in a riparian boreal forest. Can J Soil Sci 93:427–433. https://doi.org/10.4141/CJSS2012-025

Lenth RV (2022) Emmeans: Estimated marginal means, aka least-squares means. R package version 1.7-5

Liu M, Sui X, Hu Y, Feng F (2019) Microbial community structure and the relationship with soil carbon and nitrogen in an original Korean pine forest of Changbai Mountain, China. BMC Microbiol 19:218. https://doi.org/10.1186/s12866-019-1584-6

Liu QJ (1997) Structure and dynamics of the subalpine coniferous forest on Changbai Mountain, China. Plant Ecol 132:97–105. https://doi.org/10.1023/A:1009705319128

Looby CI, Martin PH (2020) Diversity and function of soil microbes on montane gradients: The state of knowledge in a changing world. FEMS Microbiol Ecol 96:fiaa122. https://doi.org/10.1093/femsec/fiaa122

Männistö MK, Tiirola M, Häggblom MM (2007) Bacterial communities in arctic fjelds of Finnish Lapland are stable but highly pH-dependent. FEMS Microbiol Ecol 59:452–465. https://doi.org/10.1111/j.1574-6941.2006.00232.x

Massaccesi L, De Feudis M, Leccese A, Agnelli A (2020) Altitude and vegetation affect soil organic carbon, basal respiration and microbial biomass in Apennine forest soils. Forests 11:710. https://doi.org/10.3390/f11060710

Melillo JM, Aber JD, Linkins AE, et al (1989) Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. Plant Soil 115:189–198. https://doi.org/10.1007/BF02202587

Moore-Kucera J, Dick RP (2008) PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microb Ecol 55:500–511. https://doi.org/10.1007/s00248-007-9295-1

Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction. Ecol Monogr 76:151–174. https://doi.org/10.1890/0012-9615(2006)076[0151:ATMOLD]2.0.CO;2

Noguchi S, Nishizono T (2010) The comparison of throughfall between evergreen coniferous and deciduous broad-leaved forests during the snow cover period. J Jpn For Soc 92:29–34. https://doi.org/10.4005/jjfs.92.29

Oksanen AJ, Kindt R, Legendre P, et al (2020) vegan: Community ecology package. R package version 2.5-7

Pollierer MM, Ferlian O, Scheu S (2015) Temporal dynamics and variation with forest type of phospholipid fatty acids in litter and soil of temperate forests across regions. Soil Biol Biochem 91:248–257. https://doi.org/10.1016/j.soilbio.2015.08.035

Potapov AM, Tiunov AV, Scheu S (2019) Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biol Rev 94:37–59. https://doi.org/10.1111/brv.12434

Prescott CE (2010) Litter decomposition: What controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101:133–149. https://doi.org/10.1007/s10533-010-9439-0

Price MF, Gratzer G, Duguma LA, et al (2015) Mountain forests in a changing world - realizing values, addressing challenges. https://doi.org/10.13140/2.1.2386.5283

R Core Team (2021) R: A language and environment for statistical computing

Raich JW, Russell AE, Kitayama K, et al (2006) Temperature influences carbon accumulation in moist tropical forests. Ecology 87:76–87. https://doi.org/10.1890/05-0023

Ratledge C, Wilkinson SG (1988) Microbial lipids Vol. 1. Academic Press, London

Russel NJ (2008) Membrane components and cold sensing. In: Psychrophiles - from biodiversity to biotechnology, Springer Berlin, Heidelberg, Germany

Salinas N, Malhi Y, Meir P, et al (2011) The sensitivity of tropical leaf litter decomposition to temperature: Results from a large-scale leaf translocation experiment along an elevation gradient in Peruvian forests. New Phytol 189:967–977. https://doi.org/10.1111/j.1469-8137.2010.03521.x

Scheu S (1992) Automated measurement of the respiratory response of soil microcompartments: Active microbial biomass in earthworm faeces. Soil Biol Biochem 24:1113–1118. https://doi.org/10.1016/0038-0717(92)90061-2

Schimel JP, Bilbrough C, Welker JM (2004) Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. Soil Biol Biochem 36:217–227. https://doi.org/10.1016/j.soilbio.2003.09.008

Schimel JP, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386–1394. https://doi.org/10.1890/06-0219

Shen C, Xiong J, Zhang H, et al (2013) Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. Soil Biol Biochem 57:204–211. https://doi.org/10.1016/j.soilbio.2012.07.013

Šnajdr J, Valášková V, Merhautová V, et al (2008) Activity and spatial distribution of lignocellulose-degrading enzymes during forest soil colonization by saprotrophic basidiomycetes. Enzyme Microb Technol 43:186–192. https://doi.org/10.1016/j.enzmictec.2007.11.008

Tang L, Li A, Shao G (2011) Landscape-level forest ecosystem conservation on Changbai Mountain, China and North Korea (DPRK). Mt Res Dev 31:169–175. https://doi.org/10.1659/MRD-JOURNAL-D-10-00120.1

Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as predictors of litter decay rates: A microcosm test. Ecology 70:97–104. https://doi.org/10.2307/1938416

Tian D, Guo Y, Dong W (2015) Future changes and uncertainties in temperature and precipitation over China based on CMIP5 models. Adv Atmospheric Sci 32:487–496. https://doi.org/10.1007/s00376-014-4102-7

Uchida M, Mo W, Nakatsubo T, et al (2005) Microbial activity and litter decomposition under snow cover in a cool-temperate broad-leaved deciduous forest. Agric For Meteorol 134:102–109. https://doi.org/10.1016/j.agrformet.2005.11.003

Vikhamar D, Solberg R (2003) Snow-cover mapping in forests by constrained linear spectral unmixing of MODIS data. Remote Sens Environ 88:309–323. https://doi.org/10.1016/j.rse.2003.06.004

Wild B, Alaei S, Bengtson P, et al (2017) Short-term carbon input increases microbial nitrogen demand, but not microbial nitrogen mining, in a set of boreal forest soils. Biogeochemistry 136:261–278. https://doi.org/10.1007/s10533-017-0391-0

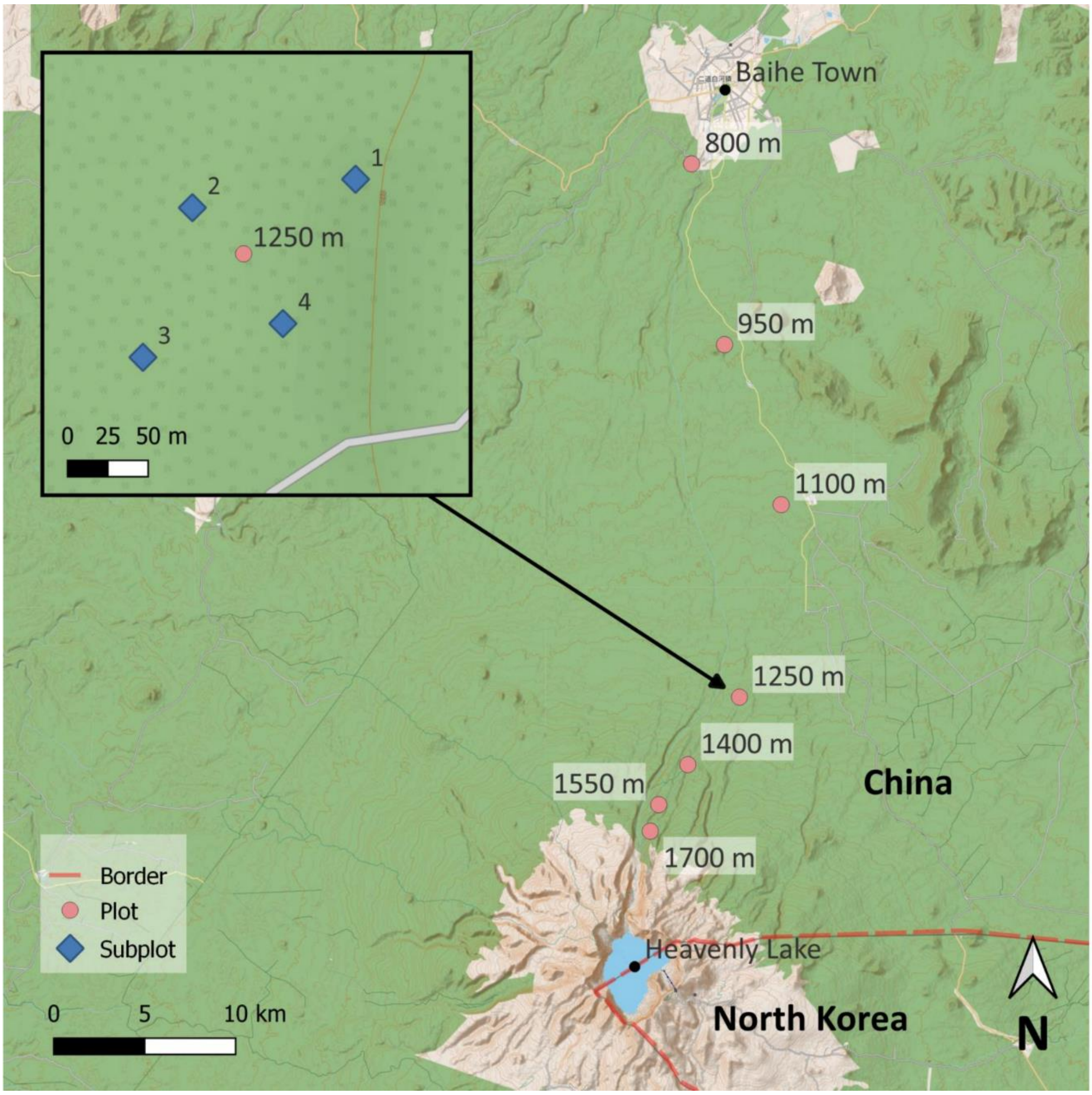
Xu M, Li X, Cai X, et al (2014) Soil microbial community structure and activity along a montane elevational gradient on the Tibetan Plateau. Eur J Soil Biol 64:6–14. https://doi.org/10.1016/j.ejsobi.2014.06.002

Yan B, Liang X, Xiao C (2018) Hydrogeochemical characteristics and genesis model of Jinjiang and Julong hot springs in Changbai Mountain, Northeast China. Geofluids 2018:1694567. https://doi.org/10.1155/2018/1694567

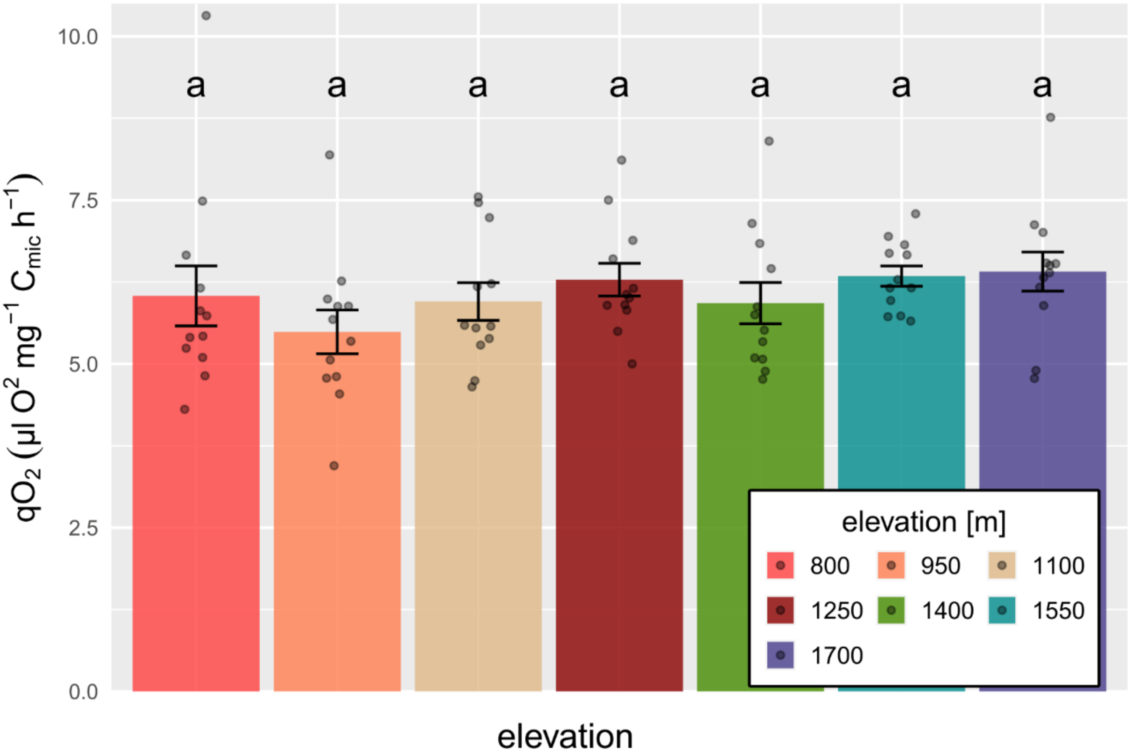
Yu D, Liu J, Lewis BJ, et al (2013) Spatial variation and temporal instability in the climate – growth relationship of Korean pine in the Changbai Mountain region of Northeast China. For Ecol Manag 300:96–105. https://doi.org/10.1016/j.foreco.2012.06.032

Zhou X, Guo Z, Chen C, Jia Z (2017) Soil microbial community structure and diversity are largely influenced by soil pH and nutrient quality in 78-year-old tree plantations. 14:2101–2111. https://doi.org/10.5194/bg-14-2101-2017

2.8 Appendix



Appendix Figure 1: Map of the sampled transect at the northern slope of Changbai Mountain. The enlarged section displays the subplot structure for one of the elevations. Map data from "OpenTopoMap" (Erhardt et al. 2022).



Appendix Figure 2: Changes in microbial specific respiration (qO_2) with elevation. Error bars represent the standard error of the mean, the dots the real data points. Letters mark significant differences between means (Tukey's HSD test at $P < 0.05$). For results of linear mixed effect model see text.

Appendix Table 1: Pairwise group dissimilarities of PLFA profiles between soil layers. (Dis-)similarity percentages (SIMPER analysis) between groups representing the contribution of each PLFA to total dissimilarity (as percentages). The averages of PLFAs of contrasts are given in mole percent. The cumsum displays the additive contribution of PLFAs to the group dissimilarity as percentage of group-dissimilarity (up to 70%).

Contrast	PLFA	group dissimilarity [%]	dissimilarity [%]	mean a [mole percent]	mean b [mole percent]	cumsum [%]
0-5 vs 5-10	18:1ω7	9.55	1.61	23.68	22.90	16.89
	18:1ω9		1.43	12.95	10.47	31.84
	a15:0		1.13	6.59	8.41	43.69
	16:0		0.66	15.53	16.32	50.55
	16:1ω7		0.62	9.03	8.28	57.03
	i15:0		0.62	9.79	10.67	63.52
	18:2ω6,9		0.49	2.03	1.82	68.68
	18:00		0.44	3.65	4.35	73.30
0-5 vs litter	18:2ω6,9	24.52	5.10	2.03	11.94	20.79
	18:1ω7		4.25	23.68	15.20	38.13
	16:00		4.23	15.53	20.66	55.39
	i15:0		1.95	9.79	5.91	63.32
	18:1ω9		1.57	12.95	15.16	69.71
	a15:0		1.52	6.59	3.55	75.92
5-10 vs litter	18:2ω6,9	26.83	5.19	1.82	11.94	19.35
	16:00		3.93	16.32	20.66	33.98
	18:1ω7		3.88	22.90	15.20	48.45
	a15:0		2.43	8.41	3.55	57.49
	i15:0		2.38	10.67	5.91	66.36
	18:1ω9		2.35	10.47	15.16	75.13

Appendix Table 2: Pairwise group dissimilarities of PLFA profiles between 800 and 1700 m in litter, 0-5 and 5-10 cm soil depth. (Dis-)similarity percentages (SIMPER analysis) between groups as percentages. Dissimilarity represents the contribution in dissimilarity of each PLFA. The averages of PLFAs of 800 m and 1700 m are given in mole percent. The cumsum displays the additive contribution of PLFAs to the group dissimilarity as percentage of group-dissimilarity (up to 70%).

layer	PLFA	group dissimilarity [%]	dissimilarity [%]	Mean 800 m [mole percent]	Mean 1700 m [mole percent]	cumsum [%]
litter	16:0	19.41	4.35	14.87	23.57	22.43
	18:1ω7		3.08	18.83	12.68	38.28
	18:2ω6,9		2.09	11.32	13.98	49.08
	18:1ω9		1.48	15.49	13.77	56.71
	16:1ω7		1.33	10.63	7.97	63.56
	22:0		1.15	0.43	2.72	69.47
	a15:0		1.03	5.11	3.06	74.76
0-5 cm	18:1ω7	10.28	2.41	26.29	21.48	23.41
	a15:0		1.83	9.34	5.68	41.21
	18:1ω9		1.52	12.08	15.11	55.96
	i15:0		0.98	8.66	10.62	65.48
	16:1ω7		0.59	7.48	8.66	71.19
5-10 cm	a15:0	10.82	2.29	11.99	7.40	21.21
	18:1ω7		1.40	19.89	22.68	34.10
	18:1ω9		1.00	11.63	9.64	43.32
	12:0		0.87	0.00	1.73	51.32
	i16:0		0.72	4.47	3.03	57.98
	22:0		0.72	0.24	1.67	64.59
	16:1ω7		0.59	7.23	8.37	70.08

Chapter 3: Trophic Positions of Soil Microarthropods in Forests Increase with Elevation, but Energy Channels Remain Unchanged

The neanurid Collembola *Neanura* sp.

3.1 Abstract

Mountain Mountain forests are at risk as the consequences of climate change will likely lead to altered tree species boundaries. Characterizing food webs along elevation gradients in primary forests may help to predict the potential consequences of such changes, for example with regard to the decomposition of dead organic matter. Here, for the first time, we studied trophic variations in two species-rich microarthropod taxa, Collembola and Oribatida, along an elevation gradient of primary forest at Changbai Mountain, China. Samples were taken at seven elevations of 150 m elevational difference between 800 and 1700 m. At each elevation, Collembola and Oribatida were extracted from litter samples of eight subplots. We applied three state-of-the-art methods to elucidate trophic positions and basal resource use at community level: Bulk stable isotope analysis of nitrogen ($\Delta^{15}N_{bulk}$) and carbon ($\Delta^{13}C_{bulk}$), compound-specific stable isotope analysis of amino acids (CSIA-AA), and dietary routing of neutral lipid fatty acids (NLFAs). Trophic positions calculated using Δ^{15}Nbulk and CSIA-AA (TP_{CSIA}) in both taxa increased similarly with elevation by about half and one third of a trophic position, respectively. Stable isotope mixing models and linear discriminant analysis bootstrapping using $\delta^{13}C$ of essential amino acids indicated fungi as the most important resource at all elevations for both taxa. Also, proportions of marker NLFAs changed little across elevations in both taxa; overall high proportions of linoleic acid indicated high fungal contributions, but in Collembola the contribution of bacterial markers was generally higher than in Oribatida. $\Delta^{13}C_{bulk}$ did not respond linearly to the elevation gradient; however, changes in elevation differed between Collembola and Oribatida. A strong linear relationship between $\delta^{15}N$ of phenylalanine and $\delta^{15}N$ of litter indicated litter as the basis of energy channels in both taxa. Overall, food web functioning likely changes with changing forest types along elevation gradients, with microarthropods switching from feeding closer to the base of the food web at lower elevations to feeding at higher trophic levels at higher elevations, potentially compromising their role in litter decomposition and nutrient cycling.

3.2 Introduction

Climate change poses a threat to mountain forests; Dakhil et al. (2021) projected range declines of between 40% and 100% for eight Chinese montane conifer species under climate change by 2070. Climate change may therefore lead to irreversible shifts in tree species boundaries in mountain habitats, for example through transitions of coniferous to deciduous forests (Albrich et al., 2020). Such shifts in forest types are likely to fundamentally alter the composition and functioning of animal communities. Investigating elevation gradients representing such forest transition zones may help to uncover responses of forest-dwelling organisms to climate driven forest conversion.

While changes in community composition along elevation gradients have been investigated for a variety of taxa (Samson et al., 1997; Blake & Loiselle, 2000; Hodkinson, 2005; McCain, 2005; Bhardwaj et al., 2011), including litter and soil dwelling animals (Xie et al., 2022, Pan et al., 2023), data about trophic shifts of soil organisms with elevation are scarce (Fischer et al., 2014; Maraun et al., 2014). Soil arthropod food webs are compartmentalized based on traits such as body size, sclerotization or digestive abilities (Pollierer et al., 2009; Potapov et al., 2019a), and rely either directly on dead or living plants/roots or on microbial resources such as bacteria and fungi. The relative fluxes of energy and nutrients from these resources to consumers are assumed to differ between compartments and are influenced by abiotic conditions such as nutrient availability, moisture and temperature (Berg et al., 1998; Moore et al., 2005). The fungal energy channel often dominates in systems that are stable and rather nutrient-poor and is termed ‘slow-cycling’ due to the slower growth of fungi compared to bacteria, whereas the ‘fast-cycling’ bacterial energy channel is more pronounced in moist, disturbed and/or nutrient-rich habitats (Moore & Hunt, 1988; Moore, 1994; de Vries et al., 2006).

Trophic niches of soil organisms, and thereby the relative fluxes via different energy channels, are likely influenced by changing tree species composition with elevation, as litter chemistry varies considerably between tree species (Hättenschwiler et al., 2008). For instance, at Changbai Mountain, China, the transition between mixed broadleaf and coniferous towards pure coniferous tree species with increasing elevation is accompanied by decreasing calcium content and increasing C/N ratios of litter (Lux et al., 2022).

Although litter decomposition and recycling of nutrients is predominantly driven by microorganisms, microarthropods also contribute to these processes, albeit the strength and direction of this interaction remains disputed (Seastedt, 1984; Kampichler & Bruckner, 2009). Collembola and Oribatida are the most abundant microarthropods in forest soils (Seastedt, 1984) and both taxa occupy a wide range of trophic niches, but predominantly function as primary and secondary decomposers (Scheu & Falca, 2000; Chahartaghi et al., 2005; Schneider et al., 2004).

Fujii et al. (2018) showed that microarthropods contribute less to the decomposition of recalcitrant compared to nutrient-rich litter. However, microbial stress, which likely compromises the production of microbial defensive compounds, may counteract effects of low litter quality on microarthropod nutrition at higher elevations (Lux et al., 2022). Since fungi form the major food resource for both Collembola and Oribatida (Pollierer & Scheu, 2021, Li et al., 2022), and fungi are the main decomposer microorganisms of low-quality litter, the diet of microarthropods at higher elevations may shift towards more microbial food; however, shifts in trophic niches of microarthropods with elevation are little studied (Fischer et al., 2014; Maraun et al., 2014).

Bulk stable isotope analysis of nitrogen and carbon allows to investigate shifts in trophic niches of soil invertebrates along environmental gradients (Potapov et al., 2019a; Maraun et al., 2023). However, delineating trophic positions and food resources of soil invertebrates using stable isotope analysis of bulk animal tissue has limitations, especially when including primary and secondary decomposers, as bulk stable isotopes reflect both, trophic positions as well as isotopic enrichment in dead organic matter itself (Potapov et al., 2019b). These problems are overcome by using compound-specific stable isotope analysis of amino acids (CSIA-AA; Chikaraishi et al., 2007, 2010, 2014; Larsen et al., 2013, 2016; Ohkouchi et al., 2017; Pollierer et al., 2019) or biomarker neutral lipids in consumers (‘dietary routing’; Ruess & Chamberlain, 2010).

$^{15}N/^{14}N$ ratios of amino acids allow baseline independent estimates of the trophic position of consumers (TP_{CSIA}) by using source and trophic amino acids such as phenylalanine (Phe) and glutamic acid (Glu), respectively. Glu is enriched in ^{15}N with each trophic transfer, while $^{15}N/^{14}N$ ratios of Phe change little and thereby reflect the ratio in the basal resource (Chikaraishi et al., 2009). Further, TP_{CSIA} can be used to evaluate the reliability of estimates of trophic positions based on bulk $^{15}N/^{14}N$ ratios.

Stable isotope ratios of $^{13}C/^{12}C$ of amino acids, on the other hand, allow identifying major basal resources of food chains, such as plants, bacteria and fungi (Larsen et al., 2009, Pollierer et al., 2019), as these phylogenetically old groups feature some distinct or modified synthesis pathways for amino acids (Hagelstein et al., 1997; Velasco et al., 2002; Kohlhaw, 2003; Hudson et al., 2005). These distinct ^{13}C patterns can be traced in essential amino acids ($\delta^{13}C_{eAA}$) of consumers (‘stable isotope fingerprinting’; Larsen et al., 2009), and thereby allow to quantify energy fluxes via the major energy channels of soil food webs.

Neutral lipid fatty acid (NLFA) analysis complements CSIA-AA by allowing to trace the origin of lipids and consequently major carbon sources of consumers. Similar to amino acids, the fatty acid composition of consumers reflects basal resources due to characteristic pathways of de novo lipid synthesis in e.g., plants, fungi and bacteria. Certain fatty acids are only synthesized by e.g., bacteria and serve as absolute markers to trace their contribution to the diet of consumers. Other NLFAs are preferentially synthesized by e.g., fungi and plants and thereby serve as relative markers which occur in high concentrations in consumers of food chains based on these basal resources (Ruess & Chamberlain, 2010; Kühn et al. 2020). By providing complementary information on dietary fatty acid and protein uptake, the combination of NLFA analysis and CSIA-AA may allow more detailed insights into trophic niches and carbon and nutrient fluxes in soil food webs (Potapov, 2022).

Here we used bulk stable isotope, CSIA-AA and NLFA analysis to investigate changes in the trophic niches of and the channeling of energy through Collembola and Oribatida as major soil mesofauna taxa along an elevation gradient from 800 to 1700 m at Changbai Mountain, China. This gradient represents a continuous forest gradient from primary deciduous and coniferous mixed forests towards pure coniferous forests at higher elevation (Liu, 1997; Tang et al., 2011), which is accompanied by changes in local litter characteristics and microbial decomposers (Lux et al., 2022) likely leading to shifts in trophic niches of microarthropods. We hypothesized that (i) the trophic position of both Collembola and Oribatida increases with increasing elevation due to more extensive feeding on microorganisms (rather than litter at lower elevations), (ii) the trophic positions as measured by TP_{CSIA} and bulk $^{15}N/^{14}N$ ratios are closely correlated, and (iii) both groups feed predominantly on fungi and the contribution of fungi to their diet increases with elevation, which is reflected by $\delta^{13}C_{eAA}$ fingerprinting and dietary routing through NLFAs.

3.3 Material and Methods

3.3.1 Study Site and Sampling

We studied the northern slope of Changbai Mountain (42°8’25.4004”N, 128°7’36.2352”E), located at the borders of the Chinese provinces Jilin and Liaoning to North Korea (Fig. 1). With 2750 m asl Changbai Mountain is the highest mountain in the Changbaishan National Nature Reserve. The geological groups represented at the sampled transect comprise mainly stomatal and laminated basalt, alkali pumice, trachyte and tuff (Yan et al, 2018). The climate is characterized by long and cold winters, and warm and wet summers. From 1982 to 2017, the mean annual temperature ranged from 2 to 5°C and annual precipitation ranged from 600 to 1000 mm (Wang et al., 2020).

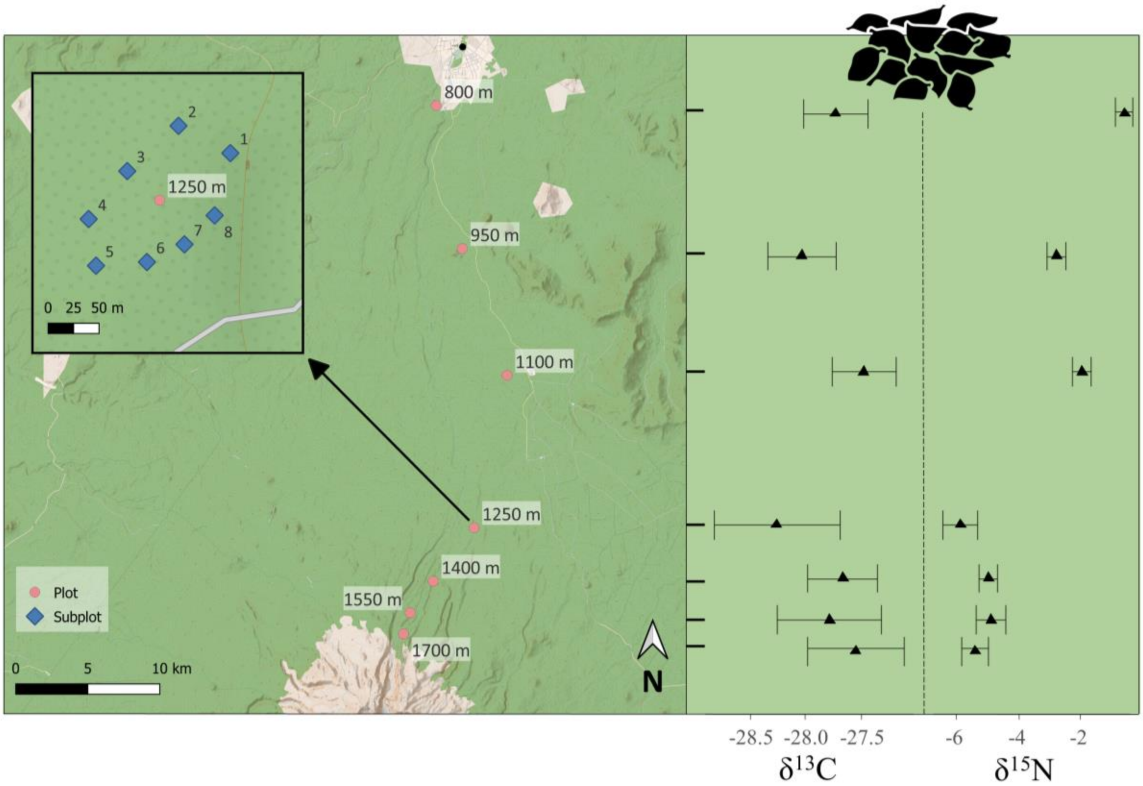


Figure 1: Map of the sampled transect at the northern slope of Changbai Mountain, China. The enlarged section displays the subplot structure for one of the elevations. The right panel displays the natural abundances of ^{13}C and ^{15}N of the litter for each elevation (means ± SD). Map data from “OpenTopoMap” (Erhardt et al., 2022).

The forests at the northern slope of Changbai Mountain are characterized by mixed forests between 800 and 1100 m dominated by Korean pine (*Pinus koraiensis* Siebold & Zucc.), pure coniferous spruce and fir forests dominated by dark-bark spruce (*Picea jezoensis* var. *komarovii* Siebold & Zucc.) between 1100 and 1700 m, followed by birch forests (*Betula ermanii* Cham.) at elevations above 1700 m. The forests have not been managed for decades or even centuries and therefore comprise mostly primary forest (Tang et al., 2011). We focused on forests between 800 and 1700 m asl, where we established seven plots of an elevational difference of 150 m with every plot subdivided into eight subplots (Fig. 1). The sampling took place in early September 2019. Three litter samples were taken at every subplot using a 10 x 10 cm frame, one for each type of analysis (bulk stable isotope, CSIA-AA, NLFA). The samples were transported to the laboratory in cooling boxes. Litter microarthropods were extracted into 75% ethanol at room temperature using Berlese funnels equipped with 2 mm mesh for five days. After extraction, animals were stored in 75% ethanol. For neutral lipid fatty acid (NLFA) analysis animals were transferred into 99.5% glycerol, following the recommendations of Zieger & Scheu (2018). All animals were stored at -26°C. Additional litter samples were taken to measure the stable isotope baseline as described in Lux et al. (2022). In short, three soil cores with a diameter of 5.5 cm were taken randomly at every second subplot at each elevation, leaf litter (L layer) from the soil cores was pooled, dried and milled, and aliquots were used for stable isotope measurements.

3.3.2 Bulk Stable Isotopes

Stable isotope ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ of Collembola, Oribatida and litter were measured using an isotopic mass spectrometer (Delta XP, Thermo Electron, Bremen, Germany) coupled via an interface (Conflo IV, Thermo Eclectron, Bremen, Germany) to an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific, Rodano, Milan, Italy). The abundance of ^{15}N and ^{13}C was expressed as δ values, calculated as $\delta\ (‰) = ((R_{sample} - R_{standard}) / R_{standard}) \times 1000$, with R_{sample} and $R_{standard}$ being the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio in the sample and standard, respectively. Atmospheric N was the standard for ^{15}N, while Vienna Pee Dee belemnite was the primary standard for ^{13}C. Acetanilide (C_8H_9NO) was used as internal standard. The calculated δ values were calibrated using the δ values of litter from the respective elevation and subplot as baseline (Fig. 1) and expressed as $\Delta^{15}N_{bulk}$ and $\Delta^{13}C_{bulk}$. Mean stable isotope values of litter from the two closest subplots were used as baseline for the subplots where litter stable isotope values were not measured.

3.3.3 Stable Isotope Analysis of Amino Acids

Collembola and Oribatida were lyophilized prior to amino acid extraction. Amino acids were extracted as described in Larsen et al. (2013) and Pollierer et al. (2019). Prior to hydrolysis and in all subsequent reaction steps, samples were flushed with N_2 to avoid oxidation. Samples were hydrolyzed in 6 N HCl at 110°C for 20 h and cleaned of lipophilic compounds using 2 ml hexane/dichloromethane solution. The aqueous phase was filtered through heat-sterilized glass wool (450°C) to remove remaining solid tissue. Fifty µl nor-leucine (5 µmol ml^{-1}) was added as internal standard. A mixture of amino acids with known $\delta^{13}C$ and $\delta^{15}N$

values was prepared as external standard. The samples and the standard mixture were dried at 110°C and stored at -18°C prior to derivatization. For derivatization, we followed the procedure of Corr et al. (2007). Amino acids were methylated with 680 µl of acidified methanol at 75°C for 1 h. Subsequently, the methanol was evaporated and samples were acetylated for 10 min at 60°C with a mixture of acetic anhydride, triethylamine and acetone. Precipitates, salts and remaining water were removed from the samples using ethyl acetate, NaCl and dichloromethane. Derivatized amino acids were dissolved in 80-100 µl ethyl acetate.

Amino acid isotopic composition was measured using a ThermoTrace gas-chromatograph coupled via a GP interface to a DeltaPlus mass spectrometer (Thermo, Bremen, Germany), equipped with an Agilent J&W VF-35ms GC column (30 m × 0.32 mm; film thickness 1 µm). Samples were measured in triplicate for both $\delta^{13}C$ and $\delta^{15}N$. N isotope composition was expressed relative to atmospheric N; measured values were corrected with the slope of the regression line between amino acid $\delta^{15}N$ values of measured standard mixtures (vs. reference gas) and the known $\delta^{15}N$ values of standard mixtures (vs. atmospheric N). To account for the additional C atoms of derivatized amino acids in C isotope analyses we corrected the measurements as described in O’Brien et al. (2002).

3.3.4 Neutral Lipid Fatty Acids

Animals were transferred to 10 ml reaction vials. To remove remaining glycerol, the vials were filled up with demineralized water and thoroughly mixed. Samples were then centrifuged to separate the animals from the storage medium; the water-glycerol mixture was discarded. Neutral lipids of Collembola and Oribatida were extracted and separated as described in Haubert et al. (2004) and Zelles (1999). For extraction of lipids, animals were shaken overnight in 5 ml extraction solvent (chloroform, methanol, 0.05 M, phosphate buffer at a ratio of 1:2:0.8). The extraction solvent was collected and the step was repeated with 2.5 ml of extraction solvent for 2 h. The lipid-containing phase was transferred to a fresh vial and 0.8 ml $CHCl_3$ and 0.8 ml H_2O were added for phase separation. The upper phase was discarded. The lower phase was transferred to $CHCl_3$ conditioned silica columns, washed with another 5 ml of $CHCl_3$, and the $CHCl_3$ was evaporated. For saponification we followed the Sherlock Microbial Identification System (MIDI, Newark, USA). In short, 1 ml of sodium hydroxide/methanol solution was added and samples were incubated for 30 min at 100°C. Then, 2 ml of HCl-methanol solution was added. NLFAs were methylated at 80°C for 10 min, methylation was stopped by fast cooling of samples. The resulting fatty acid methyl esters (FAMEs) were extracted by adding 1.25 ml of 1:1 hexane methyl tert-butyl ether solution. The hexane phase containing the FAMEs was frozen until measurement. Samples were measured in a gas-chromatograph (GC-FID Clarus 500; PerkinElmer Corporation, Norwalk, USA) equipped with an Elite 5 column (30 m × 0.32 mm inner diameter, film thickness 0.25 µm, PerkinElmer Corporation, Norwalk, USA), helium was used as carrier gas. The abundance of lipids was transformed to percentages of total lipids. NLFAs with percentages <1% were excluded from the analyses. NLFAs serving as relative and absolute markers were used to estimate the biosynthetic origin of consumer NLFAs (Table 1). We calculated the oleic (18:1ω9) to linoleic acid (18:2ω6,9) ratio in consumers (plant/fun$_{cons}$); the ratio between these relative markers for plants and fungi is used to indicate shifts between plant and fungal based diets (Sechi et al., 2014). We further calculated the ratio of linoleic acid (18:2ω6,9) to specific bacterial markers (i15:0, a15:0, i16:0, i17:0, cy17:0), henceforth referred to as fun/bac$_{cons}$.

Table 1: Relative and absolute marker neutral lipid fatty acids (NLFAs) that contributed >1% to total NLFAs of Collembola and Oribatida and their main biosynthetic origin.

NLFA	Marker	Reference
16:2ω6,9; 16:3ω3,6,9	green algae	(Buse et al., 2013)
18:2ω6,9	Fungi	(Ruess et al., 2005; Pollierer et al., 2012; Kühn et al., 2019)
16:1ω7; 18:1ω7	general bacteria	(Ruess & Chamberlain, 2010)
i15:0, a15:0, i16:0, i17:0	Gram$^+$ bacteria	(Ruess & Chamberlain, 2010)
cy17:0	Gram$^-$ bacteria	(Ruess & Chamberlain, 2010)
18:1ω9	Plants	(Ruess & Chamberlain, 2010)
18:3ω6,9,12	Plants	(Ruess et al., 2007; Ruess & Chamberlain, 2010)

3.3.5 Environmental Factors

We measured the fungal-to-bacterial PLFA ratio (fun/bac$_{litter}$), microbial biomass (C$_{mic}$), the cyclopropyl-to-monoenoic precursor PLFA ratio (cyclo/pre) and the C/N ratio in the litter layer as described in Lux et al. (2022). Environmental factors were measured at every second subplot and we used the mean of the measured factors of the two closest subplots for the subplot in between (Fig. 1). For a brief summary of the main results from Lux et al. (2022) see Appendix S1: Table S1.

3.3.6 Statistical Analyses

Statistical analyses were performed in R v 4.0.4 (R Core Team, 2021). TP$_{CSIA}$ was calculated from δ^{15}N values of Glu and Phe as follows: $TP_{CSIA} = 1 + (\delta^{15}N_{Glu} - \delta^{15}N_{Phe} + \beta) / TDF_{Glu\text{-}Phe}$, with β representing the difference in Glu and Phe in the primary producers, being 8.4 ± 1.6 ‰ in terrestrial C$_3$ plants (Chikaraishi et al., 2010, 2014). As TDF (trophic discrimination factor) we applied 7.6 ± 1.2 ‰ as suggested by Chikaraishi et al. (2009, 2014) and Pollierer et al. (2019). Measurements of $\delta^{15}N$ of Glu and Phe of each sample were performed in triplicate and the mean of these measurements was used for the TP calculation. We propagated the error of the equation above as described in Blum et al. (2013) and Ohkouchi et al. (2017), taking into account the uncertainty of $\delta^{15}N_{Phe}$, $\delta^{15}N_{Glu}$, β and the TDF (see Appendix S1: Table S2).

Linear relationships of $\Delta^{15}N_{bulk}$, $\Delta^{13}C_{bulk}$, TP_{CSIA}, $\delta^{15}N_{phe}$ and $plant/fun_{cons}$, as well as fun/bac_{cons} ratios with elevation were analyzed using linear mixed effects models with elevation (continuous and centered) and taxa as well as their interaction as predictors. Plot ID was included as random intercept. If no linear relationship was found, we used elevation as categorical variable to check for general variations across elevations. All linear mixed effect models were conducted using the “lme4” package (Bates et al., 2015); if necessary, data were $\log_{10}$ transformed to approximate Gaussian distribution of residuals as indicated in the results section. Linear models met the assumption of homoscedasticity and Gaussian distribution of residuals.

To identify the biosynthetic origin of essential amino acids (eAAs) isoleucine (Ile), leucine (Leu), methionine (Met), threonine (Thr) and valine (Val) in consumer taxa across elevations we ran a stable isotope mixing model with the $\delta^{13}C$ of the five amino acids described above per taxon using the “simmr” package (Parnell, 2021), with $\delta^{13}C$ values of the five eAAs normalized to the sample mean in both training and consumer data. Phenylalanine was excluded from the mixing model as the normalized $\delta^{13}C$ values of consumers did not fall within the range of the normalized resource values. We ran the models with 10,000 total iterations and a burn-in of 1000 iterations, we further investigated the model convergence as suggested by Phillips et al. (2014) using Gelman diagnostic values with a threshold < 1.1. We used training data from three different resources (plants, fungi, bacteria) (Larsen et al., 2013, 2016, Pollierer et al., 2020).

Since Manlick and Newsome (2022) showed that different fingerprinting approaches may yield different results, we also checked for the estimations of the linear discriminant analysis (LDA) bootstrapping approach as described by Fox et al. (2019) and Manlick & Newsome (2022). Therefore, LDAs were conducted using the “MASS” package (Venables & Ripley, 2002). To train the model, we used the same dataset as described above (Larsen et al., 2013, 2016; Pollierer et al., 2020). We then used this model to predict group membership of consumers with LDA bootstrapping. In short, the two-step bootstrapping approach included 10,000 iterations of the training data set via random draws (with replacement), followed by classification of samples for both taxa.

Besides the origin of amino acids, we investigated variations in marker NLFAs with elevation and taxa. To analyze differences in the marker NLFA composition, a Bray-Curtis dissimilarity-based PERMANOVA was performed using the “adonis2” function of the “vegan” package (Oksanen et al., 2020) with 10,000 iterations. The input matrix included relative amounts of marker NLFAs as dependent variable, and elevation, taxa and their interaction as independent factors. Preceding the PERMANOVA, multivariate variances of marker NLFA profiles were investigated using the “betadisper” function (Oksanen et al., 2020). Multivariate variances did not significantly differ between taxa or elevations. Therefore, significant differences in the PERMANOVA can be interpreted as compositional differences between groups. This analysis was followed by Bray-Curtis dissimilarity-based analysis of similarity percentages (“SIMPER”) with 10,000 iterations, to identify the marker NLFAs responsible for most group dissimilarity (Oksanen et al., 2020).

To further inspect observed changes, we correlated measures which depended on elevation with the $\text{fun/bac}_{\text{litter}}$, C_{mic}, cyclo/pre and C/N ratios from litter using Spearman rank correlation to account for non-linear relationships revealed by visual inspection. We also tested for linear relationships between $\Delta^{15}N_{\text{bulk}}$ and TP_{CSIA} as well as $\delta^{15}N_{\text{Phe}}$ and $\delta^{15}N$ of litter using Pearson correlations.

3.4 Results

3.4.1 Changes in Trophic Position across Elevations

$\Delta^{15}N_{bulk}$ in Collembola and Oribatida increased significantly from 800 (0.44 ± 0.92 ‰) to 1700 m (2.27 ± 1.58 ‰; $\chi^2 = 14.57$, $P < 0.001$; Fig. 2A). Generally, $\Delta^{15}N_{bulk}$ in Oribatida (overall mean 1.69 ± 1.42 ‰) was significantly higher than in Collembola (overall mean 1.09 ± 1.12 ‰; $\chi^2 = 9.47$, $P = 0.002$). The response of $\Delta^{15}N_{bulk}$ to elevation did not depend on taxon as indicated by non-significant elevation × taxon interaction. $\Delta^{15}N_{bulk}$ in Collembola correlated positively with the cyclo/pre PLFA ratio in litter (Table 2). $\Delta^{15}N_{bulk}$ in Oribatida correlated positively with the fun/bac$_{litter}$ and the cyclo/pre PLFA ratios in litter as well as the litter C/N ratio.

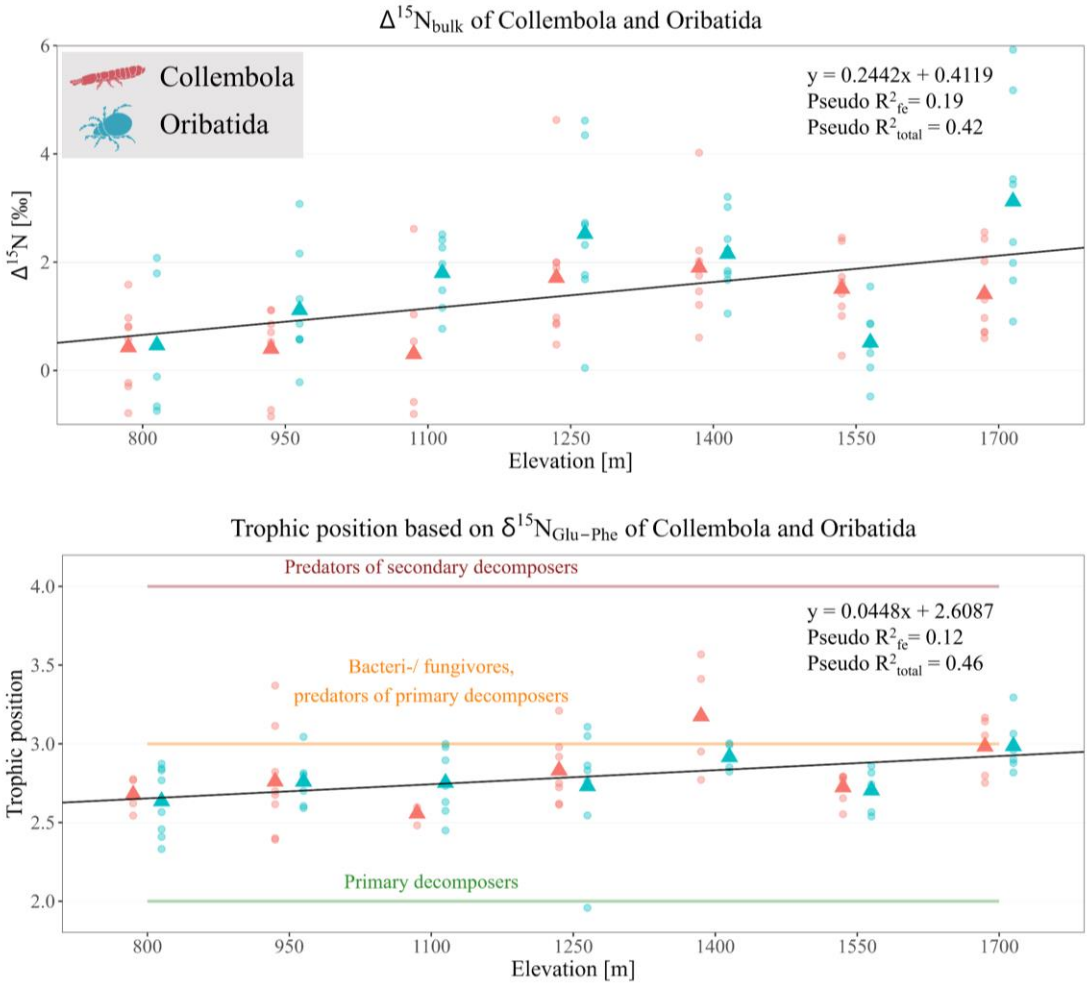


Figure 2: Linear mixed effect regressions of (A) Δ15Nbulk and elevation as well as (B) trophic position (TPCSIA) and elevation. TPCSIA was calculated from the difference in δ15N of glutamic acid (Glu) and phenylalanine (Phe). Dots represent individual data points, triangles the elevational mean for the respective taxon. Taxa are represented by different colors. Total pseudo R2 values of linear mixed effect regression models as well as pseudo R2 of fixed effects (see results section) are displayed. Equations correspond to the respective regression line, with x being one elevational step of 150 m. Colored horizontal lines in (B) represent trophic levels.

TP_{CSIA}, calculated from $\delta^{15}N$ of Glu and Phe, followed a similar pattern as $\Delta^{15}N_{bulk}$, as $\Delta^{15}N_{bulk}$ and TP_{CSIA} of Collembola and Oribatida across elevations also correlated linearly ($\rho = 0.29$, $P = 0.009$): TP_{CSIA} of Collembola and Oribatida increased significantly from 800 (2.65 ± 0.18 TP_{CSIA}) to 1700 m (2.98 ± 0.17 TP_{CSIA}; $\chi^2 = 8.89$, $P = 0.003$; Fig. 2B). However, there was no significant difference in the TP_{CSIA} between the two taxa. Further, the response of TP_{CSIA} to elevation did not depend on taxon as indicated by non-significant elevation × taxon interaction. TP_{CSIA} in both taxa correlated positively with C_{mic} (Table 2). Further, in Collembola TP_{CSIA} was significantly positively correlated with the cyclo/pre ratio.

Table 2: Spearman rank correlations between consumer (Collembola, Oribatida) trophic niche parameters [$\Delta^{15}N_{bulk}$, trophic position calculated from $\delta^{15}N$ values of glutamic acid and phenylalanine (TP_{CSIA}), oleic-to-linoleic acid ratio ($plant/fun_{cons}$), fungal-to-bacterial ratio (fun/bac_{cons}), $\Delta^{13}C_{bulk}$, $\delta^{15}N_{phe}$] and litter characteristics [cyclopropyl-to-monoenoic (cyclo/pre) PLFA ratios, fungal-to-bacterial PLFA ratios in litter (fun/bac_{litter}), litter C/N ratio, microbial biomass (C_{mic})] across elevations. Values represent Spearman's rho, values given in boldface indicate significant correlations ($P < 0.05$).

Taxon	Parameter	cyclo/pre	fun/bac_{litter}	C/N	C_{mic}
Collembola	$\Delta^{15}N_{bulk}$	**0.47**	0.05	0.10	0.14
	TP_{CSIA}	**0.40**	0.03	0.17	**0.37**
	$plant/fun_{cons}$	0.18	0.13	0.01	0.12
	fun/bac_{cons}	-0.11	0.12	0.15	-0.09
	$\Delta^{13}C_{bulk}$	**0.34**	0.14	-0.06	-0.29
	$\delta^{15}N_{Phe}$	**-0.60**	0.09	**-0.39**	-0.30
Oribatida	$\Delta^{15}N_{bulk}$	**0.32**	**0.36**	**0.50**	0.27
	TP_{CSIA}	0.15	0.03	0.25	**0.36**
	$plant/fun_{cons}$	-0.04	-0.05	-0.06	0.20
	$\Delta^{13}C_{bulk}$	0.23	0.08	0.29	0.01
	$\delta^{15}N_{Phe}$	**-0.64**	-0.23	**-0.33**	**-0.56**

3.4.2 Basal Resources

$\delta^{13}C$ fingerprinting of essential amino acids. Fungi were estimated as major source of eAAs at all elevations, according to both Bayesian mixing models and LDA bootstrapping based on the training data of Larsen et al. (2013, 2016) and Pollierer et al. (2020) (Fig. 3, Appendix S1: Fig. S1). For Collembola, the estimated contribution of fungi ranged between 53.2 ± 21.6 % (1400 m) and 87.3 ± 8.0 % (800 m) (Fig. 3A). The contributions of plants ranged

from 7.0 ± 6.0 % (800 m) to 26.1 ± 17.8 % (1400 m) and bacterial contributions ranged from 5.8 ± 4.9 % (800 m) to 19.8 ± 15.5 % (1400 m). For Oribatida, the mean contribution of fungi ranged between 56.1 ± 23.7 % (1400 m) and 85.5 ± 8.2 % (1100 m) (Fig. 3B). The contribution of plants ranged from 7.2 ± 5.4 % (1100 m) to 26.9 ± 19.7 % (1400 m) and the contribution of bacteria from 7.3 ± 5.9 % (800 m) and 17.0 ± 14.1 % (1400 m).

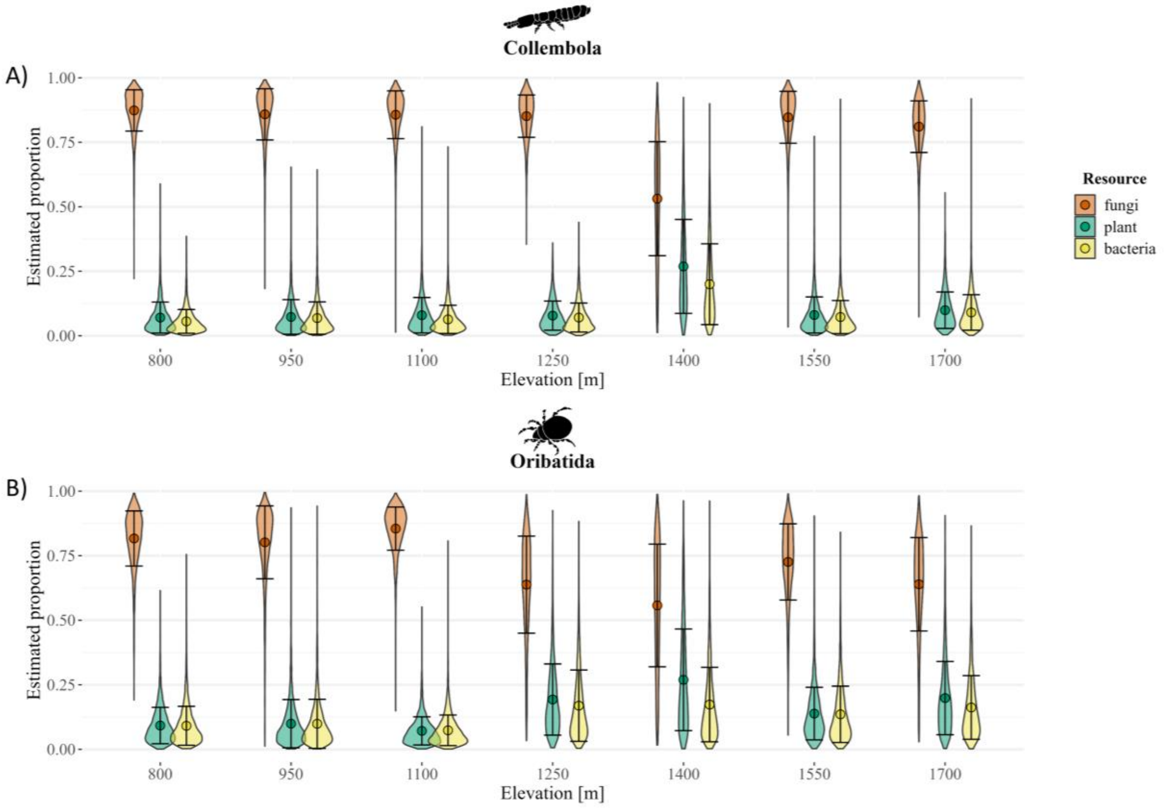


Figure 3: Variations in mean proportional resource contribution estimates for (A) Collembola and (B) Oribatida along an elevational gradient from 800 to 1700 m as indicated by Bayesian mixing models with δ13C values of isoleucine, leucine, methionine, threonine and valine, based on training data of plants, fungi and bacteria (Larsen et al., 2013, 2016, Pollierer et al., 2020). Colored areas represent the posterior distributions of the estimated proportional resource contribution of the respective resources. Error bars represent the standard deviation of proportional resource contribution.

Origin of neutral lipids in consumers. Marker NLFA composition differed significantly between Collembola and Oribatida (PERMANOVA: Pseudo-$F_{1,95}$ = 126.66, P < 0.001), but not between elevations, with the interaction between elevation and taxon also being non-significant (Appendix S1: Fig. S2). 18:2ω6:9 accounted for most dissimilarity between Collembola and Oribatida, at over 35 %, followed by 18:1ω9, at over 31% (“SIMPER” analysis). Accordingly, the plant/fun_{cons} ratio did not significantly differ between elevations, with the interaction between elevation and taxon also being non-significant. However, the plant/fun_{cons} ratio differed between taxa (χ^2 = 42.77, P = < 0.001, log-transformed), with a ratio of 1.90 ± 0.37 in Collembola and 1.49 ± 0.36 in Oribatida (non-transformed means; Appendix S1: Fig. S3). Similarly, the fun/bac_{cons} ratio did not differ between elevations in Collembola. The fun/bac_{cons} ratio could not be calculated for Oribatida, since most Oribatida samples did not contain any specific bacterial marker (Appendix S1: Fig. S2). The plant/fun$_{cons}$ ratio in both taxa as well as the fun/bac$_{cons}$ ratio in Collembola did not correlate significantly with any of the litter characteristics (Table 2).

Shifts in the use of basal resources. $\Delta^{13}C_{bulk}$ values did not respond in a linear way to the elevation gradient (Fig. 4A). Variations in $\Delta^{13}C_{bulk}$ values with elevation depended on taxon (significant elevation × taxon interaction; $\chi^2 = 44.08$, $P < 0.001$), $\Delta^{13}C_{bulk}$ also generally differed between taxa ($\chi^2 = 34.97$, $P < 0.001$). $\Delta^{13}C_{bulk}$ in Collembola was lowest at 1700 m (3.51 ± 0.70 ‰) and highest at 1550 m (4.21 ± 0.60 ‰), while in Oribatida $\Delta^{13}C_{bulk}$ was lowest at 950 m (3.10 ± 0.46 ‰) and highest at 1250 m (4.36 ± 0.92 ‰). $\Delta^{13}C_{bulk}$ in Collembola correlated positively with the cyclo/pre ratio (Table 2). $\delta^{15}N_{Phe}$ decreased significantly from 800 (overall mean 2.47 ± 2.00 ‰) to 1700 m (over all mean -4.43 ± 1.37 ‰; $\chi^2 = 46.30$, $P < 0.001$; Fig. 4), with the slope being slightly larger in Oribatida (elevation x taxon interaction, $\chi^2 = 4.28$, $P = 0.039$). However, the average $\delta^{15}N_{Phe}$ value in Oribatida (-0.67 ± 3.06 ‰) was significantly higher than in Collembola (-2.39 ± 2.68 ‰; $\chi^2 = 22.00$, $P < 0.001$). $\delta^{15}N_{Phe}$ correlated negatively with the cyclo/pre as well as C/N ratio of litter in both Collembola and Oribatida (Table 2). Additionally, in Oribatida $\delta^{15}N_{Phe}$ correlated negatively with C$_{mic}$. Overall, $\delta^{15}N_{Phe}$ in both taxa showed a close linear relationship with bulk $\delta^{15}N$ of litter ($\rho = 0.73$, $P < 0.001$).

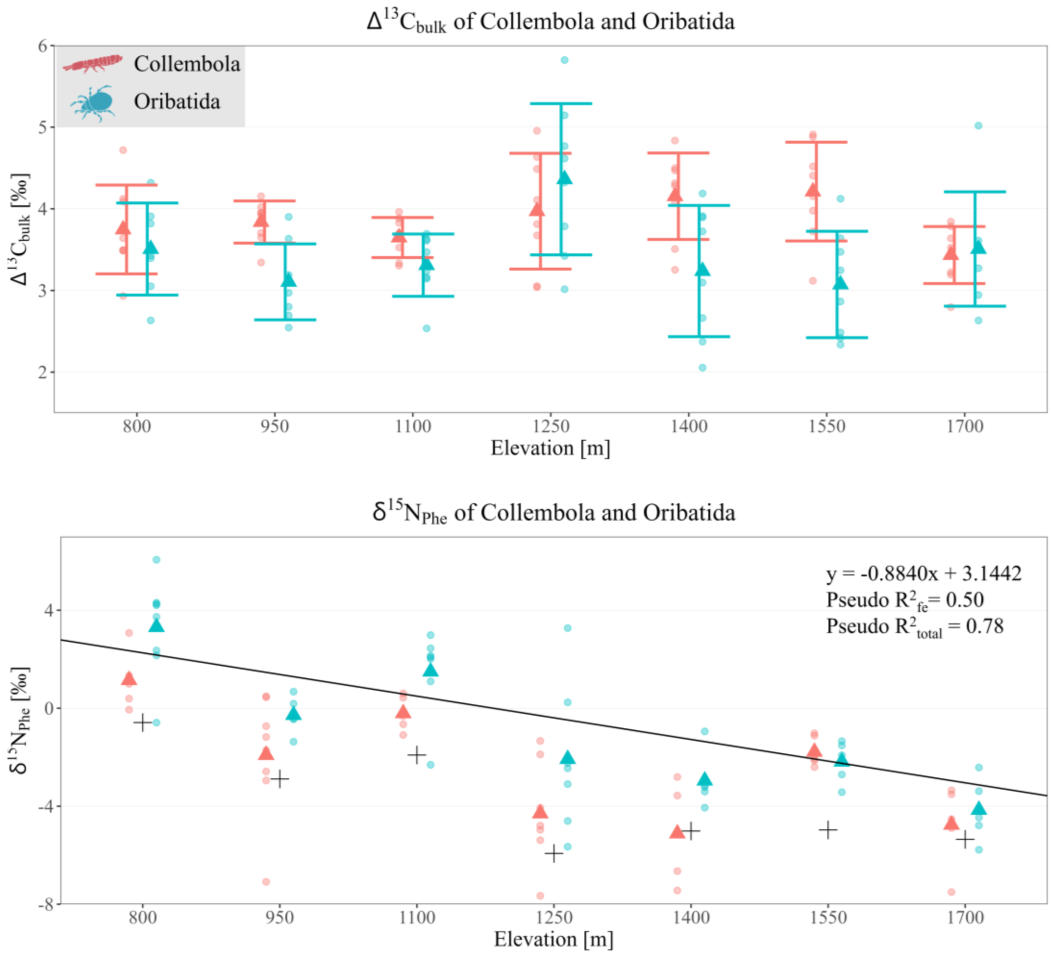


Figure 4: Changes in $\Delta^{13}C_{bulk}$ and $\delta^{15}N$ values of phenylalanine ($\delta^{15}N_{Phe}$) along an elevational gradient from 800 to 1700 m. Dots represent individual data points, triangles the elevational mean for the respective taxon. Taxa are color coded. Total pseudo R^2 values of linear mixed effects regression models as well as pseudo R^2 values of fixed effects (see result section) are given; equations correspond to the respective regression line, with x being one elevational step of 150 m. Crosses mark mean $\delta^{15}N$ bulk values of litter at the respective elevation.

3.5 Discussion

Here, we traced the shift in basal resource use and trophic positions of Collembola and Oribatida as major mesofauna detritivores along an elevation gradient comprising primary mixed broadleaf-coniferous and pure coniferous forests. Using a combination of bulk stable isotope analyses, fatty acid analyses and compound-specific stable isotope analyses of amino acids, we showed that trophic positions increase with elevation and are related to increases in microbial biomass and microbial stress. By contrast, the utilization of fungi as main basal resource did not change significantly along the studied elevation gradient.

3.5.1 Trophic Position

$^{15}N_{bulk}$ as well as TP_{CSIA}, calculated from $\delta^{15}N$ of Phe and Glu, both increased from 800 to 1700 m, indicating that the trophic position of both Collembola and Oribatida increases towards higher elevations. Collembola and Oribatida communities at Changbai Mountain shifted about one-third of a trophic level in TP_{CSIA}, from a mixture of primary and secondary decomposers at 800 m to secondary decomposers or predators/scavengers at 1700 m. This is in line with our first hypothesis and is also supported by the increase in $\Delta^{15}N_{bulk}$ values with elevation. $\Delta^{15}N_{bulk}$ indicated a somewhat higher increase in trophic position across elevations compared to TP_{CSIA}; both taxa increased by 1.9 ‰ from 800 to 1700 m, which is equivalent to approximately half a trophic level assuming an enrichment of 3.4 ‰ per trophic level (Post, 2002; Potapov et al., 2019c). Shifts in trophic positions of Collembola and Oribatida may be due to changes in species composition towards more secondary decomposer or predatory species at higher elevations. In fact, species composition of both Collembola and Oribatida changes with elevation at Changbai Mountain (Xie et al., 2022; Pan et al., 2023). For instance, in Collembola the relative abundance of epedaphic Tomoceridae and Entomobryidae, typically comprising species of low trophic position (Potapov et al., 2016), decreases from 950 to 1700 m (Xie et al., 2022). In addition, bulk stable isotopes of Collembola species at Changbai Mountain also indicate that shifts toward higher trophic positions at higher elevations are also occurring within common species (J. Lux, unpubl. data). Fischer et al. (2014) reported a similar pattern for Oribatida in the European Alps, where trophic positions of Oribatida communities also increased along the studied elevation gradient.

Forests at lower elevations at the northern slope of Changbai Mountain consist mainly of deciduous and coniferous mixed forests, with increasing abundance of pine towards 1100 m, while at elevations above 1100 m the abundance of spruce species increases (Liu, 1997). Decomposition rates of spruce litter are often slower than those of deciduous litter, potentially due to high polyphenol content in spruce needles (Albers et al., 2004; Berger & Berger, 2012). Further, calcium content is lower and C/N ratio as well as microbial biomass are higher in litter at higher elevations at Changbai Mountain (Lux et al., 2022). Fujii et al. (2018) found that microarthropods have a greater effect on the decomposition of nutrient-rich litter. Decreased nutrient availability towards higher elevations and increased C_{mic} (Lux et al., 2022) may contribute to the shift in Collembola and Oribatida towards consumption of more microbial or animal-based resources at higher elevations.

This is further supported by the positive relationship between C_{mic} and TP_{CSIA} across elevations in both Collembola and Oribatida. Even though $\Delta^{15}N_{bulk}$ followed a similar pattern as TP_{CSIA} along the elevation gradient, the relationship between $\Delta^{15}N_{bulk}$ and C_{mic} neither was significant in Collembola nor in Oribatida; potentially, TP_{CSIA} more accurately traces microbial contributions to the diet of consumers than $\Delta^{15}N_{bulk}$, which also reflects isotopic enrichment of organic matter (Steffan et al., 2017, Potapov 2019b). $\Delta^{15}N_{bulk}$ as well as TP_{CSIA} in Collembola further correlated positively with the cyclo/pre ratio, indicating trophic positions of Collembola to increase with increasing nutritional and substrate-based stress in microorganisms (Guckert et al., 1986; Moore-Kucera & Dick, 2008; Lux et al., 2022). However, the correlations may also reflect the decline in litter quality with elevation causing in parallel stress-induced changes in microbial communities as well as dietary and species shifts in Collembola communities. Extending tree species boundaries due to global warming may lead to transitions of mountain coniferous to deciduous forests (Albrich et al., 2020). Climate driven conversion towards more broad-leaved species is likely to be associated with increased litter quality and therefore decreased trophic positions of Collembola and Oribatida.

While $\Delta^{15}N_{bulk}$ of Oribatida correlated positively with the fun/bac_{litter}, cyclo/pre and C/N ratios of litter, TP_{CSIA} of Oribatida only positively correlated with C_{mic} in litter. The discrepancies between TP_{CSIA} and $\Delta^{15}N_{bulk}$ were stronger in Oribatida than in Collembola. Assuming that TP_{CSIA} provides a more baseline-independent measure of trophic position compared to $\Delta^{15}N_{bulk}$, the observed differences may indicate that Oribatida use a somewhat different basal resource than Collembola, for which litter seems to be a more suitable baseline, as also supported by the significantly higher $\delta^{15}N$ values of Phe in Oribatida.

Despite these minor mismatches for Oribatida, $\Delta^{15}N_{bulk}$ and TP_{CSIA} of both Collembola and Oribatida correlated positively, indicating that both reflect changes in the trophic position of species and communities, thereby supporting our second hypothesis. Potapov et al. (2019b) found a similar relationship between $\Delta^{15}N_{bulk}$ and TP_{CSIA} in earthworm species. Assuming that TP_{CSIA} more reliably reflects trophic positions than $\Delta^{15}N_{bulk}$, the enrichment in $^{15}N_{bulk}$ per trophic level in our study was higher than the typically used 3.4 ‰ per trophic level (Post, 2002; Potapov et al., 2019c).

Most studies use leaf litter for normalizing $\delta^{15}N_{bulk}$ values of consumers (Klarner et al., 2014; Potapov et al., 2019a; Maraun et al., 2023). Our study, however, demonstrates that $\delta^{15}N$ values of litter may vary considerably even between neighboring forests. In fact, choosing the correct baseline is one of the most critical points in bulk stable isotope analysis (Martínez Del Rio et al., 2009; Potapov et al., 2019a). This problem is overcome by using the $^{15}N/^{14}N$ ratios of essential AAs such as Phe allowing to reliably delineate trophic positions of consumers even though basal resources remain unknown or are variable (Chikaraishi et al., 2009; 2010; Pollierer et al., 2019). $\delta^{15}N_{Phe}$ closely correlated with $\delta^{15}N_{litter,}$ supporting our assumption of litter being the major basis of energy channels and therefore the valid baseline for calculating $\Delta^{15}N_{bulk}$. Further, $\delta^{15}N_{Phe}$ negatively correlated with C_{mic}, cyclo/ pre and litter C/N ratio, again reflecting the close link with litter, as C_{mic} and the stress indicator ratio increase along the elevation gradient likely due to increased carbon but lower nitrogen availability as indicated by increasing C/N ratios (Lux et al., 2022).

Compared to $\Delta^{15}N_{bulk}$ analysis, measuring TP_{CSIA} is more time consuming and needs more sophisticated machinery. Further, the minimum amount of tissue needed for TP_{CSIA} is considerably higher than in $\Delta^{15}N_{bulk}$ analyses (ca. 500 µg and 10 µg dry weight, respectively), limiting its use especially when analyzing small invertebrates such as soil microarthropods. Therefore, as indicated by our study, $\Delta^{15}N_{bulk}$ analyses remains a viable option to uncover variations in trophic positions, but the results need to be interpreted with caution.

3.5.2 Basal Resources

Bayesian mixing models as well as LDA bootstrapping identified fungi as the main source of essential amino acids for both Collembola and Oribatida across all elevations, while plants and bacteria were of low importance. Generally, our results are in line with earlier studies which identified fungi, mainly saprotrophic fungi, as major basal resources of both Collembola and Oribatida especially in forest ecosystems (Pollierer & Scheu, 2021; Li et al., 2022). High amounts of oleic (18:1ω9) as well as linoleic acid (18:2ω6,9) in both taxa also point to fungi as major basal resource. Even though oleic acid is commonly used as relative plant marker (Ruess & Chamberlain, 2010), it also accumulates in fungi (Jansa, 1999). NLFAs of Collembola feeding on fungi therefore may contain large amounts of oleic acid. Ruess et al. (2005b) found the relative amounts of oleic and linoleic acid to be similar in the collembolan species *Folsomia candida* when fed with the fungus *Chaetomium globosum*. In our study the relative amounts of oleic acid were higher than the amounts of linoleic acid in both taxa at all elevations, indicating that plant derived resources may also contribute to storage lipids in both Collembola and Oribatida. Even though plants may play a larger role as resource than indicated by amino acids, NLFA analysis generally supports the high contributions of fungal resources as estimated by mixing models. Potentially, results of mixing models may be biased when based on non-local resources (Manlick & Newsome, 2022), however, both NLFA analysis as well as a TP_{CSIA} close to three support high fungal contributions to the diet of both taxa.

Essential amino acids and lipid profiles in both taxa suggest that the high contribution of fungi to the diet of Collembola and Oribatida remains constant across the studied elevation gradient. This contradicts our third hypothesis and indicates that the shift in litter quality and increasing microbial biomass with elevation is not associated with increased consumption of fungi. Higher trophic positions of Collembola and Oribatida at higher elevations therefore are likely rather due to an increasing abundance of individuals that feed to a larger extent on resources of higher trophic position (Lux et al., 2024). In fact, using molecular tools, Collembola and Oribatida have been found to feed on a wide range of nematodes both dead and alive (Heidemann et al., 2014a, b). Further, Collembola have been shown to feed on eggs of other microarthropods including those of other species of Collembola (Longstaff, 1976; Walsh and Bolger, 1993). Both may have contributed to the increase in trophic level with constant fungal dietary content. However, as microorganisms are trophic analogs of animals (Steffan et al. 2015), feeding on microorganisms of higher trophic level may also have contributed to the increase in trophic level in Collembola and Oribatida at higher elevations.

In contrast to the constant high contribution of fungi to the diet of Collembola and Oribatida, $\Delta^{13}C_{bulk}$ significantly varied with elevation, but the variation depended on taxon, likely reflecting differences in the use of basal resources. However, the variation in $\Delta^{13}C_{bulk}$ across elevations was generally small, indicating only small resource shifts in both Collembola and Oribatida with elevation as also shown by CSIA-AA and NLFAs.

3.5.3 Differences between Taxa

$\Delta^{15}N_{bulk}$ and $\delta^{15}N_{phe}$ values were significantly higher in Oribatida than in Collembola suggesting that basal resources of the two taxa differ in their $\delta^{15}N$ values. Further, $\Delta^{13}C_{bulk}$ correlated with the cyclo/pre ratio in litter in Collembola but not in Oribatida, indicating trophic shifts in Collembola at sites where microorganisms are stressed (Guckert et al., 1986; Moore-Kucera & Dick 2008).

Although fingerprinting of eAAs indicated little differences in the use of basal resources in both Collembola and Oribatida, marker NLFAs differed between the two taxa. The relative abundance of bacterial markers was higher in Collembola, and the relative abundance of plant and fungal marker NLFAs was higher in Oribatida suggesting that on Changbai Mountain the contribution of bacteria to the diet of Collembola exceeds that of Oribatida. Differences between the two methods may generally be due to the fact that fingerprinting of eAAs reflects basal resources used for protein synthesis and growth, whereas NLFA analysis reflects basal resources used for storage lipids, i.e. energy metabolism. Higher contribution of bacteria to the diet of Collembola than to the diet of Oribatida, therefore may reflect that Collembola more intensively use bacterial lipids for their storage lipids, with Collembola generally building more storage lipids than Oribatida (J. Lux, unpubl. data). Bacterial marker

NLFAs in Collembola suggest a preference for Gram$^+$ bacteria since cyclopropyl NLFAs, representing Gram$^-$ bacteria, were almost absent. Ruess et al. (2007) also reported preferences for Gram$^+$ over Gram$^-$ bacteria in four Collembola species of German forests. Compared to Gram$^-$ bacteria, Gram$^+$ bacteria are more intensively involved in the degradation of complex organic compounds (Fanin et al., 2019). Borkott & Insam (1990) found the Collembola species *Folsomia candida* to preferentially feed on chitin colonized by microorganisms compared to sterile chitin, pointing to the exploitation of microbial enzymes for their own nutrition, but potentially also to the digestion of these microorganisms themselves.

3.5.4 Conclusions

Collembola and Oribatida as abundant microarthropods involved in litter decomposition live mostly as secondary decomposers, feeding on microbially colonized resources. Despite a constantly high contribution of fungi as basal resource, trophic positions increased with elevation, likely indicating microarthropods to feed on resources of higher trophic position at higher elevations. These shifts were linked to increased microbial biomass as well as decreased litter quality with elevation. Overall, the combined analyses of bulk stable isotopes, CSIA-AA and NLFAs allowed detailed insight into changes in trophic positions and energy channels of soil microarthropods with elevation. In particular, the combination of methods provided evidence for differential utilization of basal resources for growth (protein synthesis) and for energy metabolism (storage lipids). The uniform increase in trophic positions of Collembola and Oribatida with elevation indicates important functional shifts of microarthropod communities contributing more directly to decomposition processes at lower elevation. Future climate change and related vegetational changes therefore may be associated by increased direct contributions of microarthropods to decomposition processes.

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3.7 References

Albers D, Migge S, Schaefer M, Scheu S (2004) Decomposition of beech leaves (*Fagus sylvatica*) and spruce needles (*Picea abies*) in pure and mixed stands of beech and spruce. Soil Biol Biochem 36:155–164. https://doi.org/10.1016/j.soilbio.2003.09.002

Albrich K, Rammer W, Seidl R (2020) Climate change causes critical transitions and irreversible alterations of mountain forests. Glob Change Biol 26:4013–4027. https://doi.org/10.1111/gcb.15118

Bates D, Maechler M, Bolker B, et al (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Berg MP, Kniese JP, Verhoef HA (1998) Dynamics and stratification bacteria and fungi in the organic layers of a Scots Pine forest. Biol Fert Soils 26:313–322. https://doi.org/10.1007/s003740050382

Berger TW, Berger P (2012) Greater accumulation of litter in spruce (*Picea abies*) compared to beech (*Fagus sylvatica*) stands is not a consequence of the inherent recalcitrance of needles. Plant Soil 358:349–369. https://doi.org/10.1007/s11104-012-1165-z

Bhardwaj M, Uniyal VP, Sanyal AK, Singh AP (2011) Butterfly communities along an elevational gradient in the Tons Valley, Western Himalayas: Implications of rapid assessment for insect conservation. J Asia-Pac Entomol 15:207–217. https://doi.org/10.1016/j.aspen.2011.12.003

Blake JG, Loiselle BA (2000) Diversity of birds along an elevational gradient in the Cordillera Central, Costa Rica. The Auk 117:663–686. https://doi.org/10.1642/0004-8038(2000)117[0663:DOBAAE]2.0.CO;2

Blum JD, Popp BN, Drazen JC, et al (2013) Methylmercury production below the mixed layer in the North Pacific Ocean. Nat Geosci 6:879–884. https://doi.org/10.1038/ngeo1918

Borkott H, Insam H (1990) Symbiosis with bacteria enhances the use of chitin by the springtail, *Folsomia candida* (Collembola). Biol Fertil Soils 9:126–129. https://doi.org/10.1007/bf00335794

Buse T, Ruess L, Filser J (2013) New trophic biomarkers for Collembola reared on algal diets. Pedobiologia 56:153–159. https://doi.org/10.1016/j.pedobi.2013.03.005

Chahartaghi M, Langel R, Scheu S, Ruess L (2005) Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biol Biochem 37:1718–1725. https://doi.org/10.1016/j.soilbio.2005.02.006

Chikaraishi Y, Kashiyama Y, Ogawa NO, et al (2007) Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: Implications for aquatic food web studies. Mar Ecol Prog Ser 342:85–90. https://doi.org/10.3354/meps342085

Chikaraishi Y, Ogawa NO, Kashiyama Y, et al (2009) Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. Limnol Oceanogr Methods 7:740–750. https://doi.org/10.4319/lom.2009.7.740

Chikaraishi Y, Ogawa NO, Ohkouchi N (2010) Further evaluation of the trophic level estimation based on nitrogen isotopic composition of amino acids. In: Earth, life, and isotopes. Kyoto University Press, Kyoto

Chikaraishi Y, Steffan SA, Ogawa NO, et al (2014) High-resolution food webs based on nitrogen isotopic composition of amino acids. Ecol Evol 4:2423–2449. https://doi.org/10.1002/ece3.1103

Corr LT, Berstan R, Evershed RP (2007) Optimisation of derivatisation procedures for the determination of $\delta^{13}C$ values of amino acids by gas chromatography/combustion/ isotope ratio mass spectrometry. Rapid Commun Mass Spectrom 21:3759–3771. https://doi.org/10.1002/rcm.3252

Dakhil MA, Halmy MWA, Liao Z, et al (2021) Potential risks to endemic conifer montane forests under climate change: integrative approach for conservation prioritization in southwestern China. Landscape Ecol. 36:3137–3151. https://doi.org/10.1007/s10980-021-01309-4

De Vries FT, Hoffland E, Van Eekeren N, et al (2006) Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Bio Biochem 38:2092–2103. https://doi.org/10.1016/j.soilbio.2006.01.008

Erhardt S, Hochreuther P, Schütz M, Berger M (2022) OpenTopoMap - Topographische Karten aus Openstreetmap. https://opentopomap.org

Fanin N, Kardol P, Farrell M, et al (2019) The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. Soil Biol Biochem 128:111–114. https://doi.org/10.1016/j.soilbio.2018.10.010

Fischer BM, Meyer E, Maraun M (2014) Positive correlation of trophic level and proportion of sexual taxa of oribatid mites (Acari: Oribatida) in alpine soil systems. Exp Appl Acarol 63:465–479. https://doi.org/10.1007/s10493-014-9801-3

Fox MD, Elliott Smith EA, Smith JE, Newsome SD (2019) Trophic plasticity in a common reef-building coral: Insights from $\delta^{13}C$ analysis of essential amino acids. Funct Ecol 33:2203–2214. https://doi.org/10.1111/1365-2435.13441

Fujii S, Cornelissen JHC, Berg MP, Mori AS (2018) Tree leaf and root traits mediate soil faunal contribution to litter decomposition across an elevational gradient. Funct Ecol 32:840–852. https://doi.org/10.1111/1365-2435.13027

Guckert JB, Hood MA, White DC (1986) Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: Increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. Appl Environ Microbiology 52:794–801. https://doi.org/10.1128/aem.52.4.794-801.1986

Hagelstein P, Sieve B, Klein M, et al (1997) Leucine synthesis in chloroplasts: Leucine/isoleucine aminotransferase and valine aminotransferase are different enzymes in spinach chloroplasts. J Plant Physiol 150:23–30. https://doi.org/10.1016/S0176-1617(97)80176-9

Hättenschwiler S, Aeschlimann B, Coûteaux MM, et al (2008) High variation in foliage and leaf litter chemistry among 45 tree species of a neotropical rainforest community. New Phytol 179:165–175. https://doi.org/10.1111/j.1469-8137.2008.02438.x

Haubert D, Häggblom MM, Scheu S, Ruess L (2004) Effects of fungal food quality and starvation on the fatty acid composition of *Protaphorura fimata* (Collembola). Comp Biochem Physiol - B Biochem Mol Biol 138:41–52. https://doi.org/10.1016/j.cbpc.2004.02.009

Heidemann K, Hennies A, Schakowske J, et al (2014a) Free-living nematodes as prey for higher trophic levels of forest soil food webs. Oikos 123:1199–1211. https://doi.org/10.1111/j.1600-0706.2013.00872.x

Heidemann K, Ruess L, Scheu S, Maraun M (2014b) Nematode consumption by mite communities varies in different forest microhabitats as indicated by molecular gut content analysis. Exp Appl Acarol 64:49–60. https://doi.org/10.1007/s10493-014-9807-x

Hodkinson ID (2005) Terrestrial insects along elevation gradients: Species and community responses to altitude. Biol Rev Camb Philos Soc 80:489–513. https://doi.org/10.1017/S1464793105006767

Hudson AO, Bless C, MacEdo P, et al (2005) Biosynthesis of lysine in plants: Evidence for a variant of the known bacterial pathways. Biochim Biophys Acta 1721:27–36. https://doi.org/10.1016/j.bbagen.2004.09.008

Jansa J, Gryndler M, Matucha M (1999) Comparison of the lipid profiles of arbuscular mycorrhizal (AM) fungi and soil saprophytic fungi. Symbiosis 26:247–264

Kampichler C, Bruckner A (2009) The role of microarthropods in terrestrial decomposition: A meta-analysis of 40 years of litterbag studies. Biol Rev 84:375–389. https://doi.org/10.1111/j.1469-185X.2009.00078.x

Klarner B, Ehnes RB, Erdmann G, et al (2014) Trophic shift of soil animal species with forest type as indicated by stable isotope analysis. Oikos 123:1173–1181. https://doi.org/10.1111/j.1600-0706.2013.00939.x

Kohlhaw GB (2003) Leucine biosynthesis in fungi: entering metabolism through the back door. Microbiol Mol Biol Rev 67:1–15. https://doi.org/10.1128/mmbr.67.1.1-15.2003

Kühn J, Schweitzer K, Ruess L (2019) Diversity and specificity of lipid patterns in basal soil food web resources. PLoS ONE 14: e0221102. https://doi.org/10.1371/journal.pone.0221102

Kühn J, Tobias K, Jähngen A, Ruess L (2020) Shifting systems: prerequisites for the application of quantitative fatty acid signature analysis in soil food webs. Phil Trans R Soc B 375:20190650. https://doi.org/10.1098/rstb.2019.0650

Larsen T, Lee Taylor D, Leigh MB, O’brien DM (2009) Stable isotope fingerprinting: a novel method for identifying plant, fungal, or bacterial origins of amino acids. Ecology 90:3526–3535. https://doi.org/10.1890/08-1695.1

Larsen T, Ventura M, Andersen N, et al (2013) Tracing carbon sources through aquatic and terrestrial food webs using amino acid stable isotope fingerprinting. PLoS ONE 8: e73441. https://doi.org/10.1371/journal.pone.0073441

Larsen T, Pollierer MM, Holmstrup M, et al (2016) Substantial nutritional contribution of bacterial amino acids to earthworms and enchytraeids: A case study from organic grasslands. Soil Biol Biochem 99:21–27. https://doi.org/10.1016/j.soilbio.2016.03.018

Li Z, Bluhm SL, Scheu S, Pollierer MM (2022) Amino acid isotopes in functional assemblages of Collembola reveal the influence of vertical resource heterogeneity and root energy supply on trophic interactions in soil food webs. Soil Biol Biochem 174:108815. https://doi.org/10.1016/j.soilbio.2022.108815

Liu QJ (1997) Structure and dynamics of the subalpine coniferous forest on Changbai Mountain, China. Plant Ecol 132:97–105. https://doi.org/10.1023/A:1009705319128

Longstaff BC (1976) The dynamics of collembolan populations: Competitive relationships in an experimental system. Can J Zool 54:948–962

Lux J, Xie Z, Sun X, et al (2022) Changes in microbial community structure and functioning with elevation are linked to local soil characteristics as well as climatic variables. Ecol Evol 12:e9632. https://doi.org/10.1002/ece3.9632

Lux J, Xie Z, Sun X, et al (2024) Trophic niches of Collembola communities change with elevation, but also with body size and life form. Oecologia. https://doi.org/10.1007/s00442-023-05506-7

Manlick PJ, Newsome SD (2022) Stable isotope fingerprinting traces essential amino acid assimilation and multichannel feeding in a vertebrate consumer. Methods Ecol Evol. https://doi.org/10.1111/2041-210X.13903

Maraun M, Augustin D, Müller J, et al (2014) Changes in the community composition and trophic structure of microarthropods in sporocarps of the wood decaying fungus *Fomitopsis pinicola* along an altitudinal gradient. Appl Soil Ecol 84:16–23. https://doi.org/10.1016/j.apsoil.2014.06.004

Maraun M, Thomas T, Fast E, et al (2023) New perspectives on soil animal trophic ecology through the lens of C and N stable isotope ratios of oribatid mites. Soil Biol Biochem 177:108890. https://doi.org/10.1016/j.soilbio.2022.108890

Martínez Del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111. https://doi.org/10.1111/j.1469-185X.2008.00064.x

Mccain CM (2005) Elevational gradients in diversity of small mammals. Rep Ecol 86:366–372. https://doi.org/10.1890/03-3147

Moore JC (1994) Impact of agricultural practices on soil food web structure: Theory and application. Agriculture, Ecosystems & Environment 51:239–247. https://doi.org/10.1016/0167-8809(94)90047-7

Moore JC, Hunt HW (1988) Resource Compartmentation and the Stability of Real Ecosystems. Nature 333:261–263. https://doi.org/10.1038/333261a0

Moore JC, McCann K, De Ruiter PC (2005) Modeling trophic pathways, nutrient cycling, and dynamic stability in soils. Pedobiologia 49:499–510. https://doi.org/10.1016/j.pedobi.2005.05.008

Moore-Kucera J, Dick RP (2008) PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microb Ecol 55:500–511. https://doi.org/10.1007/s00248-007-9295-1

O'Brien DM, Fogel ML, Boggs CL (2002) Renewable and nonrenewable resources: Amino acid turnover and allocation to reproduction in Lepidoptera. Proc Natl Acad Sci 99:4413–4418. https://doi.org/10.1073/pnas.072346699

Ohkouchi N, Chikaraishi Y, Close HG, et al (2017) Advances in the application of amino acid nitrogen isotopic analysis in ecological and biogeochemical studies. Org Geochem 113:150–174. https://doi.org/10.1016/j.orggeochem.2017.07.009

Oksanen AJ, Kindt R, Legendre P, et al (2020) vegan: Community ecology package. R package version 2.5-7

Pan X, Xie Z, Sun X, et al (2023) Changes in oribatid mite community structure along two altitudinal gradients in Asia and Europe as related to environmental factors. Appl Soil Ecol 189:104912. https://doi.org/10.1016/j.apsoil.2023.104912

Parnell A (2021) simmr: A Stable Isotope mixing model. R package version 0.5-1.212

Phillips DL, Inger R, Bearhop S, et al (2014) Best practices for use of stable isotope mixing models in food-web studies. Can J Zool 92:823–835. https://doi.org/10.1139/cjz-2014-0127

Pollierer MM, Langel R, Scheu S, Maraun M (2009) Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$). Soil Biol Biochem 41:1221–1226. https://doi.org/10.1016/j.soilbio.2009.03.002

Pollierer MM, Dyckmans J, Scheu S, Haubert D (2012) Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific ^{13}C fatty acid analysis. Funct Ecol 26:978–990. https://doi.org/10.1111/j.1365-2435.2012.02005.x

Pollierer MM, Larsen T, Potapov AM, et al (2019) Compound-specific isotope analysis of amino acids as a new tool to uncover trophic chains in soil food webs. Ecol Monogr 89:e01384. https://doi.org/10.1002/ecm.1384

Pollierer MM, Scheu S, Tiunov AV (2020) Isotope analyses of amino acids in fungi and fungal feeding Diptera larvae allow differentiating ectomycorrhizal and saprotrophic fungi-based food chains. Funct Ecol 34:2375–2388. https://doi.org/10.1111/1365-2435.13654

Pollierer MM, Scheu S (2021) Stable isotopes of amino acids indicate that soil decomposer microarthropods predominantly feed on saprotrophic fungi. Ecosphere 12:e03425. https://doi.org/10.1002/ecs2.3425

Post DM (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83:703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Potapov AM, Tiunov AV, Scheu S (2019a) Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biol Rev 94:37–59. https://doi.org/10.1111/brv.12434

Potapov AM, Tiunov AV, Scheu S, et al (2019b) Combining bulk and amino acid stable isotope analyses to quantify trophic level and basal resources of detritivores: a case study on earthworms. Oecologia 189:447–460. https://doi.org/10.1007/s00442-018-04335-3

Potapov AM, Brose U, Scheu S, Tiunov AV (2019c) Trophic position of consumers and size structure of food webs across aquatic and terrestrial ecosystems. Am Nat 194:823–839. https://doi.org/doi.org/10.1086/705811

Potapov AM, Beaulieu F, Birkhofer K, et al (2022) Feeding habits and multifunctional classification of soil-associated consumers from protists to vertebrates. Biol Rev 97:1057–1117. https://doi.org/10.1111/brv.12832

R Core Team (2021) R: A language and environment for statistical computing

Ruess L, Chamberlain PM (2010) The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biol Biochem 42:1898–1910. https://doi.org/10.1016/j.soilbio.2010.07.020

Ruess L, Schütz K, Migge-Kleian S, et al (2007) Lipid composition of Collembola and their food resources in deciduous forest stands-Implications for feeding strategies. Soil Biol Biochem 39:1990–2000. https://doi.org/10.1016/j.soilbio.2007.03.002

Ruess L, Tiunov A, Haubert D, et al (2005) Carbon stable isotope fractionation and trophic transfer of fatty acids in fungal based soil food chains. Soil Biol Biochem 37:945–953. https://doi.org/10.1016/j.soilbio.2004.09.015

Ruess L, Schütz K, Haubert D, et al (2005) Application of lipid analysis to understand trophic interactions in soil. Ecology 86:2075–2082. https://doi.org/10.1890/04-1399

Samson DA, Rickart EA, Gonzales PC (1997) Ant diversity and abundance along an elevational gradient in the Philippines. Biotropica 29:349–363. https://doi.org/10.1111/j.1744-7429.1997.tb00436.x

Scheu S, Falca M (2000) The soil food web of two beech forests (*Fagus sylvatica*) of contrasting humus type: stable isotope analysis of a macro- and mesofauna-dominated community. Oecologica 123:285–286. https://doi.org/10.1007/s004420051015

Schneider K, Renker C, Maraun M (2005) Oribatid mite (Acari, Oribatida) feeding on ectomycorrhizal fungi. Mycorrhiza 16:67–72. https://doi.org/10.1007/s00572-005-0015-8

Seastedt TR (1984) The role of microarthropods in decomposition and mineralization processes. Annu Rev Entomol 29:25–46. https://doi.org/10.1146/annurev.en.29.010184.000325

Sechi V, D'Annibale A, Ambus P, et al (2014) Collembola feeding habits and niche specialization in agricultural grasslands of different composition. Soil Biol Biochem 74:31–38. https://doi.org/10.1016/j.soilbio.2014.02.019

Steffan SA, Chikaraishi Y, Currie CR, et al (2015) Microbes are trophic analogs of animals. Proceedings of the National Academy of Sciences of the United States of America 112:15119–15124. https://doi.org/10.1073/pnas.1508782112

Steffan SA, Chikaraishi Y, Dharampal PS, et al (2017) Unpacking brown food-webs: Animal trophic identity reflects rampant microbivory. Ecol Evol 7:3532–3541. https://doi.org/10.1002/ece3.2951

Tang L, Li A, Shao G (2011) Landscape-level forest ecosystem conservation on Changbai Mountain, China and North Korea (DPRK). Mt Res Dev 31:169–175. https://doi.org/10.1659/MRD-JOURNAL-D-10-00120.1

Velasco AM, Leguina JI, Lazcano A (2002) Molecular evolution of the lysine biosynthetic pathways. J Mol Evol 55:445–459. https://doi.org/10.1007/s00239-002-2340-2

Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Springer, New York

Walsh MI, Bolger T (1993) Effect of diet on the interaction between *Hypogastrura denticulate* Bagnall and *Onychiurus furcifer* Börner in Laboratory cultures. Eur J Soil Biol 29:155–160

Wang H, Xu J, Sheng L, et al (2020) Study on the characteristics of climate change in Changbai Mountain National Natural Reserve from 1958 to 2017. Arab J Geosci 13:777. https://doi.org/10.1007/s12517-020-05808-7

Xie Z, Sun X, Lux J, et al (2022) Drivers of Collembola assemblages along an altitudinal gradient in Northeast China. Ecol Evol 12:e8559. https://doi.org/10.1002/ece3.8559

Yan B, Liang X, Xiao C (2018) Hydrogeochemical characteristics and genesis model of Jinjiang and Julong hot springs in Changbai Mountain, Northeast China. Geofluids 2018:1694567. https://doi.org/10.1155/2018/1694567

Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: A review. Biol Fertil Soils 29:111–129. https://doi.org/10.1007/s003740050533

Zieger SL, Scheu S (2018) Effects of storage and handling on neutral lipid fatty acid profiles of two woodlice (Isopoda, Crustacea) species differing in size. Appl Soil Ecol. https://doi.org/10.1016/j.apsoil.2018.04.009

3.8 Appendix

Table S2: Summary of major results from Lux et al. (2022) on phospholipid fatty acid (PLFA) and substrate induced respiration (SIR) measurements of litter at the same sampling sites and dates as the present study. For measurements of soils see Lux et al. (2022).

Study site	Sampling date	Method	Focal factor	Results (litter layer)	Interpretation
Northern slope at Changbai Mountain, China	Early Sep 2019	PLFA	Microbial community composition	Clear separation between lower (800-1100 m) and higher elevations (1250-1700 m); temperature seasonality structures microbial communities	Major changes of microbial communities likely related to major forest type transitions across elevations; litter layer most strongly exposed to climatic changes
		PLFA	Microbial stress, indicated by e.g., cyclo/pre PLFA ratio	Increased at higher elevations (1250-1700 m), correlated negatively with calcium concentrations and positively with C/N ratios	Increased microbial biomass is accompanied by an increased demand for nitrogen at higher elevations, elevating microbial stress; potential use of urease reaction for N acquisition at lower elevations
		PLFA	Fungal/bacterial PLFA ratio	No significant variation with elevation	Elevation does not influence proportions of these two major microbial decomposer groups
		SIR	Microbial biomass	Increased at higher elevations (1250-1700 m) correlated negatively with calcium concentrations and positively with C/N ratios	Increased microbial biomass due to climate and forest type related accumulation of organic C, even under N deficiency

Table S3: Mean trophic positions (TP) of Collembola and Oribatida at different elevations on Changbai Mountain and their respective standard deviation (σ_1) as well as the mean of potential uncertainty of TP (σ_2) calculated after Blum et al. (2013) and Ohkouchi et al. (2017). N represents the number of replicates.

Taxon	Elevation [m]	N	Mean TP	σ_1	mean σ_2
Collembola	800	5	2.68	0.10	0.40
	950	8	2.76	0.34	0.38
	1100	5	2.56	0.04	0.35
	1250	7	2.83	0.22	0.38
	1400	4	3.18	0.38	0.48
	1550	6	2.72	0.10	0.37
	1700	5	2.98	0.19	0.45
Oribatida	800	8	2.64	0.22	0.40
	950	6	2.76	0.17	0.39
	1100	7	2.75	0.21	0.43
	1250	7	2.73	0.39	0.41
	1400	5	2.92	0.08	0.40
	1550	5	2.70	0.15	0.36
	1700	6	2.99	0.17	0.42

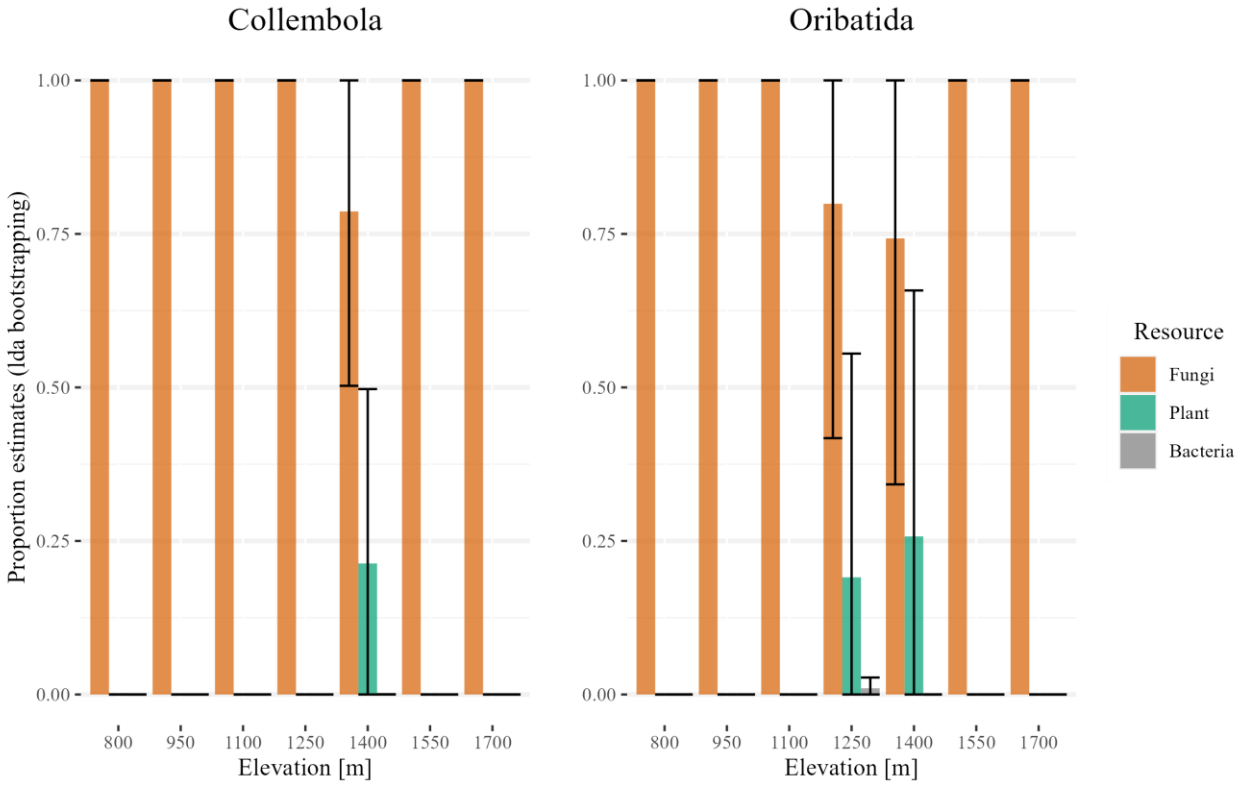

Figure S1: Variations in the relative contribution of fungi, plants and bacteria to the diet of Collembola and Oribatida at different elevations on Changbai Mountain as indicated by LDA bootstrapping of $\delta^{13}C$ values of isoleucine, leucine, methionine, threonine and valine based on data published by Larsen et al. (2013, 2016) and Pollierer et al. (2020).

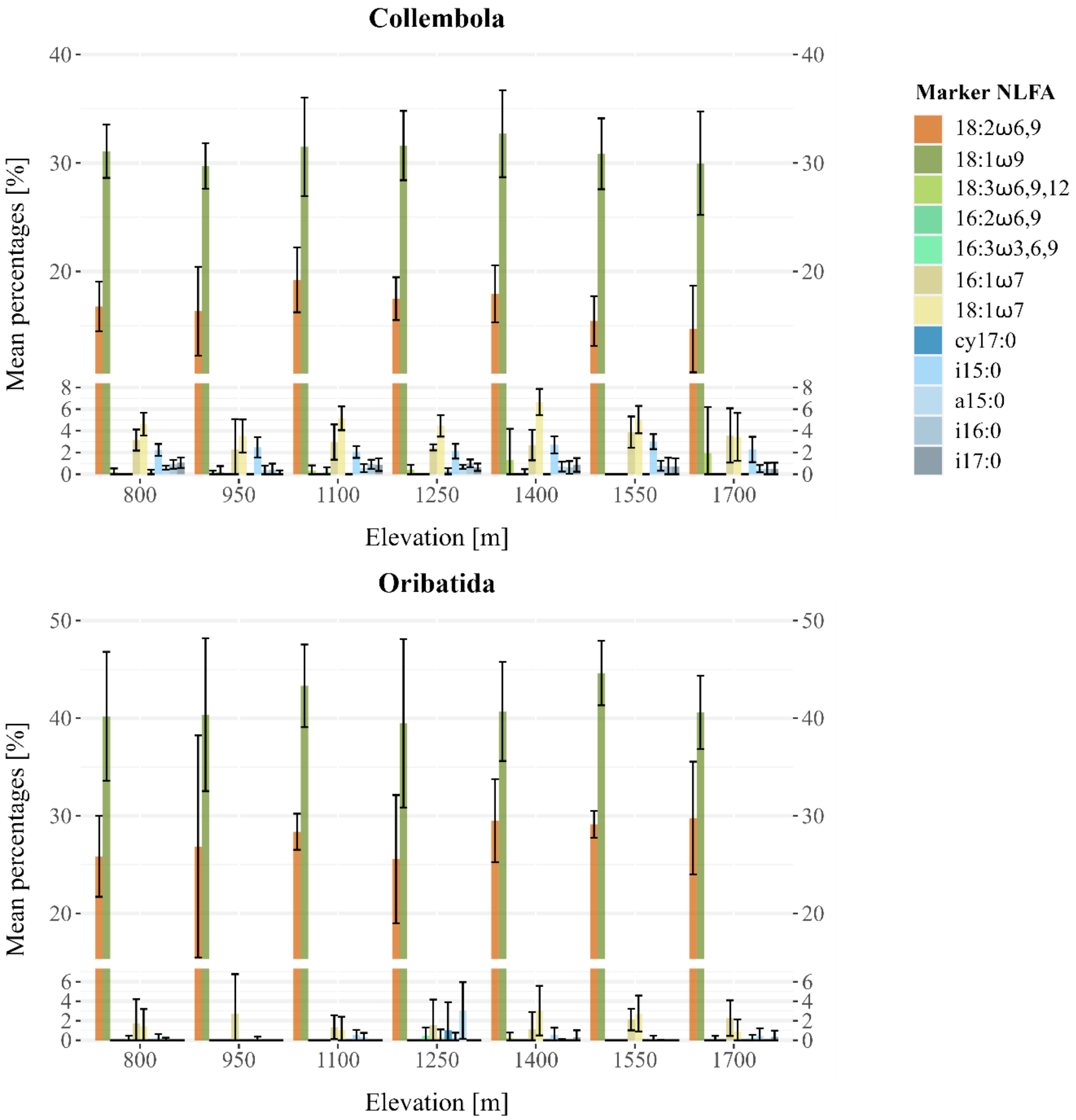

Figure S2: Variations in absolute and relative marker NLFAs in Collembola and Oribatida at different elevations on Changbai Mountain. Coloured bars represent the mean percentages (of total NLFAs) of the respective markers as given in Table 1 per elevation. Error bars represent standard deviations. Note: Bars contain a y-axis break for visualization purposes.

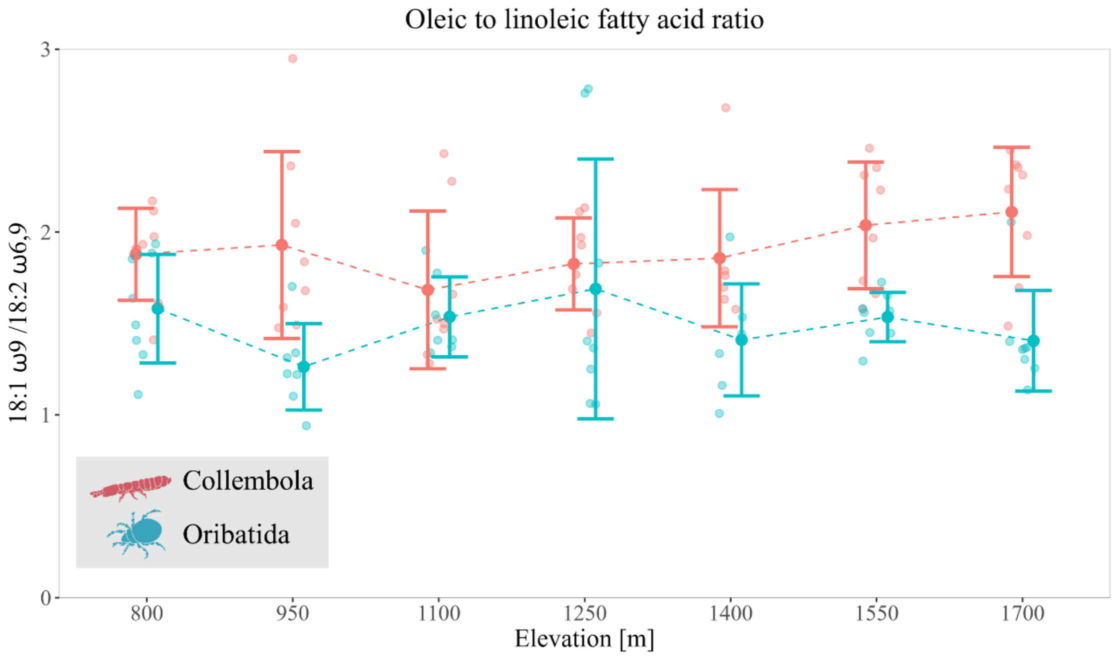

Figure S3: Changes in the oleic (18:1ω9) to linoleic acid (18:2ω6,9) ratio in Collembola (red) and Oribatida (blue) across elevations on Changbai Mountain. Error bars represent standard deviations.

Chapter 4: Trophic Niches of Collembola Communities Change with Elevation, but Also with Body Size and Life Form

Tomocerus sp. on deciduous leaf litter

4.1 Abstract

Climate change will likely increase habitat loss of endemic tree species and drives forest conversion in mountainous forests. Elevation gradients provide the opportunity to predict possible consequences of such changes. While species compositions of various taxa have been investigated along elevation gradients, data on trophic changes in soil-dwelling organisms are scarce. Here, we investigated trophic changes of the Collembola communities along the northern slope of Changbai Mountain, China. We sampled Collembola in primary forests at seven elevations (800-1700 m asl). We measured individual body lengths and bulk stable isotopes on species level. We further categorized Collembola species into life forms. The community-weighted means of Δ^{15}N and Δ^{13}C as well as minimum Δ^{15}N and isotopic uniqueness of Collembola communities increased with increasing elevation, while the range of Δ^{15}N decreased. Maximum and minimum of Δ^{13}C differed between elevations but showed no linear trend. Further, Δ^{15}N of Collembola species occurring across all elevations increased with elevation. Changes in Δ^{15}N with elevation were most pronounced in hemiedaphic species, while Δ^{13}C increased strongest with elevation in euedaphic species. Δ^{15}N increased with decreasing body size in hemiedaphic and euedaphic species. Overall, the results suggest that Collembola species functioning as primary decomposers at lower elevations shift towards functioning as secondary decomposers or even predators or scavengers at higher elevation forests. The results further indicate that access to alternative food resources depends on Collembola life form as well as body size and varies between ecosystems.

4.2 Introduction

Global climate change strongly affects forest systems across the world by accelerating extreme climate events resulting in increased tree mortality (Kharuk et al. 2017; 2021, Obladen et al. 2021). Among worldwide forest biomes mountain forest account for 23% of forest cover (Price et al. 2015). Climate change likely has detrimental consequences for mountain forests through climate driven forest conversion (Albrich et al. 2020), decreased tree growth (Matskovsky et al. 2021) and shrinking boundaries of endemic tree species (Dakhil et al. 2021). Further, Albrich et al. (2020) predicted climate change driven replacement of coniferous by deciduous forests in the European Alps. Changes in tree species composition can affect biodiversity in many taxa, the direction of the response, however, depends on taxon (Leidinger et al. 2021). Investigating elevation gradients in natural forests may therefore help to predict the response of different forest-dwelling taxa to changing climate and the resulting change in tree species composition. Changbai Mountain in North-Eastern China represents such a gradient as it comprises undisturbed montane forests including the transition between broadleaf-coniferous mixed and pure coniferous forests (Liu 1997; Tang et al. 2011).

Responses of a variety of plant, animal and microbial taxa across elevation gradients have been studied (Samson et al. 1997; Blake & Loiselle 2000; Hodkinson 2005; McCain 2005; Bhardwaj et al. 2011), including belowground animal communities (Fischer et al. 2014; Maraun et al. 2014; Bokhorst et al. 2018; Xie et al. 2022; Pan et al. 2023a). However, few studies focus on changes in trophic niches across elevation gradients, where data on belowground communities are especially scarce. Fischer et al. (2014) as well as Pan et al. (2023b) found varying responses in trophic niches of oribatid mites with elevation, indicating changes in soil food web interactions along elevation gradients.

Like oribatid mites, Collembola are among the most abundant soil microarthropods in temperate forests (Seastedt 1984), where they occupy a wide range of trophic levels (Scheu & Falca 2000; Chahartaghi et al. 2005). They predominantly feed on litter resources (Rusek 1998) and microorganisms, in particular fungi (Caravaca & Ruess 2014; Pollierer & Scheu 2021), thereby directly and indirectly affecting litter decomposition and nutrient cycling. The contribution of soil mesofauna to litter decomposition has been found to depend on litter characteristics, such as nutrient concentrations, which differ between tree species (Fujii et al. 2018). At Changbai Mountain, litter characteristics such as C/N ratio have been shown to vary strongly between the lower elevation broadleaf-coniferous mixed forests, where C/N ratio were rather low, and high elevation pure coniferous forests with higher C/N ratios. Additionally, microbial biomass increases with increasing elevation at Changbai Mountain (Lux et al. 2022). Therefore, direct contributions of the Collembola community to decomposition of litter might be higher at lower elevations, while Collembola may feed more intensively on the more abundant microbial resources at higher elevations. Collembola in forest ecosystems have been found to predominantly rely on saprotrophic fungi (Pollierer & Scheu 2021, Li et al. 2022); consequently, the trophic positions of Collembola communities may increase with elevation.

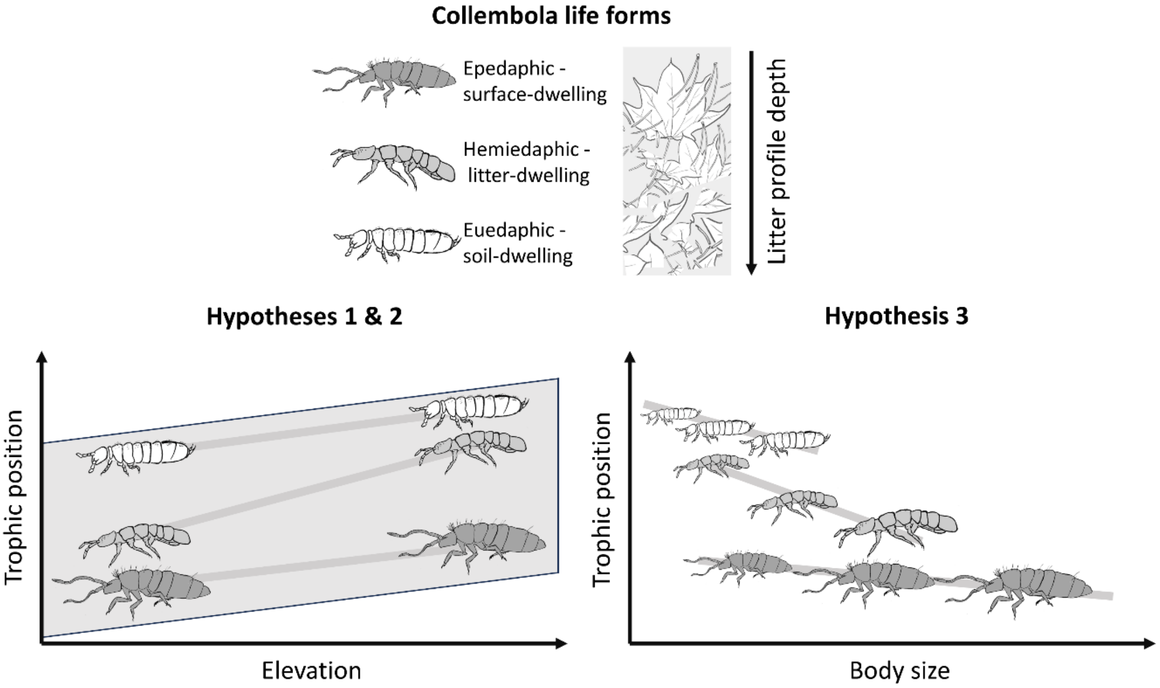

Figure 1: Conceptualized relationship between trophic positions of Collembola communities, life forms and elevation (Hypotheses 1 & 2), and between trophic positions of life forms and body size (Hypothesis 3).

Resources in forest soil systems are known to vary across microhabitats in the litter/soil matrix (Erktan et al. 2020). The ability of Collembola to access different resources within the litter/soil matrix across elevation gradients may mitigate but also aggravate trophic responses to elevation. Access to resources by Collembola may vary in particular with Collembola life forms (Gisin 1943; Rusek 2007). While epedaphic (surface-dwelling) Collembola predominantly colonize the litter surface, hemiedaphic (litter-dwelling) and euedaphic (soil-dwelling) Collembola can forage deeper in soil (Fujii & Takeda 2012). Potapov et al. (2016) found the trophic positions of life forms to increase from epedaphic to hemiedaphic to euedaphic Collembola, reflecting the different foraging strategies in the soil matrix (Fig. 1). Hemiedaphic Collembola for instance can forage on top of the litter as well as in deeper layers. This may provide hemiedaphic species access to a larger variety of resources compared to e.g., epedaphic species and therefore allows increased trophic plasticity along environmental gradients. Even though body size is among the traits differing between the three life forms in general (Rusek 2007), within life form body size variations may further influence foraging strategies. For example, the ability of euedaphic Collembola species to access smaller soil pores deeper in soil is likely determined by their body size (Rusek 2007). Stable isotopes are the major method for characterizing trophic niches in soil food webs (Potapov et al. 2019a; Maraun et al. 2023). Litter-normalized $^{15}N/^{14}N$ ratios

($\Delta^{15}N$) of consumers are commonly used to determine trophic positions, while litter-normalized $^{13}C/^{12}C$ ratios ($\Delta^{13}C$) provide information on the basal resource used by soil animals (Klarner et al. 2014; Potapov et al. 2019a; Maraun et al. 2023). However, especially when investigating organisms varying in vertical distribution within the litter/soil matrix, both $\Delta^{15}N$ and $\Delta^{13}C$ values can provide additional information about the resources used since organic matter in deeper soil layers is enriched in ^{15}N and ^{13}C compared to litter material on top of the soil (Garten et al. 2000; Ponsard & Arditi 2000, Wallander et al. 2004, Potapov et al. 2019a), which is likely due to the accumulation of microbial residues.

Bulk stable isotopes of nitrogen and carbon further allow calculation of isotope metrics to characterize trophic niches of consumer in more detail (Villéger et al. 2008; Cucherousset & Villéger 2015). In this study we use abundance weighted and unweighted bulk stable isotope metrics to characterize variations in trophic niches of Collembola communities (measured at species level) at Changbai Mountain (China). We further investigate changes in trophic niches of different Collembola life forms and species with elevation. Additionally, we study variations in trophic niches of Collembola with body size. We hypothesized that (1) abundance weighted $\Delta^{15}N$ values linearly increase with elevation, while $\Delta^{13}C$ values only slightly increase with elevation; (2) $\Delta^{15}N$ values strongly and $\Delta^{13}C$ values slightly differ among Collembola life forms, being highest in euedaphic and lowest in epedaphic Collembola, while the changes in $\Delta^{15}N$ values with elevation are strongest in hemiedaphic Collembola; and (3) smaller Collembola occupy higher trophic positions as reflected in high $\Delta^{15}N$ values and this relationship is most pronounced in hemiedaphic Collembola.

4.3 Material and Methods

4.3.1 Study Site and Sampling

The study was conducted at the northern slope of Changbai Mountain at the borders of the Chinese provinces Jilin and Liaoning to North Korea within the boundaries of the Changbaishan National Nature Reserve (Fig. 2). Mean annual temperature between 1959 to 1988 ranged between -7 and -3°C and mean annual precipitation between 700 and 1400 mm (Chen et al. 2011). The major geological groups at the sampled transect are alkalic pumice, trachyte, tuff and stomatal as well as laminated basalt.

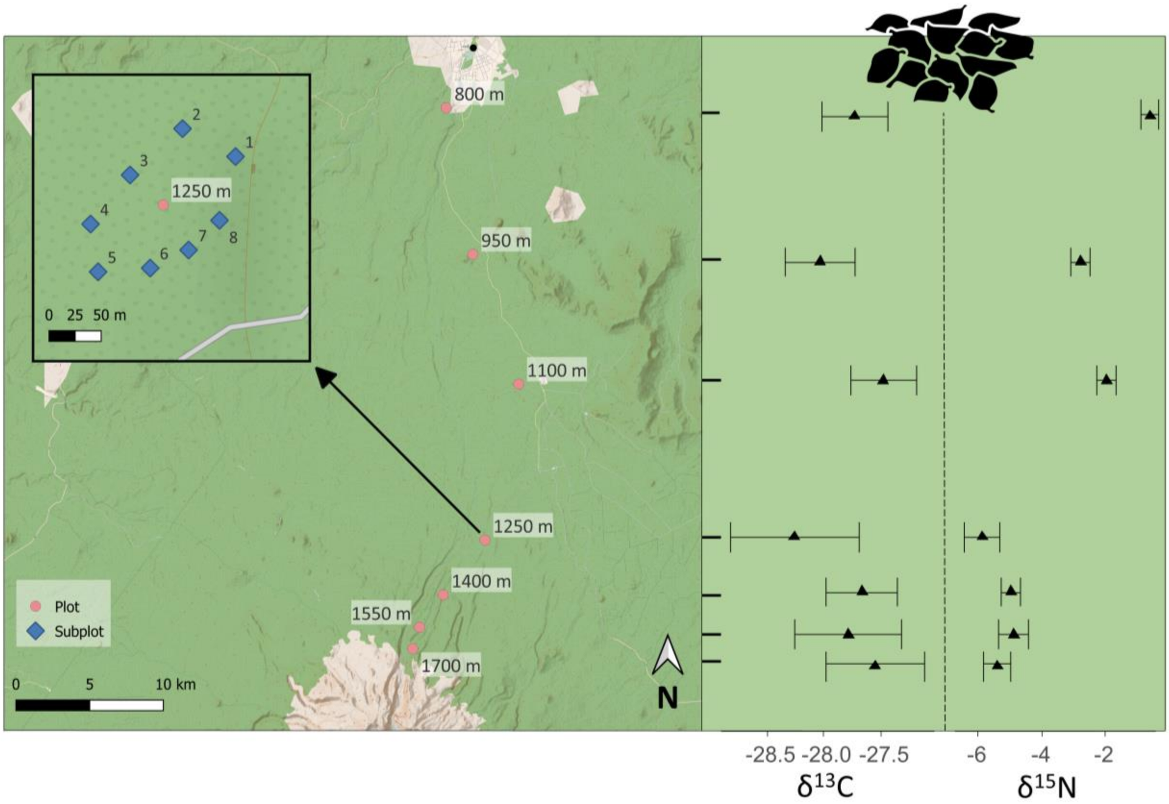

Figure 2: Map of the sampled transect at the northern slope of Changbai Mountain. The enlarged section displays the subplot structure for one of the elevations. The right panel displays the natural abundances of ^{13}C and ^{15}N of the litter for each elevation (means ± SD). Map data from "OpenTopoMap" (Erhardt et al. 2022).

The forests at the northern slope of Changbai Mountain have rarely been managed and therefore comprise mostly primary forest (Tang et al. 2011). Korean pine (*Pinus koraiensis* Siebold & Zucc.) intermixed with deciduous trees dominates between 800 and 1100 m. Above 1100 m up to 1700 m the dominant tree species is dark-bark spruce (*Picea jezoensis* var. *komarovii* Siebold & Zucc.), followed by Erman's birch (*Betula ermanii* Cham.) at higher elevation. We established seven plots of an elevation difference of 150 m between plots, along this forest transect with every plot subdivided into eight subplots (Fig. 2). The sampling took place in early September 2019. From each subplot a 10 x 10 cm^2 litter sample was taken, including the L- and F-layer. Microarthropods were extracted at room temperature using Berlese funnels (2 mm mesh size) for five days. After extraction, animals were stored in 75% ethanol.

4.3.2 Species Selection and Body Size Measurement

Collembola were determined under the microscope (Axio A1, Zeiss, Oberkochen, Germany). Specimens that needed further inspection, were either mounted in Hoyer's solution or bleached in a 4:1 glycerine-lactic acid solution. Bleached individuals were not included in bulk stable isotope measurements. Individuals were determined at species or morpho-species level using relevant literature (Potapov 1991, 2001; Thibaud et al. 2004; Jordana 2012; Sun & Wu 2012; Yu et al. 2016; Potapov et al. 2018, 2020; Weiner et al. 2019; Sun et al. 2020, 2021; Xie et al. 2019, 2022). Species accounting for the top 80 % of total abundance per elevation were used for stable isotope analysis resulting in a total of 19 species. These species were assumed to be functional representatives of Collembola communities at our study sites. Prior to isotope measurements the body size of the specimens used was measured; if more than 10 specimens were bulked only 10 specimens representing the variation in body size within the respective bulk sample were measured. However, bulked specimens were of similar body length. This allowed calculating the mean body size of the Collembola measured, resulting in multiple measurements of the same species at the same plot as indicated in Fig. 3. Further, Collembola species were grouped into three life forms (Bitzer et al. 2005; Song et al. 2016; Xie et al. 2022), i.e. surface dwelling (epedaphic), litter dwelling (hemiedaphic) and soil dwelling (euedaphic) based on Gisin (1943), Hopkin (1997), Potapov (2001) and Widenfalk et al (2015). Regardless of their contribution to 80 % total abundance, bulk stable isotopes of the three species that occurred at each of the seven elevations (*Desoria choi* Lee, *Folsomia octoculata* Handschin, *Tomocerina varia* Folsom) were measured to evaluate changes in stable isotope values of individual species with elevation.

4.3.3 Stable Isotope Measurement

Stable isotope ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ were measured using an isotopic mass spectrometer (Delta V Advantage, Thermo Electron, Bremen, Germany) coupled via an interface (Conflo III, Thermo Eclectron, Bremen, Germany) to an elemental analyser (Euro EA 3000, EuroVector S. p. A. Milano, Italy). The relative abundances of ^{15}N and ^{13}C were expressed as $\delta\ (‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$, with R_{sample} and $R_{standard}$ the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio in the sample and standard, respectively. Atmospheric N was used as standard for ^{15}N, while Vienna Pee Dee belemnite for ^{13}C. Acetanilide (C_8H_9NO) was used as internal standard.

While stable isotopes of Collembola species were measured at every subplot, stable isotope values of litter were measured at every second subplot at every elevation; only leaf / needle litter without visible damage was used. To account for different types of litter we selected a representative fraction of the litter sample, dried and milled it, then an aliquot of this homogenized sample was measured. Stable isotope values were normalized to these δ values of litter from the respective elevation and subplot (Fig. 2) and expressed as Δ^{15}N and Δ^{13}C values. We used the mean δ values of litter of the two closest subplots to normalize Collembola from the subplots where δ values were not measured. This considered the high variability of the baseline among elevations and the (minor) variations between subplots within elevations (Fig. 2).

4.3.4 Statistical Analysis

All statistical analyses were performed in R v 4.0.4 (R Core Team 2021). Four one-dimensional metrics were calculated for each Δ^{15}N and Δ^{13}C values. Metrics included the abundance weighted isotopic positions (IPos Δ^{15}N and IPos Δ^{13}C), the isotopic range (Δ^{15}N range and Δ^{13}C range) as well as the maximum (Δ^{15}N max and Δ^{13}C max) and minimum (Δ^{15}N min and Δ^{13}C min) isotopic values at subplot level. Further, five multi-dimensional stable isotope metrics were calculated including both Δ^{15}N and Δ^{13}C values as described in Cucherousset and Villéger (2015). Prior to calculating multi-dimensional metrics, Δ^{15}N and Δ^{13}C values of Collembola species were abundance weighted as described above and then scaled between 0 and 1 to equalize contributions of the two isotopes. Five multidimensional metrics were calculated. (1) Isotopic divergence (IDiv) which approaches 1 if Collembola species with extreme diverging isotopic signatures are abundant and approaches 0 if they are rare. (2) Isotopic dispersion (IDis) which approaches 1 if abundant species have diverging isotopic signatures, whereas it approaches 0 if abundant species have similar isotopic signatures. (3) Isotopic evenness (IEve) which approaches 1 if species are evenly distributed in the isotopic space, whereas it approaches 0 if species cluster in a small area of the isotopic space. (4) Isotopic uniqueness (IUni) which approaches 1 if species occupy unique positions in the isotopic space, whereas it approaches 0 if species share similar isotopic niches. (5) Isotopic richness (IRic) representing the convex hull area spanning the total isotopic space of all species; IRic was not weighted by abundance since it represents functional diversity (Villéger et al. 2008). IRic approaches 1 if the hull area is large, whereas it approaches 0 if the hull area is small.

Linear relationships of the calculated multi- and one-dimensional metrics with elevation were analysed using linear models with the respective metric as response variable and elevation as ordered categorial variable. This way we tested for a linear trend using polynomial contrasts, if the test indicated a linear relationship elevation was transformed into a continuous independent variable. Elevation was left categorial if no such trend was found to test for general differences between elevations. Metrics were square-root-transformed, if necessary (indicated in the results section).

Further, “trait flex anovas” (Lepš et al. 2011) were calculated for $\Delta^{15}N$ and $\Delta^{13}C$ values. In short, this procedure allows to decompose the variation in community weighted $\Delta^{15}N$ and $\Delta^{13}C$ values across the elevation gradient explained by species turnover and intraspecific variation by calculating three linear models with varying response variables: The first contained the community weighted isotopes per subplot per elevation, henceforth termed specific averages, as response variable (similar to IPos $\Delta^{15}N$ and IPos $\Delta^{13}C$, see above). Here, variation in the response variable may be caused by species turnover or intraspecific variability (or both). The second model contained the averages of isotopic averages of species across elevations weighted by their relative abundances per subplot per elevation, henceforth termed fixed averages. Here, variations in the response variable are only caused by species turnover. For the response variable of the third model, the fixed averages were subtracted from the specific averages. Here, variations in the response variable are only caused by intraspecific variability. Then, the sum of squares of the three models were decomposed as described in Lêps et al. (2011) to distinguish between the contribution due to species turnover, intraspecific variability and the covariation between the two.

Spearman Rank correlations of all metrics with six variables measured in the litter layer potentially linked to Collembola nutrition were tested. The fungal-to-bacterial PLFA ratio (fun/bac_{litter}), the microbial biomass per gram organic carbon (C_{mic}), the $Gram^{+}$-to-$Gram^{-}$ bacterial PLFA ratio ($Gram^{+}/Gram^{-}$), the saturated-to-monounsaturated PLFA ratio (sat/mono) and the cyclic-to-monoenoic precursor PLFA ratio (cyclo/pre) and the C/N ratio of the litter layer were included. Factors and their measurements are further characterized in Lux et al. (2022).

Linear mixed effect models were calculated using the “lme4” package (Bates et al. 2015) to analyse three common species present across all elevations. Unweighted $\Delta^{15}N$ and $\Delta^{13}C$ values of the respective species were used as response variable and elevation (continuous, selected as described above), body size and their interaction as independent variables. Body size and elevation were centred around their mean. To account for multiple measures at the same sampling location plotID was included as random intercept. Further, the influence of body size and Collembola life form on the isotopic values across the elevation gradient was investigated, including unweighted $\Delta^{15}N$ and $\Delta^{13}C$ values as response variable and elevation (continuous, selected as described above), mean body size, life from and their interaction as independent variables, again body size and elevation were centred around their mean. To account for multiple measurements of the same species at the same sampling location, plotID and species nested in plotID were included as random intercepts. To evaluate if mean body size of Collembola varies along the elevation gradient another linear mixed effect model was fitted, with body size as response variable and elevation (categorial, selected as described above) and life form as independent variables. The random intercept again included plotID and species nested in plotID. Body size (response variable) was log-transformed to increase homoscedasticity.

Significances of fixed effects were tested using type III sums of squares in the Anova function of the “car” package (Fox & Weisberg 2019). Adjusted R^2 values are given in the respective regression figures. For linear mixed effect models Pseudo R^2 of fixed effects (R^2_{fe}) and the whole model Pseudo R^2 including the random effect (R^2_{total}) are given in the respective regression figures. Values and errors given in text represent the mean ± standard deviation. All linear models met the assumptions of homoscedasticity and Gaussian distribution of residuals.

4.4 Results

4.4.1 Stable Isotope Metrics along the Elevational Gradient

Three one-dimensional metrics of $\Delta^{15}N$ values, calculated from mean species stable isotope values (Fig.3) at subplot level, differed significantly across the elevation gradient (Fig. 4a). The abundance weighted IPos $\Delta^{15}N$ ($F_{1,51} = 23.32$, $P < 0.001$) increased from 800 (0.45 ± 1.21 ‰) to 1700 m (1.76 ± 0.76 ‰); 57.5% of the total variation explained by elevation was contributed by intraspecific variability and only 5.9 % by species turnover, the other 36.7 % were contributed by the covariation between the two. Further, $\Delta^{15}N$ min increased with elevation ($F_{1,51} = 20.98$, $P < 0.001$), increasing from 800 (-1.70 ± 1.66 ‰) to 1700 m (0.15 ± 0.73 ‰, respectively). By contrast, $\Delta^{15}N$ range decreased ($F_{1,51} = 5.53$, $P = 0.023$) from 800 (4.61 ± 1.56 ‰) to 1700 m (3.39 ± 1.25 ‰) (Fig. 4a). Further, three one-dimensional metrics of $\Delta^{13}C$ values varied significantly with elevation (Fig. 4b).

The abundance weighted IPos $\Delta^{13}C$ increased significantly ($F_{1,51} = 6.70$, $P = 0.013$) from 800 (3.43 ± 0.47 ‰) to 1700 m (3.70 ± 0.72; with values at 1250, 1400 and 1550 m higher than the value at 1700 m). As in $\Delta^{15}N$, intraspecific variability contributed most to the variation in $\Delta^{13}C$ explained by elevation (35.6 %), while species turnover only contributed 16.3 % and the covariation between the two 48.1 %. Additionally, $\Delta^{13}C$ max and $\Delta^{13}C$ min varied significantly with elevation but the response was not linear ($F_{6,46} = 2.42$, $P = 0.041$, $F_{6,46} = 2.57$, $P = 0.031$, respectively); both were lowest at 800 m (4.23 ± 0.87 and 2.30 ± 0.96 ‰, respectively), but $\Delta^{13}C$ max was highest at 1550 m and $\Delta^{13}C$ min at 1250 m (5.49 ± 0.78 and 3.52 ± 0.85 ‰, respectively).

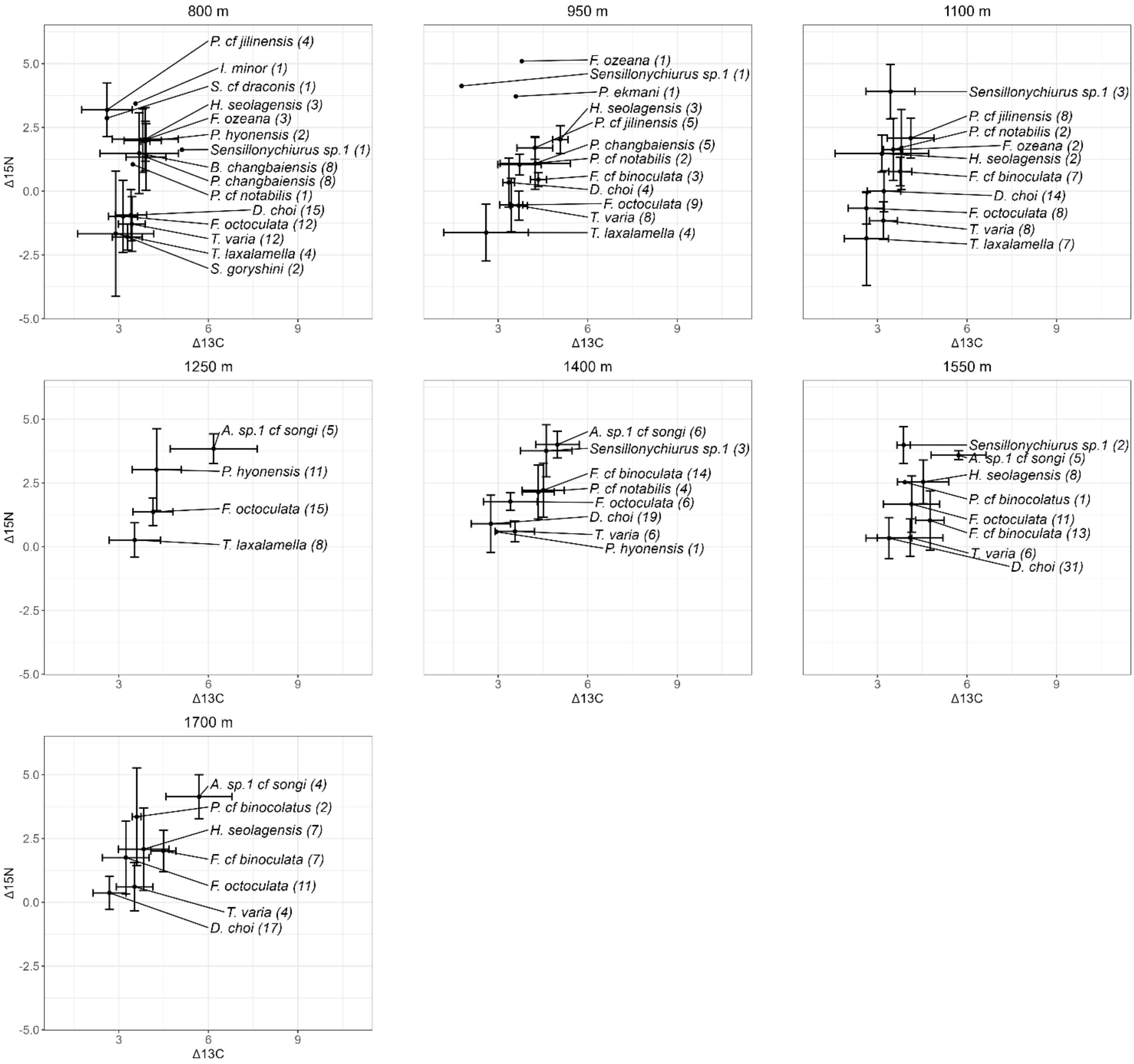

Figure 3: Stable isotope (Δ^{15}N and Δ^{13}C) biplots of 19 Collembola species comprising 80% of the total abundance of Collembola at the respective elevation. Error bars represent standard deviations. Number of isotope measurements of species at the respective elevations are given in brackets.

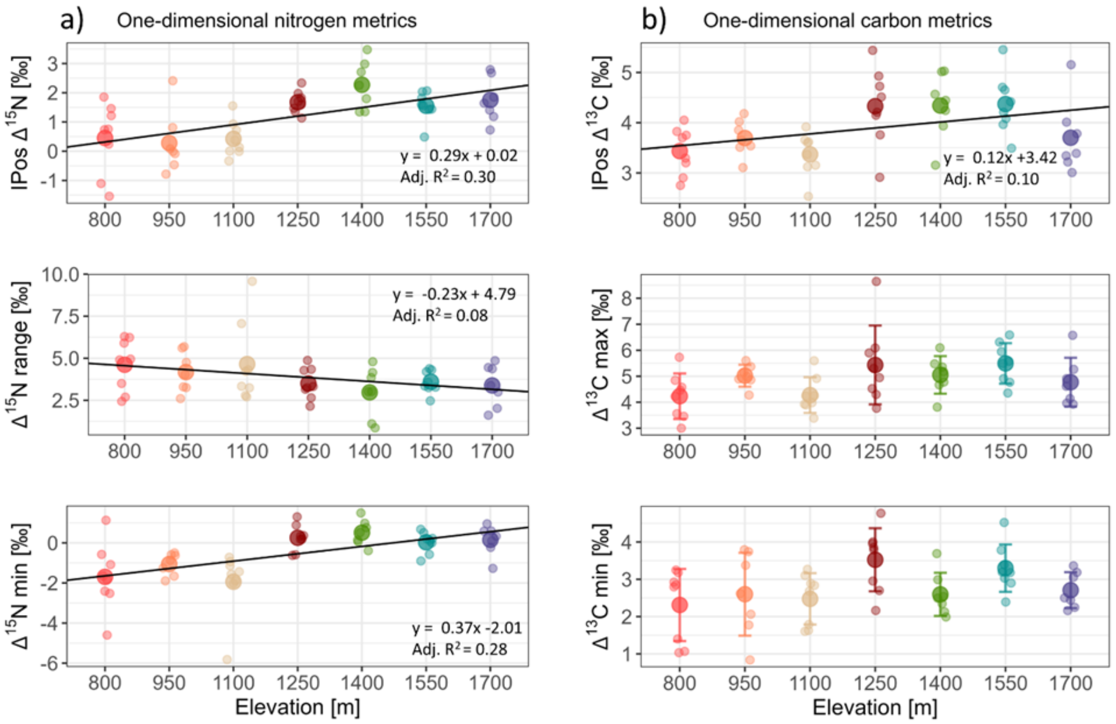

Figure 4: One- dimensional stable isotope metrics of nitrogen (a) and carbon (b), which significantly varied with elevation. Elevations are color coded; large dots represent the means and error bars standard deviations. Error bars are only displayed if the metric showed a non-linear response to elevation. Regression line formulas and adjusted R^2 values (Adj. R^2) are displayed for linear responses to elevation.

Two multidimensional metrics of Δ^{15}N and Δ^{13}C values significantly varied with elevation (Fig. 5). IRic varied marginally significantly in a non-linear way with elevation ($F_{6,46} = 2.08$, $P = 0.074$, square-root-transformed data); it was lowest at 1250 m (0.02 ± 0.02; non-transformed mean) and highest at 1100 m (0.05 ± 0.04; non-transformed mean). IUni showed a linear trend, it increased with elevation from 800 m (0.41 ± 0.15) to 1700 m (0.61 ± 0.23) ($F_{1,51} = 8.00$, $P = 0.007$). A figure including non-significant isotopic metrics is given in the Appendix (Appendix Fig. 1).

IPos Δ^{15}N and Δ^{15}N min correlated significantly positive with the C/N ratio of the litter layer as well as C_{mic}, sat/mono and cyclo/pre PLFA ratios (Table 1). Δ^{15}N max on the other hand only correlated positively to cyclo/pre ratios across the elevation gradient. IPos Δ^{13}C correlated significantly positive with C_{mic}, sat/mono and cyclo/pre PLFA ratios. Δ^{13}C min and Δ^{13}C max both correlated positively with sat/mono and cyclo/pre ratios. Of the multidimensional metrics IRic correlated negatively with the litter C/N ratio and IUni correlated negatively with the fun/bac$_{litter}$ across the elevation gradient.

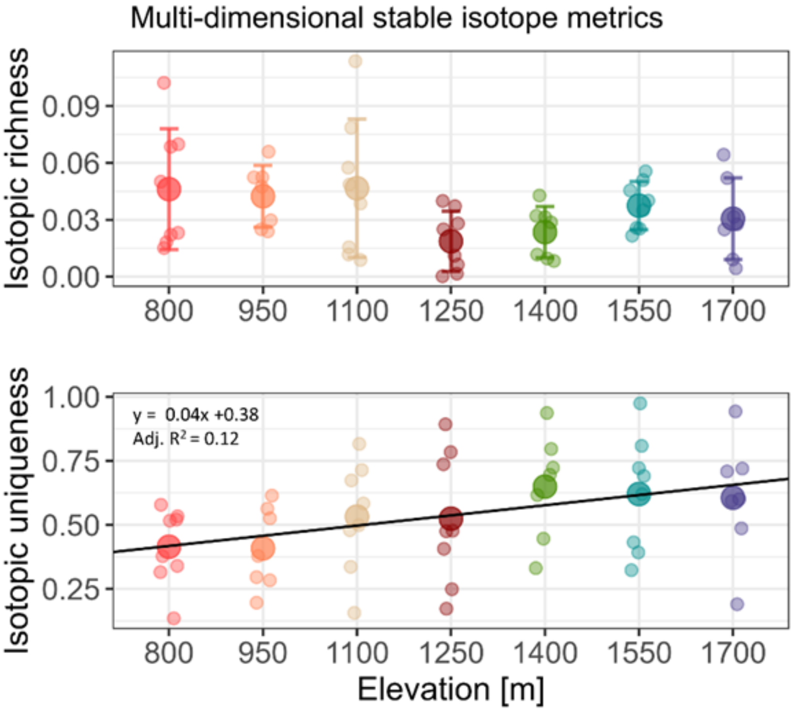

Figure 5: Multidimensional stable isotope metrics, which significantly varied with elevation. Elevations are color coded; large dots represent means and error bars standard deviations. Regression line formula and adjusted R^2 value (Adj. R^2) are displayed for the linear response to elevation. Isotopic richness was square-root transformed to approximate Gaussian distribution of residuals.

4.4.2 Response of Δ^{15}N and Δ^{13}C Values of Species to Elevation

Underlining the high contribution of intraspecific variability to increased weighted IPos Δ^{15}N with elevation, the unweighted Δ^{15}N values of three species which occurred across the studied elevation gradient (*Desoria choi*, *Folsomia octoculata* and *Tomocerina varia*) increased significantly with elevation ($\chi^2 = 10.19$, $P = 0.001$; $\chi^2 = 44.25$, $P < 0.001$; $\chi^2 = 22.70$, $P < 0.001$, respectively). Δ^{15}N values of *D. choi*, *F. octoculata* and *T. varia* increased from 800 (-0.94 ± 1.00 ‰, -0.78 ± 1.42 ‰ and -1.29 ± 1.07 ‰, respectively) to 1700 m (0.37 ± 0.65 ‰, 1.75 ± 1.43 ‰ and 0.61 ± 0.95 ‰, respectively; Appendix Fig. 2). Δ^{15}N significantly decreased with body size only in *D. choi* ($\chi^2 = 10.39$, $P = 0.001$), it decreased by 0.48 ‰ per 1000 µm body size (Appendix Fig. 2). Unweighted Δ^{13}C values of the three species, on the other hand, showed no linear trend with elevation nor body size. Even though showing no linear response, Δ^{13}C values generally varied with elevation in *D.choi and F. octoculata* ($\chi^2 = 13.08$, $P = 0.042$; $\chi^2 = 28.81$, $P < 0.001$, respectively). *D. choi* was lowest at 1700 m (2.69 ± 0.55 ‰) and highest at 800 m (3.41 ± 0.51 ‰). *F. octoculata* on the other hand was lowest at 1100 m (2.63 ± 0.60 ‰) and highest at 1250 m (4.15 ± 0.67 ‰).

Table 1: Spearman rank correlations between one- as well as multi-dimensional isotope metrics and litter characteristics [fungal-to-bacterial PLFA ratios in litter (fun/bac$_{\text{litter}}$), microbial biomass (C$_{\text{mic}}$), Gram$^+$-to-Gram$^-$, saturated-to-monounsaturated (sat/mono), and cyclic-to-monoenoic (cyclo/pre) PLFA ratios, C/N ratio] across elevations. Values represent Spearman's rho, values given in bold indicate Spearman's rho to significantly differ from zero (P < 0.05).

	factor	C/N	C$_{\text{mic}}$	fun/bac$_{\text{litter}}$	Gram$^+$/Gram	sat/mono	cyclo/pre
Nitrogen metrics	IPos Δ^{15}N	**0.34**	**0.46**	0.02	0.04	**0.44**	**0.59**
	Δ^{15}N range	-0.20	-0.15	-0.02	0.07	-0.19	-0.23
	Δ^{15}N min	**0.47**	**0.51**	0.07	-0.06	**0.46**	**0.67**
	Δ^{15}N max	0.20	-0.25	0.09	-0.05	0.16	**0.31**
Carbon metrics	IPos Δ^{13}C	0.19	**0.30**	0.03	-0.09	**0.40**	**0.52**
	Δ^{13}C range	-0.01	0.19	0.00	-0.02	0.00	0.09
	Δ^{13}C min	0.10	0.13	0.15	-0.07	**0.36**	**0.39**
	Δ^{13}C max	0.05	0.23	0.08	-0.04	**0.27**	**0.37**
Multidimensional metrics	Isotopic diversity	< 0.01	-0.06	0.08	-0.04	-0.14	-0.06
	Isotopic richness	**-0.32**	-0.04	-0.04	0.12	-0.22	-0.24
	Isotopic dispersion	-0.10	0.10	-0.11	0.27	< 0.01	-0.04
	Isotopic evenness	-0.10	0.09	-0.21	0.11	0.02	-0.09
	Isotopic uniqueness	0.03	0.23	**-0.34**	0.25	0.23	0.17

4.4.3 Changes in Δ^{15}N and Δ^{13}C Values of Collembola with Body Size and Life Form

The unweighted Δ^{15}N values of Collembola significantly increased with elevation ($\chi^2 = 32.69$, $P < 0.001$), varied with Collembola life form ($\chi^2 = 130.15$, $P < 0.001$) and decreased with mean body size of individuals per sample, henceforth body size ($\chi^2 = 30.98$, $P < 0.001$). Further, the response of Δ^{15}N values to elevation depended on life form (significant elevation × life from interaction, $\chi^2 = 28.98$, $P < 0.001$; Fig. 6a). Δ^{15}N values increased strongest between 800 and 1700 m in hemiedaphic Collembola (-0.52 ± 1.67 ‰ to 1.75 ± 1.42 ‰, respectively) and the increase was weakest in epedaphic Collembola (-1.09 ± 1.39 ‰ to 0.41 ± 0.70 ‰, respectively). The strength of the decrease of Δ^{15}N with body size also depended on life form (significant life form × body size interaction, $\chi^2 = 17.67$, $P < 0.001$; Fig. 6a). Δ^{15}N values decreased strongest with body size in hemiedaphic Collembola by 2.02 ‰ per 1000 µm body size, while there was no decrease in epedaphic Collembola with body size (<0.01 ‰ per 1000 µm body size). Additionally, the interaction between body size and life form depended on elevation (significant elevation × life from × body size interaction, $\chi^2 = 10.74$, $P = 0.005$). Variations in the response of Δ^{15}N to body size with elevation were most pronounced in hemiedaphic Collembola where Δ^{15}N decreased by 2.26 ‰ per 1000 µm body size at 800 m, but only by 0.49 ‰ per 1000 µm body size at 1700 m.

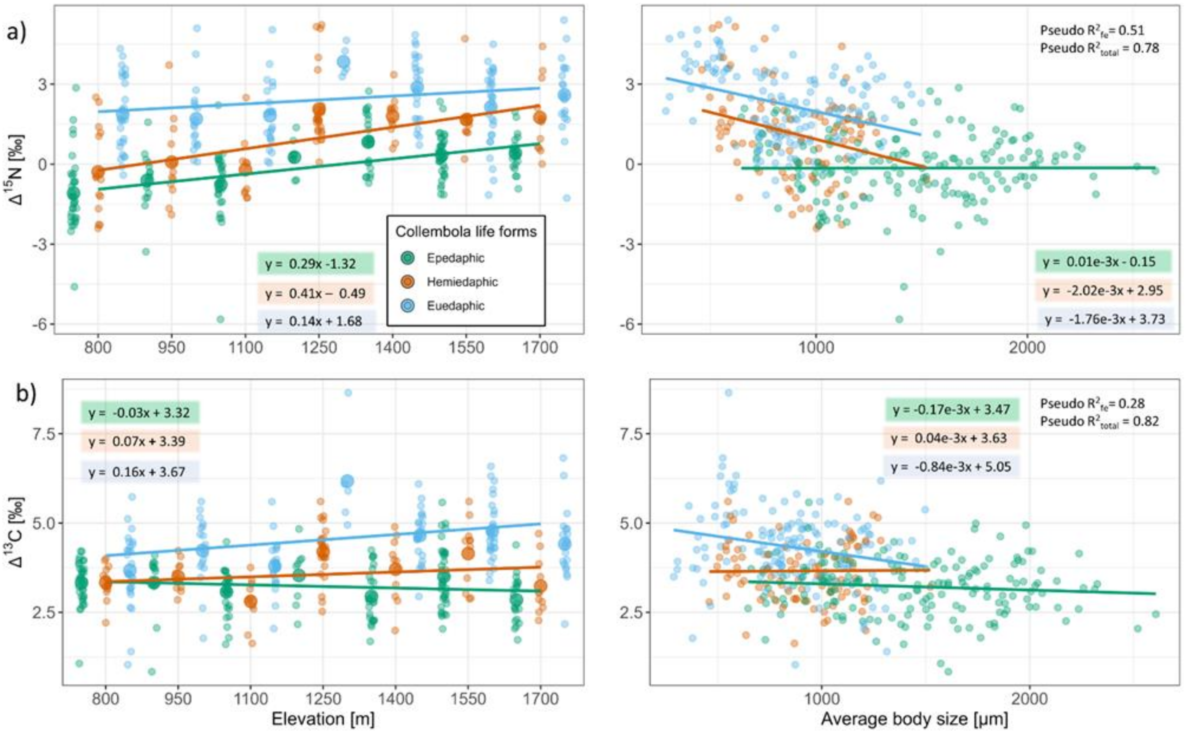

Figure 6: Δ^{15}N (a) and Δ^{13}C (b) of different Collembola life forms (epedaphic, hemiedaphic and euedaphic) across elevations and body size. Colors mark life forms; means (large dots) and individual measurements (small dots). Pseudo R^2 values refer to linear mixed effects models including Δ^{15}N or Δ^{13}C as dependent variable, elevation (continuous), body size and life form as well as their interactions as fixed effects and plot ID as well as species nested in plotID as random effect. For significant fixed effects and interactions see text.

Overall, the unweighted Δ^{13}C values of Collembola significantly varied with Collembola life form ($\chi^2 = 57.76$, $P < 0.001$). Further, the linear response of unweighted Δ^{13}C values to elevation depended on Collembola life form (significant elevation × life from interaction, $\chi^2 = 8.01$, $P = 0.018$). Δ^{13}C increased strongest from 3.67 ± 1.02 ‰ to 4.41 ± 1.01 ‰ between 800 and 1700 m in euedaphic Collembola, while there was a slight decrease in epedaphic Collembola from 800 to 1700 m (3.33 ± 0.62 ‰ to 2.85 ± 0.64 ‰, respectively; Fig. 6b). Δ^{13}C decreased marginally significantly with increasing body size ($\chi^2 = 3.45$, $P = 0.063$) by 1.04 ‰ per 1000 µm body size. The interaction between life form and body size further depended on elevation (marginally significant elevation × life form × body size interaction, $\chi^2 = 5.46$, $P = 0.065$). Variations in the response of $\Delta^{13}C$ to body size with elevation were most pronounced in hemiedaphic Collembola where $\Delta^{13}C$ decreased by 0.65 ‰ per 1000 µm body size at 800 m, while showing an increase by 1.02 ‰ per 1000 µm body size at 1700 m.

Generally, body size varied with Collembola life form ($\chi^2 = 207.97$, $P < 0.001$, log-transformed data); epedaphic Collembola were larger (1452 ± 434 µm) than hemiedaphic (920 ± 237 µm) and euedaphic Collembola (857 ± 275 µm; untransformed means). Collembola body size varied across elevations followed a non-linear response ($\chi^2 = 13.19$, $P = 0.040$). However, differences in body size of life forms varied with elevation (significant elevation × life from interaction, $\chi^2 = 32.81$, $P = 0.001$; Appendix Fig. 3). The difference in body size with life form was highest at 1250 m, where epedaphic Collembola were largest (1704 ± 210 µm), followed by hemiedaphic (883 ± 282 µm) and euedaphic Collembola (570 ± 35 µm).

4.5 Discussion

Here, we investigated trophic shifts of Collembola communities across an elevation gradient using bulk stable isotope analysis at species level. The results showed that the trophic level of Collembola communities as well as selected Collembola species increased with elevation as indicated by IPos $\Delta^{15}N$ values. Both life form (epedaphic, hemiedaphic and euedaphic) and body size influenced $\Delta^{15}N$ and $\Delta^{13}C$ values of Collembola, but their relative influence varied with elevation. Isotopic metrics including both $\Delta^{15}N$ and $\Delta^{13}C$ indicated that IRic was low at high elevation plots (1250-1700 m) and IUni increased with increasing elevation. Overall, our results indicate a decrease in Collembola acting as primary decomposers towards higher elevations where microbial resources prevail.

4.5.1 Influence of Elevation on Trophic Niches of Collembola

The trophic position of (functional) Collembola communities increased by about one third trophic level across the studied elevation gradient from 800 to 1700 m assuming an enrichment factor of 3.4 ‰ $\Delta^{15}N$ per trophic level (Post 2002, Potapov 2019b), which is in line with our first hypothesis. This shift is driven predominantly by intraspecific variations rather than species turnover. Large Collembola, such as *Tomocerus laxalamella* and *Tomocerina varia*, likely feed on litter and this is reflected by their negative $\Delta^{15}N$ values at lower elevations. Negative $\Delta^{15}N$ values of primary decomposers have been reported before for Collembola, Diplopoda and Oribatida (Scheu & Falca 2000, Pollierer et al. 2009, Schneider & Maraun 2011, Maraun et al. 2023) and presumably reflect that they feed on certain litter components depleted in ^{15}N compared to bulk litter material, as shown for ^{13}C of e.g., lignin or lipids (Pollierer et al. 2009). Even though lower $\Delta^{15}N$ values may also indicate algal or lichen feeding (Maraun et al. 2023), NLFA analyses at the same sampling location and date indicate that algae are negligible as food resource for Collembola at Changbai Mountain (Lux et al. 2023). Lichen feeders usually have even lower $\Delta^{15}N$ values as shown by Chahartaghi et al. (2005) who grouped Collembola species with $\Delta^{15}N$ values similar to those of *T. laxalamella* and *T. varia* as primary decomposers. As $\Delta^{15}N$ values of these putative primary decomposer species were most negative at lower elevations, the significant increase in minimum $\Delta^{15}N$ values and the decreasing range in $\Delta^{15}N$ values indicate a decrease in primary decomposers along the elevation gradient. Even though generally low across all elevations, IRic was especially low at higher elevations (1250-1700 m), which are characterized by pure coniferous forests. Decreases in primary decomposers consequently led to a smaller isotopic hull area.

IPos $\Delta^{15}N$ values and $\Delta^{15}N$ min positively correlated with C/N ratio of litter as well as microbial biomass, indicating that the shift from living predominantly as primary decomposer at lower elevation to living more as secondary decomposers at higher elevation, feeding either on living microorganisms or microbial residues, was driven by lower litter quality (i.e., higher C/N ratio). The negative correlation between IRic and C/N ratio also reflects this shift. Conform to this conclusion, Fujii et al. (2018) found the contribution of soil microarthropods to litter decomposition to be higher in nutrient rich than nutrient poor litter. By contrast, Ma et al. (2019) concluded the contribution of soil fauna to litter (lignin) decomposition to be higher in more recalcitrant litter at Changbai Mountain. These contrasting results may implicate that soil fauna taxa other than Collembola are more involved in fragmentation of recalcitrant compounds such as lignin at higher elevations. The contradictory results could also be due to seasonal variations, which were not considered in our study and may be important for resource availability, especially at lower elevations where deciduous trees are more prevalent.

Generally, Collembola have been shown to preferentially feed on fungi (Pollierer & Scheu 2021, Li et al. 2022, Lux et al. 2023). However, as indicated by results of our study, a large fraction of the (functional) Collembola community may also predominantly feed on litter and only switch to feeding on resources of higher trophic level if nutrient limitation increases. This is also reflected by the correlation between Δ^{15}N min (as well as IPos Δ^{15}N) values of Collembola and the sat/mono and the cyclo/pre PLFA ratio in the litter layer, both reflecting nutritional and substrate-induced stress of microorganisms (Bossio & Scow 1997, Moore-Kucera & Dick 2008). Forests at higher elevations at Changbai Mountain are dominated by dark bark spruce (Liu 1997) and decomposition processes in spruce forests typically are slower than in deciduous forests (Albers et al. 2004; Berger & Berger 2012). Slow litter decomposition and associated microbial stress may have promoted the trophic shift in Collembola towards increased feeding on microbial resources at higher elevations. In plants it is well documented that increased stress results in increased herbivory (White 1993) and the same may hold true for microorganisms.

In addition to the increase in IPos Δ^{15}N values with elevation, also Δ^{15}N values of individual Collembola species, such as *Desoria choi*, *Folsomia octoculata* and *Tomocerina varia*, increased at higher elevation indicating that both Collembola communities, but also individual species, shifted their diet towards feeding more on microorganisms, microbial residues or other isotopic enriched resources at higher elevation. In fact, our study shows that most of the shift in community weighted trophic positions across elevations is due to intraspecific variations rather than species turnover. This is likely due to few very abundant species; for example, the trophic shift was particularly strong in *F. octoculata* indicating high trophic plasticity in this species. This is in line with findings of Hishi et al. (2007), who found this species to colonize different successional stages of litter. At Changbai Mountain, *F. octoculata* is among the most dominant Collembola species in forests across the studied elevation gradient (Xie et al. 2022). Its dominance across elevations likely also reflects its high trophic plasticity living as primary decomposer at lower elevations and as secondary decomposer at higher elevations, consuming microbes, microbial residues and/or other isotopic enriched resources. In fact, *F. octoculata* was found to incorporate root-derived carbon (Fujii et al. 2016) and the higher trophic position at higher elevations therefore may be related to increased feeding on mycorrhizal fungi.

Besides these variations within species, the reported trophic shifts of Collembola communities were, at least to a certain extent, also due to species turnover. As shown recently, Collembola communities at Changbai Mountain are structured by the availability of food resources as indicated by litter and soil C/N ratios (Xie et al. 2022). Further, Collembola at high elevations likely occupy more unique trophic positions in the isotopic space, as indicated by the increases in IUni (Cucherousset and Villéger 2015). IUni was negatively correlated with the fun/bac_{litter} indicating that trophic niches additionally show a larger overlap if fungal resources become more abundant.

Therefore, our results suggest that trophic changes with elevation are driven by resource availability. The ability of Collembola to access resources in different microhabitats in the litter/soil matrix might consequently alter their trophic response to elevation (Erktan et al. 2020). The ability of accessing resources in the soil matrix is determined by Collembola life forms and their respective foraging strategies (Fig. 1). Among Collembola life forms, the shift in $\Delta^{15}N$ values with elevation was strongest in hemiedaphic Collembola (including *F. octoculata*), with the values shifting from close to the ones in epedaphic species at 800 m towards the ones of euedaphic species at 1700 m, which is in line with our second hypothesis. Hemiedaphic Collembola likely are able to access similar resources as those used by euedaphic Collembola as body size in both groups was overall similar. Supporting the overlap in resource use by hemi- and euedaphic species, Fujii & Takeda (2012) found epedaphic Collembola species to predominantly colonize leaf litter placed on top of the soil in a coniferous forest, while leaf litter placed into the soils were colonized by both hemi- and euedaphic Collembola.

Even though Collembola life forms usually differ in their body size (Rusek 2007), body size variation within life forms likely also plays a role especially for species migrating vertically (Fig. 1). Accordingly, $\Delta^{15}N$ values of hemiedaphic and euedaphic, but not in epedaphic Collembola decreased with body size. Presumably, this reflects that foraging strategies differ among Collembola life forms supporting our third hypothesis. As stressed repeatedly, traits of epedaphic Collembola, such as large body size, a long furca and long antennae, hamper access to deeper soil layers, whereas smaller body size, and shorter (or absent) furca and shorter antennae of hemi- and euedaphic species allow access to food resources in smaller soil pores deeper in soil (Hopkin 1997, Erktan et al. 2020). Consequently, hemiedaphic Collembola may be able to shift resources towards those of euedaphic Collembola if necessary, i.e. if other food resources are lacking or are of poor food quality. Organic matter in deeper soil layers typically is enriched in ^{15}N and ^{13}C compared to litter material on top of the soil and this also likely is true for microorganisms (Wallander et al. 2004, Potapov et al. 2019a). Consequently, higher $\Delta^{15}N$ values in hemi- and euedaphic than in epedaphic

Collembola may, at least in part, be due to feeding on microorganisms or their residues deeper in soil (Potapov et al. 2019a). Supporting our conclusion that the switch of hemiedaphic Collembola to resources deeper in soil is due to poor litter resources at higher elevation, Xie et al. (2022) found the relative abundance of epedaphic species to decrease with increasing elevation at Changbai Mountain.

Feeding on litter resources may be facilitated by increased mandible size which is known to correlate with body size allowing to chew litter materials to get access to litter resources (Raymond-Léonard et al. 2019). Even though the relationship between body size and mandible function is more complex, the negative relationship between Δ^{15}N values and Collembola body size support this inference, reflecting that litter material is more likely consumed by larger Collembola, while smaller Collembola preferentially consume microorganisms or microbial residues. However, epedaphic Collembola had low Δ^{15}N values irrespective of body size, indicating that body size related variations in litter consumption are more important in hemi- and euedaphic species. This is also supported by the fact that Δ^{13}C values of epedaphic Collembola were lowest among Collembola life forms and varied little with body size. However, as indicated by the interaction between body size, life form and elevation for both Δ^{15}N and Δ^{13}C, the response of trophic niches of Collembola to body size in different lifeforms varies between ecosystems, suggesting that it depends on local habitat conditions.

Interestingly, in some species, such as *F. octoculata* and *T. varia*, neither Δ^{15}N nor Δ^{13}C values significantly varied with body size, indicating that the trophic niche of these species does not change during development. However, in other species, such as *D. choi*, Δ^{15}N values, but not Δ^{13}C values, decreased significantly with body size indicating a shift from a more microbial-based diet in juveniles towards including more litter resources in adults. However, this shift in Δ^{15}N was small despite the body size of *D. choi* varied considerably. Generally, the results indicate that trophic positions within Collembola species vary little with body size and thus developmental stages.

The increase in IPos Δ^{13}C, Δ^{13}C min and Δ^{13}C max values along the studied elevation gradient was higher than expected from the increase in trophic position by one third trophic level (Post et al. 2002) indicating that Collembola at higher elevations use resources more enriched in ^{13}C. Similar to Δ^{15}N values, Δ^{13}C values of Collembola correlated positively with microbial biomass supporting the conclusion above that they more intensively consume microorganisms. In fact, Collembola at Changbai Mountain have been found to predominantly feed on fungi (Lux et al. 2023) and increased consumption of fungi deeper in soil by hemiedaphic Collembola may have contributed to the shift in Δ^{15}N and Δ^{13}C values of the Collembola community at higher elevation. Additionally, the increase in IPos Δ^{13}C, Δ^{13}C min and Δ^{13}C max values of Collembola with increasing microbial stress underlines that adverse conditions in the litter layer at higher elevations contributed to the shift in resource use of Collembola.

4.5.2 Conclusions

Results of the present study indicate that (functional) Collembola communities, as major decomposer animals in soil involved in litter decomposition, shift towards more feeding on microbes, microbial residues or even living as predators or scavengers at higher elevations. Trophic plastic species occurring at all elevations shifted towards higher trophic position and decomposing variations in $\Delta^{15}N$ values indicated that the shift in Collembola communities towards higher trophic positions was mainly due to intraspecific shifts. The results further showed that the ability to access alternative food resources depends on Collembola life form and body size. The shift from lower to higher trophic positions with elevation suggests that global climate change, driving forest conversions, may be associated by a shift from decomposer soil microarthropods functioning as secondary decomposers towards functioning as primary decomposers in a warmer future climate, with potentially pronounced ramifications for decomposition processes, humus formation and carbon sequestration.

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4.7 References

Albrich K, Rammer W, Seidl R (2020) Climate change causes critical transitions and irreversible alterations of mountain forests. Glob Change Biol 26:4013–4027. https://doi.org/10.1111/gcb.15118

Bates D, Maechler M, Bolker B, et al (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Bhardwaj M, Uniyal VP, Sanyal AK, Singh AP (2011) Butterfly communities along an elevational gradient in the Tons Valley, Western Himalayas: Implications of rapid assessment for insect conservation. J Asia-Pac Entomol 15:207–217. https://doi.org/10.1016/j.aspen.2011.12.003

Bitzer RJ, Rice ME, Pilcher CD, et al (2005) Biodiversity and community structure of epedaphic and euedaphic springtails (Collembola) in transgenic rootworm Bt corn. Environ Entomol 34:1346–1376. https://doi.org/10.1093/ee/34.5.1346

Blake JG, Loiselle BA (2000) Diversity of birds along an elevational gradient in the Cordillera Central, Costa Rica. The Auk 117:663–686. https://doi.org/10.1642/0004-8038(2000)117[0663:DOBAAE]2.0.CO;2

Bokhorst S, (Ciska) Veen GF, Sundqvist M, et al (2018) Contrasting responses of springtails and mites to elevation and vegetation type in the sub-Arctic. Pedobiologia 67: 57-64. https://doi.org/10.1016/j.pedobi.2018.02.004

Bossio DA, Scow KM (1997) Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb Ecol 35:265–278. https://doi.org/10.1007/s002489900082

Caravaca F, Ruess L (2014) Arbuscular mycorrhizal fungi and their associated microbial community modulated by collembola grazers in host plant free substrate. Soil Biol Biochem 69:25–33. https://doi.org/10.1016/j.soilbio.2013.10.032

Chahartaghi M, Langel R, Scheu S, Ruess L (2005) Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biol Biochem 37:1718–1725. https://doi.org/10.1016/j.soilbio.2005.02.006

Chen L, Wu S, Pan T (2011) Variability of climate-growth relationships along an elevation gradient in the Changbai Mountain, Northeastern China. Trees - Struct Funct 25:1133–1139. https://doi.org/10.1007/s00468-011-0588-0

Cucherousset J, Villéger S (2015) Quantifying the multiple facets of isotopic diversity: New metrics for stable isotope ecology. Ecol Indic 56:152–160. https://doi.org/10.1016/j.ecolind.2015.03.032

Dakhil MA, Halmy MWA, Liao Z, et al (2021) Potential risks to endemic conifer montane forests under climate change: integrative approach for conservation prioritization in southwestern China. Landsc Ecol 36:3137–3151. https://doi.org/10.1007/s10980-021-01309-4

Erhardt S, Hochreuther P, Schütz M, Berger M (2022) OpenTopoMap - Topographische Karten aus Openstreetmap. In: https://opentopomap.org

Erktan A, Or D, Scheu S (2020) The physical structure of soil: Determinant and consequence of trophic interactions. Soil Biol Biochem 148:107876. https://doi.org/10.1016/j.soilbio.2020.107876

Fischer BM, Meyer E, Maraun M (2014) Positive correlation of trophic level and proportion of sexual taxa of oribatid mites (Acari: Oribatida) in alpine soil systems. Exp Appl Acarol 63:465–479. https://doi.org/10.1007/s10493-014-9801-3

Fox J, Weisberg S (2019) An R companion to applied regression. Sage, Thousand Oaks, United States

Fujii S, Takeda H (2012) Succession of collembolan communities during decomposition of leaf and root litter: Effects of litter type and position. Soil Biol Biochem 54:77–85. https://doi.org/10.1016/j.soilbio.2012.04.021

Fujii S, Mori AS, Kominami Y, et al (2016) Differential utilization of root-derived carbon among collembolan species. Pedobiologia 59: 225–227. https://doi.org/10.1016/j.pedobi.2016.05.001

Garten CT, Cooper LW, Post III WM, Hanson PJ (2000) Climate controls on forest soil C isotope rations in the southern Appalachian Mountains. Ecology 81:1108–1119. https://doi.org/10.1890/0012-9658(2000)081[1108:ccofsc]2.0.co;2

Gisin H (1943) Ökologie und Lebensgemeinschaften der Collembolen im schweizerischen Exkursionsgebiet Basels. Zoologische Anstalt Basel, Basel, Switzerland

Hishi T, Hyodo F, Saitoh S, Takeda H (2007) The feeding habits of Collembola along decomposition gradients using stable carbon and nitrogen isotope analyses. Soil Biol Biochem 39:1820–1823. https://doi.org/10.1016/j.soilbio.2007.01.028

Hodkinson ID (2005) Terrestrial insects along elevation gradients: Species and community responses to altitude. Biol Rev Camb Philos Soc 80:489–513. https://doi.org/10.1017/S1464793105006767

Hopkin SP (1997) Biology of Springtails. Oxford University Press, Oxford, Great Britain

Jordana R (2012) Synopses on Palearctic Collembola: Capryinae & Entomobryini. Museum of Natural History Görlitz, Görlitz, Germany

Klarner B, Ehnes RB, Erdmann G, et al (2014) Trophic shift of soil animal species with forest type as indicated by stable isotope analysis. Oikos 123:1173–1181. https://doi.org/10.1111/j.1600-0706.2013.00939.x

Klarner B, Maraun M, Scheu S (2013) Trophic diversity and niche partitioning in a species rich predator guild –Natural variations in stable isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$) of mesostigmatid mites (Acari, Mesostigmata) from Central European beech forests. Soil Biol Biochem 57:327–333. https://doi.org/10.1016/j.soilbio.2012.08.013

Leidinger J, Blaschke M, Ehrhardt M, et al (2021) Shifting tree species composition affects biodiversity of multiple taxa in Central European forests. For Ecol Manag 498:1119552. https://doi.org/10.1016/j.foreco.2021.119552

Lepš J, De Bello F, Šmilauer P, et al (2011) Community trait response to environment: disentangling species turnover vs intraspecific trait variability effects. Ecography 34: 856–863. https://doi.org/10.1111/j.1600-0587.2010.06904.x

Li Z, Bluhm SL, Scheu S, Pollierer MM (2022) Amino acid isotopes in functional assemblages of Collembola reveal the influence of vertical resource heterogeneity and root energy supply on trophic interactions in soil food webs. Soil Biol Biochem 174:108815. https://doi.org/10.1016/j.soilbio.2022.108815

Liu QJ (1997) Structure and dynamics of the subalpine coniferous forest on Changbai Mountain, China. Plant Ecol 132:97–105. https://doi.org/10.1023/A:1009705319128

Lux J, Xie Z, Sun X, et al (2022) Changes in microbial community structure and functioning with elevation are linked to local soil characteristics as well as climatic variables. Ecol Evol 12:e9632. https://doi.org/10.1002/ece3.9632

Lux J, Xie Z, Sun X, et al (2024) Trophic positions of soil microarthropods in forests increase with elevation, but energy channels remain unchanged. Ecosphere (in press)

Maraun M, Augustin D, Müller J, et al (2014) Changes in the community composition and trophic structure of microarthropods in sporocarps of the wood decaying fungus *Fomitopsis pinicola* along an altitudinal gradient. Appl Soil Ecol 84:16–23. https://doi.org/10.1016/j.apsoil.2014.06.004

Maraun M, Thomas T, Fast E, et al (2023) New perspectives on soil animal trophic ecology through the lens of C and N stable isotope ratios of oribatid mites. Soil Biol Biochem 177:108890. https://doi.org/10.1016/j.soilbio.2022.108890

Matskovsky V, Venegas-González A, Garreaud R, et al (2021) Tree growth decline as a response to projected climate change in the 21st century in Mediterranean mountain forests of Chile. Glob Planet Change 198:. https://doi.org/10.1016/j.gloplacha.2020.103406

Mccain CM (2005) Elevational gradients in diversity of small mammals. Rep Ecol 86:366–372. https://doi.org/10.1890/03-3147

Moore-Kucera J, Dick RP (2008) PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microb Ecol 55:500–511. https://doi.org/10.1007/s00248-007-9295-1

Pan X, Xie Z, Sun X, et al (2023a) Changes in oribatid mite community structure along two altitudinal gradients in Asia and Europe as related to environmental factors. Appl Soil Ecol 189:104912. https://doi.org/10.1016/j.apsoil.2023.104912

Pan X, Xie Z, Zhou Z, et al (2023b) Variations in trophic niches of soil microarthropods with elevation in two distant mountain regions in Eurasia as indicated by stable isotopes (^{15}N, ^{13}C). Soil Biology and Biochemistry 185: 109162. https://doi.org/10.1016/j.soilbio.2023.109162

Pollierer MM, Langel R, Scheu S, Maraun M (2009) Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios (^{15}N/^{14}N and ^{13}C/^{12}C). Soil Biol Biochem 41:1221–1226. https://doi.org/10.1016/j.soilbio.2009.03.002

Pollierer MM, Scheu S (2021) Stable isotopes of amino acids indicate that soil decomposer microarthropods predominantly feed on saprotrophic fungi. Ecosphere 12:e03425. https://doi.org/10.1002/ecs2.3425

Ponsard S, Arditi R (2000) What can stable isotopes (^{15}N and ^{13}C) tell about the food web of soil macro-invertebrates? Ecology 81:852–864. https://doi.org/10.2307/177382

Post DM (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83:703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Potapov AM, Semenina EE, Korotkevich AY, et al (2016) Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. Soil Biol Biochem 101:20–31. https://doi.org/10.1016/j.soilbio.2016.07.002

Potapov AM, Scheu S, Tiunov AV (2019a) Trophic consistency of supraspecific taxa in below-ground invertebrate communities: Comparison across lineages and taxonomic ranks. Funct Ecol 33:1172–1183. https://doi.org/10.1111/1365-2435.13309

Potapov AM, Brose U, Scheu S, Tiunov AV (2019b) Trophic Position of Consumers and Size Structure of Food Webs across Aquatic and Terrestrial Ecosystems. Am Nat 194:823–839. https://doi.org/doi.org/10.1086/705811

Potapov AM, Tiunov AV, Scheu S (2019c) Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biol Rev 94:37–59. https://doi.org/10.1111/brv.12434

Potapov M (1991) Species of the genus *Isotoma* subgenus *Parisotoma* and *Sericeotoma* subgen. nov. (Collembola, Isotomidae) of USSR fauna. Acta Zool Cracoviensia 34:267–301

Potapov M (2001) Synopses on Palearctic Collembola: Isotomidae. Museum of Natural History Görlitz, Görlitz, Germany

Potapov M, Hasegawa M, Kuznetsova N, et al (2018) Isotomidae of Japan and the Asiatic part of Russia. I. *Folsomia ‘inoculata’* group. ZooKeys 750:1–40. https://doi.org/10.3897/zookeys.750.22764

Potapov M, Xie Z, Kuprin A, Sun X (2020) The genus *Semicerura* (Collembola; Isotomidae) in Asia. Zootaxa 4751:105–118. https://doi.org/10.11646/zootaxa.4751.1.5

R Core Team (2021) R: A language and environment for statistical computing

Raymond-Léonard LJ, Gravel D, Handa IT (2019) A novel set of traits to describe Collembola mouthparts: taking a bite out of the broad chewing mandible classification. Soil Biol Biochem 138:107608. https://doi.org/10.1016/j.soilbio.2019.107608

Rusek J (1998) Biodiversity of Collembola and their functional role in the ecosystem. Biodivers Conserv 7:1207–1219. http://dx.doi.org/10.1023/A:1008887817883

Rusek J (2007) A new classification of Collembola and Protura life forms. Contrib Soil Zool Cent Eur II:109–115

Samson DA, Rickart EA, Gonzales PC (1997) Ant diversity and abundance along an elevational gradient in the Philippines. Biotropica 29: 349-363. https://doi.org/10.1111/j.1744-7429.1997.tb00436.x

Scheu S, Falca M (2000) The soil food web of two beech forests (*Fagus sylvatica*) of contrasting humus type: stable isotope analysis of a macro- and mesofauna-dominated community. Oecologica 123:285–286. https://doi.org/10.1007/s004420051015

Song L, Liu J, Yan X, et al (2016) Euedaphic and hemiedaphic Collembola suffer larger damages than epedaphic species to nitrogen input. Environ Pollut 208:413–415. https://doi.org/10.1016/j.envpol.2015.10.008

Sun X (2021) Biodiversity of Jilin Province: Collembola. Jilin Education Publishing House, Changchun, China

Sun X, Deharveng L, Bedos A, et al (2020) Changes in diversity and body size of Onychiurinae (Collembola: Onychiuridae) along an altitudinal gradient in Changbai Mountain, China. Soil Ecol Lett 2:230–239. https://doi.org/10.1007/s42832-020-0040-8

Sun X, Wu DH (2012) Two new species of the genus *Sensillonychiurus* Pomorski et Sveenkova, 2006 (Collembola: Onychiuridae) from Changbai Mountains, China. Ann Zool 62:563–570. https://doi.org/10.3161/000345412X659623

Tang L, Li A, Shao G (2011) Landscape-level forest ecosystem conservation on Changbai Mountain, China and North Korea (DPRK). Mt Res Dev 31:169–175. https://doi.org/10.1659/MRD-JOURNAL-D-10-00120.1

Thibaud J, Schulz H, da Gama Assalino MM (2004) Synopses on Palearctic Collembola: Hypogastruridae. Museum of Natural History Görlitz, Görlitz, Germany

Villéger S, Mason NWH, Mouillot D (2008) New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecology 89:2290–2301. https://doi.org/10.1890/07-1206.1

Wallander H, Göransson H, Rosengren U (2004) Production, standing biomass and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types. Oecologia 139:89–97. https://doi.org/10.1007/s00442-003-1477-z

Weiner WM, Xie Z, Li Y, Sun X (2019) *Ceratophysella* species from mushrooms in China (Collembola, Hypogastruridae). ZooKeys 822:67–77. https://doi.org/10.3897/zookeys.822.30880

White TCR (1993) The inadequate environment - nitrogen and abundance of animals. Springer Berlin, Heidelberg, Germany

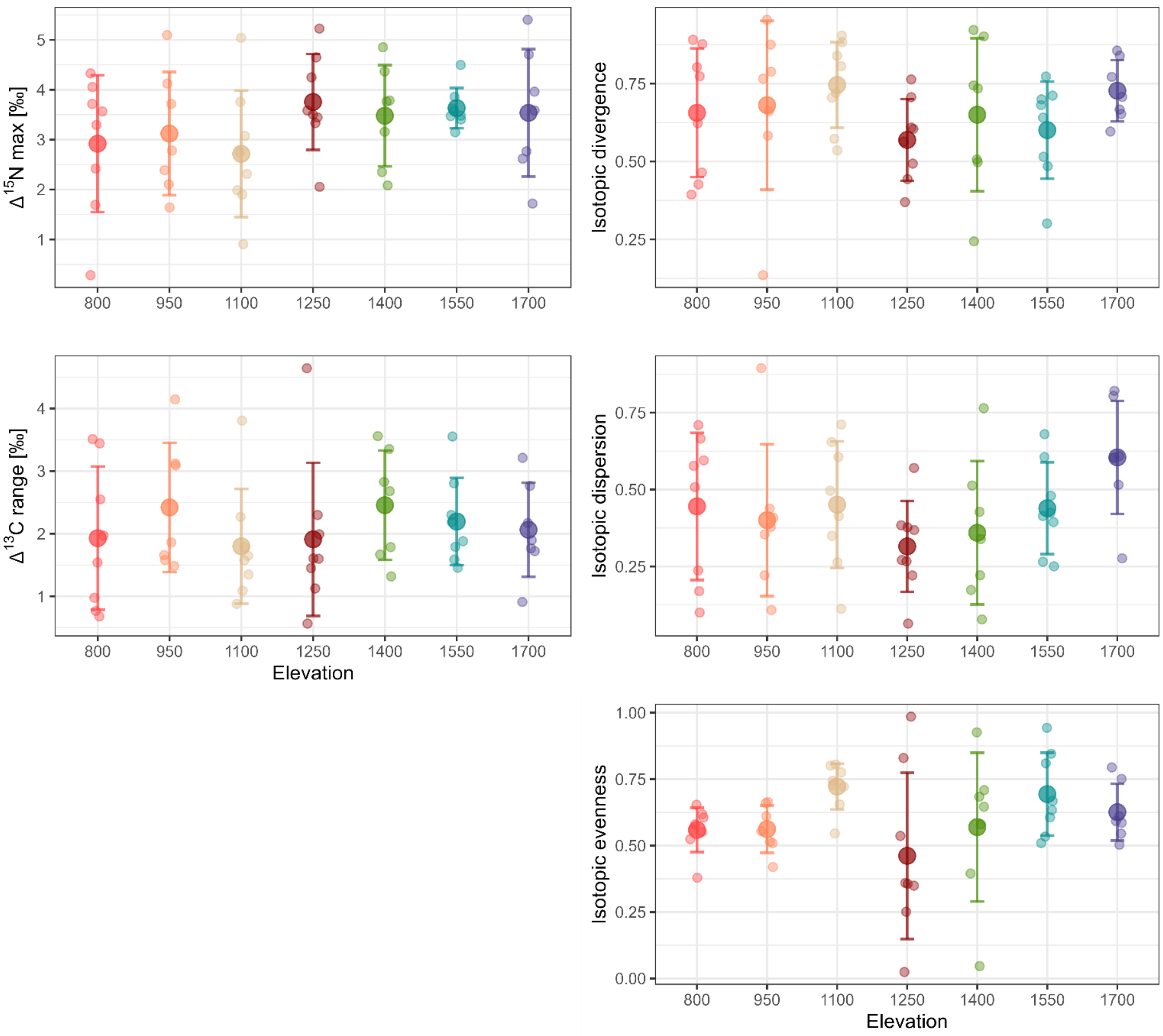
Widenfalk LA, Bengtsson J, Berggren Å, et al (2015) Spatially structured environmental filtering of collembolan traits in late successional salt marsh vegetation. Oecologia 179:537–549. https://doi.org/10.1007/s00442-015-3345-z

Xie Z, Potapov M, Sun X (2019) Two new species of the genus *Tetracanthella* (Collembola; Isotomidae) from China. Zootaxa 4585:573–580. https://doi.org/10.11646/zootaxa.4585.3.11

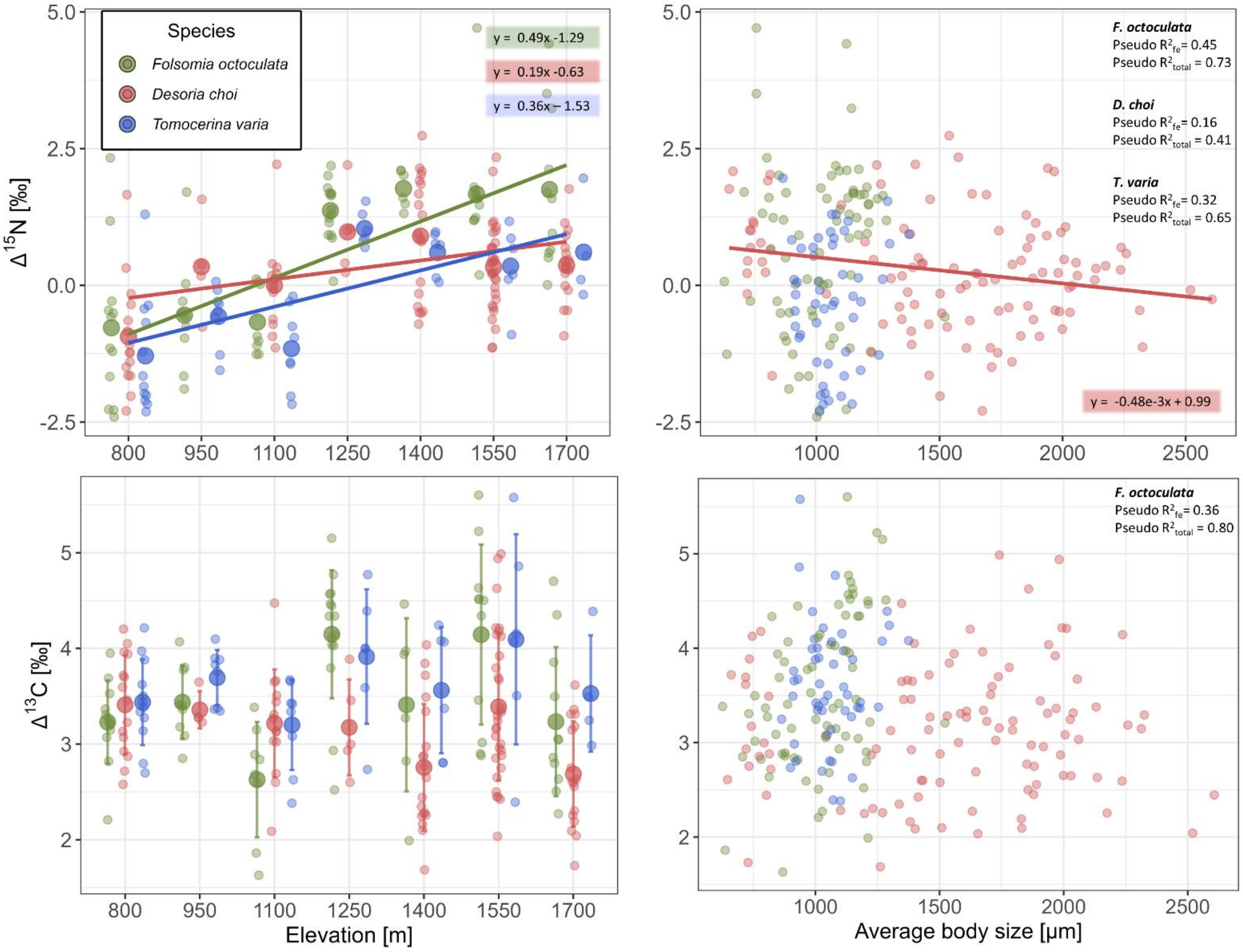
Xie Z, Sun X, Lux J, et al (2022) Drivers of Collembola assemblages along an altitudinal gradient in Northeast China. Ecol Evol 12:e8559. https://doi.org/10.1002/ece3.8559

Yu D, Zhang F, Stevens MI, et al (2016) New insight into the systematics of Tomoceridae (Hexapoda, Collembola) by integrating molecular and morphological evidence. Zool Scr 45:286–299. https://doi.org/10.1111/zsc.12149

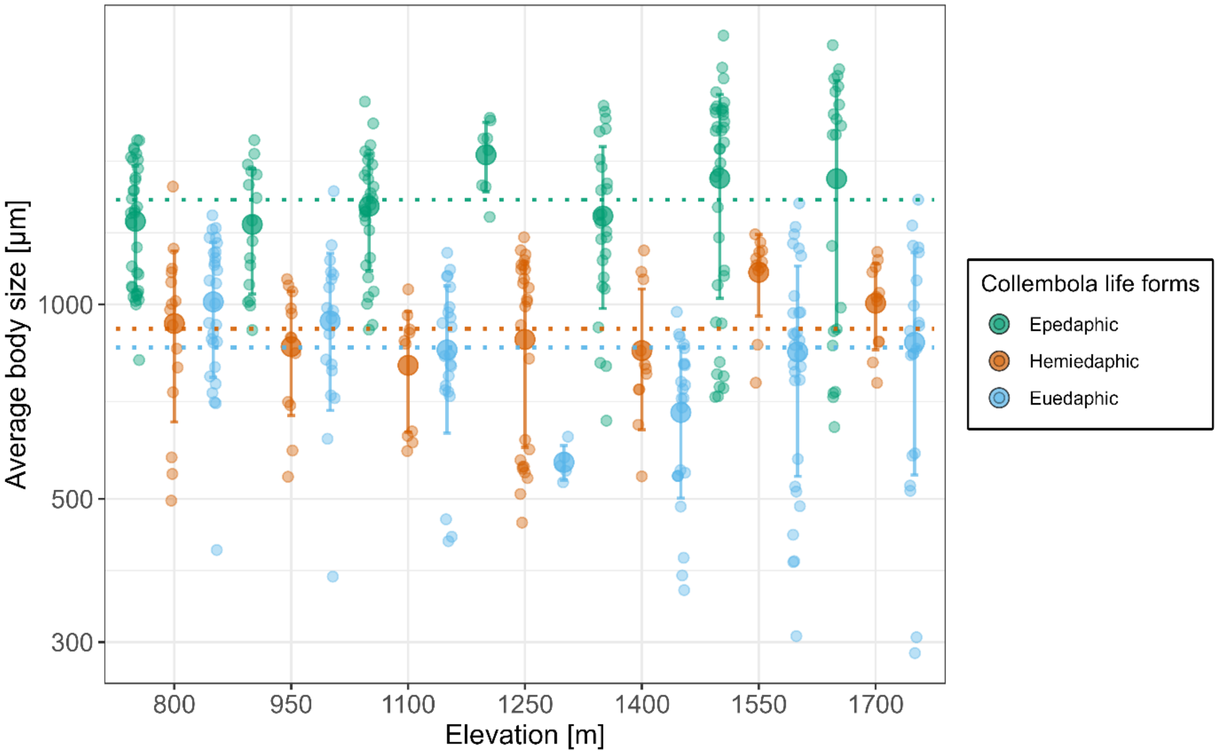
4.8 Appendix



Appendix Figure 1: Non-significant one- and multidimensional metrics across elevation. Elevations are color coded; large dots represent the means and error bars standard deviations.



Appendix Figure 2: Δ^{15}N of Folsomia octoculata, Desoria choi and Tomcerina varia (marked in color) across the studied elevation gradient. Larger dots represent means, small dots individual measurements. Regression lines were plotted only for species in which a significant linear relationship was found. The pseudo R^2 values refer to linear mixed effects models including Δ^{15}N values of the respective species as dependent variable, elevation (continuous) and body size as fixed effect and subplot nested in elevation as random effect. For significant fixed effects see main text.



Appendix Figure 3: Body size [µm] of Collembola life forms (epedaphic, hemiedaphic and euedaphic; marked in color) across the studied elevation gradient. Large dots represent means, error bars standard deviations and small dots individual measurements. Dotted lines mark the mean body size across elevations for the respective life form. Note: The Y-axis displays the log-scale.

Chapter 5: General Discussion

Primary forest at Changbai Mountain

The work at hand focused the question of “how do trophic interactions of different components of the soil food web change with elevation”. In the previous three chapters we reported strong changes in microbial communities and biomass, trophic positions of microarthropods in general and trophic changes within the Collembola community along the elevational gradient in the primary forests of Changbai Mountain, China.

5.1 Soil Food Webs at Changbai Mountain

Our results suggest that the microbial community, as the major primary decomposers in the soil food web, is heavily influenced by elevation. The microbial community changed strongest in the litter layer between elevations 1100 m and 1250 m, this was also true for microbial biomass which was high above 1100 m. The area between 1100 m and 1250 m represents the transition zone between the pine deciduous mixed forests and spruce dominated forest (Liu 1997). We identified Ca content of litter to be one of the major drivers of microbial community composition in upper soil and found a strong relationship between Ca content and microbial biomass as well as microbial stress in litter. Ca plays a major role in various plant processes such as the formation of cell walls and membranes as well as defense mechanism (White 2003). Plant leaves can store Ca in the form of e.g., Ca oxalate crystals, serving as protection from herbivory but also as Ca reservoir under Ca deficit conditions (Volk et al. 2002). Therefore, Ca oxalate can be found in the foliage of both deciduous and coniferous tree species (Minocha et al. 2015). The Ca content of the litter is likely determined by tree species identity as well as Ca availability within the habitat. Tree leaves vary considerably in their Ca content between species (Ricklefs and Matthew 1982), which can explain the strong changes in Ca concentrations in the litter between the two major forest types investigated. Even though the major geological groups are metamorphic alkaline rocks, they cover bedrock of dolomitic marble which contains Ca (Yan et al. 2018), it can be transported through springs and is likely the main source of Ca at Changbai Mountain.

As discussed in detail in Chapter 2 the increased Ca content at lower elevations likely promotes N uptake by microbes as it allows urease driven transformation of urea towards the easily processable ammonium (NH_4^+) under Ca mineralization (as calcium carbonate) (Krajewska 2018). This availability of N is reflected in low microbial stress ratios under high Ca availability (Fig. 1). However, the high availability of carbon through larger litter accumulations at higher elevations likely increases microbial biomass. This habitat of high carbon but low nutrient availability likely led to major differences between the microbial community compositions at the lower (800 – 1100 m) and higher (1250 – 1700 m) elevations.

This signal is again reflected in both trophic positions of Collembola and Oribatida as presented in Chapter 3. Microarthropods occupied higher trophic positions at sites of high microbial stress and biomass as well as high C/N ratio. In general, there was a shift of Collembola and Oribatida communities from trophic positions between primary and secondary decomposers towards more secondary decomposers or predators/scavengers at higher elevations. The trophic position, however, was calculated from bulk samples representing the body mass weighted mean of the community. To understand which species and functional groups are truly responsible for this shift, we further assessed trophic variations of Collembola at species level. In Chapter 4 we in fact identified, besides minor changes in energy channels ($\Delta^{13}C$), increasing trophic positions ($\Delta^{15}N$) within Collembola species acting as primary decomposers at lower elevations. The decrease in the frequency of Collembola functioning as primary decomposers with elevation is further indicated by the increased $\Delta^{15}N_{min}$ towards 1700 m. Supporting the implications of Chapter 2 & 3, the role of primary decomposers may in fact be compromised by decreasing litter quality and increasing microbial stress. $\Delta^{15}N_{min}$ was strongest influenced by the cyclo/pre stress indicator ratio (Fig. 1), which is an indicator for substrate and nutritional stress in bacteria (Knivett and Cullen 1965; Guckert et al. 1986; Bossio and Scow 1997). Even though the cyclo/pre ratio is bacteria specific, it is likely that nutrient shortage also influences the fungal community. Structural equation modelling indicated that the effect of microbial stress on $\Delta^{15}N_{min}$ of Collembola was stronger than the effect of microbial biomass (Fig. 1), with the two, however, being closely correlated. As indicated by the results presented in Chapter 3, soil microarthropods at Changbai Mountain predominantly feed on fungi and bacteria. Like plants, microbes can produce secondary metabolites against microbivores (predators). Döll et al. (2013) found an increase in toxic secondary metabolites of *Aspergillus nidulans* exposed to Collembola grazing. Under nutritional stress the production of costly defenses by fungi and bacteria might be limited, leading to more intense grazing by microarthropods which is consequently displayed in their trophic positions. The fungal to bacterial ratio in the litter layer increased with elevation and further led to an increase in the isotopic uniqueness (IUni) within the Collembola community (Fig. 1). This suggests that the trophic niches of Collembola species show stronger overlap if fungi are more abundant, which supports the indications of Chapter 3 that fungi are the preferred resource of soil microarthropods.

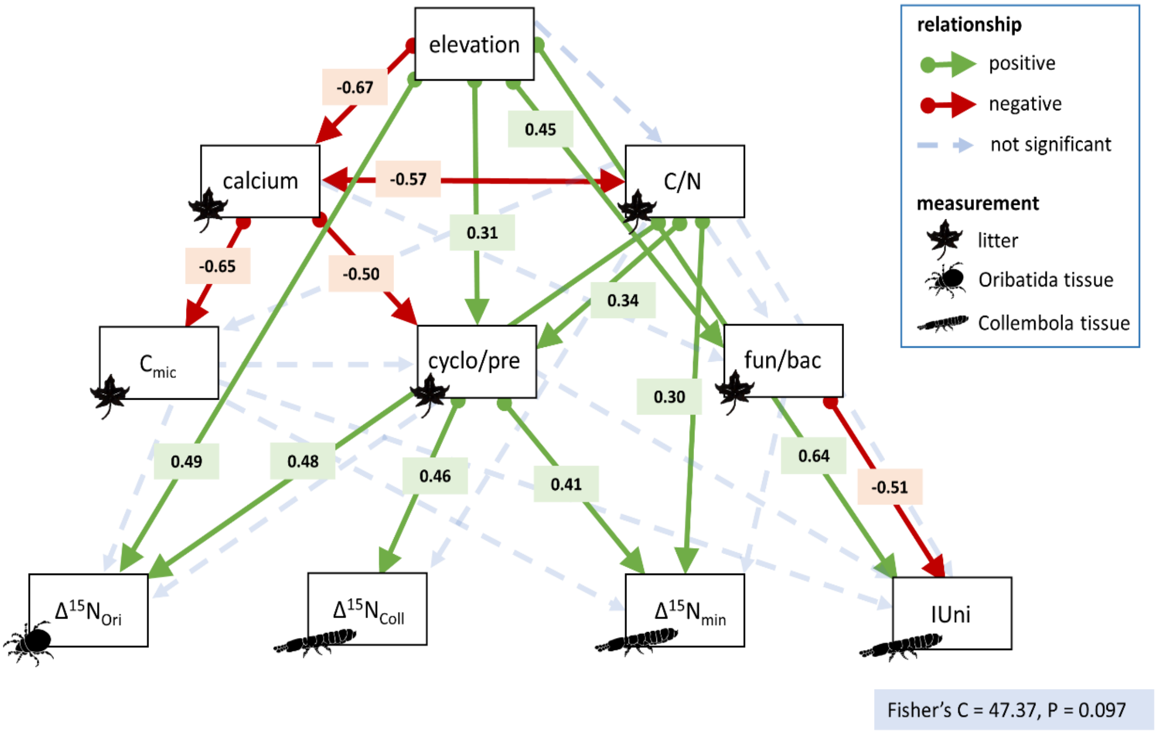


Figure 1: Structural equation model on direct and indirect effects of elevation and litter related factors [calcium content, C/N ratio, microbial biomass (C_{mic}), microbial stress (cyclo/pre), fungal to bacterial ratio (fun/bac)] on trophic positions of Collembola and Oribatida ($\Delta^{15}N$) as well as mean minimum trophic positions ($\Delta^{15}N_{min}$) and isotopic uniqueness (IUni) of Collembola. The numbers represent the standardized path coefficients. Fisher's C of 47.37 with P = 0.097 indicates a solid overall model fit.

The results of the study presented in Chapter 4 further indicated that the ability to switch from living as primary towards living as secondary decomposer along the elevational gradient is likely linked to Collembola traits. This trophic plasticity was most pronounced in hemiedaphic species, which possess medium large antennae, pronounced ocelli and body pigments, but antennae are usually shorter than in the surface dwelling epedaphic species (Rusek 2007). Therefore, euedaphic species can move between the upper and the lower litter layer. This is further supported by the steep decline in $\Delta^{15}N$ values with body size in hemiedaphic Collembola, smaller hemiedaphic Collembola will likely access deeper litter layers (Rusek 2007), where they feed on microbes enriched in ^{15}N.

Overall, our studies underline that the availability of nutrients and their influence on microbial communities along an elevational gradient determines the role of soil microarthropods within the soil food web. Consequently, it is likely that the trophic roles of microarthropods changes with forest types and accompanying litter characteristics. On the other hand, how Collembola communities at Changbai Mountain respond to such resource changes is influenced by Collembola traits such as life form and body size which allow access to alternative resources.

Like in many other mountain systems the temperature at Changbai Mountain increased over the last decades, while the annual precipitation decreased (Wang et al. 2020). Such climatic trends will likely facilitate transitions of the coniferous forests at higher elevations as species boundaries of (deciduous) tree species of lower elevations will extend, as predicted for the European Alps (Albrich et al. 2020). As repeatedly stressed, altered litter characteristics between different forest types will likely lead to changes in soil food web functioning. The direct participation of soil microarthropods to litter decomposition at higher elevations at Changbai Mountain will likely increase under global climate change. However, since a somehow similar trend of increasing trophic positions of Oribatida with increasing elevation was detected before in the European Alps (Fischer et al. 2014), although not being the main focus of the study, the expected changes in soil food webs may well be a global phenomenon. Accordingly, there is a call for more studies focusing on changes in the structure and functioning of soil food webs with elevation in montane forest ecosystems.

5.2 Trophic Analyses of Soil Food Webs

In Chapter 3 we used multiple methods of food web analyses to identify major energy channels and trophic positions of soil microarthropods. Even though there are studies which review the advantages and disadvantages of methods for the analysis of food webs (Nielsen et al. 2018), only few studies combined and compared methods used in soil food webs in a single experiment (Potapov et al. 2019a, 2021). Our studies underline the complementarity of compound-specific ^{13}C analysis of amino acids (CSSIA-AA; Larsen et al. 2013, 2016; Pollierer et al. 2019) and biomarker neutral lipids (NLFA) in consumers (Ruess and Chamberlain 2010). NLFA patterns in consumers indicate, besides large fungal contributions, bacterial NLFAs to considerably contribute to storage fat in Collembola, with their relative contribution being higher in Collembola than in Oribatida. CSSIA-AA, on the other hand, revealed similar contributions of bacteria to the diet of both Collembola and Oribatida. Therefore, bacteria seem to be more important for Oribatida growth (protein synthesis) than for short-term energy metabolism (indicated by storage fat), where fungi are the preferred resource. At this point it may be stressed that by excluding juvenile microarthropods, as is usually unavoidable at high taxonomic level analyses (as juveniles often cannot be determined to species), NLFA analyses alone may provide an incomplete picture of general energy fluxes as it ignores the developmental history of the consumer. Therefore, we suggest the combination of both methods if a complete picture of basal resource use is needed.

Further our study indicates that even though both TP_{CSSIA} and $\Delta^{15}N$ show similar trends, the more precise method of TP_{CSSIA} only weakly correlated with $\Delta^{15}N$, supporting the findings of Potapov et al. (2019). Further, $\delta^{15}N$ of litter varied considerably between our two major forest types again stressing the importance of correct baseline measurements to produce reliable results from bulk stable isotope measurements (Gannes et al. 1997; Martínez Del Rio et al. 2009; Potapov et al. 2019a). Still, considering limitations in time and budget bulk stable isotope analysis often is the only option to measure trophic niches of microarthropods at high taxonomic resolution (species). However, we suggest bulk stable isotope analyses to be accompanied by TP_{CSSIA}, as it, even though conducted on lower taxonomic resolution, helps to verify baseline measurements and ease interpretation of bulk stable isotope analyses. As shown in Chapter 3, $\delta^{15}N$ of phenylalanine can be used to verify the baseline for bulk stable isotope measurements.

5.3 Outlook

We identified Ca to be one of the major drivers of changes in the microbial community, which likely is linked to urease activity. However, upcoming studies investigating microbial communities in forests may consider to directly measure urease-activity within in the sampled soils to confirm this hypothesis. This relationship may shed new light on often reported influences of pH on microbial communities and secondary decomposers in forest systems.

Our study is the first to report in detail trophic changes in soil microarthropods along elevational gradients. Based on the implications of this study, comparable experiments along other gradients of montane forests will help to come up with more generalizable statements on how soil food web structure and functioning change with increasing elevation. Such studies should also include multiple transects on different facing slopes to account for effects of exposure. Further, sampling multiple seasons may help to identify season interactions with focal factors, which may be especially pronounced in deciduous forests.

However, as such studies are based on field samples it is impossible to account for all environmental factors changing with elevation. This is especially true for climatic variables. Therefore, manipulative laboratory experiments need to be conducted to explore consequences of different climate change scenarios on decomposition processes and trophic interactions in soil. Intact soil cores excavated from different elevations could be manipulated under different climate scenarios in the laboratory. Based on the example of Changbai Mountain we suggest focusing at least on one lower (<1100 m) and one higher elevation (>1100 m). Manipulations should focus on temperature, precipitation, temperature seasonality and precipitation seasonality as well as CO_2 levels. This may allow to uncover the responses of soil food webs to climate change factors in different forest types. Such experiments are needed to understand and predict how climate change will influence soil food webs and the functions and services they provide.

5.4 References

Albrich K, Rammer W, Seidl R (2020) Climate change causes critical transitions and irreversible alterations of mountain forests. Glob Change Biol 26:4013–4027. https://doi.org/10.1111/gcb.15118

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 10:215–221. https://doi.org/10.1016/0038-0717(78)90099-8

Anderson TH, Domsch KH (1993) The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol Biochem 25:393–395. https://doi.org/10.1016/0038-0717(93)90140-7

Bani A, Pioli S, Ventura M, et al (2018) The role of microbial community in the decomposition of leaf litter and deadwood. Appl Soil Ecol 126:75–84. https://doi.org/10.1016/j.apsoil.2018.02.017

Bossio DA, Scow KM (1997) Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb Ecol 35:265–278. https://doi.org/10.1007/s002489900082

Chahartaghi M, Langel R, Scheu S, Ruess L (2005) Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biol Biochem 37:1718–1725. https://doi.org/10.1016/j.soilbio.2005.02.006

Chapin FS, Matson PA, Vitousek PM (2011) Principles of terrestrial ecosystem ecology. Springer New York, New York, United States

Chikaraishi Y, Kashiyama Y, Ogawa NO, et al (2007) Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: Implications for aquatic food web studies. Mar Ecol Prog Ser 342:85–90. https://doi.org/10.3354/meps342085

Chikaraishi Y, Ogawa NO, Ohkouchi N (2010) Further evaluation of the trophic level estimation based on nitrogen isotopic composition of amino acids. In: Earth, life, and isotopes. Kyoto University Press, Kyoto, Japan

Chikaraishi Y, Steffan SA, Ogawa NO, et al (2014) High-resolution food webs based on nitrogen isotopic composition of amino acids. Ecol Evol 4:2423–2449. https://doi.org/10.1002/ece3.1103

Döll K, Chatterjee S, Scheu S, et al (2013) Fungal metabolic plasticity and sexual development mediate induced resistance to arthropod fungivory. Proc R Soc B Biol Sci 280:20131219. https://doi.org/10.1098/rspb.2013.1219

Eisenhauer N, Reich PB (2012) Above- and below-ground plant inputs both fuel soil food webs. Soil Biol Biochem 45:156–160. https://doi.org/10.1016/j.soilbio.2011.10.019

Fischer BM, Meyer E, Maraun M (2014) Positive correlation of trophic level and proportion of sexual taxa of oribatid mites (Acari: Oribatida) in alpine soil systems. Exp Appl Acarol 63:465–479. https://doi.org/10.1007/s10493-014-9801-3

Frostegård Å, Tunlid A, Bååth E (2011) Use and misuse of PLFA measurements in soils. Soil Biol Biochem 43:1621–1625. https://doi.org/10.1016/j.soilbio.2010.11.021

Fujii S, Cornelissen JHC, Berg MP, Mori AS (2018) Tree leaf and root traits mediate soil faunal contribution to litter decomposition across an elevational gradient. Funct Ecol 32:840–852. https://doi.org/10.1111/1365-2435.13027

Gannes LZ, O'brien DM, Martínez del Rio C (1997) Stable isotopes in animal ecology: Assumptions, caveats and a call for more laboratory experiments. Ecology 78:1271–1276. https://doi.org/10.1890/0012-9658(1997)078[1271:siiaea]2.0.co;2

Guckert JB, Hood MA, White DC (1986) Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: Increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. Appl Environ Microbiol 52:794–801. https://doi.org/10.1128/aem.52.4.794-801.1986

Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annu Rev Ecol Evol Syst 36:191–218. https://doi.org/10.1146/annurev.ecolsys.36.112904.151932

Kim JB, Kerns BK, Drapek RJ, et al (2018) Simulating vegetation response to climate change in the Blue Mountains with MC2 dynamic global vegetation model. Clim Serv 10:20–32. https://doi.org/10.1016/j.cliser.2018.04.001

Knivett VA, Cullen J (1965) Some factors affecting cyclopropane acid formation in *Escherichia coli*. Biochem J 96:771–776. https://doi.org/10.1042/bj0960771

Korotkevich AYu, Potapov AM, Tiunov AV, Kuznetsova NA (2018) Collapse of trophic-niche structure in belowground communities under anthropogenic disturbance. Ecosphere 9:e02528. https://doi.org/10.1002/ecs2.2528

Krajewska B (2018) Urease-aided calcium carbonate mineralization for engineering applications: A review. J Adv Res 13:59–67. https://doi.org/10.1016/j.jare.2017.10.009

Larsen T, Ventura M, Andersen N, et al (2013) Tracing carbon Sources through aquatic and terrestrial food webs using amino acid stable isotope fingerprinting. PLoS ONE 8:e73441. https://doi.org/10.1371/journal.pone.0073441

Larsen T, Pollierer MM, Holmstrup M, et al (2016) Substantial nutritional contribution of bacterial amino acids to earthworms and enchytraeids: A case study from organic grasslands. Soil Biol Biochem 99:21–27. https://doi.org/10.1016/j.soilbio.2016.03.018

Li MM, Xu ZT, Ventura G, et al (2021) Geochronology and petrogenesis of early pleistocene dikes in the Changbai Mountain volcanic field (NE China) Based on geochemistry and Sr-Nd-Pb-Hf isotopic compositions. Front Earth Sci 9:729905. https://doi.org/10.3389/feart.2021.729905

Liu M, Sui X, Hu Y, Feng F (2019) Microbial community structure and the relationship with soil carbon and nitrogen in an original Korean pine forest of Changbai Mountain, China. BMC Microbiol 19:218. https://doi.org/10.1186/s12866-019-1584-6

Liu QJ (1997) Structure and dynamics of the subalpine coniferous forest on Changbai mountain, China. Plant Ecol 132:97–105. https://doi.org/10.1023/A:1009705319128

Maraun M, Thomas T, Fast E, et al (2023) New perspectives on soil animal trophic ecology through the lens of C and N stable isotope ratios of oribatid mites. Soil Biol Biochem 177:108890. https://doi.org/10.1016/j.soilbio.2022.108890

Martínez Del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111. https://doi.org/10.1111/j.1469-185X.2008.00064.x

Minocha R, Chamberlain B, Long S, et al (2015) Extraction and estimation of the quantity of calcium oxalate crystals in the foliage of conifer and hardwood trees. Tree Physiol 35:574–580. https://doi.org/10.1093/treephys/tpv031

Moore-Kucera J, Dick RP (2008) PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microb Ecol 55:500–511. https://doi.org/10.1007/s00248-007-9295-1

Nielsen JM, Clare EL, Hayden B, et al (2018) Diet tracing in ecology: Method comparison and selection. Methods Ecol Evol 9:278–291. https://doi.org/10.1111/2041-210X.12869

Pan X, Xie Z, Sun X, et al (2023) Changes in oribatid mite community structure along two altitudinal gradients in Asia and Europe as related to environmental factors. Appl Soil Ecol 189:104912. https://doi.org/10.1016/j.apsoil.2023.104912

Perez-Harguindeguy N, Dıaz S, Cornelissen JHC, et al (2000) Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. Plant Soil 218:21–30

Pollierer MM, Langel R, Körner C, et al (2007) The underestimated importance of belowground carbon input for forest soil animal food webs. Ecol Lett 10:729–736. https://doi.org/10.1111/j.1461-0248.2007.01064.x

Pollierer MM, Langel R, Scheu S, Maraun M (2009) Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$). Soil Biol Biochem 41:1221–1226. https://doi.org/10.1016/j.soilbio.2009.03.002

Pollierer MM, Larsen T, Potapov AM, et al (2019) Compound-specific isotope analysis of amino acids as a new tool to uncover trophic chains in soil food webs. Ecol Monogr 89:e01384. https://doi.org/10.1002/ecm.1384

Ponsard S, Arditi R (2000) What can stable isotopes (^{15}N and ^{13}C) tell about the food web of soil macro-invertebrates? Ecology 81:852–864. https://doi.org/10.2307/177382

Potapov AM, Tiunov AV, Scheu S, et al (2019a) Combining bulk and amino acid stable isotope analyses to quantify trophic level and basal resources of detritivores: a case study on earthworms. Oecologia 189:447–460. https://doi.org/10.1007/s00442-018-04335-3

Potapov AM, Tiunov AV, Scheu S (2019b) Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biol Rev 94:37–59. https://doi.org/10.1111/brv.12434

Potapov AM, Pollierer MM, Salmon S, et al (2021) Multidimensional trophic niche revealed by complementary approaches: Gut content, digestive enzymes, fatty acids and stable isotopes in Collembola. J Anim Ecol 90:1919–1933. https://doi.org/10.1111/1365-2656.13511

Price MF, Gratzer G, Duguma LA, et al (2015) Mountain forests in a changing world - realizing values, addressing challenges. https://doi.org/10.13140/2.1.2386.5283

Ricklefs RE, Matthew KK (1982) Chemical characteristics of the foliage of some deciduous trees in Southeastern Ontario. Can J Bot 60:2037–2045. https://doi.org/10.1139/b82-251

Ruess L, Chamberlain PM (2010) The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biol Biochem 42:1898–1910. https://doi.org/10.1016/j.soilbio.2010.07.020

Rusek J (2007) A new classification of Collembola and Protura life forms. Contrib Soil Zool Cent Eur II:109–115

Scheu S (1992) Automated measurement of the respiratory response of soil microcompartments: Active microbial biomass in earthworm faeces. Soil Biol Biochem 24:1113–1118. https://doi.org/10.1016/0038-0717(92)90061-2

Scheu S, Falca M (2000) The soil food web of two beech forests (*Fagus sylvatica*) of contrasting humus type: stable isotope analysis of a macro- and mesofauna-dominated community. Oecologica 123:285–286. https://doi.org/10.1007/s004420051015

Schneider K, Migge S, Norton RA, et al (2004) Trophic niche differentiation in soil microarthropods (Oribatida, Acari): Evidence from stable isotope ratios (^{15}N/^{14}N). Soil Biol Biochem 36:1769–1774. https://doi.org/10.1016/j.soilbio.2004.04.033

Shen C, Xiong J, Zhang H, et al (2013) Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. Soil Biol Biochem 57:204–211. https://doi.org/10.1016/j.soilbio.2012.07.013

Tang L, Li A, Shao G (2011) Landscape-level forest ecosystem conservation on Changbai Mountain, China and North Korea (DPRK). Mt Res Dev 31:169–175. https://doi.org/10.1659/MRD-JOURNAL-D-10-00120.1

Volk GM, Lynch-Holm VJ, Kostman TA, et al (2002) The role of druse and raphide calcium oxalate crystals in tissue calcium regulation in *Pistia stratiotes* leaves. Plant Biol 4:34–45. https://doi.org/10.1055/s-2002-20434

Wang H, Xu J, Sheng L, et al (2020) Study on the characteristics of climate change in Changbai Mountain National Natural Reserve from 1958 to 2017. Arab J Geosci 13:. https://doi.org/10.1007/s12517-020-05808-7

White PJ (2003) Calcium in Plants. Ann Bot 92:487–511. https://doi.org/10.1093/aob/mcg164

Xie Z, Sun X, Lux J, et al (2022) Drivers of Collembola assemblages along an altitudinal gradient in Northeast China. Ecol Evol 12:e8559. https://doi.org/10.1002/ece3.8559

Yan B, Liang X, Xiao C (2018) Hydrogeochemical characteristics and genesis model of Jinjiang and Julong hot springs in Changbai Mountain, Northeast China. Geofluids 2018:1694567. https://doi.org/10.1155/2018/1694567

List of Publications

Peer-reviewed articles:

Lux J, Xie Z, Sun X, Wu D, Scheu S (2022) Changes in microbial community structure and functioning with elevation are linked to local soil characteristics as well as climatic variables. Ecol Evol 12:e9632. https://doi.org/10.1002/ece3.9632

Xie Z, Sun X, **Lux J**, Chen TW, Potapov M, Wu D, Scheu S (2022) Drivers of Collembola assemblages along an altitudinal gradient in Northeast China. Ecol Evol 12:e8559. https://doi.org/10.1002/ece3.8559

Lux J, Xie Z, Sun X, Wu D, Pollierer MM, Scheu S (2024) Trophic positions of soil microarthropods in forests increase with elevation, but energy channels remain unchanged. Ecosphere 15: e4747. https://doi.org/10.1002/ecs2.4747

Lux J, Xie Z, Sun X, Wu D, Scheu S (2024) Trophic niches of Collembola communities change with elevation, but also with body size and life form. Oecologia. https://doi.org/10.1007/s00442-023-05506-7

Xie Z, **Lux J**, Wu Y, Sun X, Chen TW, Zhang R, Zhu J, Wu D, Scheu S (2023) Intraspecific variability and species turnover drive the variation of Collembola body size along a temperate-boreal elevation gradient in northern China. Geoderma 441: 116731. https://doi.org/10.1016/j.geoderma.2023.116731

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Thesis Declaration

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

Chapter 2: Changes in microbial community structure and functioning with elevation are linked to local soil characteristics as well as climatic variables.

Johannes Lux, Zhijing Xie, Xin Sun, Donghui Wu and Stefan Scheu

I am the first author of the manuscript. I conducted the sampling, measurements, data curation and visualization, statistical analyses and wrote the manuscript, I further assisted in the study conceptualisation. Zhijing Xie and Xin Sun reviewed and edited the manuscript. Xin Sun further assisted in the study conceptualization. Donghui Wu conceptualized the study, raised funds, administrated the project and reviewed the manuscript. Stefan Scheu conceptualized the study, raised funds, administrated the project, supervised the work and reviewed and edited the manuscript.

Chapter 3: Changes in Microbial Community Structure and Functioning with Elevation Are Linked to Local Soil Characteristics as Well as Climatic Variables

Johannes Lux, Zhijing Xie, Xin Sun, Donghui Wu, Melanie M Pollierer and Stefan Scheu

I am the first author of the manuscript. I conducted the sampling, measurements, data curation and visualization, statistical analyses and wrote the manuscript, I further assisted in the study conceptualisation. Zhijing Xie and Xin Sun reviewed and edited the manuscript. Xin Sun further assisted in the study conceptualization. Donghui Wu conceptualized the study, raised funds, administrated the project and reviewed the manuscript. Melanie M Maraun (née Pollierer) supervised the work and formal analysis and reviewed and edited the manuscript. Stefan Scheu conceptualized the study, raised funds, administrated the project, supervised the working process and reviewed and edited the manuscript.

Chapter 4: Trophic Niches of Collembola Communities Change with Elevation, but Also with Body Size and Life Form

Johannes Lux, Zhijing Xie, Xin Sun, Donghui Wu and Stefan Scheu.

I am the first author of the manuscript. I conducted the sampling, measurements, data curation and visualization, statistical analyses and wrote the manuscript, I further assisted in the study conceptualisation. Zhijing Xie and Xin Sun reviewed and edited the manuscript.

Xin Sun further assisted in the study conceptualization. Donghui Wu conceptualized the study, raised funds, administrated the project and reviewed the manuscript. Stefan Scheu conceptualized the study, raised funds, administrated the project, supervised the work and reviewed and edited the manuscript.

Plagiarism declaration

I declare that I have written this doctoral thesis independently. All persons who contributed to the manuscripts have been named. All passages and quotes from other people's work have been specifically acknowledged by clear cross-referencing. I have not submitted any part of this thesis in any form for another degree at any university and institution.

Johannes Lux, Göttingen July 2023