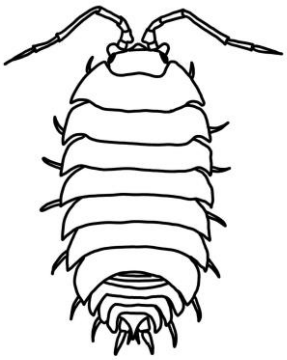
Significance of intraspecific variation for decomposition processes



Dissertation

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Universität Bremen

Significance of intraspecific variation for decomposition processes

Hanno Müller

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“What is easy to measure is not always what is important.”

John Green

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Summary

Over the last decades a growing awareness for the role of biodiversity in an ecological context has grown. Species richness, among other and more complex definitions and perceptions of biodiversity, has been found to influence ecosystem processes like production and decomposition – often in a positive way, though this seems to depend on different factors like the ecosystem or the identity of the involved species. In the context of decomposition mainly the diversity of the decaying litter has been in the focus of interest – and like for other studies on diversity, on the level of interspecific diversity. As the concept of biodiversity shifted from "number of species" to other definitions and measurements of variation and diversity like "functional diversity", intraspecific variation was more and more recognized as another, so far neglected and potentially ecologically significant level of biodiversity. However, very little is known about the influence of intraspecific variation and diversity in the context of decomposition and up to now, nothing is known regarding the ecological role of this level of diversity in a detritivore species in the context of decomposition. Consequently, the aim of this thesis is to study the influence of intraspecific variation in a detritivore species on consumption and on decomposition of different litter mixes.

In order to test if an increased diversity within such a detritivore species results in a higher consumption (as it e.g. often does with litter diversity), isopods of the species *Porcellio scaber* (Latreille 1804, Isopoda, Oniscidea) from several European populations were combined into artificial groups of three different diversity-levels. Groups were tested with newly developed genetic markers to confirm differences in diversity and fed on different leaf litter species and litter mixtures with different levels of interspecific diversity in a microcosm-experiment. A positive effect of intraspecific diversity on litter consumption only occurred in single litter treatments, but – other than expected – not in litter mixtures. However, these effects were considerably lower than the also measured "mixed litter effects" (effects of increased interspecific litter diversity). In addition to the results, due to the developed genetic marker some insights about the variation and population differentiation of the widely distributed Isopod species *P. scaber* could be gained.

To test the effect of an (expected) decreased diversity on consumption, two populations of the Isopod *Trachelipus ratzeburgii* (Brandt 1833, Isopoda, Oniscidea) living on two different altitudes of the same mountain were fed on different leaf litter species and litter mixtures – hypothesizing that the fluctuation in environmental parameters like temperature at high

altitude reduced the diversity in a high-altitude population. In addition, genetic variation within and differentiation between both populations was tested with newly developed genetic markers and stress resistance (HSP70-expression) was measured to see if a (under such conditions) reduced variation would go along with an increased level of stress resistance due to positive selection towards this trait. However, results show that the chosen populations did not differ in variation (along with being not differentiated) and had no differences in stress resistance. They also did not differ in absolute consumption, despite a strong difference in size, as the isopods from high altitude were much smaller than those from the low altitude population. Accordingly, the relative consumption of the isopods of the high-altitude population was significantly higher. The reasons for this remain unknown, but these findings show a high plasticity for this species - which in this case compensates for reduced size.

As consumption rate is only one element of the direct and indirect influences detritivores have on decomposition, the variation in digestion of *P. scaber* was measured in an additional approach. Animals from several populations were fed with standardized food, whereafter the chemical structure of the feces was compared using Pyrolysis-GC-MS. The isopods showed (also in comparison to specimen of other Oniscidea species) a substantial amount of variation in the chemical fingerprint of the feces, whereas the differences could only partly be explained by genetic proximity. Other factors, such as the microbiome of the digestive tract, appear to have an additional influence. Although with this methodology it was not possible to measure any exact qualitative differences in digestion, the quantitative scale of the differences indicates that digestion is a rather variable trait in *P. scaber*.

In summary, the results provide indications of an importance of intraspecific variation and diversity for decomposition processes, but its influence was (with respect to consumption of leaf litter) considerably less strong compared to the influence of the interspecific variation of the leaf litter. Nevertheless, the results show in many respects that intraspecific variation (and plasticity) should not be neglected in the context of decomposition.

Zusammenfassung

In den letzten Jahrzehnten ist ein wachsendes Bewusstsein für die Rolle der Biodiversität im ökologischen Kontext entstanden. Neben anderen und komplexeren Definitionen und Auffassungen von Biodiversität wurde festgestellt, dass die Anzahl der beteiligten Arten Ökosystemprozesse wie Produktion und Dekomposition beeinflusst - oft auf positive Weise, obwohl dies von verschiedenen Faktoren wie dem Ökosystem oder der Identität der beteiligten Arten abzuhängen scheint. Im Zusammenhang mit Dekomposition stand vor allem die Diversität der verrottenden Streu im Mittelpunkt des Interesses - und zwar, wie bei anderen Studien zur Diversität, auf der Ebene der interspezifischen (zwischenartlichen) Diversität. Als sich das Konzept der Biodiversität von der "Anzahl der Arten" zu anderen Definitionen und Messungen von Variation und Diversität wie der "funktionellen Diversität" verschob, wurde die intraspezifische Variation mehr und mehr als eine weitere, bisher vernachlässigte und potenziell ökologisch bedeutsame Ebene der Biodiversität erkannt. Es ist jedoch nur sehr wenig über die Rolle der intraspezifischen Variation und Diversität im Kontext von Dekomposition bekannt und bisher ist nichts über die ökologische Rolle dieses Diversitätsniveaus bei einer detritivoren Art bekannt. Ziel dieser Arbeit ist es daher, den Einfluss von intraspezifischer Variation bei einer Detritivorenart auf die Konsumption und die Zersetzung verschiedener Laubstreuarten bzw. Streumischungen zu untersuchen.

Um zu testen, ob eine erhöhte Diversität innerhalb einer Population einer Detritivorenart zu einer höheren Laubstreukonsumption führt (wie es z.B. oft bei einer erhöhten Streudiversität der Fall ist), wurden Isopoden der Art *Porcellio scaber* (Latreille 1804, Isopoda, Oniscidea) aus verschiedenen europäischen Populationen zu künstlichen Gruppen mit drei verschiedenen Diversitätsniveaus kombiniert. Die Gruppen wurden mit neu entwickelten genetischen Markern auf Unterschiede in der Diversität getestet und in einem Versuch in Mikrokosmen mit verschiedenen Laubstreu-Arten bzw. Streu-Mischungen (mit unterschiedlichem Grad an interspezifischer Diversität) gesetzt. Ein positiver Effekt der intraspezifischen Diversität auf den Streuverbrauch trat nur bei den Mikrokosmen mit Einzelstreu auf, aber - anders als erwartet - nicht bei den Streu-Mischungen. Diese Effekte waren jedoch deutlich geringer als die ebenfalls gemessenen "mixed litter effects" (Effekte der erhöhten interspezifischer Laubstreudiversität). Zusätzlich zu den Ergebnissen konnten aufgrund der entwickelten genetischen Marker

einige Erkenntnisse über die Variation und Populationsdifferenzierung des weit verbreiteten Isopoden *P. scaber* gewonnen werden.

Um die Auswirkung einer (erwarteten) verringerten Diversität auf die Konsumption zu testen, wurden zwei Populationen des Isopoden *Trachelipus ratzeburgii* (Brandt 1833, Isopoda, Oniscidea), die auf zwei verschiedenen Höhenlagen desselben Berges leben, in einem Mikrokosmenexperiment mit verschiedenen Blattstreuarten und Streumischungen gefüttert - unter der Hypothese, dass die Schwankung von Umweltparametern wie der Temperatur in großer Höhe die Diversität in einer Population auf größerer Höhe reduziert. Zusätzlich wurde die genetische Variation und Differenzierung zwischen beiden Populationen mit neu entwickelten genetischen Markern getestet und die Stressresistenz (anhand von HSP70-Expression) gemessen, um zu testen, ob eine (unter solchen Bedingungen) reduzierte Variation mit einer erhöhten Stressresistenz aufgrund einer positiven Selektion bezüglich dieses Merkmals einhergehen würde. Die Ergebnisse zeigen jedoch, dass die ausgewählten Populationen sich nicht in ihrer Variation unterschieden (und genetisch nicht differenziert sind) und im Versuch auch keine Unterschiede in der Stressresistenz aufwiesen. Sie unterschieden sich auch nicht in der absoluten Konsumption, trotz eines starken Größenunterschieds, da die Isopoden aus der Hochlage ein geringeres Körpergewicht aufwiesen als die der Tieflagenpopulation - entsprechend ist die relative Konsumption der Hochlagenisopoden deutlich höher. Die Gründe dafür bleiben unbekannt, aber diese Ergebnisse zeigen eine hohe Plastizität für diese Art, die in diesem Fall die geringere Größe kompensiert.

Da die Konsumptionsrate nur ein Aspekt der direkten und indirekten Einflüsse von Detritivoren auf die Zersetzung von Streu ist, wurde in einem zusätzlichen Ansatz die Variation der Verdauung von *P. scaber* gemessen. Tiere aus mehreren Populationen wurden mit einem standardisierten Futter gefüttert, woraufhin die chemische Struktur des Kots mittels Pyrolyse-GC-MS verglichen wurde. Die Asseln zeigten (auch im Vergleich zu Individuen anderer Oniscidea-Arten) eine erhebliche Variation im chemischen Fingerabdruck des Kots, wobei die Unterschiede nur teilweise durch genetische Nähe erklärt werden konnten. Andere Faktoren, wie das Mikrobiom des Verdauungstraktes, scheinen einen zusätzlichen Einfluss zu haben. Obwohl mit dieser Methodik keine exakten qualitativen Unterschiede in der Verdauung gemessen werden konnten, deutet das quantitative Ausmaß der Unterschiede darauf hin, dass die Verdauung ein variables Merkmal bei *P. scaber* ist.

Zusammenfassend liefern die Ergebnisse Hinweise auf eine Bedeutung der intraspezifischen Variation und Diversität für Dekompositionsprozesse, aber ihr Einfluss war (in Bezug auf die Konsumption von Laubstreu) im Vergleich zum Einfluss der interspezifischen Variation der Laubstreuarten deutlich geringer. Dennoch zeigen die Ergebnisse in vielerlei Hinsicht, dass intraspezifische Variation (und Plastizität) im Zusammenhang mit der Zersetzung nicht vernachlässigt werden sollte.

Contributions

Hanno Müller developed the general idea of this theses, the main hypothesis, and the general methodology, with contributions by Martin Zimmer. All data analysis, figure preparation and writing were done by H. Müller. Martin Zimmer contributed to all chapters with advice regarding experimental design and data analysis.

Chapter 2: Similar, but not identical – Influence of intraspecific variation on leaf litter consumption in a microcosm experiment

Contributions of the candidate in % of the total work load:

Experimental concept and design:	ca. 90%
Experimental work and acquisition of experimental data:	ca. 90%
Data analysis and interpretation:	ca. 100%
Preparation of Figures and Tables:	ca. 100%
Drafting of the manuscript:	ca. 100%

Chapter 3: North and South - Comments on the population genetics of the Porcellio scaber populations used in the experiment

Contributions of the candidate in % of the total work load:

Experimental concept and design:	ca. 90%
Experimental work and acquisition of experimental data:	ca. 90%
Data analysis and interpretation:	ca. 100%
Preparation of Figures and Tables:	ca. 100%
Drafting of the manuscript:	ca. 100%

Chapter 4: Up and down – a case study on the influence of altitudinal environmental fluctuation on intraspecific variation, HSP70 gene expression and leaf litter consumption

Contributions of the candidate in % of the total work load:

Experimental concept and design:	ca. 90%
Experimental work and acquisition of experimental data:	ca. 95%
Data analysis and interpretation:	ca. 100%
Preparation of Figures and Tables:	ca. 100%
Drafting of the manuscript:	ca. 100%

Chapter 5: In and out – differences and variation in Py-GC-MS patterns of feces from several isopod populations as an estimate of variation in digestion

Contributions of the candidate in % of the total work load:

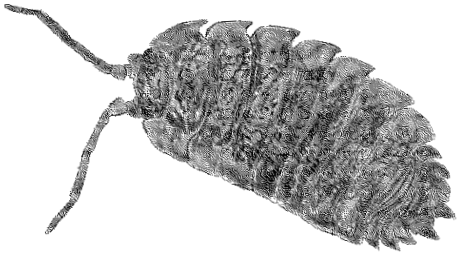
Experimental concept and design:	ca. 80 %
Experimental work and acquisition of experimental data:	ca. 80%
Data analysis and interpretation:	ca. 100%
Preparation of Figures and Tables:	ca. 100%
Drafting of the manuscript:	ca. 100%

Chapters 2, 4 and 5 are to be published separately, but have not yet been submitted at the time of the submission of this thesis. H. Müller will be the main author of all publications.

This page was adjusted after submitting the thesis (usage of percentages instead of text).

Chapter 1

General introduction



The ecological role and significance of biodiversity

While the extinction of species and the possible impact on ecosystems already received increased attention since the middle of the last century (in general society and in biological research) an increasing awareness of a more general, broader significance of biodiversity was added in the following decades (Naeem et al. 1994, Chapin et al. 1998, Loreau et al. 2001, Hooper et al. 2005, 2012, Worm et al. 2006, Cardinale et al. 2006).

Though our understanding regarding the question which aspects of this diversity are how important for ecosystem processes und ecosystem functioning (also in relation to other factors) is still limited today (Frainer et al. 2014) there are up to now countless lab and field studies showing this influence – and consequences of its loss (Hooper et al. 2012). One of the main explanations of these effects is rather simple: different species do not only use (limited) resources differently (niche complementarity), but also effect their surrounding environment in different ways (positive species interactions), which leads to a relatively low competition (relative to communities with less species) and complementary effects (Hector et al. 1999). However, there are of course numerous factors that influence these dynamics and up to now it is unclear if a general relation between species richness and ecosystem functioning across ecosystems and trophic levels and can be found (O'Connor et al. 2017).

Despite that, one major finding of the last decades is that species richness, being a rather simple way of perceiving biodiversity, often positively affects ecosystem functioning such as nutrient uptake (Cardinale 2011), production (Hector et al. 1999, Tilman et al. 2001, Ruijven and Berendse 2005), decomposition (Hättenschwiler et al. 2005, Gessner et al. 2010) and resilience (Oliver et al. 2015).

Especially production has been in the focus of ecological studies for many years, since a deeper understanding of the underlining connection between biodiversity and biomass production is not only of scientific interest, but might also be of direct and indirect economic interest. But while many studies conducted in grasslands clearly show a positive influence of plant diversity on biomass production (Hector et al. 1999, Tilman et al. 2001), these findings cannot be transferred to agro-ecosystems. In fact, although Letourneau et al. (2011) found a general positive effect of diversification on herbivore and crop damage suppression in their review, crop yield was slightly negatively effected on average.

However, a different picture emerges for forest ecosystems and especially for silviculture. Although the different advantages of mixed stands have been discussed for centuries, Keltys (mainly theoretical) application of the fundamental niche concept (Kelty 1992) was among others a starting point for a variety of experiments comparing the productivity of monocultures and mixed stands (e.g. Debell et al. (1997) and Forrester et al. (2004)) and many of these plantations are still under constant survey. This first generation of studies and results was summarized by Kelty (2006), and it is showing a distinct higher productivity of many tree-species-mixtures in forest plantations. A connection between biodiversity and productivity on a global scale was shown by Liang et al. (2016), pointing out that the effect of biodiversity on ecosystem productivity can be measured across a variety of forest ecosystems all over the world.

Another ecosystem process, however, was less in focus of diversity-studies, although it is not only as omnipresent as production, but due to nutrient cycling also directly connected to it: decomposition. The fundamental importance of decomposition as an ecosystem process is obvious and well studied (Swift et al. 1979), especially as up to 90% of the mass of primary producers is decomposed as dead organic matter in a variety of processes (Cebrian 1999, Gessner et al. 2010). The analysis of the connection between diversity and decomposition can be divided into two sections: The importance of the diversity of the species forming the dead organic material (e.g. leaf litter) and the importance of the diversity of decomposers, whereas the former is much more well studied so far – and mainly in forest ecosystems (Krishna and Mohan 2017).

Though - in the context of biodiversity - being much less studied than production, the first (published) awareness that mass loss during decomposition can be higher in litter mixtures compared to single litter was printed almost 80 years ago (Gustafson 1943, Hättenschwiler et al. 2005). Gustavson concludes his paper with the finding that "A mixture of pine and hardwood leaves increases the rate of decay of both kinds of leaves." The approach to combine and compare the decomposition of monocultures and leave mixtures was reintroduced e.g. by Thomas (1968) and Staaf (1980). Since than several studies regarding that matter have been made, summarized by Gartner and Cardon (2004), Hättenschwiler et al. (2005) and Gessner et al. (2010). These findings show a diverse picture of the influence of litter diversity on litter mass loss and subsequently on decomposition. Many studies found no effect, e.g. Blair et al. (1990) and Ashton et al. (1999) or even a slower mass loss of litter mixtures (McArthur et al. 1994) or concluded

the effect of mixed litter to be "less important than that of life plants" (Wardle et al. 1997). But many studies found accelerated litter decay rates of litter mixtures compared to the predicted decay rates of the included litter species – especially in certain mixtures (e.g. Hector et al. (2000)).

While nice differentiation is likely to be the main driver of the connection between biodiversity and mass production, Hättenschwiler et al. (2005) named four mechanisms to explain the observed "mixed-litter-effects": 1. nutrient transfer among litter types, 2. influences of specific litter compounds, 3. habitat diversity and improved microclimate conditions, 4. effects due to interactions across trophic levels.

Consequently, the presence of mixed litter effects often depends on the specific litter mix, especially regarding the contents of nutrients and (stimulating or inhibiting) secondary compounds (Hättenschwiler et al. 2005, Gessner et al. 2010). In addition, Vos et al. (2011) explain the sometimes conflicting results with the insufficient consideration of macro-detritivores in these processes.

While the mechanisms and magnitude of mixed litter effects are increasingly understood due to the growing number of studies, the role of biodiversity in microbes, fungi and the animal-detritivore community has received much less attention. This is unfortunate, as the composition of the decomposer community is among the litter quality and the physiochemical environment one of the main drivers of litter decomposition (Berg et al. 1993, Cadisch and Giller 1995, Coûteaux et al. 1995, Hättenschwiler et al. 2005, Bradford et al. 2016).

Assumingly, there is an extremely high number of bacterial and fungal species involved in decomposition processes. Both the total respective local number and diversity of soil-microorganisms are hard to determine, but estimations are quite high, as Schloss and Handelsman (2006) found a range from 4,000 to 10,000,000 bacterial genome equivalents per 10 to 30 g of soil in their review. The number of fungal species can be assumed to be considerably lower, but O'Brien et al. (2005) also found over 400 fungal OTUs in 1 g of soil - estimating a global number of 3.5 to 5.1 million fungal species. However, up to now it is hard to estimate how many of these bacterial and fungal species are involved in the different steps of decomposition. In addition, despite the actual number of species, the importance of microbial biodiversity in comparison to e.g., the presence of some key species or groups is relatively complicated to measure in the field and to test

experimentally so far. This is especially true for bacteria, although some early studies used toxic substances like chloroform (Griffiths et al. 2000) or uranium (Meyer et al. 1998) to artificially lower microbial diversity of soil samples - with overall inconsistent results regarding the ecological consequences for decomposition (Hättenschwiler et al. 2005).

Regarding fungi Setälä and McLean (2004), as one of the first studies experimentally controlling fungal diversity in a decomposition experiment, showed a clear positive influence of fungal species number on litter decomposition (measured as CO2-release), but only for a relatively low diversity. Nielsen et al. (2011) later found this to be a general pattern for many diversity–function relationship studies in soil: positive relationships between soil biodiversity and ecosystem processes regarding carbon cycling were much more often found up to a species richness of 10 included species – and less beyond that number.

So up to now, no consensus has been found regarding the importance of soil microbial diversity for decomposition processes. While Nannipieri et al. (2017) summon the research of the last decades to the statement that "no relationship has been shown to exist between microbial diversity and decomposition of organic matter", Delgado-Baquerizo et al. (2016) found an overall positive relation of microbial diversity to multifunctionality (including decomposition) in terrestrial ecosystems using data from large-scale databases.

The microbial soil community plays a fundamental and key role in the decomposition of organic matter, as microbes enzymatically break down many molecules, including hard-decaying components as lignin (Nielsen et al. 2011). In addition to these communities, detritivorous animals such as earthworms, nematodes, isopods, millipeds and springtails (in terrestrial ecosystems), have a vast influence on decomposition processes by feeding on and digesting dead organic matter. These effects are assumed to be mainly indirect effects like litter fragmentation (Yang et al. 2012) and many forms of interactions with microbial community (Hassall et al. 1987, Gómez-Brandón et al. 2010, Nielsen et al. 2011, David 2014), but also many direct influences by digestion have been shown (Kadamannaya and Sridhar 2009, Coulis et al. 2009, David 2014). In addition, several studies showed that the mixed litter effects described above can depend on or are amplified by the presence of (macro-)detritivores (Hättenschwiler and Gasser 2005, Oliveira et al. 2010, Vos et al. 2011).

The role of biodiversity of detritivorous animals for decomposition processes and nutrient cycling however has relatively scarcely and only lately been studied. In an early study by

Faber and Verhoef (1991) on three collembola species the authors concluded that nutrient mineralization is rather influenced by the ecological characteristics of the dominant species than by diversity (within one specific functional group). Naeem et al. (1994) included different soil biodiversity levels, but did not separate if from plant diversity in their setup (and showed only short-term effects of ecosystem-biodiversity on decomposition).

One of the fist surveys studying potential effects of the biodiversity of animal detritovores in the decomposition process was Heemsbergen et al. (2004), who manipulated species composition in microcosms with increasing numbers of the detritivores (up to eight species). Species number had an effect on decomposition, but - in compliance with Nielsens et al. (2011) later generalization - saturation occurred very early. However, functional dissimilarity had a significant positive relation with both litter mass loss and soil respiration. A study focusing on specific interactions of two macro-detritivores feeding on different litter and litter mixtures was done by Zimmer et al. (2005a), showing synergistical effects of woodlice and earthworms, but only when fed on high quality litter. The same approach was repeated relatively recently by Patoine et al. (2017), but with several more litter species and litter mixtures. They found no synergistic effects, but non-additive effects on decomposition and also showed that effects of mixed macro-detritivore treatments on litter mass loss are highest at high diversity of litter traits. This supports the hypothesized importance of the presence of (macro-)detritivores for mixed-litter-effects described above. Oliveira et al. (2010) showed complementarity-effects for a snail and a millipede-species, also with a stronger effect in litter mixtures than in single litter. In a wider approach, including terrestrial and aquatic ecosystems, Srivastava et al. (2009) compiled the results of 28 studies to answer the question weather consumer diversity (species richness) has the same effects as the up to that point way more often hypothesized effect of detritus diversity. They found relatively strong “top-down” effects of consumer diversity, but only weak and not consistent effects of “bottom-up” detrital diversity, which is congruent with the Vos et al. (2011) results regarding the importance of (diverse) detritivores for mixed-litter-effects.

Whereas Srivastava et al. (2009) – treating biodiversity as species richness – included studies of several ecosystems in their analysis, Handa et al. (2014) conducted field experiments in several terrestrial and aquatic ecosystems in different climatic zones. They found that both a reduced diversity of decomposers and of plant litter types results in

slower C and N cycling. However – in contrast to most other studies – they used variation in body size as factor of diversity for decomposers and variation in litter quality as factor of diversity for leaf litter. Hence, they used a completely different concept of diversity than number of species: functional diversity. In addition to other studies (Heemsbergen et al. 2004, Hedde et al. 2010, Coulis et al. 2015) these results might indicate an important point regarding biodiversity and decomposition: functional (trait) dissimilarity and thus functional diversity might be more important than number of species.

The concept of functional traits (McGill et al. 2006, Violle et al. 2007, Nock et al. 2016), functional groups (a groups of organisms being similar regarding a trait) and functional diversity (the variability of traits within ecological communities – Tilman 1997, 2001, Petchey and Gaston 2006, Cianciaruso et al. 2009, Cadotte et al. 2011, Laureto et al. 2015) became more common in ecology from the late 1990s and is nowadays well established within community ecology. This concept had – among others – two consequences for the understanding and studying of communities and ecosystem processes:

1. The consideration of "diversity" changed from "number of species" to the functional properties of species (or individual organisms) and the classification into functional groups. 2. It was increasingly challenged that trait means of species are sufficient (or even appropriate) when studying communities and ecological relations (Bolnick et al. 2003, 2011, Siefert 2012, Barabás and D'Andrea 2016).

Thus, it became apparent that, though species from different taxa are still often combined into functional groups, intraspecific trait variation can be of immense ecological importance (Hughes et al. 2008, Cianciaruso et al. 2009, Violle et al. 2012, Barabás and D'Andrea 2016, Des Roches et al. 2018, Raffard et al. 2019). In many cases within-species (trait) variation can be comparable with between-species (trait) variation (Albert et al. 2010, Jung et al. 2010, Messier et al. 2010, Auger and Shipley 2013, Siefert et al. 2015, Des Roches et al. 2018). Consequently, studies including or specially focusing on intraspecific variation and its impact on species relations and ecosystem functions became more frequent.

Notably, the main focus and the terminology apparently was changed during the shift from exclusively considering species-diversity to the inclusion of within-spices-effects: While studies on the effects of multiple species almost exclusively focused on (and used the terms) "biodiversity" or "interspecific diversity", many studies including within-species

measurements used the term “variation”. Surprisingly, it seems that the terminology is not always used in a strictly standardized way. Traditionally the term "variation" is (coming from evolutionary biology) often used synonymously for within-species, as “diversity” is used for between-species – this definition can still be found in ecological dictionaries (Allaby 2010). But as the understanding of interspecific diversity has evolved from “number of species” to more complex (and diverse) interpretations of the diversity-concept, so has “intraspecific variation”. Consequently, the term “diversity” is often used in an intraspecific context as well (Johnson et al. 2012, Koricheva and Hayes 2018, Raffard et al. 2019). However, despite the sometimes imprecise use of the terms, most often the term “intraspecific variation” refers to the observation or measurement of differences among individuals within a populations or species, whereas “intraspecific diversity” is the result of this variation on the level of the population or species. In other words: the diversity of a population or species is the extend of the variation within a population or species.

Many examples of the first generation of these intraspecific diversity-studies were reviewed and meta-analyzed by Des Roches et al. (2018) – focusing on phenotypic trait variation –, Raffard et al. (2019) – focusing on genetic diversity – and Koricheva and Hayes (2018) – focusing on plant-arthropod-interactions.

Though showing a broad and diverse spectrum of results and interpretations, this first generation of studies already allows to hypothesize some general patterns:

1. The overall ecological impact of interspecific diversity and intraspecific diversity is comparable for some ecosystem processes.
2. For some ecological functions, the importance of intraspecific variation can exceed the importance of interspecific variation, especially for indirect effects.
3. The importance of intraspecific variation differs by trophic level (stronger in primary producers than in consumers).
4. The relationship between the strength of diversity and the influence on an ecosystem process is often - as with interspecific diversity - not linear but seems to follow a saturating curve.

Thereby intraspecific diversity has an influence on a variety of different ecosystem processes like productivity (Crutsinger et al. 2006, Lojewski et al. 2009) – including crop yield (Reiss and Drinkwater 2018), ecosystem stability and recovery (Hughes and

Stachowicz 2004, Reusch et al. 2005, Prieto et al. 2015, Reiss and Drinkwater 2018), communitiy structures (Crutsinger et al. 2006, Howeth et al. 2013, Koricheva and Hayes 2018) – and decomposition (described in detail further below).

However, despite these described first clear indications of the importance of intraspecific diversity, much more research is needed to understand the amount of diversity in a variety of (key) species and the importance of this diversity for ecosystem processes and ecosystem resilience. This is especially important since, as for interspecific diversity, the study of the importance of intraspecies diversity is not exclusively of scientific interest, since the ongoing loss of interspecific diversity due to global change is accompanied by a loss of intraspecific diversity as well (Pauls et al. 2013). In addition to global change, many other anthropogenic influences like habitat modification, intense agriculture and species introduction also have an impact on intraspecific diversity (Mimura et al. 2017). In conclusion, the anticipated loss of diversity is not only likely to be strongly underestimated, but at the same time there is rising evidence for the importance of this level of diversity for ecosystem processes.

The influence of intraspecific variation and diversity on decomposition

As the consideration of intraspecific variation has been introduced in several areas of ecological research, this has also been the case for soil ecology and decomposition. Consequently, the question "Does intraspecific genetic diversity contribute to variation in ecosystem functioning?" was stated as one of the major knowledge gaps to be prioritized by Eisenhauer et al. (2017). Nevertheless, up to now relatively few studies have addressed this question.

Plant litter can show considerable phenotypic variation, which is at least partly based on genetic differences. This phenotypic variation can directly result in variation in decomposition of this litter (Madritch and Hunter 2005, Lecerf and Chauvet 2008). The same was shown for genetic variation (Madritch et al. 2006, Silfver et al. 2007, LeRoy et al. 2007). Consistently - as for interspecific litter diversity - a mixture of different phenotypes (resulting in phenotypic intraspecific litter diversity) can differ from single-phenotype decomposition (Madritch and Hunter 2002, 2004). The same applies for a mixture of

different genotypes (resulting in intraspecific genetic diversity – Madritch et al. 2006, Semchenko et al. 2017).

While there is initial evidence regarding the importance of intraspecific variation or diversity in decomposition processes with respect to leaf litter, little is known about the importance of these factors in detritivorous species so far. To my knowledge, there are up to now only two studies which have addressed this issue, following quite different approaches:

1. Fontana et al. (2019) examine the influences of trait variation (size) on consumption for an isopod species (*Oniscus asellus*) in a microcosm-experiment, hypothesizing that mixed-litter effects are enhanced by intraspecific feeding niche partitioning of the detritivore. Their results support the hypothesis: Litter mass loss was highest for combined litter with a combination of small and big isopods.

2. Mustonen et al. (2020) examine the impact of genetic diversity on decomposition for an earthworm species (*Dendrobaena octaedra*) in a microcosm-experiment, hypothesizing that nutrient mineralization is higher when combining animals with four different genotypes compared to a single genotype treatment (and that possible impacts of this diversity are modified by the diversity of the decomposer community). Their results do not support these hypotheses, but the amount of the genetic variation between the used animals (and therefore the diversity in the "high diversity"-treatments) was not quantified.

Thus, this study aims to expand the knowledge about the influence of a macro-detritivore's intraspecific variation on decomposition processes – while also quantifying the amount of genetic variation when using different treatments regarding diversity.

On the measurement of diversity

As described above, many ways have been used to define and measure interspecific variation and diversity, but there is a clear pattern from "number of species" to consideration (and counting) of functional traits and/or functional groups. The latter has not only imposed and promoted the inclusion of intraspecific variation (and consequently intraspecific diversity), but the use of functional traits can also directly be transferred to measuring intraspecific diversity and possible impacts on ecosystem processes. However, some traits are more complicated (and laboratory- and cost-intensive) to measure,

especially those that concern biochemical processes and certain aspects of physiology. To compensate for that problem, more quantifiable (but less function-correlated) "soft traits" are often used as proxy for stronger function-correlated "hard traits" (Hodgson et al. 1999, Nock et al. 2016). Body size for example is a common "soft trait" for estimating growth rate, but also nutrient uptake and clearance rates (Nock et al. 2016). Alternatively – especially when measuring the trait of interest is difficult – the origin of phenotypic variation in the trait can be used as proxy. When looking at intraspecific variation as phenotypic trait variation, there are (in addition to the in this context less relevant stages of ontogeny and sexual dimorphism) two origins for this diversity: phenotypic plasticity and genetic variation.

Phenotypic plasticity is the ability of a genotype to produce different phenotypes in response to abiotic and biotic conditions (Bradshaw 1965, Pigliucci 2001). Plastic responses thereby allow different individuals to form different phenotypes – potential leading to intraspecific phenotypic diversity regarding certain traits. However, despite the undoubtably enormous role of plasticity for evolutionary processes (Moczek 2015) and niche partitioning (Lipowsky et al. 2015), plastic responses are likely to be very similar among individuals living in the same environment. The more decisive origin for phenotypic trait variation is accordingly much more often likely to be genetic variation.

The ecological effects of genetic variation and diversity have been summarized by Hughes et al. (2008), pointing out the effects of genetic diversity on several ecological processes.

Hence, variation of the genotype can not only be used as proxy for the variation of phenotypic (genetically mediated) traits, but it might also even be the more suitable way to measure intraspecific diversity when focusing on potential ecological consequences of an ongoing and continuing diversity loss.

There is no unanimous definition of genetic diversity, so Hughes et al. (2008) describe it very broadly as "any measure that quantifies the magnitude of genetic variability within a population", which is a feasible approach. Numerous ways have been developed in order to quantify genetic diversity, with different advantages and disadvantages. Before other molecular markers were developed and became affordable, protein polymorphism ("allozymes") was often used for quantifying genetic diversity (e.g. by Bilton et al. 1999), but the resolution is relatively low in comparison to (most) DNA-based markers and requires a sufficient polymorphism regarding the proteins of the organism of interest.

Hence, over the last two decades in the study of genetic variation and diversity, mainly the three following techniques have been used:

RFLP (restriction fragment length polymorphism, used for example by Schweitzer et al. (2005) was among the first techniques allowing not only to measure differences between individuals, but also allowing to get information about the diversity of a population. However, the resolution of this technique is also rather low. A PCR-based variation of this technique – AFLP (used for example by Crutsinger et al. (2008)) has – despite the dominant nature of this marker – a higher resolution, but also requires more intensive lab-work. Microsatellites (tandem repeated sequences, used for example by Barbour et al. (2009) and Abbott et al. (2017)) are often highly polymorphic and widely distributed - and hence have become a very popular and often used molecular marker since the late 1990s until today (Hoshino et al. 2012, Madesis et al. 2013, Granevitze et al. 2014). One of their main disadvantages – the complicated and cost-intensive development – has been compensated using next generation sequencing techniques in the development process (Abdelkrim et al. 2009, Gardner et al. 2011). In a more direct approach of sequencing, both Sanger- and next generation sequencing is used for comparing different genetic regions of mtDNA or cDNA. A newer approach based on this technique is the usage of single nucleotide polymorphisms (SNPs), which became (with a variety of genotyping techniques (Wit et al. 2015)) a major molecular marker for population genetics next to microsatellites (Helyar et al. 2011).

In this study, microsatellites were chosen as genetic marker as they are (when using NGS sequencing) a good compromise in terms of gained information, development effort, cost, resolution, and the possibility to study many individuals. The use of both of these markers and phenotypical functional traits should allow to study the influence of intraspecific variation on decomposition processes and add some valuable content on the field of diversity studies.

Aims and approach of this study

The aim of this thesis is to study the importance of intraspecific variation and diversity in a macro-detritivore on decomposition processes – including a potential loss of diversity in the future. Regarding the latter this work focuses on the effects of strong environmental fluctuations on intraspecific variation to estimate possible impacts of extreme weather events. Since digestion is one of the critical functional traits of a macro-detritivore, the third step is to examine the degree of intraspecific variation in this trait.

Accordingly, the issues to be examined are:

1. The influence of intraspecific variation in an ecologically relevant soil macro-detritivore on, and its significance for decomposition of leaf litter (Chapter 2 and 3).

As mentioned above, little is known about this impact of intraspecific variation in a detritivore species on decomposing processes. Therefore, the first step in this project is to study the amount of this effect. Based on the results of previous research on the influence of interspecific variation and diversity, the following hypothesis can be derived:

Populations with variable digestive phenotypes are more efficient in consuming diverse litter mixtures and the importance of this effect increases with increasing litter diversity.

The isopod species *Porcellio scaber* (Latreille 1804, Isopoda, Oniscidea) was chosen as the macro-detritivore, as this species is of high importance for the decomposition of leaf litter due to its wide distribution. It is native to almost all of Europe and was introduced in many other parts of the world (Wang and Schreiber 1999, Schmalfuss 2003) with a high abundance in many ecosystems. In addition, due to its wide distribution it is also well suited for the chosen study design. Like most terrestrial isopods, it feeds mainly on dead organic material (detritus) and plays a role in nutrient- and carbon-cycling (Zimmer 2002). Many morphological and physiological, but also ecotoxicological studies have been performed with this species (Donker and Bogert 1991, Drobne 1997), but few on the matter of diversity or the role of intraspecific variation (but see Wang and Schreiber (1999) and Schmalfuss (2003)). The main idea in this approach is to increase the variation in lab populations artificially by mixing individuals from different populations of across Europe and to measure whether this increased variation influences leaf litter consumption (Chapter 2). In chapter 3, the properties of the microsatellites developed for this species and the selected populations are examined in more detail.

2. The influence of environmental fluctuation (as a proximate of extreme weather events) on the intraspecific variation in a soil macro-detritivore (Chapter 4).

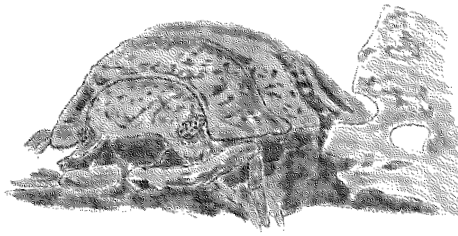
There is much evidence that the ongoing climate change as well as many other human influences lead to a decline in biodiversity – including intraspecific diversity (Pauls et al. 2013, Mimura et al. 2017). One of the predicted effects of climate change is the increase in extreme weather events such as heat waves, droughts, or floods - which potentially have a major impact on biodiversity (Paaijmans et al. 2013, Vasseur et al. 2014, Stott 2016). These factors are expected to select for those individuals, that within the variation of a population are best able to deal with stress and leading to a reduced diversity within the population. In Order to examine the effects of environmental extremes on a) diversity within a population - and subsequently on decomposition – and b) reaction to stress caused by environmental extremes in chapter 4 I compare two populations that are geographically close but live at two different elevations and are thus exposed to very different environmental fluctuations. Here, two populations of the terrestrial isopod species *Trachelipus ratzeburgii* (Brandt 1833) at Unterberg near Salzburg (Austria) were chosen for comparison regarding their diversity using newly developed microsatellites and - as in Chapter 2 – regarding their consumption on different leaf litter mixtures. In this context, the hypothesis is stated that high environmental fluctuation alters the diversity of a population, which has a positive effect on stress resistance, but a negative effect on decomposition.

3. The extend of variation in digestion in a soil macro-detritivore (Chapter 5).

If a trait functional for an ecosystem process cannot be measured directly or only be measured with great difficulty, other, more quantifiable traits are often used, which are less directly connected to certain functions (Nock et al. 2016). However, for the context of this study, this approach cannot be used, as the influence of the variation in traits which are directly connected to the ecosystem process decomposition would be of interest. The use of other traits or genetic variation is a pragmatic approach when the influence of variation or diversity on the ecosystem process alone is of interest. This approach is followed in the chapters described so far. In addition to this, the aim is to test how much variation there really is in a trait that is linked to the process of decomposition. Here, we used pyrolysis GC-MS to test for chemical changes in leaf litter (or rather a variation in chemical change) as it passes through the digestive tract of *P. scaber*.

Chapter 2

Similar, but not identical – Influence of intraspecific variation on leaf litter consumption in a microcosm experiment



Abstract

Despite the fundamental role of intraspecific variation for evolutionary processes, the direct impact of this variation on ecosystem processes has rarely been studied in the context of decomposition. Such studies, however, would be of vital importance since intraspecific diversity is as threatened by global change and other human impacts as interspecific diversity. Due to the well-studied influence of interspecific or trait diversity on litter consumption and decomposition processes, we tested the role of intraspecific variation in the terrestrial isopod species *Porcellio scaber* on litter consumption in a microcosm experiment. Isopod diversity, being measured with newly developed microsatellite markers, was increased experimentally by combining isopods from different field populations. The results indicate an influence of intraspecific variation on decomposition by increasing litter consumption, but this effect only occurred in single-litter treatments, not in mixed-litter treatments and was relatively weak compared to the also measured mixed-litter-effects. We argue that further research on this matter is necessary and recommended, since the potential direct impact of a diversity loss within species on ecosystem processes might have been neglected so far.

Introduction

The importance of biodiversity and the actual and potential consequences of its loss have been subject to scientific research for decades (Loreau et al. 2001, Cardinale et al. 2012, Naeem et al. 2012, Tilman et al. 2014), and have been studied in detail regarding several kinds of ecosystems like marine systems (Worm et al. 2006), grasslands (Tilman and Downing 1994) and soils (Bardgett and Van Der Putten 2014).

Whereas many details remain controversial, two general assumptions can be made: 1. Two of the main factors of biodiversity loss are direct human impacts on various ecosystems (e.g. habitat modification, intense agriculture, overfishing, fertilization) and the ongoing climate change. 2. The loss of biodiversity had, has, and will have a substantial effect on ecosystem functioning and thereby on humans.

Those aspects of biodiversity that influence ecosystem functioning are generally referred to as “functional diversity” (Tilman 2001, Petchey and Gaston 2006). Earlier measurements of (functional) diversity were total number of species or number of functional groups, with

different species assigned to different groups (Naeem and Li 1997, Tilman 2001), but several other measurement techniques have been proposed and tested since then (Petchey and Gaston 2006, McGill et al. 2006, Cianciaruso et al. 2009, Petchey et al. 2014, Laureto et al. 2015). Whereas - as stated by Petchey and Gaston (2006) - most concepts stick to taxonomic scales when measuring functional diversity, the concept of ecological traits allows to focus on individuals (Pachepsky et al. 2007, Violle et al. 2007, Cianciaruso et al. 2009, Cadotte et al. 2011), thereby potentially also including intraspecific variation in the measurement. Focusing on individuals, this concept was used over the last years in many studies (e.g. Albert et al. 2010, Hulshof and Swenson 2010, Messier et al. 2010). However, most studies including functional traits still focus on the species level. Until generally accepted specimen-based trait measurement techniques are implemented (as e.g. Pérez-Harguindeguy et al. 2013 proposed for plants) genetic variation serves as proxy for trait variation in many studies (Araújo et al. 2011). The potential influence of intraspecific variation (summarized by Bolnick et al. (2011)) is examined in studies assessing the ecological consequences of genetic variation (Reusch et al. 2005, Gamfeldt et al. 2005, Hughes et al. 2008, Münzbergová et al. 2009, Crawford and Whitney 2010, Chateil et al. 2013, Fischer et al. 2014). This research is of fundamental importance, since intraspecific variation is also likely to decline in many species due to the same reasons as the decline of species diversity (Bálint et al. 2011, Pfenninger et al. 2012, Pauls et al. 2013).

Despite the underlying measurement and the focused level, it is generally accepted that a loss of functional diversity in many cases leads to changes in ecosystem functioning and ecosystem services, e.g. production, respiration and nutrient cycling (Naeem et al. 1994, Tilman 1997, Hooper et al. 2005). Nutrient cycling is driven by the decomposition of dead organic plant material by the interaction of various organisms (e.g., Zimmer 2008). Naturally, the role of biodiversity for this ecosystem process was examined by many studies in various systems (Gartner and Cardon 2004, Hättenschwiler et al. 2005, Gessner et al. 2010, Handa et al. 2014). Although depending on site-specific conditions, including litter types and decomposer species (or traits) (Wardle at al. 1997), high litter- and decomposer-diversity often leads to accelerated decomposition (e.g. Hector et al. (2000) and Heemsbergen et al. (2004). Handa et al. (2014) specifically showed that reducing functional diversity of decomposers and leaf litter retarded C- and N-cycling across various locations and ecosystems. Whereas, due to the widespread use of litterbag-experiments (Graça et al. 2005), the in- and exclusion of detritivores is often already trait-based (body

size), and some studies focus on the influence of genetic diversity on decomposition regarding litter (Madritch and Hunter 2003, Schweitzer et al. 2004, 2005, Madritch et al. 2006 p. 206, LeRoy et al. 2007, 2012, Crutsinger et al. 2009), the specific role of intraspecific detritivore diversity has scarcely been considered. This is surprising, given the facts that a) detritivore community composition and diversity can have a huge effect on leaf litter consumption (Heemsbergen et al. 2004, Gessner et al. 2010, Handa et al. 2014) and b) animal genetic diversity has been shown to effect other ecological processes, e.g. settlement (Gamfeldt et al. 2005).

Hence, we hold that intraspecific variation can also influence leaf litter consumption and thus on decomposition. Many groups of detritivore species with different niches are involved in the decomposition of dead organic material. One of these groups are terrestrial isopods (Oniscidea), which occur worldwide in a variety of terrestrial ecosystems (Warburg 1993, Schmalfuss 2003). Although depending on isopod species (Zimmer and Topp 2000, Zimmer et al. 2002), food source (Hasall and Rushron 1984) and other factors e.g. degradation stage (Van Wensem et al. 1993), most species of this group play an important role in decomposition by ingesting, digesting and modifying large amounts of leaf litter and other dead organic material. Substantial differences between food sources and selective feeding behaviors have been shown for several isopod species (Rushton and Hassall 1983, Loureiro et al. 2006, Ihnen and Zimmer 2008), but nothing is known about intraspecific resource-use variation and potential non-additive effects in this context.

Therefore, we experimentally manipulated the intraspecific diversity of artificially combined groups of a detritivorous isopod species, *Porcellio scaber* (Latreille 1804), by combining specimens from several European populations. In order to test whether this approach actually increases genetic diversity, we developed microsatellite markers for *P. scaber* to examine this diversity within groups of isopods coming from one natural population and groups of (combined) isopods coming from several geographically distinct locations. The resulting isopod groups were offered different litter types and mixtures in order to study the effects of intraspecific variation on leaf litter consumption. Since a variation in digestive traits should become more important for more diverse mixtures of food sources due du a potential mutual facilitation (Hättenschwiler et al. 2005), we not only hypothesize that a) populations with more variable digestive phenotypes (approximated via genetic variation) are more efficient in consuming leaf litter, but also that b) the relevance of intraspecific variation increases with increasing litter diversity.

Material and Methods

Leaf litter samples of *Acer pseudoplatanus L., Betula pendula ROTH, Fagus sylvatica L., Fraxinus excelsior L., Picea abies (L.) H.KARST, Pinus sylvestris L., Quercus robur L.* and *Sorbus aucuparia L.* were collected in autumn 2012 in areas near Kalmar (Sweden), Lund (Sweden), Kiel (Germany), Cologne (Germany) and Salzburg (Austria) (Fig. 1). Litter of deciduous trees was shaken off trees after senescence, litter of conifers was collected from the ground directly under the trees. Subsamples of leaf litter were grinded in a ZM100 Centrifugal Grinding Mill (Retsch) and analyzed for C and N on an EA3000 Elemental Analyser (using Acetanilid as standard). C/N was calculated as a parameter for litter quality as food source (Swift et al. 1979, Melillo et al. 1982, Schadler et al. 2003, Schädler and Brandl 2005).

Specimens of *P. scaber* were collected in summer 2014 in corresponding areas to the leaf litter. Specimens near Kiel, Kalmar, Cologne and Lund were collected in several forest patches within a small area, specimens from Salzburg were collected in a private garden near a forest.

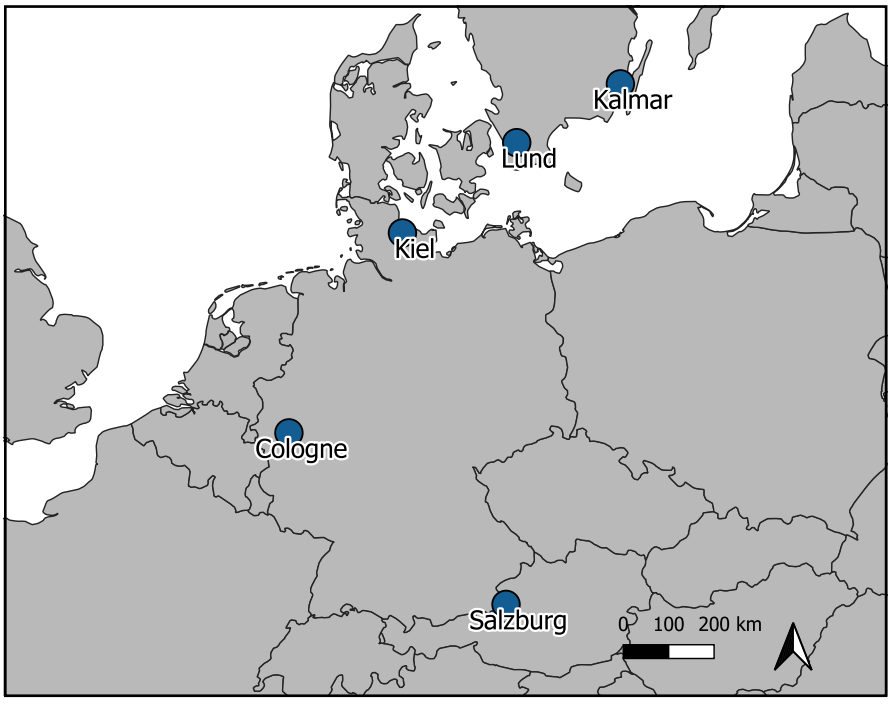


Figure 1: Map showing the five sampling sites within Europe.
(Based on "World border map" by Björn Sandvik. CC BY-SA 3.0)

Development and testing of microsatellite markers

Genomic DNA was extracted from one specimen of *P. scaber* sampled near Cologne, Germany using the DNeasy Blood & Tissue Kit (QIAGEN). A 450-500bp shotgun-library was prepared using the NebNext Kit (New England Biolabs) and sequenced on an Illumina-MiSeq platform with paired-end 250bp reads. Reads were quality controlled, quality-trimmed (Phred quality score < 30) with trimmomatic 0.32 (Bolger et al. 2014) and merged with PEAR (Zhang et al. 2014). The dataset can be accessed in the European Nucleotide Archive (PRJEB14179). A resulting total number of 1,497,465 sequences were screened for microsatellites using QDD3.1 (Meglécz et al. 2014). The screening pipeline included primer development, using primer3 (Untergasser et al. 2012), and a contamination check, using repeatmasker (Smit et al. 2013). Out of the resulting 12,106 sequences containing suitable microsatellites, 48 primer pairs for sequences with a minimum of 12 repeats and an estimated fragment length between 120 bp and 320 bp were chosen for laboratory testing. All primer pairs were tested with DNA from 7 specimens of 4 different populations for successful amplification and loci-polymorphism. DNA was extracted from isopod legs by 4-hour incubation in 500 ml lysis buffer (100 mM EDTA, 10 mM Tris-HCl pH 7.5, adjusted to pH 8) with 50 µl of 10% SDS and 2 µl of Proteinase K (20 mg/ml) followed by protein precipitation using the protein precipitation solution offered by Promega and isopropanol precipitation. (This extraction method was used for all further specimens.) PCR was performed with an annealing temperature of 58 °C for all primer pairs, and the products were separated by gel electrophoresis on a 3.5 % agarose gel. 18 primer pairs for loci showing amplification success and polymorphism were selected for fragment length analysis on a capillary electrophoresis system. One primer of each pair was labeled with one of the fluorescent dyes FAM, HEX or TAMRA and pooled in three multiplex sets according to the estimated fragment length range and dye. Multiplex sets were tested with several specimens of three populations, sampled near Kiel (Germany), Bremen (Germany) and Salzburg (Austria). For eight loci, peaks could be scored reliably. Primer pairs for these loci were rearranged in three final multiplex sets (Table 1). For each marker one amplicon was sequenced (Sanger sequencing) to verify the repeat motives. Multiplex PCR reactions were performed with the QIAGEN multiplex PCR kit, using the standard microsatellite amplification protocol with 30 cycles and an annealing temperature of 58 °C.

Table 1: Developed microsatellites primer pairs for *Porcellio scaber*. MS = Multiplex set

Locus	MS	Primer Sequence (label)	Motive	Size Range
Posca11	3	F: [HEX] GCAAGTCACTCTACTTAGTTTGCC R: GGGTTAGGGTCCTGCTACCA	TAA	121 - 190
Posca17	3	F: [TAM] GCAGCAGGCATACATTTGGG R: TTGACAAGGGCGGTTCTTCT	TG	164 - 194
Posca19	1	F: [TAM] CGCGTCCTTTGAGCCCTATA R: CGAGGCTTTCGTGTACACATC	ATA	154 - 226
Posca34	2	F: [TAM] TGATGCGTTCCTTCGAGGTT R: ACTTTGAGGCCGGCTAACTG	TAT	192 - 288
Posca39	1	F: [HEX] AGGATTGCTAGCCTGTTTAGTG R: AACAGGCTAGCGCTTAAGGG	TTA	212 - 404
Posca40	1	F: [FAM]GGCAGTTAAGAACGCCAGGA R: TCGGTAGGATACGCATTACATCA	TAA	233 - 287
Posca45	2	F: [HEX] CCAGAGGTAATATTCTGTATTCCTGC R: GACACCCACGAGATGTCACA	ATA	268 - 328
Posca48	3	F: [FAM] GGGATGCTATGTTCACAAGTACAT R: AGGTAACGTCAAACCACGCT	AAT	268 - 383

PCR products were run on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) with an ILS600 standard (Promega). The results were scored using Geneious 8.1.6 (Biomatters). To obtain a first overview over the amount of variation appraisable with these markers and to estimate population differentiation of *P. scaber* 183 specimens of all populations sampled for the experiment were used for the population genetics study: Kalmar (41 specimens), Lund (30), Kiel (60), Cologne (28) and Salzburg (24).

The results regarding the natural populations are shown and discussed in chapter 3.

Experimental setup

Isopods were combined in several groups (contaning six specimens) of three variation-levels: Low variation (specimens from one field population), intermediate variation (specimens from two field populations in three different combinations) and high variation (specimens from four field populations in three different combinations) (for details regarding combinations of litter species and isopods see Appendix p. 126).

For litter diversity levels, leaf litter from one species, two species (eight different combinations) and four species (eight different combinations) was combined in equal amounts of litter totalling 2 g (air-dry weight). For treatments with isopods from different

field-populations, litter from all corresponding areas was used in equal amounts to compensate for site-specific adaptations. Additionally, 2 g of each litter type from each area was dried for 24 h at 60 °C and weighed to calculate a correction factor for air-dried litter. Each isopod diversity level was set up with each litter diversity level, resulting in nine different treatments (Fig. 2). Each treatment was set up using at least eight replicates. For paired comparison of all increased isopod diversity and increased litter diversity treatments with corresponding single-litter and single-population isopod treatments, the number of replicates for low isopod diversity and single litter treatment was higher (e.g. all five field population with all eight litter species, resulting in 40 replicates of "single litter, low isopod diversity). The experiment was setup in 192 960 ml transparent plastic boxes (128 x 128 x 90 mm, bikapack kg) with a 100 g plaster-ground in two climate cabinets (MIR-254, Panasonic) with a 12-hour day (light provided by 2600 K LED striped over each shelf) and 16 °C – 14 °C night cycle. The boxes were sealed with transparent lids containing 5 holes (4mm Ø).

All isopods were labeled with two dots (posterior and anterior dorsal pereion) of water-based porcelain ink (Marabu GmbH & Co. KG, Bietigheim-Bissingen) with different colours corresponding to different field populations to identify specimens in case of necessary replacements upon death. All populations were weighed for calculating relative consumption rate (RCR). Relative consumption rate was calculated according to Waldbauer (1968) L/(I*T) (with L = dry weight of consumed leaf litter in gram (g), A = mean weight of isopods in gram (g) and T = time in days (d)).

At the start of the experiment, 15 ml of water was added to the plaster. Every two weeks, 5 ml were added to compensate for evaporation (as determined in pre-experiments under the same conditions). Once a week, dead animals were replaced with specimens from the same source populations and molted animals were relabeled (the source population could be identified by ink on the exuviae or on the other specimens). Laboratory populations were weighed again after replacement of dead animals and the new weight was used for calculating the relative consumption of remaining time until the end of the experiment or the next replacement. After eight weeks litter, was taken out, dried at 60 °C for 24 h and weighed.

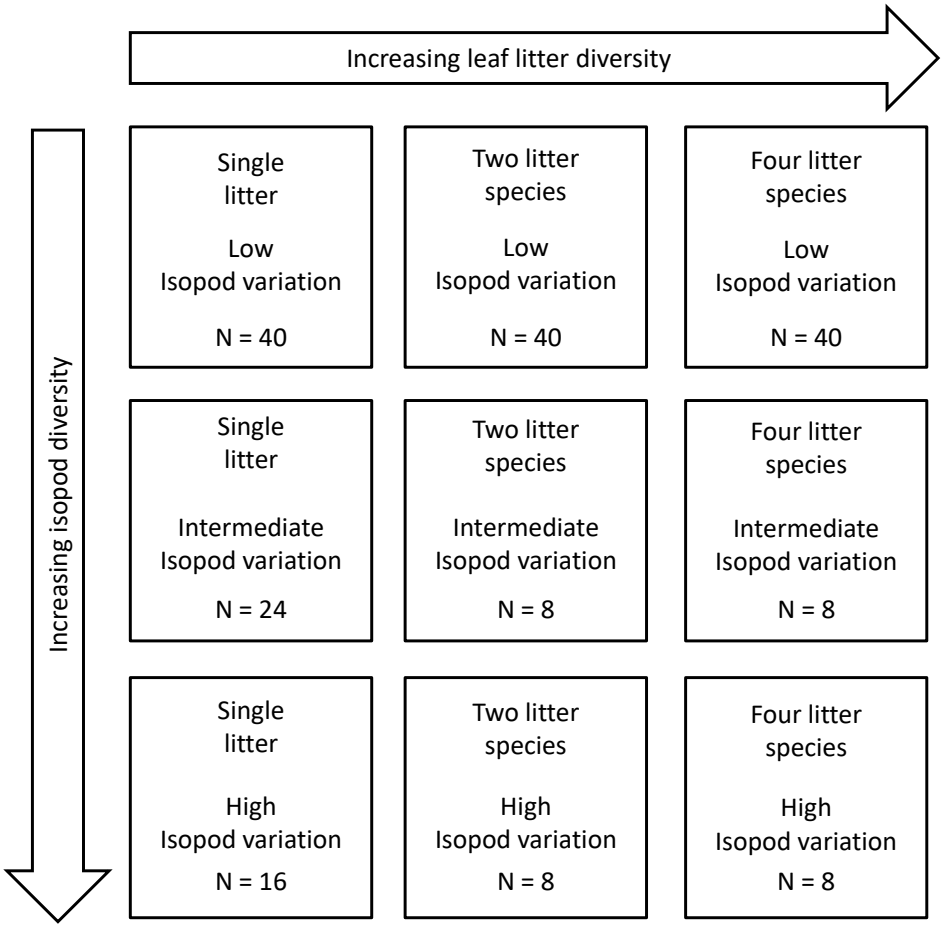


Figure 2: Scheme of experimental setup with factor 1: Leaf litter diversity (horizontally) and factor 2: Isopod diversity (vertically), resulting in nine different treatments. N = Number of replicates.

Genetic diversity of natural and combined isopd groups

For the three diversity levels 10 (natural population), 6 (combination of two populations) and 6 (combination of four populations) groups were chosen for microsatellite analysis (different numbers account for coverage of all populations and combinations at least twice, whereas one high variation laboratory population in the high isopod variation treatment had to be excluded from the analysis due to insufficient DNA quality of two specimens). Since laboratory populations of the two increased variation levels represent artifical populations, total mean number of alleles per marker was chosen as parameter for genetic variation ("allelic diversity") instead of heterozygosity. The parameters were calculated using GenAlEx 6.5 (Peakall and Smouse 2006, 2012).

Data analysis

The total number of alleles was compared among isopod diversity levels with Kruskal-Wallis-Test (KWT) and Mann-Whitney-Test (MWT) as Post-hoc-Test.

Total litter mass loss was calculated with corrected start mass and oven dry litter mass. An average weight for every isopod laboratory population was calculated, taking all replacements into account, and used to calculate relative consumption rate (RCR). Since several assumptions for parametric tests were violated and the isopod laboratory population weight was not positively correlated with litter mass loss, separated tests for both factors (isopod variation and litter diversity) on RCR data was used instead of two factorial ANOVA with isopod laboratory population weight as cofactor. Consequently, both factors – isopod diversity and litter diversity – were analyzed separately with KWT.

In addition, treatments with increased diversity were analyzed in a paired MWT with the corresponding low isopod diversity and single litter treatments. All tests were performed using R (R Core Team 2017).

Results

Microsatellite analysis showed significantly higher numbers of alleles in the laboratory populations containing specimens from four populations (four pop) compared to the laboratory populations containing specimens from one population (natural) and two populations (two pop). There was no significant difference between the two latter groups of laboratory populations (Fig. 3)

Litter mass loss in all 192 boxes did not correlate with isopod laboratory population weight (Fig. 4), despite the considerable variation within the latter. Due to this lack of correlation, laboratory population weight was not included as cofactor in the analysis and relative consumption rate (RCR) was used instead for the analysis.

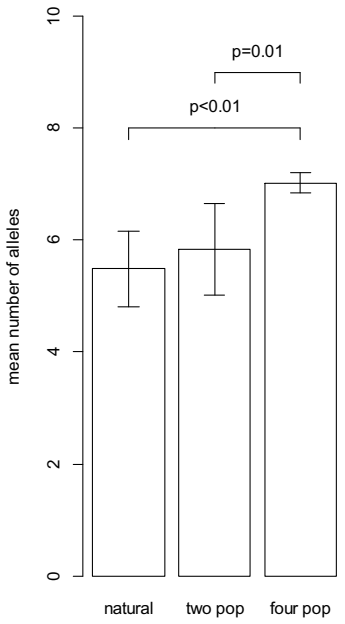


Figure 3: Mean number of alleles per marker in microcosm-groups for three diversity levels. Error bars indicate standard deviation. Significant differences (post-hoc tests, Bonferroni corrected) are marked with corresponding p-value. (N = 10, 6 and 5).

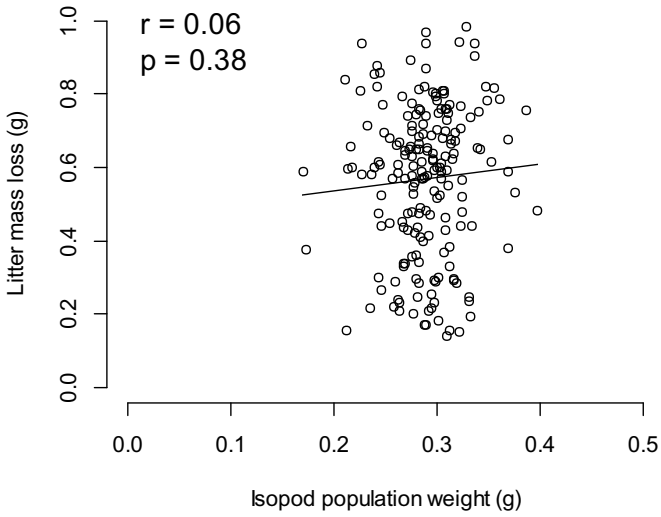


Figure 4: Litter mass loss correlated with total isopod laboratory population weight. Pearson correlation coefficient and significance level are shown in top left corner. Trend line calculated for linear model (least squares).

Within the single litter treatments, litter species had a highly significant influence on RCR (Fig. 5, $p < 0.001$, KWT), but the origin of the isopods had no such influence on the mean consumption (Fig. 6, $p = 0.75$, KWT). Although these effects were expected, the former leads to a high variation of RCR in the analysis of the experiment (see discussion) and a violation of the assumption of homogeneity of variance. Hence, nonparametric tests were used for all further statistical analysis. As leaf litter quality parameter, C/N was measured for all litter types of each origin, but RCR within the single-litter treatments did not significantly correlate with C/N (Fig. 7).

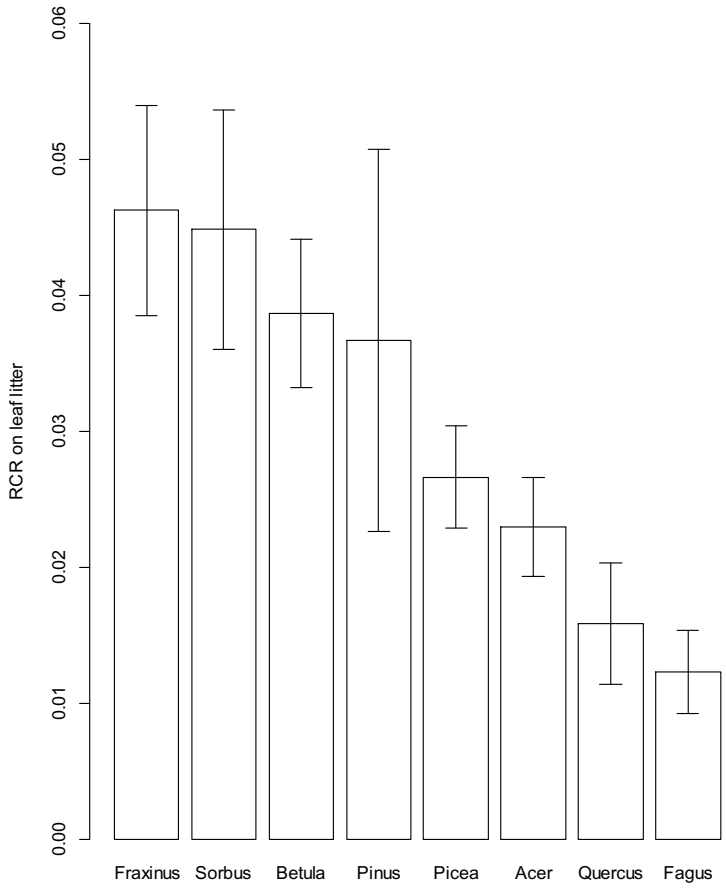


Figure 5: RCR of leaf litter for different litter species within the single-litter low-isopod-diversity treatment. Error bars indicate standard deviation.

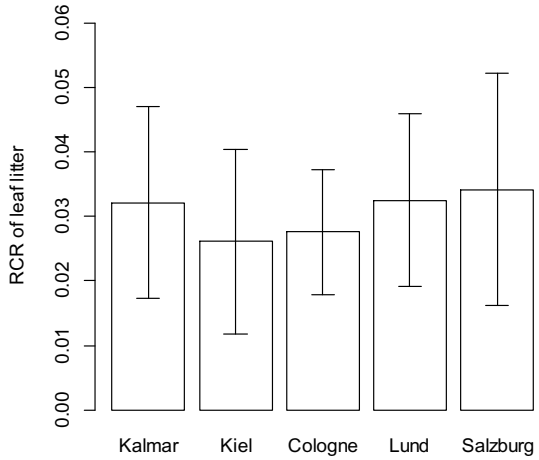


Figure 6: RCR of leaf litter for different litter species within the single-litter low-isopod-diversity treatment. Error bars indicate standard deviation.

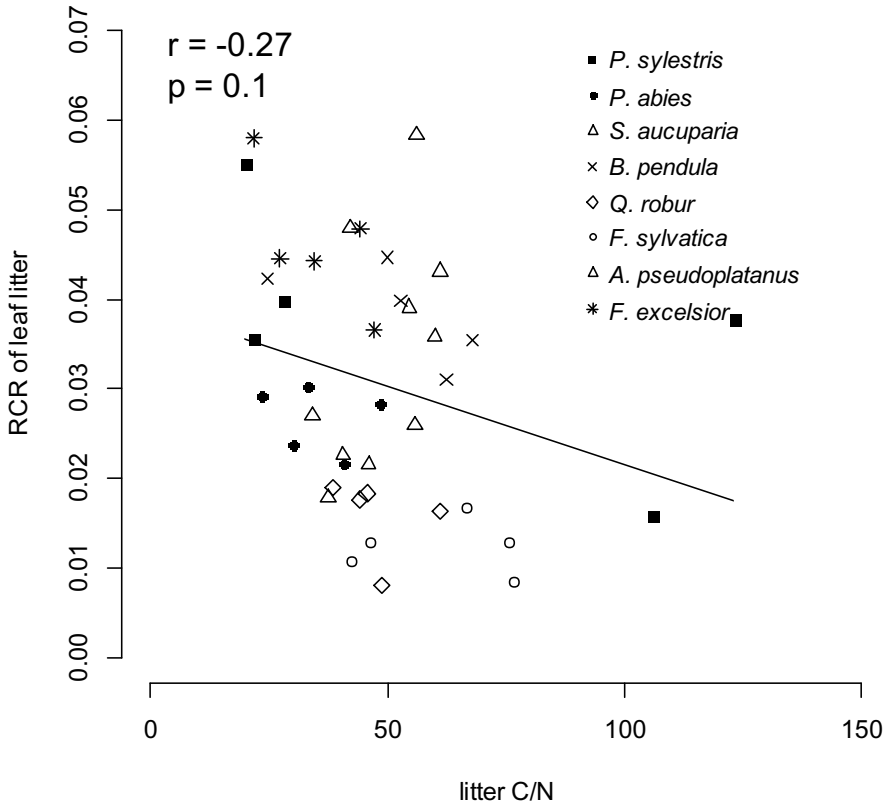


Figure 7: RCR of leaf litter within the single-litter low-isopod-diversity treatment plotted against litter C/N. Pearson correlation coefficient and significance level are shown in top left corner. Trend line calculated for linear model (least squares).

Isopod diversity had no influence on consumption of leaf litter ("intraspecific diversity", Fig. 8a), but a significant influence of leaf litter diversity was found ("mixed-litter-effects", Fig. 8b) p = 0.004 for overall KWT and p = 0.005 for subsequent post-hoc test comparing single species litter and four species mix).

Within all separated isopod diversity treatments (as well as in all treatments together), RCR rose with litter diversity, although within the separated treatments this effect was only significant for the "low diversity" setup (p = 0.01 for overall KWT and 0.02 for subsequent post-hoc test). As expected, variation within the different treatments was very high.

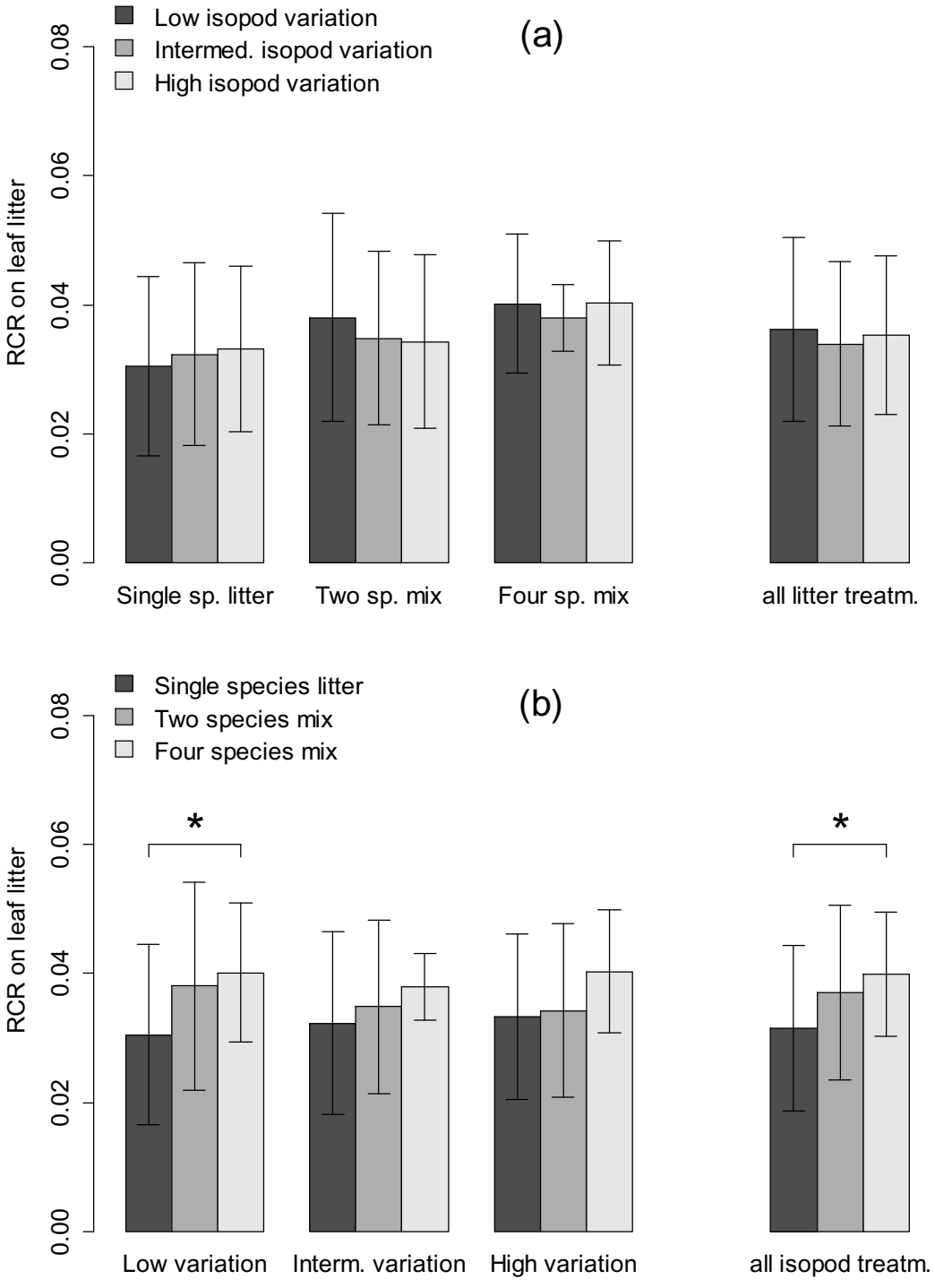


Figure 8: Consumption of leaf litter as a function of (a) isopod diversity levels for the three different litter diversity levels separately and for all litter treatments together, and (b) different litter diversity levels for the three different isopod diversity levels separately and for all isopod treatments together. Significant differences are marked with asterisk. Error bars indicate standard deviation.

However, paired comparison of treatments with increased isopod diversity and increased litter diversity with corresponding low isopod diversity and single-litter treatments showed a significant influence of both factors in some cases. However, for isopod diversity, RCR was only increased significantly in the high-isopod-diversity setup within the single-litter treatment (Fig. 9). For leaf litter diversity, RCR was highly increased in both litter mixes within the low-isopod-diversity treatment and significantly increased in the four species mix within both other isopod diversity treatments.

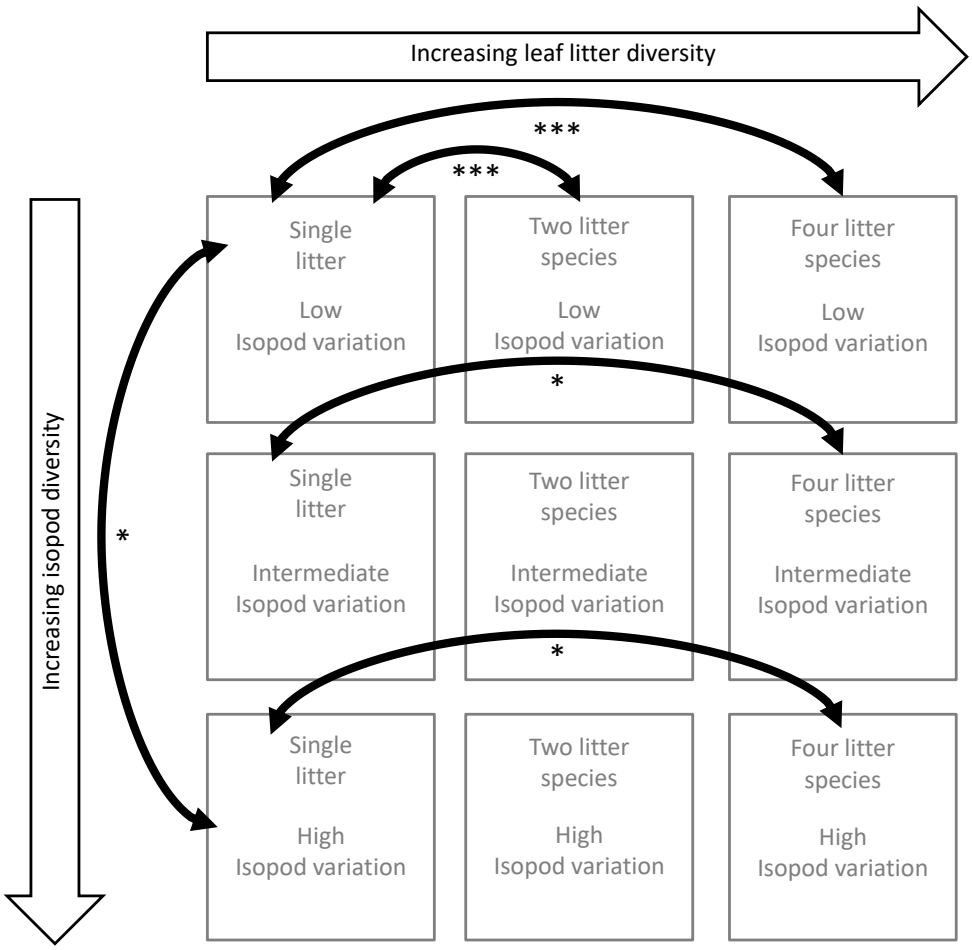


Figure 9: Paired comparison of RCR of leaf litter (paired two-sided MWT). All RCR of increased litter diversity were pairwise compared with mean RCR of corresponding single-litter treatment (horizontally), all RCR upon increased isopod diversity were pairwise compared with mean RCR of the corresponding low-isopod-diversity treatment (vertically). Solid arrows indicate significantly higher RCR in treatments with higher diversity (concordant with hypothesis). Asterisks show significance levels, *= $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Since values for single litter were calculated for each comparison individually (corresponding to the mixture), no correction for multiple comparisons was used.

Discussion

The influence of interspecific litter diversity on decomposition is clearly shown by our results (Fig. 8a), whereas the influence of intraspecific (detritivore) diversity remains inconclusive. While the overall analysis of the factor "intraspecific diversity" showed no such effect (Fig. 8b), paired comparison of RCR of leaf litter showed that this effect is significant – but only if litter diversity is low (Fig. 9). Since there was no such effect in the two treatments with increased litter diversity, the hypothesis of an increasing importance of this effect with increasing litter diversity must be rejected.

Interestingly, a comparable pattern can also be seen regarding the "mixed-litter effects". Although an overall significantly effect of mixing litter on consumption is obvious (Fig. 8b, Fig. 9) the effect decreased with increasing isopod variation: No significant effect was shown in the not paired analysis of relative consumption (Fig. 8b), and only a significant effect regarding the four litter species mix in the paired comparison (Fig. 9). Hence, the two factors a) isopod diversity and b) litter diversity do not seem to facilitate each other. On the contrary, the results indicate a mutual inhibition of these factors in this experiment.

However, for the decreased importance of mixed-litter effects another factor of statistical manner might have a substantial influence on the results: the sample size and its effect on statistical power (Cohen 1992). Due to the experimental design, the number of replicates in the increased isopod diversity (and litter diversity) treatments was much lower than in the low isopod diversity treatments (Fig. 2). Both affected the power of the overall analysis and the paired comparison, with N = 40 for comparisons within the low-isopod-diversity treatments but N=8 for comparisons within the other two isopod diversity treatments. Nevertheless, independent of the dissimilar results in the different isopod diversity treatments, our study shows clearly visible and significant mixed-litter effects as reported by many other studies (Gartner and Cardon 2004, Hättenschwiler and Gasser 2005, Gessner et al. 2010, Handa et al. 2014).

Theoretically this sample size effect also applies for the comparison of different isopod diversity treatments within different litter mixtures, but for the two-litter-species and the four-litter species treatment, the RCR in the treatments with the increased isopod diversity is not significant. However, taking the mixed-litter-effects and the high diversity into account, this effect might potentially be masked by these other factors in the

treatments where N = 8. Especially so, since the effect of mixed litter already promotes consumption in these treatments, whereby other effects might be extenuated.

Although the increased litter consumption in the single-litter high-isopod-diversity treatment can be directly connected to the estimated genetic isopod diversity (and the potential resulting diversity in traits) in this treatment, there is another factor that might be important: the intraspecific diversity of the litter. Due to the experimental design, including litter from each origin to compensate for local adaptations, intraspecific leaf litter diversity was increased in the mixed setups as well. According to our experimental design, this mixing cannot be disentangled from the isopod-diversity-treatment. Combining litter from different genotypes has the same potential influence as combining different litter species that might lead to non-additive effects: Homogenisation of litter quality and an increased niche space for detritivores (Schweitzer et al. 2005). While the latter can be disregarded under these controlled lab-conditions, the former might add to the effects of isopod diversity (if any). Hence, future studies should separate these two factors by using standardized food, despite the artificiality of such an approach.

Only C/N was measured as potential indicator of litter quality but did not significantly correlate with litter consumption (Fig. 7). Nevertheless, the intraspecific as well as the interspecific variation of this parameter can be clearly shown. Homogenisation of litter quality, including nutrient-leaching and selective feeding on litter of higher quality, can therefore have increased consumption as well. But if so, this effect did not appear in the mixed-litter treatments.

Although this study indicates a small effect of intraspecific consumer diversity on consumption, this effect should be tested on a bigger scale. The microsatellite analysis clearly supports the general idea of increasing (and controlling) intraspecific diversity by combining animals from different field populations (Fig. 3). This approach, of course, assumes substantial differences between the included field populations, which was the case for this study (see chapter 3). However, the effect was only significant when combining specimens of four field populations, whereas mixing specimens from only two populations had no such effect. In addition this approach is rather artificial, time-consuming and does by design only increase, but not decrease variation compared to natural populations. However, with genetic markers as developed for *P. scaber* in the context of this study or markers of other terrestrial isopods (Grandjean et al. 2005, Verne

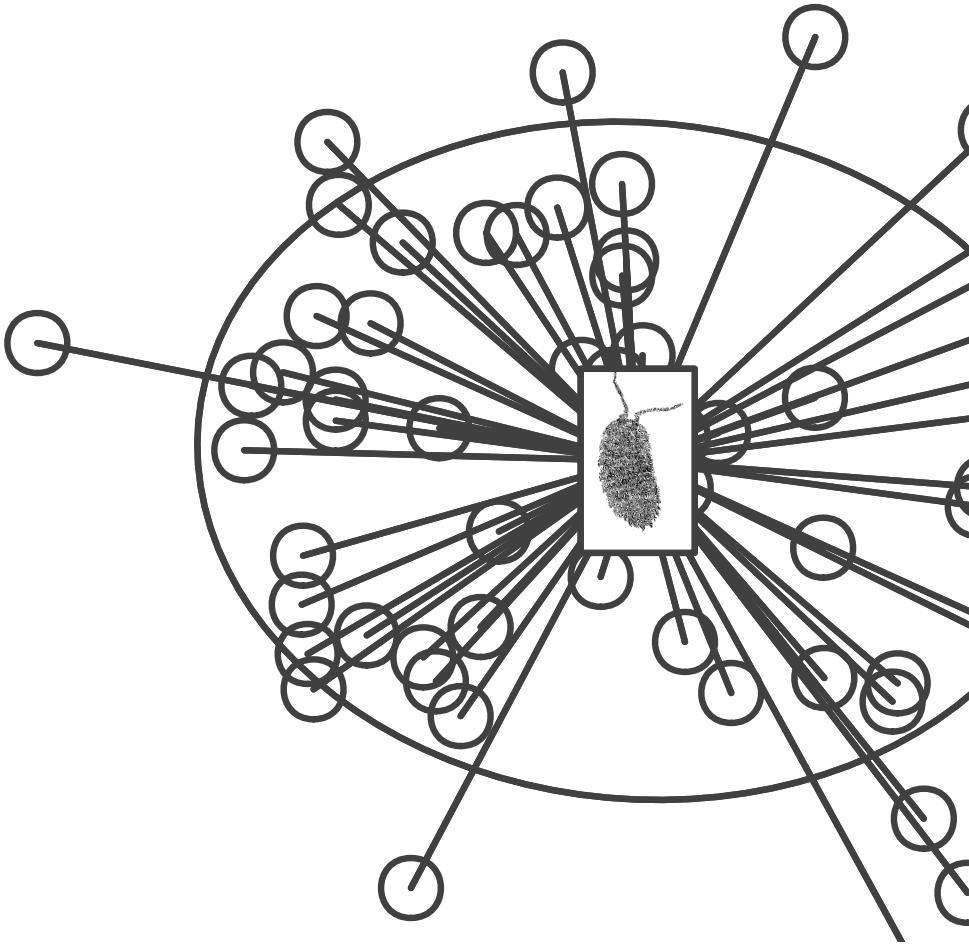
et al. 2006), several different field populations – potentially differing in intraspecific diversity – can be tested towards consumption of local or standadized food sources. Our data however does not show a significant correlation between field population diversity and litter consumption (results not shown).

Genetic diversity is, of course, still only a proxy for intraspecific diversity in (phenotypic) traits directly connected to the targeted ecosystem process and might (as well as should) be replaced by direct measurments as soon as such traits, regarding e.g. carbon- or nurtient-turnover as effect traits, can be clearly identified, defined and measured (Violle et al. 2007). However, the fundamental importance of genetic diversity as basis for any diversity in traits and plasticity should not be forgotten (Hughes et al. 2008).

Litter consumption merely is a first step in the decomposition proccess (Swift et al. 1979, Seastedt 1984, Abd El-Wakeil 2015) and the role of isopods in the entire process, at least for carbon mineralization, has been questioned (David 2014). Nevertheless, if an effect of intraspecific consumer diversity can also be shown in further studies, it will indiacte that a loss of intraspecific diversity might have not only effects on species abilities to adapt to environmental changes, but also directly affect decomposition and other ecosystem proccesses. Further research on this matter is hence warranted.

Chapter 3

North and South - Comments on the population genetics of the *Porcellio scaber* populations used in the experiment



Abstract

In order to measure the genetic variation and population differentiation, eight highly polymorphic microsatellite markers were developed for the terrestrial isopod (Isopoda: Oniscidea) *Porcellio scaber*, using Illumina MiSeq paired-end sequencing, and arranged in three PCR multiplex sets. The markers were tested on five European populations from Austria, Germany and Southern Sweden regarding genetic variation and population differentiation. The studied populations showed a high variability, indicating the suitability of the markers for future population genetic studies, including population assignment. The population differentiation for the studied populations, measured as G_{ST} and Jost's D, was moderate, but distinct and did – measured pairwise – significantly correlate with geographical distance. Our results support the hypothesis of a relatively recent colonialization of Central Europe and Scandinavia by *P. scaber*. The transfer of the markers to two other Oniscidea species, *Trachelipus ratzeburgii* and *Oniscus asellus*, was unsuccessful.

Introduction

Whereas *P. scaber* is well-studied regarding morphology, physiology and behavior, up to now little is known about population genetic parameters of this species, except for Sassaman's study on lactate dehydrogenase polymorphism on populations in California (Sassaman 1978) and Wang and Schreiber's study on multiple allozyme variability on populations in Central Europe (Wang and Schreiber 1999). So far, no specific genetic markers are available for *P. scaber* (apart from some transferable makers developed by Giraud et al. 2013), although its wide geographic distribution and high abundance makes it a good choice for studying population dynamics and dispersal. Genetic markers would also support future physiological and ecotoxicological studies. On that account – and to obtain genetic information necessary for the experiment describes in chapter 2 – we developed eight polymorphic microsatellite markers for *P. scaber* using Illumina MiSeq shotgun sequencing. Microsatellites can be used to answer various ecological and evolutionary questions, whereas their strength lies in their often very high variability (Ellegren 2004, Hoshino et al. 2012, Madesis et al. 2013, Granevitze et al. 2014).

Up to now, only few microsatellites have been developed for Oniscidea (with the exception of *Armadillidium vulgare*, e.g. Verne et al. 2006, Giraud et al. 2013). Thus, we also tested

the transferability of the microsatellite markers (Barbará et al. 2007) for *Oniscus asellus* (Oniscidae) and *Trachelipus ratzeburgii* (Trachelipodidae) as representatives of two other Oniscidea-families.

Using these newly developed primer pairs, five populations from Austria, Germany and Sweden were used for population genetics to obtain a first overview over the amount of variation appraisable with these markers and the population differentiation of *P. scaber.* Wang and Schreiber (1999) support the hypothesis of a postglacial recolonization of Central Europe (based on Vandel (1962)) from a single ice age refugial area, resulting in a relatively high genetic homogeneity and a relatively low genetic differentiation between populations in Central Europe. Although the extent of our study is limited, we hope – in addition to use the markers for our own study on the influence of variation on decomposition - to add some valuable information to the population genetics of *P. scaber* in general.

Material and Methods

Development of microsatellite primers are described in Chapter 2.

For population genetics 183 specimens from 5 different populations (named after a nearby city) were used for the population genetics study: Kalmar (Sweden, 41 specimens), Lund (Sweden, 30), Kiel (Germany, 60), Cologne (Germany, 28) and Salzburg (Austria, 24) (Fig. 1). Specimens near Kiel, Kalmar, Cologne and Lund were collected in several forest patches within a small area, specimens from Salzburg were collected in a private garden near a forest.

Statistical Analysis

In order to estimate the pattern of genetic diversity for the different populations with the new developed primer pairs, the total number of alleles (NA), Observed Heterozygosity (H_O) and Expected Heterozygosity (H_E) were calculated using GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Deviation from Hardy-Weinberg-proportions and Linkage disequilibrium between all pairs of loci were tested using the exact tests with Genepop 4.2 (Raymond and Rousset 1995, Rousset 2008).

As population differentiation parameters, total G_{st}, total Jost's estimate of differentiation (D_{est} - Jost 2008), pairwise G_{st} and pairwise D_{est} were calculated using GenAlEx 6.5. The terminology of G-statistics (as well as of F-statistics) is hardly standardized regarding the exact underlying mathematical procedure. We follow the calculation used in GenAlEx 6.5, where $G_{ST} = \frac{cH_T - cH_S}{1 - cH_S}$, (with H_T and H_S averaged over loci following Meirmans and Hedrick (2011) with corrections of Nei and Chesser (1983) regarding of calculation of H_T and H_S) and $D_{est} = \left(\frac{k}{k-1}\right)\left(\frac{cH_T - cH_S}{cH_S}\right)$ (following Meirmans and Hedrick (2011) eq. 2). Since G_{ST} is decreased by a high mutation rate and the resulting high polymorphism (Jost 2008, Whitlock 2011), it represents a problematic estimator for microsatellites, but still is a valuable parameter (Ryman and Leimar 2009). D_{est} does not have constraints in this respect, but shows other drawbacks (Whitlock 2011). To get a general idea of the genetic differentiation of *P. scaber*, we calculated both parameters. For discussion regarding G_{ST} / F_{ST} and D_{EST} see Jost (2008, 2009), Heller and Siegismund (2009), Ryman and Leimar (2009), Whitlock (2011) and Verity and Nichols (2014). Significance for G-statistics in GenAlEx 6.5 is tested via random permutations (we used 999 permutations), probability values P indicates probability of a random values $\leq$ data value. For population assignment, frequency-based assignment of Paetkau et al. was used via GenAlEx 6.5 (Paetkau et al. 1995, 2004). Mantel test (Mantel 1967) with 999 permutations of pairwise geographical distance and G_{ST} / D_{EST} was performed via GenAlEx 6.5 and visualized with R (R Core Team 2017).

For additional visualization and analysis of population structure, discriminant analysis of principal components (Jombart et al. 2010) was used via the R-Package adegenet (Jombart et al. 2020). Group assignment probability was calculated by using both GenAlEx 6.5 and (for visualization) the R-Package "adegenet" (Jombart et al. 2020).

Results

Developed marker and genetic diversity

Final markers are summarized in Chapter 1, Table 1. All markers were relatively polymorph (Table 2) with a mean of 12.2 alleles over all populations and marker. Heterozygosity for almost all marker / population combinations was high with a mean Expected and Observed Heterozygosity for the five populations of 0.819 and 0.622, respectively. Both variation of heterozygosity among the markers and variation of heterozygosity among the populations

was relatively low with a standard error of 0.034 over all populations and loci for observed heterozygosity and 0.009 for expected heterozygosity. The mean number of alleles for every population did positively correlate with sampling size ($r > 0.99$).

A noticeable number of null alleles occurred for loci Posca19 (for populations from Salzburg and Cologne), Posca40 (for populations from Lund and Kalmar) and Posca39 (Kiel), where over 20 % of the samples could not be amplified. Some Loci/Population-combinations showed significant deviation from Hardy-Weinberg proportions (Bonferroni-corrected, Table 2). For no marker combination linkage disequilibrium was detected in any population.

In contrast to the developed marker for other isopod species (Grandjean et al. 2005, Giraud et al. 2013), none of the primer pairs were transferable. Additional modifications may increase transferability (Barbará et al. 2007), but were not tested in this study.

Population Structure

The overall multilocus G_{ST} value was 0.069 with an SE of 0.004, individual Locus-G_{ST} were ranging from 0.052 – 0.088 (Table 3). The multilocus D_{EST} was 0.473, with an SE of 0.044; individual Locus-D_{EST} were ranging from 0.260 – 0.629 (Table 3). All P for G_{ST} and D_{EST} were 0,001 (smallest possible value with 999 permutations).

Pairwise G_{ST} and pairwise D_{EST} showed lowest differentiation between the two Swedish populations (Table 4). Highest differentiation for both estimators was measured between Lund and Salzburg. All P G_{St} and P D_{EST} were 0,001. These results are fully congruent with the results of the discriminant analysis of principal components (Fig. 10). While all other populations are plainly separated, the populations from Lund and Kalmar strongly overlap. Both populations are – together with population from Kiel – clearly separated from the populations from Salzburg and Cologne.

In Population assignment test, 84 % (154 specimens) were correctly assigned (Table 5). Most mismatches for each population (except for Kiel) were assigned to one particular other population. Although the results are not completely consistent, calculation of group assignment probability shows a very similar pattern: for the vast majority of samples the actual source population is also the population with the highest calculated membership probability (Fig. 11). Especially the similarity of the two Swedish populations is illustrated,

though – in contrast to what could be expected based on the discriminant analysis of principal components – most samples are correctly assigned. For populations from Cologne and Salzburg, membership probability is almost in all samples 100% for the actual source population – with one major exception for a specimen from Cologne that has a very high probability to belong to the population from Salzburg.

Table 2: Genetic variability per locus for different populations and in total. N = total number of scorable samples, NA = number of alleles, Ho = Observed Heterozygosity, He = Expected Heterozygosity, asterisks indicate significant deviations from Hardy-Weinber

Locus		Kalmar	Lund	Kiel	Cologne	Salzburg
Posca11	N	41	30	60	26	24
	NA	13	11	12	16	12
	H_O	0.927	0.767	0.833	0.654	0.625
	H_E	0.831	0.807	0.806	0.881*	0.883*
Posca17	N	41	30	60	28	24
	NA	7	8	8	11	8
	H_O	0.707	0.533	0.733	0.607	0.625
	H_E	0.757	0.760	0.792	0.816	0.760
Posca19	N	41	30	59	17	16
	NA	9	8	9	12	10
	H_O	0.610	0.533	0.508	0.118	0.438
	H_E	0.762	0.807*	0.748*	0.872*	0.822*
Posca34	N	41	29	49	24	22
	NA	13	7	12	15	14
	H_O	0.561	0.345	0.224	0.333	0.500
	H_E	0.807	0.766*	0.808*	0.893*	0.812
Posca39	N	41	28	47	27	24
	NA	10	11	19	15	15
	H_O	0.366	0.464	0.383	0.481	0.792
	H_E	0.766*	0.802*	0.910*	0.892*	0.893
Posca40	N	29	23	60	28	24
	NA	8	8	11	10	12
	H_O	0.276	0.304	0.667	0.893	0.875
	H_E	0.700*	0.779*	0.802	0.730	0.878
Posca45	N	41	30	60	28	24
	NA	14	12	12	14	14
	H_O	0.780	0.833	0.733	0.821	0.792
	H_E	0.794	0.788	0.713	0.888	0.891
Posca48	N	41	30	60	28	24
	NA	18	17	16	23	14
	H_O	0.878	0.667	0.867	1.000	0.833
	H_E	0.881	0.876*	0.853	0.920	0.801
Total (mean)	N	39.500	28.750	56.875	25.750	26.750
	NA	11.500	10.250	12.375	14.500	12.375
	H_O (SE)	0.638 (0.082)	0.556 (0.067)	0.619 (0.080)	0.613 (0.105)	0.685 (0.057)
	H_E (SE)	0.787 (0.019)	0.798 (0.013)	0.804 (0.021)	0.862 (0.021)	0.840 (0.018)

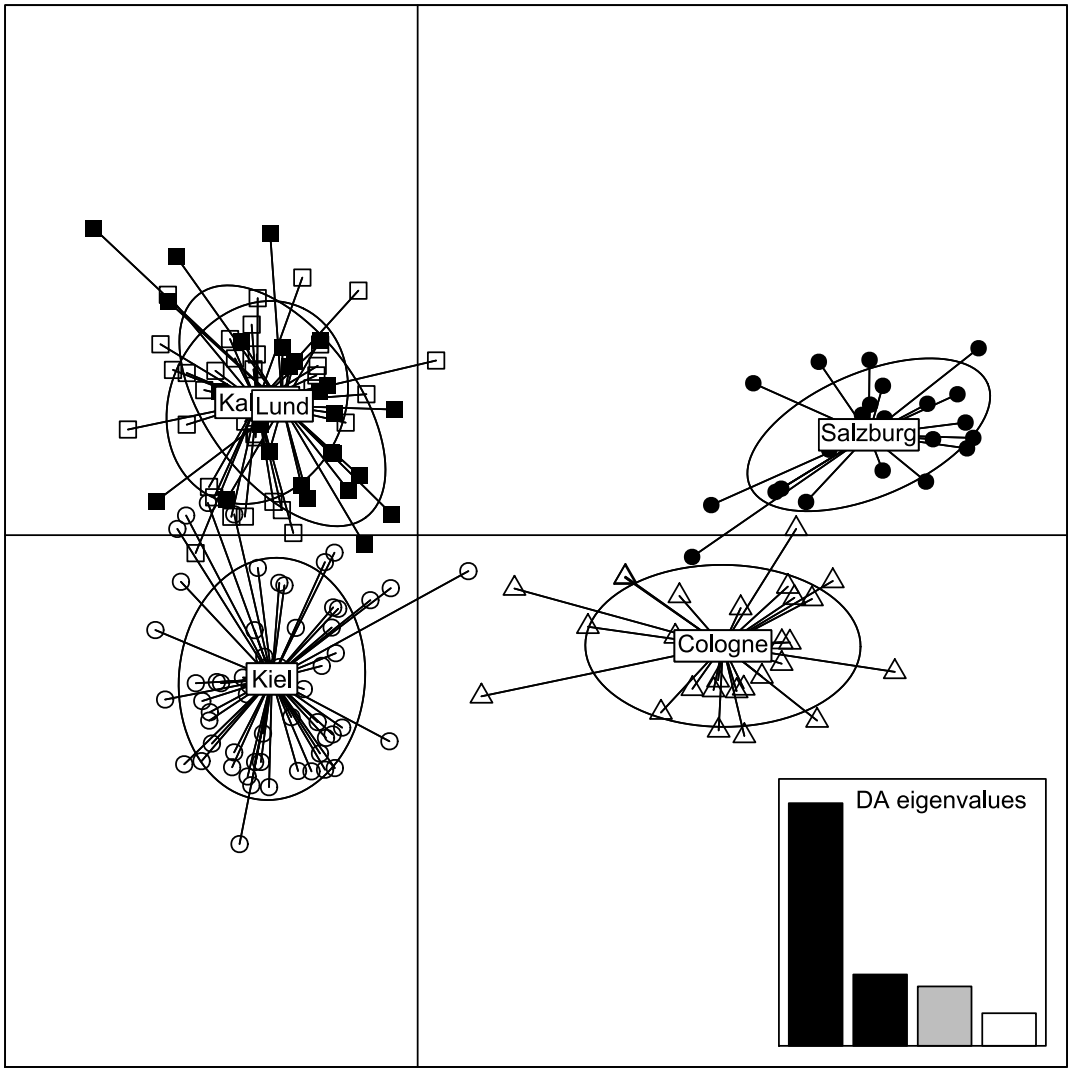


Figure 10: Discriminant analysis of principal components. White quares = Specimen from (s.f.) Kalmar, grey squares = s.f. Lund, white circles = s.f. Kiel, grey circles = s.f. Salzburg, white triangles = s.f. cologne

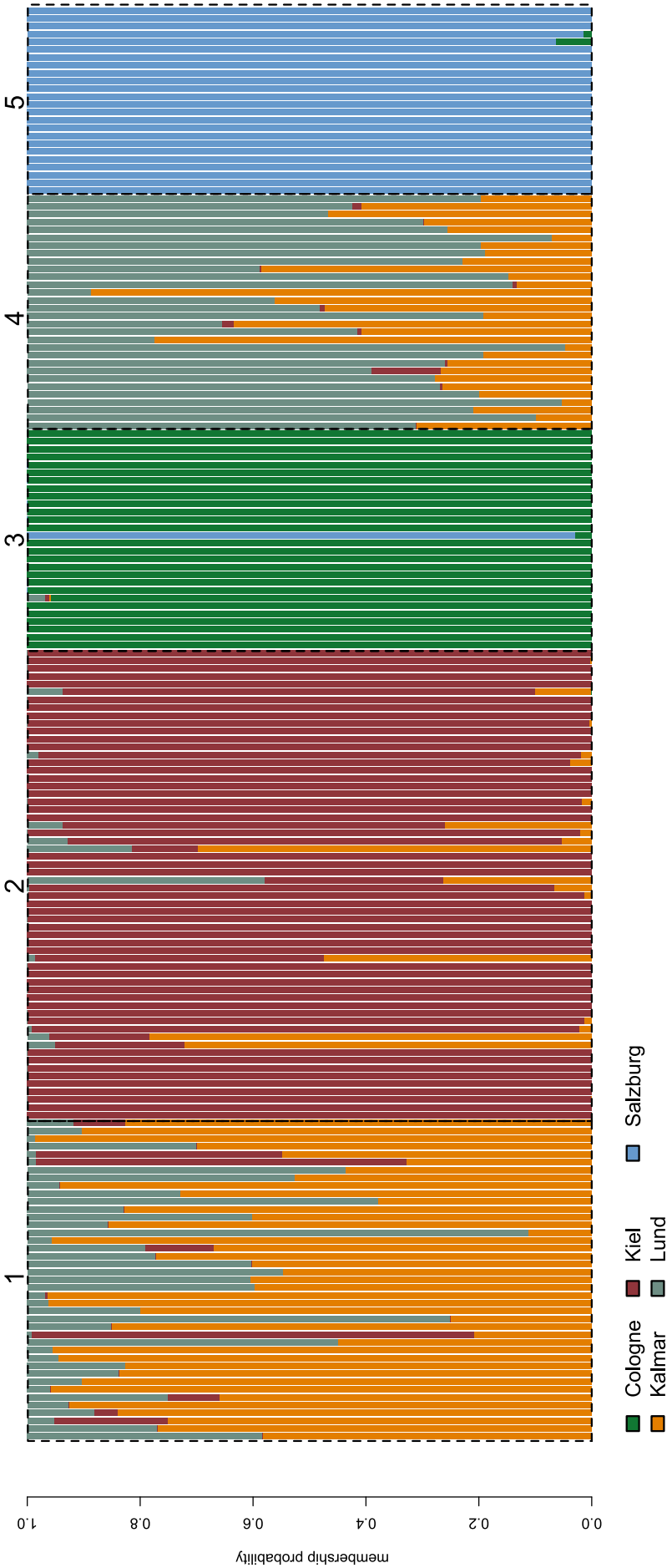


Figure 11: Group assignment probability. Top numbers show the actual origin population of the samples 1 = Kalmar, 2 = Kiel, 3 = Cologne, 4 = Lund, 5 = Salzburg. Colors show the probability of assignment to the respective population. Yellow= Kalmar, red = Kiel, green = Cologne, grey = Lund, blue = Salzburg.

Table 3: Total GST and DEST for individual markers and in total.

Locus	G_{ST}	D_{EST}
Posca11	0.066	0.523
Posca17	0.052	0.260
Posca19	0.077	0.492
Posca34	0.075	0.528
Posca39	0.065	0.597
Posca40	0.088	0.466
Posca45	0.066	0.425
Posca48	0.064	0.629
Total (SE)	0.069 (0.004)	0.473 (0.044)

Table 4: Pairwise GST (below diagonal) and DEST (above diagonal).

	Kalmar	Lund	Kiel	Cologne	Salzburg
Kalmar	-	0.111	0.226	0.653	0.675
Lund	0.013	-	0.228	0.675	0.734
Kiel	0.026	0.025	-	0.621	0.687
Cologne	0.058	0.056	0.053	-	0.257
Salzburg	0.064	0.065	0.062	0.018	-

Table 5: Population assignment.

Source Population	Assigned population				
	Kalmar	**Lund**	**Kiel**	**Cologne**	**Salzburg**
Kalmar	**37**	4	-	-	-
Lund	7	**23**		-	-
Kiel	5	4	**49**	-	2
Cologne	-	1	-	**23**	4
Salzburg	-	-	-	2	**22**

Mantel tests of pairwise geographical distance and pairwise G_{ST} / D_{EST} consistently showed a high and significant correlation between these matrices with r = 0.837 and 0.828 respectively (Fig. 12). Both correlations were highly significant with p = 0.004 and p = 0.006.

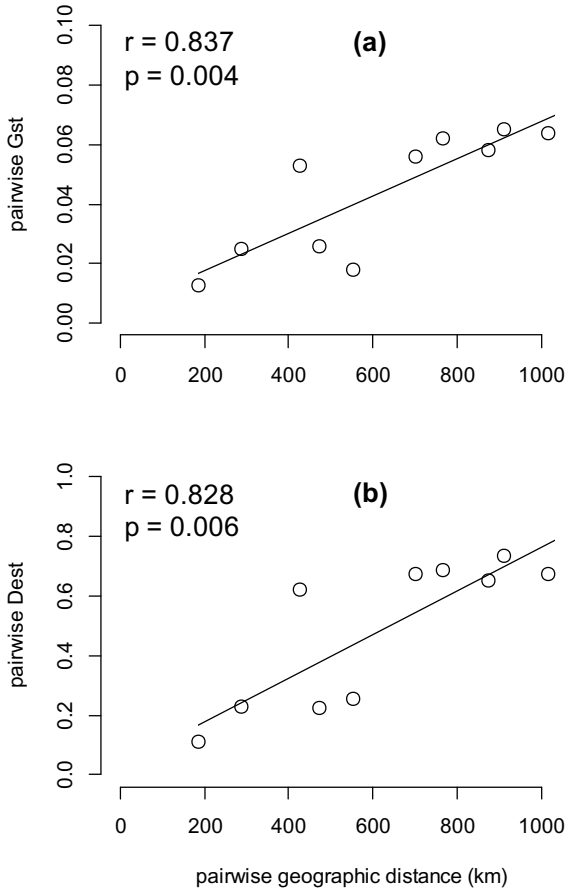


Figure 12: Correlation of (a) pairwise GST values and (b) pairwise DEST–values with pairwise geographical distance. r and p calculated by Mantel-Test. Trend line calculated for linear model (least squares).

Discussion

The eight markers developed in this study are highly polymorphic, resulting in a high number of alleles and, for the tested populations, also in a high heterozygosity. This high polymorphism may be simply explained by the specific microsatellite development process. Only sequences with a minimum of 12 repeats in the specimen used for the library were chosen for further testing, already targeting a potentially high number of alleles.

There were a considerably high number of marker/population-combinations that significantly deviated from Hardy-Weinberg-Proportions. It is, however, a general problem to test for Hardy-Weinberg proportions, if loci have many different alleles, since many genotypes will occur at low numbers, especially so, if sampling size is low. Since isopods usually have high abundances, deviations from Hardy-Weinberg proportions are highly likely to have occurred due to non-exhaustive sampling. Future studies should consider this. In addition, a considerable number of null alleles occurred for three makers, with two of these markers showing a high number of null alleles for two populations. These two population pairs (Cologne and Salzburg at locus 19 / Kalmar and Lund at locus 40) both showed a very low pairwise differentiation. There are several potential reasons for null alleles (Flores-Rentería and Krohn 2013), but this clustering explicitly indicates failed primer annealing due to mutations on the primer binding sited (though this is surprising for locus 19, since a specimen from Cologne was used to create the NGS library). This is a potential problem for future studies including other and genetically more distinct populations, but this issue might be solved by PCR-modifications.

Neither mean observed nor mean expected heterozygosity showed any substantial differences in genetic variation between the populations (and, since standard error was relatively low, between the markers) despite the different number of collected specimens. As expected, mean number of total alleles did positively correlate with sampling size.

Taking the violation of the assumption of Hardy-Weinberg-Proportions and especially the low sample number into account, the results of population differentiation tests should be interpreted with caution. Nevertheless, they can give a good first insight into the degree of differentiation. Considering all loci to have a quite high heterozygosity (reducing the maximal achievable G_{ST}), genetic differentiation overall is not exceedingly high, but significant distinctive. Pairwise G_{ST}-values were slightly higher compared to the allozyme-results of Wang and Schreiber (1999) where G_{ST} for different populations was calculated as

averaging values of population pairs and ranged from 0.016 to 0.052 (with the outstanding exception of 0.117 when a population from the island of Hiddensee, North-East Germany, was included). Jost's D displays a different estimate of differentiation, it measures the fraction of allelic variation among populations and is an absolute measure (Jost 2008, Balkenhol et al. 2015). The total differentiation for the three studied populations can therefore be interpreted as intermediate.

The pairwise comparison showed a relatively low differentiation between the three northern populations (especially between the two Swedish populations) on the one hand, and between the two southern populations on the other hand (Table 4). The differentiation between Kiel and the two Swedish populations is low despite the Baltic Sea as a natural border between these populations and is for instance much lower than the differentiations between Kiel and the other two Central European populations. This suggests two possible scenarios: A high anthropogenic exchange of specimens (passive transfer as described by Slabber and Chown (2002)) or – more likely – a relatively recent colonialization of the Scandinavian Peninsula, also most likely du to anthropogenic influences. The latter explanation would support and add to the results of Wang and Schreiber (1999), who included no Scandinavian population. Due to this lack of border-effect, population differentiation parameters could be very well correlated with geographic distance (Fig. 12). Wang and Schreiber (1999) showed the same result, although only significant when not corrected for small sample sizes according to Nei and Chesser (1983). However, to study the effect of natural borders, the spreading and the impact of anthropogenic influences, specimens from more populations from Europe and also outside Europe should be included. This might also help to understand the global distribution of *P. scaber* (very likely due to human influences), since with population assignment potential means of distribution may be comprehensible (as asked by e.g. Slabber and Chown (2002)).

In summary, the developed markers are sufficient to compare genetic variation between populations of *P. scaber* and should also suite many others purpose e.g., population assignment. Since highly variable markers were developed by design, no general conclusion about the amount of genetic variation of this species in Central Europe can be drawn. The population differentiation analysis showed a moderate, but distinct and significant differentiation between the studied populations, whereas our results are consistent with the hypothesis of a relatively recent colonialization of Central Europe. However, future studies should include specimens from more distant populations outside Europe.

Chapter 4

Up and down – a case study on the influence of altitudinal environmental fluctuation on intraspecific variation, HSP70 gene expression and leaf litter consumption

„Der Untersberg bei Salzburg" by Heinrich Bürkel (appr. 1863, public domain)

Abstract

The influence of altitude on specimen and populations has been studied intensively for decades, but few studies have been focusing on effects of high diurnal fluctuation of several abiotic parameters at high altitude – although these diurnal fluctuations are very likely to have an enormous effect on organisms living in these environments. These conditions might not only function as a selective pressure changing and potentially lowering genetic and phenotypic variation (with ecological effects), they might also be a model to estimate the effects of temporary environmental extremes and extreme weather events as part of the ongoing climate change. In this study, we examine the effects of high altitudinal diurnal fluctuation on a population of the terrestrial isopod *Trachelipus ratzeburgii* by comparing two populations living at two different altitudes of the same mountain regarding genetic variation (measured via microsatellites), consumption of litter mixtures and stress response (measured via HSP70 gene expression). Our results show neither a difference in genetic variation nor any genetic differentiation between the populations and no difference in stress response. But we observed a nearly identical absolute consumption of leaf litter despite the fact that the isopods living at high altitude were significantly smaller than the isopods at lower altitude. Potential reasons for this increased relative consumption and the size differences are discussed.

We conclude that our study did not detect any effects of altitude on the measured parameters and that the results show that altitude is only suitable as a potential model for the effects of extreme weather events when isolation by environment – in contrast to our study – is given.

Introduction

Altitude is one of the most common used gradients in the study of ecological effects of different environmental condition (Hodkinson 2005, Körner 2007, Montesinos-Navarro et al. 2011, Luquet et al. 2015). While effects like UV-radiation, shortened season and lower average temperature are well known and popular effects of high altitude (Körner 2007), diurnal fluctuation and higher amplitude of several parameters (especially temperature) are less perceived. This is incomprehensible, since a) a fluctuating environment has presumably an enormous effect on a variety of species in this environment (Folguera et al. 2011, Hallsson and Björklund 2012) and b) the effects of environmental fluctuation in other habitats were frequently examined over the last decade (Estay et al. 2011, Vasseur et al. 2014). Besides species specialized on habitats with environmental fluctuation (e.g. organisms living in tidal zones or alpine plants) many generalist species, sometimes referred to as "elevational generalists" , can be found at high altitudes with a high diurnal amplitude as well (Heaney 2001, Gadek et al. 2018). A fluctuating environment should have, besides all other influences of high altitude, a considerable impact on populations of such generalist species, since they lead to stress (Bernhardt et al. 2020). Stressful environments haven been known to alter phenotypic and genetic variation, though is still unclear if stressful conditions increase (Bubliy and Loeschcke 2000, Husby et al. 2011) or decrease (Hallsson and Björklund 2012) this variation. Rowiński and Rogell (2017) summarize various hypotheses and studies on the effect of stressful conditions on genetic variations, concluding that previous research makes predictions about variation in a particular stressful environment impossible. Another prediction, however, is more reliable: As Hoffmann et al. (1991) summarize, there are three possibilities in general how a population can react when exposed to stressful conditions: a) avoidance (movement or behavioral change) b) extinction and c) adaptation through selection or through a plastic response. If avoidance is impossible (which applies for many mountain populations) a fluctuating environment will undoubtably lead to a positive selection towards specimen being able to deal with that stress (Endler 1986, Stillman 2002, Rowiński and Rogell 2017). Therefore, these environments might function as a selective pressure, altering not only the genetic variation, but should also lead to an increase of stress resilience within this population. This form of local adaptation has been shown in many studies (Stillman 2002) and is rather driven through isolation by environment (Wang and Bradburd 2014) than geographic distance. Hence, a population from a stressful environment should be

genetically differentiated to population from an environment lacking these stressful conditions, even if geographic distance is low.

It is an appealing, but also reasonable idea to use high fluctuations of environmental parameters at high altitudes as a model to estimate some of the effects of the ongoing climatic and therefore environmental change (space-for-time approach), since one of these predicted effects is the increase of temporary environmental extremes and extreme weather events (Easterling et al. 2000, Rummukainen 2012, Stott 2016), having a comparable stress-effect on species as the rapid and immense temperature changes at higher altitudes and can affect biodiversity and ecosystem processes (Jentsch and Beierkuhnlein 2008, Bernhardt et al. 2020).

The effect of variation has been neglected in the first generation of climate-change studies focusing on changing mean temperatures (Thompson et al. 2013) – whereas recent studies and simulations suggest that an increase of extreme events and an increase of environmental variation might have a comparable or even bigger influence (Paaijmans et al. 2013, Vasseur et al. 2014, Vázquez et al. 2017). Besides these extremes events Donat and Alexander (2012) show that also daily minimum and maximum temperatures may be substantially effected.

Though these predicted effects might differ from relatively "constant" environmental extremes, they are one of few ways to look at long term effects of such temporary environmental extremes. In other words: Looking at fluctuating environments might be the most realistic way to make predictions (Lundberg et al. 2000, Hallsson and Björklund 2012).

Although populations of some species presumably will not be able to survive temporary extremes, for many other species (especially generalist species) environmental extremes will function as a selection pressure, resulting not only in a reduction of the variation within the population, but also changing the phenotypic composition of the populations.

Otherwise stated, a loss of genetic diversity and of certain phenotypes precedes the loss of the whole population or species. Unfortunately, the former is much harder to see or even predict compared to the latter, whereby the awareness for this problem is much lower. However, a lower genetic variation does not only potentially effect population and ecosystem dynamics, but also makes the population more prone to extinction (Frankham 1997). Adaptation to high altitude like altered oxygen transport (Monge and Leon-Velarde

1991) or physiological adaptations to low temperatures or high radiation (Alonso-Amelot 2008) have been shown for many species, but reduction of variation itself – both loss of traits and genetic variation - has not been described as a result of populations dynamics on high altitudes.

Comparing populations of the same species from areas that differ considerably in their diurnal fluctuation of environmental parameters – especially temperature – might thus be an opportunity to examine the hypothesis, that (generalist) populations from areas with a high diurnal environmental fluctuation might be less divers, but due to local adaptations more resistant to environmental extremes by being more resistant to stress.

In addition to the changes for the populations, the reduction of the variation and the changes of the phenotypic variation might alter the populations influences on the ecosystem. A loss of certain phenotypes or in a loss in overall variation might result in a loss of functional traits. This might change several dynamics within the ecosystem, depending on the affected species and its role in the ecosystem.

We chose two populations of the isopod species *Trachelipus ratzeburgii* (Brandt 1833), sampled at two different altitudes with different amounts of temperature fluctuation to study the effects of these fluctuations on a) genetic differentiation and diversity (measured with newly developed microsatellite markers), b) the resistance to stress, caused by two different sudden environmental extremes (measured via relative gene expression of HSP70) and c) the ability to consume (and thereby decompose) leaf litter mixtures of different complexity as an example of a potential loss of functional traits.

We chose to use microsatellite markers for measurement of genetic variation and population differentiation, since these genetic markers are not only well suitable to measure both parameters, they are also relatively easy to develop by using NGS sequencing technology (Akemi et al. 2012, Fernandez-Silva et al. 2013), have a comparatively high resolution and therefore allow to study fine-scale ecological questions like ours (Selkoe and Toonen 2006).

For measurement of stress response, we chose HSP70 gene expression measurement via qPCR. Heat shock proteins - and especially HSP70 - are known to play a fundamental role in the reaction in cells towards stressful conditions, as they protect critical metabolic enzymes (Sørensen et al. 2003, Richter et al. 2010). But as their expression is cost-intensive, in populations living under constant levels of stress a physiological stress

resistance towards this stress is achieved thorough other cellular mechanisms and hence the stress induces expression of HSP is lower. This was shown before by Bettencourt et al. (1999) and Lansing et al. (2000) for *Drosophila melanogaster* regarding heat stress and Köhler et al. (2000) for soil arthropods (including terrestrial isopods) regarding heavy metals. We expect the same effect regarding heat stress for a population adapted to the diurnal temperature fluctuation at high altitudes – however this adaptation might not be beneficial regarding oxidative stress induced by flooding. Thus flooding-induced stress was measured additionally.

As ecological trait we selected decomposition. Not only because it is a fundamental process in any ecosystem, but also since numerous studies have been conducted in the context of variation and decomposition (Gessner et al. 2010, Kou et al. 2020). Though the vast majority of studies relate to interspecific variation and mainly variation of the consumed litter, we believe that a the intraspecific diversity of the detritivore - if significantly altered und in resulting in a loss of traits – can also have an affect on decomposition by affecting litter consumption – as shown by many studies for interspecific (functional) diversity (Heemsbergen et al. 2004, Handa et al. 2014). In this context, we designed the leaf-litter consumption experiment not only to examine the assumption that a higher diversity (including diverse functional traits) leads to a faster decomposition, but also that this effect becomes more important for more diverse mixtures of food sources to a potential mutual influence of detritivore and litter diversity (Hättenschwiler et al. 2005).

Material and Methods

Sample sites and litter consumption experiment

As high altitude and low altitude populations, two populations from the Untersberg mountain near Salzburg were chosen. The low altitude population was located at 47°44'40.00"N, 13° 0'47.00"E, the high-altitude population was located at 47°43'35.00"N, 13° 0'45.00"E (Fig. 13.) To simplify terminology, the two populations will be referred to as populations “valley” and “mountain” in the following. At both sites, temperature loggers (Hobo) were placed between November 2011 and May 2014, measuring intervals of one hour.

Leaf litter of *Acer pseudoplatanus L., Betula pendula ROTH, Fagus sylvatica L), Fraxinus excelsior L., Picea abies (L.) H.KARST, Pinus sylvestris L., Quercus robur L.* and *Sorbus aucuparia L.* was collected in autumn 2012 at several sites near Salzburg. The litter was stored and air-dried until usage. Specimen for the litter consumption experiment were collected in summer 2013 at both sites on several days over the year and kept in plastic boxes in a climate cabinet (MIR-254, Panasonic) with a 12 h day-night cycle at 14 °C and 16 °C.

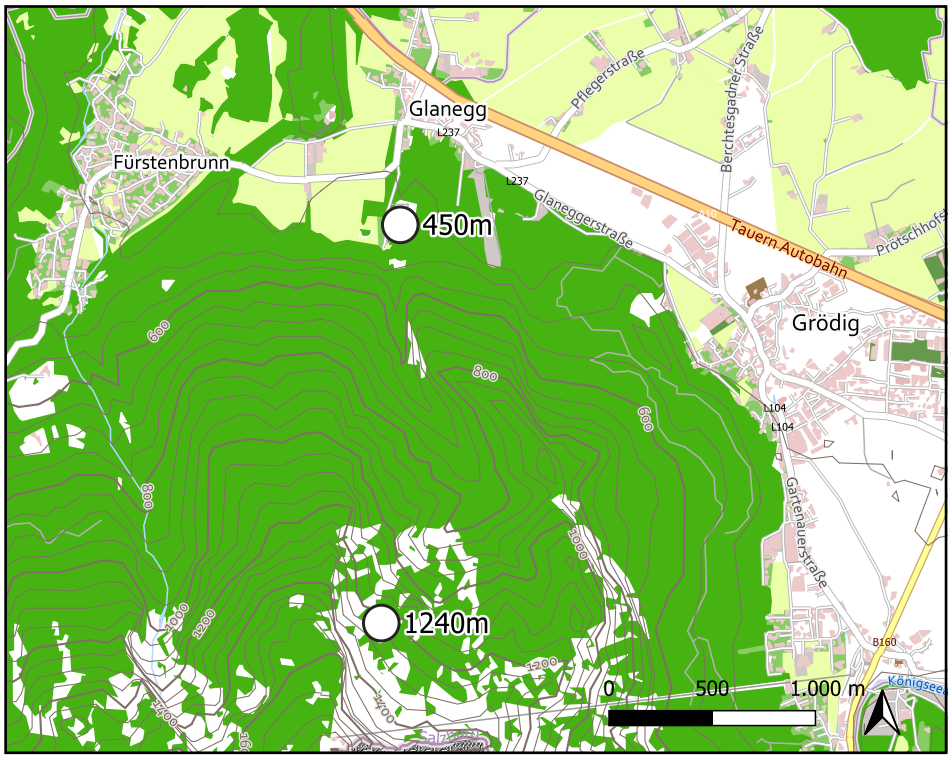


Figure 13: Map showing the sampling sites "valley" at 450m and "mountain" at 1240m at Untersberg near Salzburg, Austria (based on basemap.at, CC-BY 4.0).

Population genetics

Isopods for population genetics and the stress experiment were collected in summer 2015 and kept in plastic boxes in a climate cabinet (MIR-254, Panasonic) with a 12 h day-night cycle at 14 °C and 16 °C. Light was provided by 2600 K LED stripes.

For the development of microsatellite markers, genomic DNA was extracted from one specimen of *T. ratzeburgii* of the mountain-population using the DNeasy Blood & Tissue Kit (QIAGEN) according to the manufacturer's protocol. A 450-500bp shotgun-library was prepared using the NebNext Kit (New England Biolabs) und sequenced on an Illumina-MiSeq platform with paired-end 250bp reads. Reads were quality controlled, quality-trimmed (Phred quality score < 30) with trimmomatic 0.32 (Bolger et al. 2014) and merged with PEAR (Zhang et al. 2014). The resulting sequences were screened for microsatellites using QDD3.1 (Meglécz et al. 2014). The screening pipeline included primer development, using primer3 (Untergasser et al. 2012), and a contamination check, using repeatmasker (Smit et al. 2013). Out of the resulting 177 sequences containing suitable microsatellites, 45 primer pairs for sequences with a minimum of 12 repeats and an estimated fragment length between 120 bp and 400 bp were chosen for laboratory testing. All primer pairs were tested with DNA from 2 specimens of each population for successful amplification and loci-polymorphism. DNA was extracted from legs of specimens by overnight incubation in 500 ml lysis buffer (100 mM EDTA, 10 mM Tris-HCl pH 7.5, adjusted to pH 8) with 50 µl of 10% SDS and 2 µl of Proteinase K (20 mg/ml) followed by protein precipitation using a protein precipitation solution (Promega) and isopropanol precipitation. This extraction method was used for all samples in this study. PCR was performed with an annealing temperature of 58 °C for all primer pairs (30 cycles).Products were separated by gel electrophoresis on a 3.5 % agarose gel. 12 primer pairs for loci showing amplification success and polymorphism were selected for fragment length analysis on a capillary electrophoresis system. One primer of each pair was labeled with one of the fluorescent dyes FAM, HEX or TAMRA and pooled in four multiplex sets according to the estimated fragment length (sufficient differences), range and dye. Multiplex PCR reactions were performed with the QIAGEN multiplex PCR kit, using the standard microsatellite amplification protocol with 30 cycles and an annealing temperature of 58 °C. PCR products were run on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) with an ILS600 standard (Promega). The results were scored using Geneious 8.1.6 (Biomatters).

Table 6: Developed microsatellites primer pairs for *Trachelipus ratzeburgii*. MS = Multiplex set

Locus	MS	Primer Sequence (label)	Motive	Size Range
Trara17	1	F:[HEX]ACTGATCACACTGACAGGGC R: GCGATGTGCGTGTTCACTAA	ATC	188 – 224
Trara25	1	F: [TAM]TGACATTTCTGGCAGCGAGC R: CACTCAGGAAGAATTTCACAGTCC	TAT	197 – 227
Trara8	2	F: [FAM]GCCTAACCTGAGTGAGTCCG R: GCGCACGTCCAAACATGAAT	TAT	158 – 188
Trara18	2	F: [HEX]GCGCACTCTAATCCACTTGG R: TCCTCCCTCTAAGCTCAACCA	AAT	186 – 243
Trara34	2	F: [TAM]GTCTCAGAATACACCCTGGCA R: ACGCTATATGGATGTCACAGGC	CTAC	240 - 378
Trara33	3	F: [HEX]CGAGTCGGTCACGCTATCAA R: CTCCGCGGACTCGTTTACAA	AG	211 – 271
Trara11	4	F: [FAM]ATGACATAAGCGCGTCTGCT R: CGTCAGTGTCCATTGTAACTGC	TCT	175 - 229
Trara21	4	F: [HEX]TCAGTTGTAGTGCTGCGATT R: AGGATGCAAGGGTAGTGGTC	TTA	190 – 205
Trara44	4	F: [TAM]TCCGACAGAATGAGGAAGGC R: TCCGACAGAATGAGGAAGGC	AGA	310 - 337
Trara9	5	F: [FAM]AGAGGACTTCTGTTGGCAAC R: TGCCTGAACAGATTCAATGCA	AAT	157 – 263
Trara30	5	F: [HEX]TTTCCGCTTCCGAGGTTTGT R: TGGGTCAGCAATGTAGGGTG	ATT	215 – 263
Trara39	5	F: [TAM]ACTGGAGTTCTGAGTTGGAGAC R: GGTCAATTGGATGCTGGTGC	ATA	271 - 301

Stress Experiment, RNA extraction and Gene Expression

72 specimens of each population were weighed and separated into 960 ml transparent plastic boxes (128 x 128 x 90 mm, bikapack kg) with 100 g plaster-ground containing 15 ml water and 50 g sieved forest soil on top (1 specimen per box). Boxes were sealed with transparent lids containing 5 holes (4mm Ø). Boxes of both population were randomly assigned to one of the two stress treatments – temperature or water – and the control treatment, resulting in 12 replicates for each population-treatment combination. Boxes of control and water stress treatment were randomly distributed in a climate cabinet and kept under a day-night cycle (12 h light) with the following temperature profile: 12 AM – 6 AM 14°C, 6 AM – 7 AM 15 °C, 7 AM – 9 AM 16 °C, 9 AM – 10 AM 17 °C, 10 AM – 12 PM 19 °C, 12 PM – 2 PM 20°C, 2 PM – 4 PM 19 °C, 4 PM – 5 PM 17 °C, 5 PM – 7 PM 16°C, 7 PM – 12 AM 14 °C. For water stress treatment, 35 ml H20 were added at 12 PM to each box, completely covering the isopod.

Boxes of temperature treatment were kept in a second cabinet under the same conditions for the first day, for second day for temperature steps between 9 AM and 6 PM temperature was increased by 2 °C. For the third day, temperature was increased by 4 °C between 10 AM and 12 PM and by 10 °C between 12 PM and 14 PM. 30 °C were reached at 12.30 PM, starting the temperature stress treatment.

One hour after each stress factor was applied, all specimens were taken out of boxes and immediately frozen at -80°C.

A total of 9 randomly chosen samples of each population-treatment combination were grinded in liquid nitrogen and RNA was extracted using 750 µl Trizol Reagent (ThermoFisher) according to the manufacturer's protocol. All equipment was treated with RNaseZap (ThermoFisher) and washed with DEPC-treated Water before usage. Resulting RNA pellets were diluted in 50 µl DEPC treated water. RNA quality and integrity was estimated by photometric measurements and run in a 1% agarose gel. 7 µl of each sample were treated with DNAse I (ThermoFisher) according to the manufacturer's protocol and frozen at -80 °C until gene expression analysis.

Gene expression was measured via quantitative real-time-pcr. HSP70 was chosen as gene of interest and five genes were chosen as potential reference genes: Histone3, 18s, beta-actin, S20 and Ubiquitin. For primer development several sequences of crustaceans (and some insects) were aligned do develop degenerated primer, whereas only primers for HSP70, Histone3, beta-actin and GAPDH were able to create an amplicon that could be used for further sequencing. 18s primer were developed directly from NGS-dataset. The following sequences were used for development of degenerated primer. HSP70: AM502913.1 (*Oniscus asellus*), EU514494.1 (*Daphina magna*), JN704340 (*Gammarus lacustris*), JX913782.1 (*Scylla paramamosain*), AM410079.1 (*Cancer pagurus*), DQ301506.1 (*Procambarus clarkii*), FR693761.1 (*Porcellio laevis*). Histone 3: KC428937.1 (*Cyathura sp*), JN800702 (*Ligidium lapetum*), JX839661.1 (*Alpioniscus tuberculatus*), JX839662.1 (*Alpioniscus verhoeffi*), KC428949.1 (*Idotea sp.*). Beta actin: AY100005.1 (*Culex pipiens*), HM143924 (*Eriocheir sinensis*), HM217821 (*Scylla paramamosain*), EU825991.1 (*Mantichorula semenowi*), GQ449384.1 (*Liposcelis paeta*). GAPDH: EV248517.1 (*Uca pugilator*), AB753163.1 (*Anopheles stephensi*), AAA28560 (*Drosophila melanogaster*). Degenerated primer sequences are shown in Table 7. Amplicons generated from T. ratzeburgii DNA with degenerated primer were sequenced with the same primers and

specific primer pairs were designed from gained sequences. Specific primer pairs are shown in Table 8. Amplicons generated with specific primer and cDNA were sequenced and compared to sequences used for primer development to confirm the identity of the amplicon.

All qPCR reactions were performed on a CFX Connect Real-Time PCR Detection System (Biorad) using the SensiFAST SYBR No-ROX One-Step Kit in a 20 µl volume using 2 µl RNA (diluted 1:100) . PCR conditions were the following: 45°C for 10 min (RT), 90°C for 2 min, 40 cycles of [90°C for 10 sec., 60°C for 10 sec, 72 °C for 30 sec, plate read] followed by 95°C for 5 sec. and a melt curve from 65°C to 95°C in 0,5°C increments (5 sec. for each temperature, followed by plate read). Primer concentration was 200 nM for each primer.

Potential reference genes were tested on RNA from 12 specimens (two from each treatment and population). All potential reference genes showed sufficient stability values for heterogeneous samples (Hellemans et al. 2007): Histone 3 (CV: 0,1551, M:0,4536) < GAPDH (CV: 0.1523, M: 0.4566) < beta actin (CV: 0,2276, M: 0,6047) < 18s (CV: 0.3037, M: 0,6108).

PCR efficiency for all primer pairs was estimated by dilution series (seven dilutions, two technical replicates) of a pooled sample of all 12 samples used for reference gene testing (as recommended by Hellemans et al. 2007, Derveaux et al. (2010) . At least four dilutions for each primer pair showed a sufficient Cq and a sufficiently low replicate group Cq standard deviation (< 0.60).

Based on the reference gene testing and the PCR efficiency of the primer (Table 8), GAPDH and beta actin were chosen as reference genes. Histone 3 as well as 18s were rejected due to the high PCR efficiency. Samples were run using sample maximization method and with two technical replicates. By this design, inter-run calibration was not necessary. Only samples with a replicate group Cq standard deviation < 0.80 were used for analysis. Samples where the target or at least one control gene did not fulfil this criteria were completely excluded from the analysis. Using this approach, the following number of samples were used in the analysis: control mountain N = 7, control valley N = 7, heat mountain N = 7, heat valley N = 6, water mountain N = 6, water valley N = 5.

For data analysis the approach of Vandesompele et al. (2002) was used to calculate relative expression. Average Ct-values of the valley-control-group were used as calibrator sample to determine delta Ct.

Table 7: Degenerated PCR primer for qPCR. F = forward, r = reverse

Degenerated PCR primer for qPCR	
deg_HSP70	F: YTKCARGACTTCTTYAACGG R: CGACCYTTGTCRTTGGTGAT
deg_Histone 3	F: ATCGYCCYGGWACTGTNGCNC R: TGGCGCACAAGTTGGTRTCT
deg_Beta actin	F: ATGAAGATCCTGACNGARCG R: YTCGTTGCCGATGGTGATGA
deg_GAPDH	F: TNGTNTCCAATGCNTCNTGCAC R: GGATGATGTTCTGKNCRGCNCC

Table 8: Final qPCR primer and primer efficiency. F = forward, R = reverse. Asterisk mark final chosen genes.

Final PCR primer for qPCR		Primer efficiency (E)
HSP70* (115 bp)	F: ATGGAGCTGCAGTTCAAGCA R: ACACCTCCAGCAGTTTCGAT	100,5 %
18s (124 bp)	F: TCGGCTCTTCGCGTATAACC R: ACCATCGCTCGCTGACATAG	167,2%
Histone 3 (101 bp)	F: CGCAAATTGCCTTTCCAACG R: GAAGCCTCCTGAAGAGCCAT	143,6%
Beta actin* (81 bp)	F: CGCATAACACCGTATTGTCCG R: CGAGAGCGGTGATTTCCTTCT	109,4%
GAPDH* (138 bp)	F: CACCACAAACTGCCTTGCTC R: CCTCTCCAGTCCTTGCTTGA	108,1%

Litter consumption experiment

For the litter consumption experiment, isopods of both populations "mountain" and "valley" were fed on different leaf litter or leaf litter mixtures of three different diversity levels with eight replicates respectively. Single species litter (all eight different species), litter mix of two species (eight different combinations) and litter mix of four species (eight different combinations). For single litter, 4g of each air-dried litter was used. For litter mixtures, litter of all mixed species was combined in equal amounts totalling 4 g litter respectively. In total, 24 boxes were set up for each isopod population.

The litter was filled into 1200 ml transparent plastic boxes (192 x 128, bikapack kg) with a 100 g plaster-ground. Dried plaster was filled with 40 ml water, additional 5 ml were sprinkled over the litter. Additional 20 ml water were filled into the plaster after 4 weeks. The boxes were sealed with transparent lids containing 3 holes (4mm Ø) and placed in two climate cabinets (MIR-254, Panasonic) with a 12 hours day cycle (light provided by 2600 K LED striped over each shelf) with a temperature of 17°C during day (light on) and 12 °C during night (light off).

Isopods were randomly assigned to experimental groups of 8 specimens. Experimental groups were weighed and randomly assigned to the litter filled boxes. The experiment ran over 6 weeks. After each week isopods were weighed and dead isopods were replaced with specimens of approximately the same weight. After 6 weeks, remaining litter was removed from the boxes, oven-dried at 60° C for 48 hours and weighed. Additionally 2 g of each litter was dried and weighed to calculate a correction factor for air-dried litter.

Data analysis

All data used for statistical analysis was tested for normality using Shapiro-Wilk-Test and for equal variance using Levene-median-test. Based on these results, differences in weight of isopod-groups were tested using t-Test. Overall litter mass loss and relative consumption rates as well as relative consumption rates within groups were compared using Mann-Whitney-U-Test. HSP70 expression was compared using Mann-Whitney-U-Test and Kruskal-Wallis-Test. All tests were performed and all figures (except Fig. 14) were generated with R (R Core Team 2017).

Results

The temperature fluctuation, measured as the difference between the minimal and maximal temperature for each day, was immensely higher at 1240m compared to the site at 450m, although this effect only occurred during summer (Fig. 14.) During winter until early spring the temperature at 1240m varied between 0°C and 1°C (due to a constant snowpack) with almost no diurnal fluctuation, whereas the temperature difference at 450m varied between 0°C and 7°C. From spring till autumn, the fluctuation at 450m was not considerably higher compared to winter, but at 1240m the fluctuation was higher.

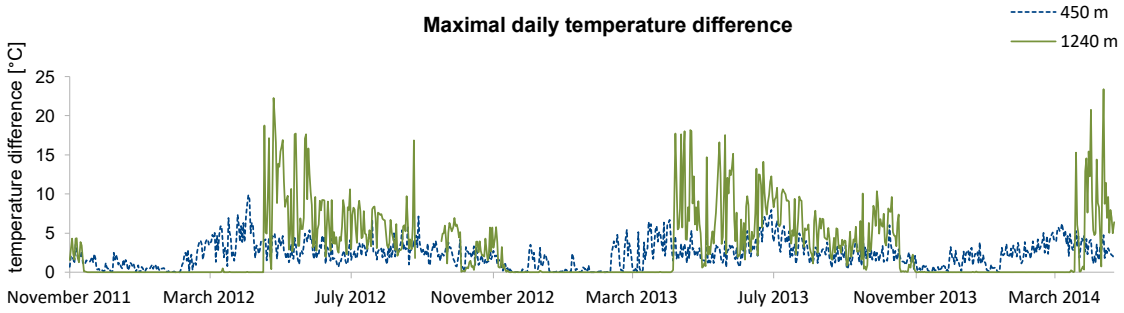


Figure 14: Diurnal temperature fluctuation at sample sites, measured as maximal temperature difference for each day. Streight green line shown data for 1240m, dotted blue line shown data for 450m

Population genetics

All samples showed good amplification results for microsatellite markers. Scoring results and subsequent analysis of population differentiation (GST and DEST) show that all isopods, though treated as two populations for this study, belong in fact to the same population, with little variation among the markers (Table 9). Thus heterozygosity in both groups is almost identical (Table 10).

Table 9: Total GST and DEST for individual markers and in total.

Locus	G_{ST}	D_{EST}
Trara_17	0,003	0,010
Trara_25	0,000	-0,003
Trara_8	-0,004	-0,012
Trara_18	0,008	0,006
Trara_34	0,000	0,004
Trara_33	0,006	0,193
Trara_11	0,009	0,070
Trara_21	-0,014	-0,046
Trara_44	0,013	0,087
Trara_9	-0,002	-0,007
Trara_30	-0,007	-0,013
Trara_39	-0,009	-0,059
Total (SE)	**0.000 (0.002)**	**0.002 (0.010)**

Table 10: Heterozygosity in both groups.

population	He	SE(He)
mountain	0.656	0.056
valley	0.663	0.049

Litter consumption experiment

For the litter consumption-experiment no difference in total mass loss (identical with total consumption of litter) between both groups was shown (Fig. 15, left), but relative consumption rate was significantly higher for group “mountain” (Fig. 15, right). However, the evaluation of the weights of randomly formed groups for the experiment, which were evaluated after the beginning of the experiment, show a very clear difference in weight between the two groups. The isopods-groups (and thus the isopods) of the “mountain” population were significantly lighter than the isopods of the “valley” population (Fig. 16).

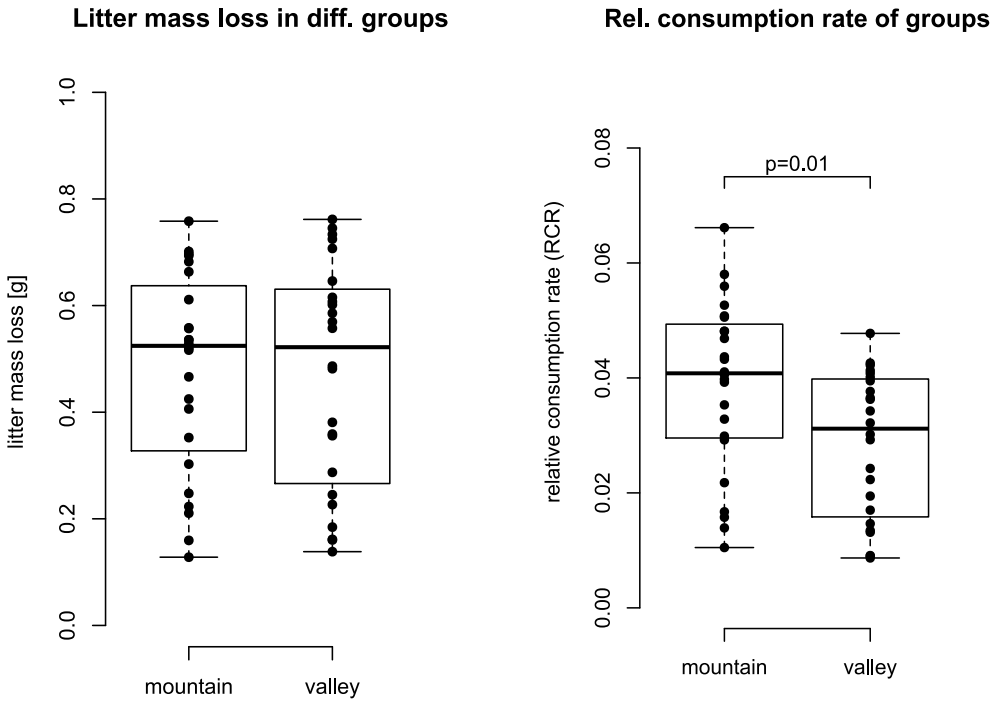


Figure 15: Litter mass loss in different groups (identical with absolute consumption, left) and relative consumption rate of both groups (right). Boxplots show median, lower and upper quartile and maximum and minimum of samples. Black dots show results of individual groups. Relative consumption rate was calculated according to Waldbauer (1968) L/(I*T) (with L = dry weight of consumed leaf litter in gram (g), A = mean weight of isopods in gram (g) and T = time in days (d)).

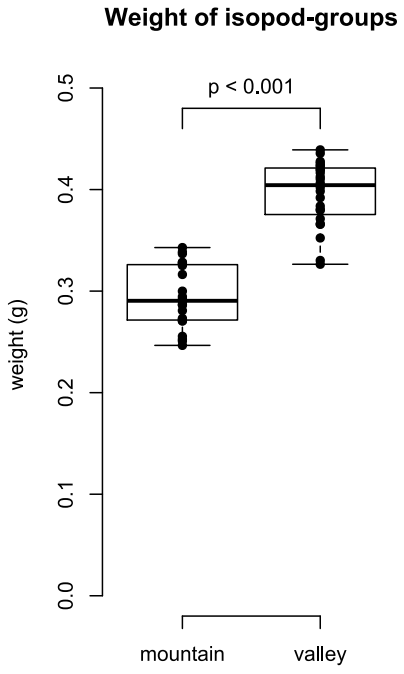


Figure 16: Weight of isopod-groups in the experiment (containing 8 specimen each). Boxplots show median, lower, and upper quartile and maximum and minimum of samples. Black dots show individual samples.

Within both groups, the consumption rate of litter mixes was higher compared to single litter. Though only for group "mountain" this effect was significant for both litter mix two and litter mix four (Fig. 17). For group "valley" it was only significant for the comparison of single litter and litter mix four. Overall, the results show noticeably clear mixed litter effects.

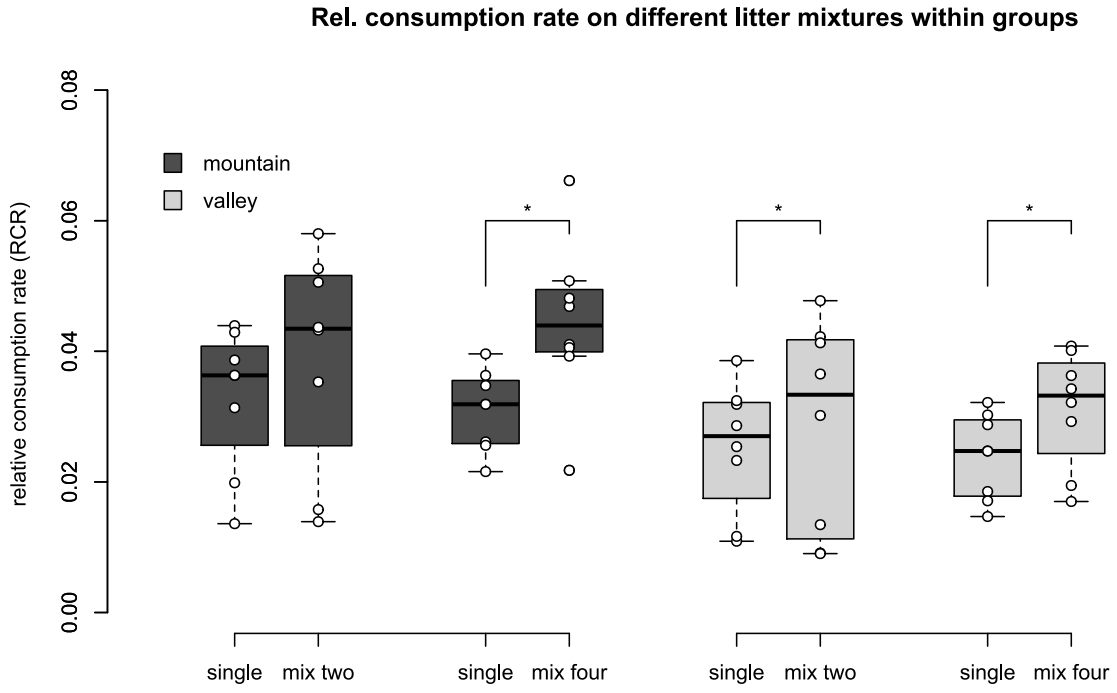


Figure 17: Comparison of relative consumption rates in single litter and litter mixtures within group "mountain" (dark grey) and group "valley" (light grey). Single litter data for comparison was calculated with all litter species used in the corresponding litter mixtures, hence values for single litter differ between comparisons. Boxplots show median, lower, and upper quartile and maximum and minimum of samples excluding outliers). White dots show individual samples. Significant differences are marked with asterisk. Relative consumption rate was calculated according to Waldbauer (1968) L/(I*T) (with L = dry weight of consumed leaf litter in gram (g), A = mean weight of isopods in gram (g) and T = time in days (d)).

Stress Experiment

The Mann-Whitney-U-Tests showed no significant influence of the population (mountain vs. valley) on gene expression for both “heat” ($p = 0.84$) and “water” ($p = 0.08$) treatment. Kruskal-Wallis-Tests showed no influence of any stress compared to the control ($p = 0.94$ for mountain and $p = 0.46$ for valley) (Fig. 18). With valley-control-group used as calibrator sample, relative gene expression rate varied between 0.3 and 3 for control, between 0.2 and 3.3 for heat stress and between 0.3 and 3.1 for water stress.

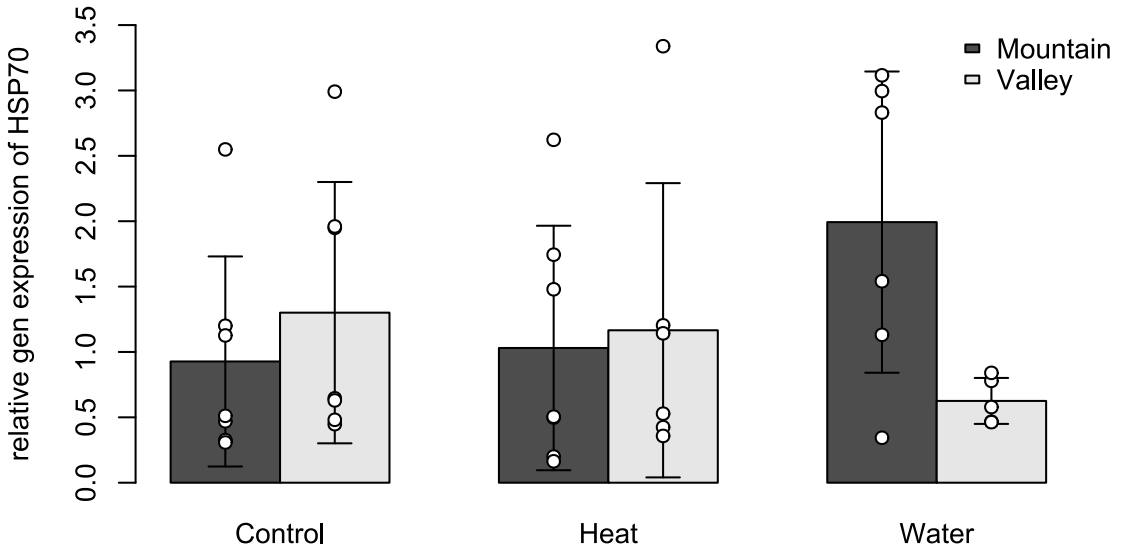


Figure 18: Relative gene expression of HSP70-gene for groups "mountain" (dark grey) and "valley" (light grey). White dots show individual samples, error bars show standard deviation.

Discussion

In this study, we aimed to examine the genetic and phenotypic effects of altitude, associated with high environmental fluctuation (especially temperature), on an isopod-population. In doing so, we expected the high altitude population to exhibit less genetic variation compared to a (geographically close) low altitude population of the same species and a genetic differentiation between the populations due to isolation by environment. Furthermore, we expected that this high-altitude population would be comparatively more stress resistant to environmental extremes, but slower to consume complex litter mixtures due to reduced (functional) variation and the los of certain phenotypes respectively.

In contrast to our hypothesized influence of high environmental fluctuations on intraspecific variation, our results show no considerable differences in heterozygosity between both populations (Table 10). In addition, results of genetic differentiation measurement strongly indicate that populations “valley” and “mountain” are in fact not differentiated at all, but belong to the same population. These findings imply two conclusions: a) The comparatively harsh climatic conditions at 1200m did not put any (sufficient) selective pressure on the individuals living in that climate. b) There is no isolation by environment and therefore a continuous geneflow between both areas. If there is selective pressure and a form of isolation by environment, the areas at high altitude could only relatively recently been colonized, which is highly unlikely. While the results are interesting from a molecular-geobiological perspective, they also mean that the assumed differences in genetic variation do not exist. All possible differences regarding litter consumption and resistance to stress (e.g. HSP70 gene expression) are therefore either based on plastic adaptation or on phenotypic differences that have no representation in genetic variation as shown by Muir et al. (2014).

At first, the results of the feeding experiment seem to suggest such a phenotypic difference, as overall relative consumption rate (RCR) significantly differs between the populations (Fig. 15), although it is in contrast to our hypotheses. However, one of the conditions for using RCR is not given, which, as noted in the results, was measured during the ongoing experiment. Although RCR is a frequently used and proven method (Farrar et al. 1989), among all other potential problems like starting weight vs. mean weight and consideration of molting (Hare 1998) RCR measurement requires differences in body mass to be equally distributed among all samples and not being substantially different between

two experimental groups. In our study, the isopods of the population "mountain" were significantly lighter and therefore smaller than the isopods of the population "valley" (Fig. 16). This interfering variable is likely to have a paramount effect on the comparability of the two groups and makes it impossible to measure any differences via relative consumption or any other method requiring values of relative consumption. In other words: it is impossible to distinguish between the effect size has on consumption and on any other effect - and the tremendous phenotypic variation in one trait (size) makes is impossible to draw conclusions in the other (consumption), despite the finding that in this case total consumption was independent from size.

In contrast to the relative consumption, the amount of absolute consumed litter is nearly the same in both groups despite the size difference, meaning the (much) smaller individuals of the group "mountain" had a much higher rate of consumption per gram of isopod biomass than group “valley”, in contrast to the general assumption (and reason for using measurement of relative consumption) that bigger animals – including invertebrates – consume (absolute) more food than smaller animals (Hare 1998). These effects have been seen in isopods (Vilisics et al. 2012) and other groups (Reichle 1968) before – although the reasons remain often unknown. In this case the (relative) higher consumption of the mountain-population might me explained by the metabolic cold adaptation hypothesis (Clarke 1993, Addo-Bediako et al. 2002): the regular expose to low temperatures at high altitudes (especially with a high diurnal temperature fluctuation) leads to a positive selection for a higher metabolic rate to counter the slowing effect of low temperatures and a shortened season (Williams et al. 2016). This might additionally indicate some form of counter-gradient variation (as described by Conover et al. (2009) and Williams et al. (2016)) with an increased metabolic rate of the mountain-population and a comparatively lower metabolic rate of the valley-population. However, since the two populations are not differentiated according to our data, the changed metabolic rate might as well be a plastic response to the high-altitude environment. But since metabolic rate was not part of this study, actual data about the metabolic rate for specimen from both populations is needed to confirm that this principle applies here – and if the consumption rate at the regarding site in the field is as high as it was under lab conditions.

This still leaves the question why the specimens of the valley population are that much smaller – especially since metabolic cold adaptation (and counter-gradient variation) is in particular discussed as a principle to accelerate growth in cold environments. However,

Williams et al. (2016) show that metabolic rates can be increased independently of growth rates.

It is still an ongoing debate if and in which cases Bergmann's rule applies for exotherms with countless examples supporting Bergmann clines, but also converse Bergmann clines or with no general pattern (for review see Shelomi (2012) and Vinarski (2014)). However, we strongly agree with Vinarski (2014) that most probably several factors covarying with altitude (and of course latitude) influence size and a universal rule cannot be found. Hence it can be assumed that in our study – especially since the high altitude population is not differentiated from the valley population (and most likely from all animals between these two sites) – the described conditions at high altitude (diurnal fluctuation, temperature extremes, short season) limit the maximum growth of isopods or slow it down to such an extent that they cannot reach the maximum size even when reaching the maximum life span – and that this limit is not compensated by potential metabolic cold adaptation or any other form of local adaptation. But since this difference in size does not result in a difference in consumption, a reduced maximum size may not necessarily have ecological consequences in the aspect of litter consumption and therefore decomposition, since a reduced size might be compensated by metabolic cold adaptation (or rather metabolic diurnal fluctuation adaptation) and therefore overall litter decomposition might not be affected in this aspect.

For any measurements within the two populations however, the described effects do not apply. Therefore, relative consumption can be used to compare consumption on different mixtures. Here, the results show significant mixed-litter-effects for the four-species-mixtures in both groups, but significant mixed-litter-effects for the two species-mixtures occurred only for the "mountain"-populations (Fig. 17). (For mixed-litter effects see Gartner and Cardon (2004), Gessner et al. (2010), Song et al. (2010) and Kou et al. (2020).) Furthermore, mixed-litter-effects for four-species-mixtures are considerably higher in this group. Since consumer-diversity-effect and mixed litter-effects might promote each other (Hättenschwiler at al. 2005), these results could indicate a higher digestive phenotypic variation for the "valley" group. But further experiments specifically designed to answer this question have to be made before any conclusions can be drawn regarding this matter.

In the stress-experiment we tried to measure the stress response to two environmental extremes: short-term heat and short-term flooding, whereas the stress response was

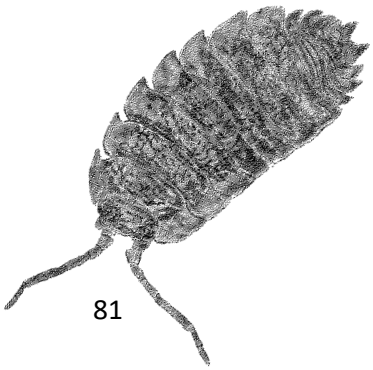
measured 60 minutes after the occurrence of the stressor. The results show an overall rather stable expression of the HSP70 gene, despite a seemingly high variation within the treatments (Fig. 18). Though the latter seems surprising. Especially in the control-groups, HSP70 expression – as all gene expression – naturally varies among individuals (Krebs and Feder 1997) and the natural differences in gene expression are relatively low compared to HSP70 expression under stress, found in other studies (Luo et al. 2015). But especially since only samples with a replicate group Cq standard deviation < 0.80 were used for analysis (for both target and reference genes) it is likely that this variation is actual biological variation rather than technical one.

This lack of any (significant) differences in gene expression may have several reasons, but since there were no differences between the control and any experimental groups, the stress a) does not seem to have been high enough to trigger an appropriate stress response or b) the isopods do not react to thermal stress by an accelerated HSP70 expression (as e.g. shown by Jensen et al. (2010) for *Drosophila melanogaster)*. Since there are few studies regarding the stress response of terrestrial isopods in general, the chosen change in abiotic conditions and the chosen time period may not have been sufficient. This would also explain why there is no difference between the two experimental groups: If higher expression of HSP70 is not required in general, there is no expected difference between the two populations.

Our results suggest that, at least under these conditions, altitude is not suited to serve as a model for the effects of future extreme weather events, since in this case there was no selection or isolation caused by the environment. Thus, it is possible that the high-altitude isopods are in permanent gene flow with the isopods at lower altitudes. In addition, isopods at high altitude were considerably smaller, but due so some mechanism – potentially genetic or plastic metabolic cold adaptation – had a higher relative consumption rate. While being a interesting discovery, the latter phenomenon can hardly be transferred with respect to a space-for-time approach.

Chapter 5

In and out – differences and variation in Py-GC-MS patterns of feces from several isopod populations as an estimate of variation in digestion



Abstract

In recent years, functional diversity and the influence of functional traits on ecosystem processes have become considered more often to be significant in the context of studying diversity and the influence of diversity on ecosystem processes. In this framework, intraspecific variation has become increasingly accepted as an important part of diversity. One of these ecosystem processes is the decomposition of dead organic material, in the context of which interspecific diversity has long been seen as an important factor. However, when looking at specific functional traits influencing ecosystem processes - such as digestion patterns - at the individual level, it is often difficult to measure these traits and a variation in these traits. In this study we use Py-GC/MS, which has been rarely used on this setting up to this point, to measure intraspecific variation in digestion of the terrestrial isopod *Porcellio scaber*. We used specimen of several European populations with known population differentiation to evaluate if genetic variation and differentiation influences patterns in digestion. The results show a high intraspecific variation in digestion patterns, but only a small prospective influence of phylogenetic relations on digestive (dis)similarities. Potential reasons such as the influence of other phenotypic traits, but especially the potential influence of the microbiome on digestion are discussed.

Introduction

Over the last decades the focus of research studying the role of biodiversity shifted to some extend from focusing on only the number of species involved in an ecosystem process to the functional diversity and dissimilarity of the individuals involved in that process (Tilman 1997, 2001, Petchey and Gaston 2006, Cianciaruso et al. 2009, Cadotte et al. 2011, Laureto et al. 2015). This is consistent as, although the number of species is a proxy for diversity in traits (Tucker et al. 2018) – assuming that these species are sufficiently different in their characteristics – it does not ultimately target the different influences that are crucial for the role of "diversity" in an ecosystem process. This new concept of functional traits and functional diversity includes that – though still very often different species are studied – the border between species becomes obsolete and among the differences between species the differences within species (intraspecific variation) is considered a relevant part of biodiversity (Cianciaruso et al. 2009).

However, studying functional diversity and its influence on ecosystem processes is far more complex than studying the influence of number of species, since very often those traits are strongly connected to precise functions (like nutrient uptake) that are complicated to find and measure. A common way of solving this problem is to use other traits that are easier to quantify (often referred to as "soft traits") as proxy (Nock et al. 2016), although they are less connected ("softer") to the ecological function of interest. This approach is however not only limited to those functions where a proxy-trait can be found, it also provides no direct information regarding the variation of the trait of interest, as a soft trait can be a proxy for different "hard" traits. Accordingly, it would be more beneficial to measure those traits as often as possible, which are more directly connected to an ecosystem process. In other words: The measured trait must be as hard as possible.

One of these ecosystem processes which is a main driver in most ecosystems is the decomposition of dead organic matter (Swift et al. 1979). This process is essential for primary production in many ecosystems as by this process nutrients from dead organic matter become available for plants again. Up to 90% of the mass of primary producers is directly decomposed as dead organic matter (Cebrian 1999, Gessner et al. 2010). Several groups of microbes (mainly soil bacteria and fungi) play a major role during this process, as they have the ability to degrade not only many, but also very complex and recalcitrant molecules such as lignin or cellulose (Nielsen et al. 2011). In combination with microbial decomposers, several groups of detritivorous animals (often referred to as "macro detritivores") have an in influence on decomposition. Besides many indirect effects like litter fragmentation (Yang et al. 2012) and different forms of microbial stimulation (Hassall et al. 1987, Gómez-Brandón et al. 2010, Nielsen et al. 2011, David 2014) some of these animals also contribute to decomposition directly by breaking down litter or reducing recalcitrant substances in their gut – either by their own enzymes or by the microbiome of the digestive tract (Zimmer and Topp 1998, Kadamannaya and Sridhar 2009, Coulis et al. 2009, Bouchon et al. 2016). However, while other aspects of decomposition have been attended in the context of diversity-studies over the last decades (Hättenschwiler et al. 2005, Gessner et al. 2010), intraspecific differences in digestion have not been examined.

In order to study changes during digestion in detritivorous animals, several techniques have been used in the past, mainly by comparing chemical parameters between food and feces. Compounds of interest were e.g. cellulose (Zimmer and Topp 1998) or tannins

(Zimmer 1999, Zimmer et al. 2005b). In addition, broader approaches have been applied, e.g. near infrared spectroscopy (NIRS) by Gillon and David (2001) or pyrolysis gas chromatography-mass spectrometry by Rawlins et al. (2006) – both studies are using the respective technique to study chemical changes in leaf litter consumed by a millipede species. The latter is a promising method, as it is easy to approach and allows a relatively accurate view on individual components. However, only the combustion products of food and feces can be compared here (the samples are burned at 900 °C prior to GC/MS analysis) and it is difficult to quantify single components. Nevertheless, this technique has – in addition to all other fields – been used to study ecological processes like degradation of wood or leaf litter by fungi (del Río et al. 2001, Steffen et al. 2007) or biochemical composition, e.g. of algae (Biller and Ross 2014).

The disadvantages of the pyrolysis are less problematic if only differences between samples are of interest, as with this method a wide metabolistic "snapshot" can be obtained and compared among samples without a mandatory need for identifying the single metabolites (Dettmer et al. 2007, Du and Zeisel 2013) – a technique usually referred to as "metabolic fingerprinting". This approach was (without pyrolysis) e.g. used by Ivanišević et al. (2011) to measure intra- and interspecific variability among sponges, (with pyrolysis) by Tianniam et al. (2010) to evaluate the quality of commercial herb-roots and (with pyrolysis) by Bakkar et al. (2017) to measure chemical changes of detritus during digestion in a crab species. Hence this technique has the potential of being a good, fast and potentially high-throughput method to measure even small chemical changes in digestive processes and reveal potential intra- and interspecific variation.

In order to test whether a) this method is generally suited for investigating intraspecific phenotypic differences between detritivores in the digestion of food, b) there is a relationship between genetic and functional (dis)similarity and c) there is a variation in digestion of standardized food among individuals of the same species (with being able to relate the distances between individuals) we used several individuals from different European populations (with known intra- and interpopulation genetic distances) of the terrestrial isopod species (woodlouse) *Porcellio scaber* Latreille 1804 (Schmalfuss 2003) (family Porcellionidae, suborder Oniscidea) in order to test not only differences between food and feces, but also variation within and differences between populations. In addition, specimens of two other terrestrial isopod spices, *Oniscus asellus* L. 1758 (Schmalfuss 2003, family Oniscidae, suborder Oniscidea) and *Trachelipus ratzeburgii* Brandt 1833 (Schmalfuss

2003, family Trachelipodidae, suborder Oniscidea) were included in the experiment in order to be able to relate the intra-species variation to an inter-species variation.

Material and Methods

As standardized food, air-dried leaves of *Fraxinus excelsior,* collected in autumn 2012 near Kalmar (Sweden), were pulverized in a ZM100 Centrifugal Grinding Mill (Retsch). Powder was pressed into 10 x 5 mm pellets with a metal tablet press, compressed in a bench vice.

Specimen of *P. scaber* were collected in summers 2012 to 2014 in areas near Kalmar (Sweden), Lund (Sweden), Kiel (Germany), Cologne (Germany) and Salzburg (Austria). In order to test for genetic differences, eight highly polymorphic microsatellite markers were developed (using Illumina MiSeq 183 specimens from all different populations) and 183 specimens from 5 different populations were used for the population genetics study (see Chapter 2). Specimen of *O. asellus* and *T. ratzeburgii* were collected in Summer 2014 near Salzburg.

Seven days before the experiment the isopods were kept in separate populations and fed only pellets of standardized food. For the experiment 10 isopods of each *P. scaber* population and of each of the two other species *O. asellus* and *T. ratzeburgii* were put into separated glass vials (with closed lid including several holes), each containing a small wet clay shard as shelter and moisturizer and one pellet of standardized food. The vials were randomized and kept in a climate cabinet (MIR-254, Panasonic) with a 12 h day-night cycle at 14 °C and 16 °C during the experiment. Light was provided by 2600 K LED stripes. For 5 days feces was collected using an exhauster and kept in glass vials at 4°C. After 5 days feces was freeze-dried for 48 hours. Samples were pulverized and subsequently stored in 2ml Eppendorf tubes vial (with closed lit) at 4°C. Between 80 an 100 µg were used for analysis.

Samples were analyzed using a Thermal Desorption Unit (Gerstel, Germany) in combination with a GC/MS (7890B GC-System, Agilent Technologies, USA) at an initial temperature of 300°C and a pyrolysis temperature of 650°C.

For the GC-unit a 30 m column with 250µm inner diameter and a 0.25µm silarylene-coating (Optima 17MS) was used. Helium was used as carrier. For the GC-unit the following settings were used: 1. an initial oven temperature of 40°C for two minutes with following increment of 4°C per min to 100°C, 2. an increment of 4°C per min to 200°C, 3. increment

of 8°C per min to 300°C, 4. hold time for 15min, 5. increment of 10°C per min to 320°C, 6. hold time for 5min.

The MS-unit detection range was m/z 45–600. Data was collected using the device-associated Gerstel Software (V. 5.04). 191 Peaks were determined on the basis of a corresponding retention time and calculated mass automatically using Agilent Masshunter (V. 7.0) and aligned manually (according to retention time) using MS Excel.

Data analysis

To normalize the samples, peak height values were correlated with sample weight. The peak with the highest correlation coefficient was chosen as standard to normalise the samples, most likely representing a compound largely unaffected by treatment. After normalization the peak used for data-normalisation was removed from the dataset for all further analysis.

An exploratory data analysis approach was used to analyze the GC/MS-Data in order to have a neutral method of evaluation since not all differences are exclusively (or at all) caused by genetic differences. We used therefore hierarchical clustering (Suzuki and Shimodaira 2006) to analyze the samples. Euclidean distance was used to create a distance matrix and Average-Group-Linkage (McQuitty), was used as agglomeration method. In addition k-means clustering was used for visualization. All tests were performed and all graphs were generated with R (R Core Team 2017).

Results

Of 70 samples, only 35 specimens had produced sufficient amounts of feces for analysis, 35 samples could not be analyzed due to no or too little feces.

After baseline removal, alignment and data normalization all samples showed clear GC-spectrograms with distinct peaks (Fig. 19).

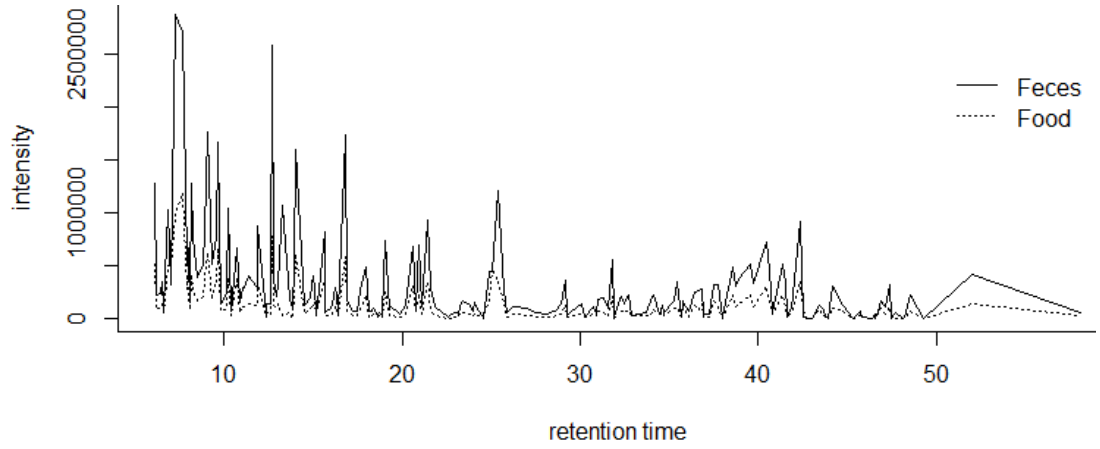


Figure 19: GC spectrograms of a food-sample (dotted line) and a faeces-sample (straight line) plotted from the peak height data (after alignment and data normalisation) used for the analysis.

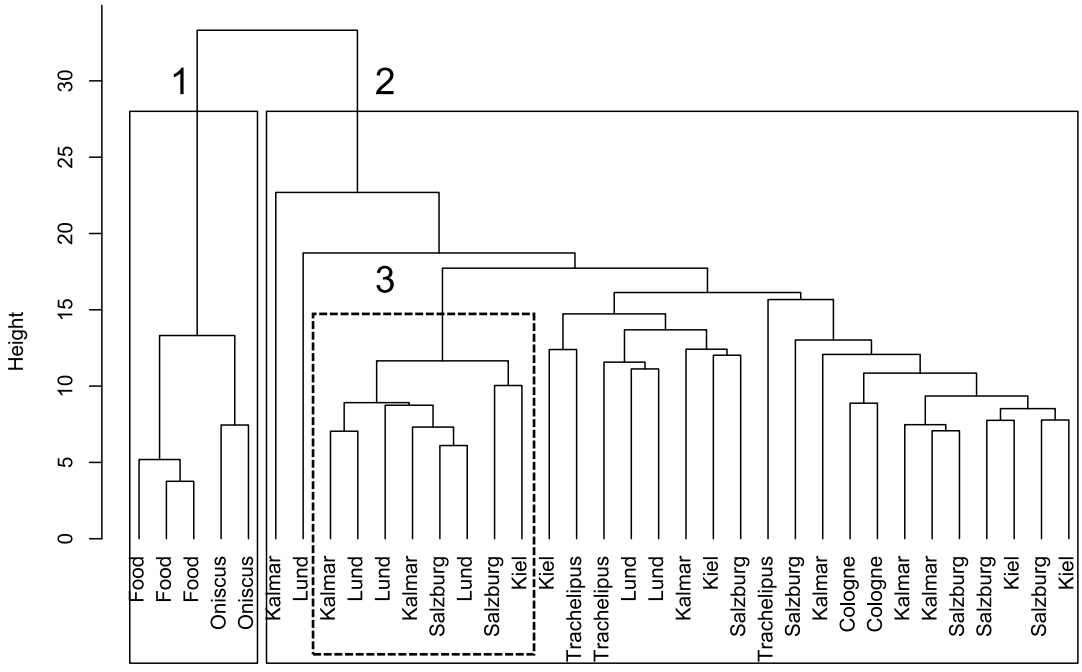


Figure 20: Hierarchical clustering analysis of food and feces samples. Euclidean distance was used to create distance matrix and Average-Group-Linkage (McQuitty), was used as agglomeration method.

The hierarchical clustering clearly shows a different clustering of the food and *O. asellus*-feces (Fig. 20, (1)). samples and all other feces samples (Fig. 20, (2)). These feces-samples (2) roughly form three clusters, but samples do not strictly cluster according to genetic similarity of the isopods. However, they do not seem to cluster fully independent of genetic

similarity, as e.g. one branch contains almost exclusively samples of the genetically very similar populations Kalmar and Lund and the two analyzable Cologne-samples are very close. Notably, bootstrap analysis of clustering shows that only the food samples, the *O. asellus* feces and the feces samples of the branch within the dotted line (Fig. 20 (3)) show sufficient probability values (> 99 %). In contract to *O. asellus* the samples of *T. ratzeburgii* do not cluster independently of the *P. scaber* samples.

K-means clustering analysis shows comparable result to the hierarchical cluster analysis (Fig. 21). The three food samples cluster close to each other and together with the feces of *O. asellus* and far away from all other feces samples. The feces samples can best be described by two clusters ("left" and "right"). With two exceptions the left cluster is formed by samples from the closely related Lund, Kalmar and Kiel populations, whereas nearly all samples from the Salzburg population and both samples from the Cologne-population are the right cluster, which also contains samples from all other populations except the Lund-Population.

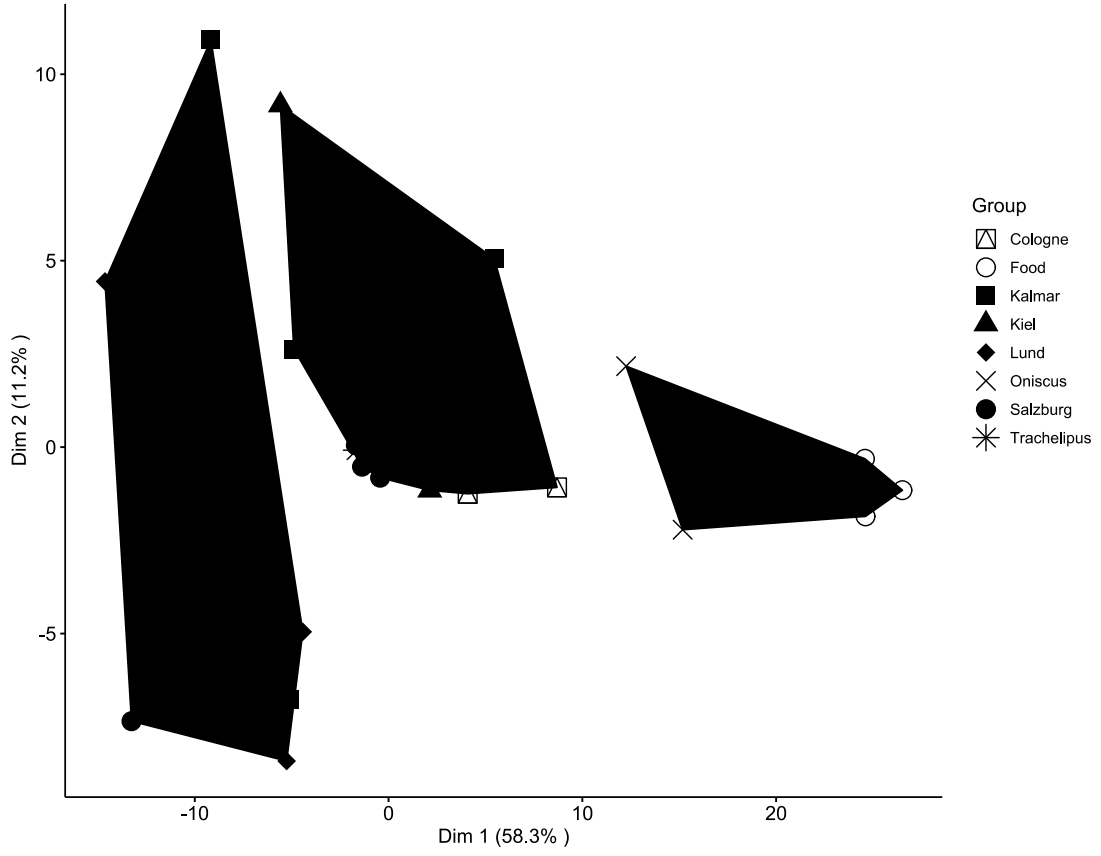


Figure 21: K-means clustering analysis of food and feces samples. Empty square and triangle down: feces samples of *P. scaber* Cologne (f.s. Cologne), empty circle: food samples, filled square: f.s. Kalmar, filled triangle: f.s. Kiel, filled diamond: f.s. Lund, crossed lines: f.s. *Oniscus asellus,* filled circle: f.s. Salzburg, star: f.s. *Trachelipus ratzeburgii*

Discussion

The aim of the study was a) to test the suitability of Py-GC/MS for measuring both changes in food composition during digestion and intraspecific variation in this digestion process, and b) to measure the extent of this variation in several individuals of *P. scaber* whose genetic variation and population differentiation is known.

The results suggest that the method is very promising for measuring intraspecific variation. The food samples, which are chemically identical, cluster closely together in both analyses and are plainly separated from all feces samples. However, due to the drop-out of many samples and the production of relatively low amounts of feces, considerably fewer samples than planned could be analysed. With the exception of the food samples no technical replicates could be included in order to evaluate the used alignment and normalisation methods more precisely.

However, the analysis shows a distinct intraspecific variation in digestion of. *P. scaber*. In both cluster analyses, the faecal samples form comparable clusters, with slight differences in the allocation of the individual samples to the clusters. The samples do not cluster consistently with regard to their genetic proximity, though the origin seem to have an influence. Regarding the interspecific variation, only one of the other species (*O. asellus*) is clearly separated (and considerable closer to the food), while the other one clusters among the intraspecific variance of *P. scaber*. This difference however can be explained by the different phylogenetic proximity.

While the phylogenetic position of the *Oniscidea* and the monophyly of the taxon *Crinocheta* (containing among others *Porcellionidae*, *Oniscidae* and *Trachelipodidae*) are well supported by many studies (Schmidt 2008), phylogenetic relationships within the *Crinocheta* are still under discussion. However, recent molecular based phylogenetic analyses by Lins et al. (2017) show a closer relation of *P. scaber* and *T. ratzeburgii* compared to *O. asellus*. This phylogenetic proximity and distance seem to be mirrored in the proximity and distance of digestive enzymes. Interestingly, the results suggest that feces of *O. asellus* has a closer proximity to the diet than to the other feces samples. However, since only two samples could be evaluated here, the results should be interpreted with caution.

Within *P. scaber* samples, phylogenetic relations can explain the results only to some extend. While the samples from Lund and Cologne (the latter with very low sample number) show intrapopulational proximity in the analysis, the clustering of most samples

cannot be explained by genetic similarity and dissimilarity. However, there are several other potential reasons for the unexpected clustering of the samples, as endogenous digestive enzymes are only one contributor to the digestion in terrestrial isopods.

Like most animals isopods have symbiotic microorganisms in their gut, which are a substantial part of the microbiome (Zimmer and Topp 1998, Zimmer 2002, Kostanjšek et al. 2006, Bouchon et al. 2016) and play a role in the digestive process. Whereas isopods have direct endogenous enzymes for substances which are more easily accessible, the existence and importance of endogenous genes for more recalcitrant substances like lignocellulose has been discussed for many years. While Kostanjšek et al. (2010) were able to show the abundance of endogenous cellulases in *P. scaber* (as Bredon et al. (2018) for the isopod species *Armadillidium vulgare*), the result of Bredon et al. (2019) clearly indicate that the role of the endogenous cellulases in isopods is much smaller than the role of the bacteria in the gut, concluding that "isopods cannot digest lignocellulose by themselves." This is however not unusual, as associations with bacteria are quite common regarding the degradation of lignocelluloses for many animals (Cragg et al. 2015). The role of ingested bacteria in this context (being a potential source for high variance in digestion processes) has been debated for a long time. Zimmer and Topp (1998) found a bigger influence of endosymbiotic bacteria in the hepatopancreas compared to ingested bacteria on the degradation of cellulose and other recalcitrant substances, but constituted a direct influence of ingested bacteria due to e.g. delivery of nutrients, influences on pH or osmoregulary features nevertheless. Recent analysis showed an additional influence of different food-sources on the taxonomical composition and biofilm-feeding on both the abundance of bacterial taxa in the isopod gut and the growth of the animals (Horváthová et al. 2016). Either way - both symbiotic and ingested bacteria are a potential source of variation, and both are independent of genetic similarity or dissimilarity of the hosting animals. This way, the microbiome could contribute to variation in digestion, depending on the identity and abundance of bacteria in the digestive tract. Bacterial contributions to the digestion might potentially also add to the position of the other isopod species in the cluster analysis.

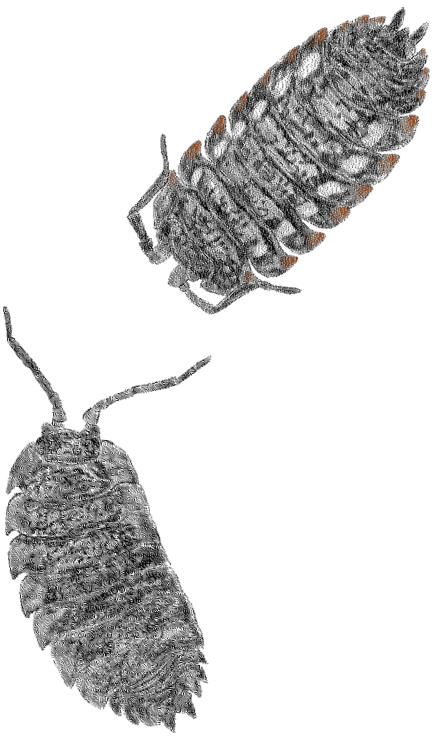
In addition to the influences of the microbiome, there are also various phenotypic functional traits that can have an impact on digestive characteristics – and which were not taken into account in this study. For example, in their study (using the same technique), Bakkar et al. (2017) found a substantial influence of the sex on digestive processes in a

detritivorous crab. Small differences in age and size could also have an influence, although comparable animals were selected for the experiment. An additional potential source of variation is coprophagy, which is quite common for isopods (Zimmer 2002). Even if the feces is reingested only partly, this could change the chemical composition of the subsequent feces substantially. This influence was tried to be prevented by the daily collection of the feces, but cannot be ruled out.

In summary (especially when including the results of *T. ratzeburgii)* this study provides clear indications of high intraspecific variance in *P. scaber* with respect to digestion. Potentially it could even exceed interspecific variation as Des Roches et al. (2018), showed for many ecological effects. Py-GC/MS seems to be a well-suited method to study chemical differences between diet and feces and chemical variation of feces samples, but future studies should include technical replicates and, if possible, substances added to the diet for normalization of the results prior to analysis. In addition to the Py-GC/MS-results, further consideration of the microbiome as well as phenotypic traits would be desirable to find potential sources of variation indigestion. If the nature and extent of the variation is confirmed in future studies, this would support the importance of intraspecific variation in the context of decomposition.

Chapter 6

Synoptic discussion



Main results

At the latest since the introduction of the concept of functional diversity, intraspecific variation has become an fundamental part in the understanding of diversity in ecology (Tilman 2001, Pachepsky et al. 2007). However, the effects of a potential loss of intraspecific diversity (as intraspecific diversity itself) has received little attention outside of ecological and evolutionary research and conservation biology. Intraspecific variation, for example, was not considered in the latest "Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES)", as Des Roches et al. (2021) point out. As the evidence grows that the importance of intraspecific diversity may be equivalent in some cases to that of interspecific diversity (Des Roches et al. 2018, Raffard et al. 2019) it gets increasingly important to study those fields in ecology, where biodiversity has been shown to matter – and where a loss of diversity would impact the ecosystem and ecosystem functions. The aim of this study was to add to this challenge and to investigate the role of intraspecific variation – and its loss – for decomposition processes.

In chapter 2 the results show, that a genetic more diverse group of isopods can have a higher consumption rate of leaf litter, though this was a) only true for consumption on single litter and – in contract to the hypothesis – not for litter mixtures, and b) only significant – in contrast to mixed litter effects – for paired analysis of RCR of leaf litter. Accordingly this effect was much weaker than the (due to the experimental design) also measurable mixed-litter-effects. The latter were also stronger when isopod diversity was low compared to mixed-litter-effects in treatments with an increased diversity. Hence, in this study isopod and litter diversity did not facilitate each other. However, this might be partly influenced by different sample size and the resulting differences in statistical power.

Some aspects of the population genetics of the *P. scaber* populations used in the experiments were shown in chapter 3 and the newly developed microsatellite markers were described in more detail. The results show that the developed markers are sufficiently polymorph and overall suitable to not only measure the genetic diversity in the natural populations, but also for the artificially combined groups used in the feeding-experiment. Natural populations did not differ much in their variation (measured as heterozygosity), but were – apart from the geographically very close Swedish populations – clearly differentiated. Genetic distance and geographical distance showed a high and significant

correlation. It was also shown that the markers are suitable for population assignment and should – despite the occurring null alleles – be applicable for future population genetics measurements on *P. scaber* and future studies on the influence of intraspecific variation.

In chapter 4 two populations of *T. ratzeburgii* from different altitudes were compared regarding their genetic variation, response to stress and consumption on different litter mixtures, hypothesizing that the different environmental conditions (especially temperature fluctuation) in combination with isolation by environment have an effect on these parameters. However, the results show that the two populations a) do not differ regarding their variation and b) are not genetically differentiated at all. Hence, neither of the two populations were isolated by the environment and influences of the environment on the variation of a population could not be measured (at least not with neutral genetic markers). Populations did dot significantly differ in their HSP-expression response to stress or (absolute) consumption of different litter mixtures – despite remarkable size differences between the specimens of the two populations. Regarding the comparable consumption despite the size and weight difference, metabolic cold adaptation hypothesis (Clarke 1993, Addo-Bediako et al. 2002) might serve as an explanation. As in chapter 2 for consumption by *P. scaber*, the results of the consumption-experiment with *T. ratzeburgii* show strong mixed litter effects.

In both summarized experiments, genetic diversity was measured and used as indicator for phenotypic variation. In chapter 5, digestive variation was directly measured via metabolic fingerprinting, using Py-GC/MS. Although the results of the analysis can only be partially compared to interspecific variation, they do show substantial intraspecific variation in digestion for *P. scaber*. The genetic proximity and the proximity in terms of a metabolic fingerprint showed no consistent similarity, although some animals from the same population also showed a vicinity regarding the digestion as well. Hence, as genetic parameters are not sufficient to explain the clustering of the metabolic fingerprints, other factors – probably the microbiome – are likely to have an influence as well.

Although no final conclusions on the matter of the influence of interspecific diversity on decomposition can be drawn so far, there is strong evidence of a positive influence of this level of diversity (Srivastava et al. 2009). As described earlier, though the amount of studies on (or including) intraspecific variation has increased over the last decade, almost no literature was published on its influence on decomposition, with some exceptions

regarding litter diversity (Madritch et al. 2006, Semchenko et al. 2017). But as the potential impact of interspecific variation and diversity was shown in the context of many other ecosystem processes, the potential influence of intraspecific diversity on decomposition processes was largely unknown. Due to the fundamental role of decomposition in all ecosystems and thus the potentially great impact of such an effect, this was a gap in the developing field of the study of the influence of intraspecific variation and diversity on ecosystem processes so far. This thesis adds some valuable information on that matter – without being able to give a clear answer to the initiating questions, however. This study was able to show an influence of intraspecific diversity on litter consumption (Chapter 2), but only in one experiment and only for paired analysis of the highest and lowest variation-level.

As some assumptions were not given, the experiment on the *T. ratzeburgii*-populations from Untersberg was not suitable to add to the question of the significance of intraspecific variation and diversity on decomposition (Chapter 4). However – though unintended – a finding not directly related to any of the initiating questions shows the independence of consumption from isopod weight and the potential impact oft a high plasticity of. *T. ratzeburgii*.

Chapter 5 shows additional support for a high intraspecific variation regarding the digestion and transformation of some dietary compounds. But this measurement was only a first step in order to estimate the overall influence of this variation on decomposition and nutrient cycling in ecosystems.

Broadening the concept of variation and diversity

The overall results from chapters 2 and 5 indicate that also in the field of decomposition, intraspecific variation should not be excluded, but instead be understood as a fundamental part of biodiversity. Even if the ecological extent of the intraspecific variation of the decomposer is lower compared to the shown (and well established) mixed litter effects, it would be a factor of great impact if further confirmed. Also – though not particularly tested here due to the undifferentiated populations in chapter 4 – it can be assumed from the results that this would be true especially for a loss of this diversity. This is of particular importance, since it can be reasonably assumed that - regardless of the type of influence -

a loss of a population or even species is preceded by a loss of intraspecific variation. Moreover, unlike the loss on other levels of diversity, at the intraspecific level, a loss of diversity is not immediately apparent.

These potential proximate ecological effects should be understood as an addition to the effects that a loss of diversity would have on the ability for future evolutionary adaptations of a population due to the loss of particular phenotypes, as this variation is the key for global and local adaptations (Davis et al. 2005, Skelly et al. 2007). The latter effect has also received little attention in terms of importance in the debate on direct climate change impacts on future ecosystem development. Hence, for a better understanding of how ecosystem processes will change in the future, it may be worthwhile to expand the perception of diversity on order to include intraspecies diversity as well.

Implications for future methodology

Another finding of this thesis, not directly related to any of the initial questions, is of methodological nature: mixing individuals from different populations can be a functional method to increase intraspecific variation, as shown in chapter 2 and 3. Although requiring relatively high effort in this study due to the verification of the method with newly developed genetic markers, it generally offers the possibility of influencing variation in experimental populations relatively fast and easily. It was not possible to measure the consequences of a reduced diversity in this study. However, future studies could additionally consider an artificial reduction of variation and diversity, either by choosing closely related individuals (if genetic data is available), inbreeding, or by obtaining a population that has undergone a reduction of variation, either by selection or gene drift.

Notably, a certain aspect of intraspecific variation was not included in this study: morphological variation. Naturally this variation is both easy to measure and obviously existing for many animals - including terrestrial isopods. Different color morphs of *P. scaber* have been described (Selby et al. 2006) and can commonly be seen in the field. But despite being easy to see and capture, few morphological characteristics are likely to influence digestion and would be of interest in this context. Hence, genetic variation and physiological variation are better approaches to measure intraspecific variation, as genetic variation is the best parameter to compare variation between populations and parameters

of physiological variation can function as a "hard trait" (Nock et al. 2016) as being connected directly to ecosystem functions.

Using altitude as a "space for time" approach did not work in this theses, as the two populations were not differentiated. Potential reasons were discussed in detail in the respective chapter 4. Hence, no conclusions can be drawn on either the influence of a fluctuation environment on intraspecific variation nor the influence of a reduced diversity on decomposition. Despite that, the approach to compare two populations of the same species living under considerably different conditions that are likely to have an influence on intraspecific variation can still be seen as a potentially valuable method to study both the type and extent of this influence – and the consequences for ecosystem processes like decomposition. However, extended genetic testing is necessary to ensure that isolation by environment (Wang and Bradburd 2014) is given.

As described in chapter 5, when studying differences between detritivores (intra- and interspecific) the microbiome should be taken into account (Bouchon et al. 2016) as it is not only a potential interfering variable, but also an additional potential sources of variation in this context. Regardless of this, it has been shown here (as by Bakkar et al. (2017)) that Py-GC-MS is a potentially very promising method to study patterns in the digestion of detritivores – also both intra- and interspecific.

After examining the results it becomes apparent how important it is to compare measurement of intraspecific with interspecific variation (such as Crutsinger et al. (2009)), as it was partly done (due to the failure of several samples) in chapter 5. If these methodological approaches are pursued further in the future, instead of examining the importance of intraspecies detritivore variation with increasing litter variation (which had no influence in this study) the methodological focus should be different. Either the influence of the intraspecific variation should be compared directly to interspecific variation or the concept of "intraspecific vs. interspecific variation" should be completely abandoned in favor of a consideration of only functional traits – independent of species borders. This implies that appropriate traits regarding digestion can be found and measured with respect to digestion. Chapter 5 certainly offers a good starting point here.

Conclusion and Outlook

Understanding the role of diversity at multiple levels in the context of different ecosystem processes is of high importance in order to assess the consequences of a loss of biodiversity. In this context, diversity is increasingly no longer understood as a "number of species", but as functional diversity, where functional characteristics play a greater role than species boundaries. However, if no hard traits can be defined (yet), an additional consideration of intraspecific variation – e.g. genetic variation – remains useful.

In the context of decomposition processes, litter diversity often has a clear influence on the consumption of detritivores, as it was the case in this study. Such a clear influence could not be detected for the intraspecific variation of a detritivore, even though this effect was also observable here under certain conditions. In contrast to the expectations, litter and detritivore diversity did not facilitate each other. That a variation in digestion – which is only one influence of a macro-detritivore on decomposition – does exist, could be shown by this study. The results provide numerous directions for further investigations of the ecological effects of this variation. This includes both a study of consumption with a reduced variation (with the described methodological possibilities) and an explicit direct comparison with interspecific variation. If possible, measurements of hard traits that are directly related to the ecological role regarding decomposition should supersede measurements of genetic diversity with neutral markers in this context.

In this study it was not possible to examine the effects of reduced variation on decomposition or the effects of the selection factor of the fluctuating environment on the stress resistance of a population, because the selection factor had no effect on variation here. Nevertheless, the approach of comparing populations from areas with different degrees of fluctuating environmental conditions and extreme events should be further pursued, since such a comparison directly allows possible statements about future long-term changes of populations due to climate changes. In this case, however, an isolation by the environment should be given – unlike it was the case here.

In summary, this study offers potential methodological ways to further investigate an influence of intraspecific variation on decomposition processes, but can only show this influence on a small scale. However, one has to bear in mind that in the context of an ecosystem process like decomposition, even small influences can have high impacts.

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List of figures and tables

The drawings on the title page and the chapter pages were made by the author (except for chapter page of chapter 3). Chapter page of chapter 3 is a decolorized version of "Der Untersberg bei Salzburg" by Heinrich Bürkel (appr. 1863, public domain). The drawing on the title page is based on the plate XIII in "The British Woodlice" by W. Webb and C. Sillem (1906, public domain). The drawing of P. scaber at the chapter pages of chapters 1,5 and 6 are based on a picture by Fritz Geller-Grimm (CC BY-SA 2.5 via Wikimedia Commons). The drawing of P. scaber at the chapter page of chapters 2 is based on a picture by Sören Franzenburg. The drawing of T. ratzeburgii at the chapter page of chapter 6 is based on a picture by Jörg Spelda (CC BY-SA 4.0 via bodentierhochvier.de).

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Versicherung an Eides statt

Ich, Hanno Müller, versichere an Eides Statt durch meine Unterschrift, dass ich die vorstehende Arbeit „Significance of intraspecific variation for decomposition processes“ selbständig und ohne fremde Hilfe angefertigt und alle Stellen, die ich wörtlich dem Sinne nach aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe, mich auch keiner anderen als der angegebenen Literatur oder sonstiger Hilfsmittel bedient habe.

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Bremen, 06. Juli 2021 _____

Ort, Datum Unterschrift

This page was adjusted after submitting the thesis (address and matriculation number have been removed from the text).

Appendix

Appendix Chapter 2

Table 11: Data of feeding experiment of chapter 2. T = Treatment. L.c.sw = Litter - corrected start weight, L.fw = Litter - final weight , wl = weight loss, IL = Isopod weight (average), RCR = relative consumption rate. The genus-names of "litter species" refer to the species described in chapter 2.

Box	T	Origin of Isopods	Litter Species	L. c.sw	L. fw	wl	IW	RCR
1	I	Kalmar	*Pinus*	1,900	1,321	0,579	0,275	0,038
2	I	Lund	*Pinus*	1,889	1,210	0,678	0,305	0,040
3	I	Kiel	*Pinus*	1,898	1,667	0,231	0,263	0,016
4	I	Cologne	*Pinus*	1,881	1,281	0,600	0,303	0,035
5	I	Salzburg	*Pinus*	1,870	1,157	0,714	0,232	0,055
6	I	Kalmar	*Picea*	1,873	1,392	0,481	0,397	0,022
7	I	Lund	*Picea*	1,833	1,382	0,451	0,265	0,030
8	I	Kiel	*Picea*	1,861	1,419	0,442	0,334	0,024
9	I	Cologne	*Picea*	1,870	1,440	0,430	0,271	0,028
10	I	Salzburg	*Picea*	1,852	1,416	0,436	0,267	0,029
11	I	Kalmar	*Sorbus*	1,881	0,987	0,894	0,274	0,058
12	I	Lund	*Sorbus*	1,874	0,970	0,904	0,337	0,048
13	I	Kiel	*Sorbus*	1,889	1,140	0,749	0,310	0,043
14	I	Cologne	*Sorbus*	1,892	1,317	0,575	0,287	0,036
15	I	Salzburg	*Sorbus*	1,867	1,262	0,605	0,277	0,039
16	I	Kalmar	*Betula*	1,910	1,431	0,479	0,276	0,031
17	I	Lund	*Betula*	1,888	1,253	0,635	0,268	0,042
18	I	Kiel	*Betula*	1,906	1,334	0,572	0,256	0,040
19	I	Cologne	*Betula*	1,902	1,334	0,569	0,286	0,035
20	I	Salzburg	*Betula*	1,893	1,293	0,599	0,239	0,045
21	I	Kalmar	*Quercus*	1,874	1,495	0,378	0,369	0,018
22	I	Lund	*Quercus*	1,845	1,552	0,293	0,297	0,018
23	I	Kiel	*Quercus*	1,851	1,712	0,139	0,309	0,008
24	I	Cologne	*Quercus*	1,841	1,511	0,331	0,312	0,019
25	I	Salzburg	*Quercus*	1,861	1,622	0,240	0,262	0,016
26	I	Kalmar	*Fagus*	1,863	1,567	0,296	0,316	0,017
27	I	Lund	*Fagus*	1,841	1,669	0,172	0,288	0,011
28	I	Kiel	*Fagus*	1,847	1,639	0,208	0,291	0,013
29	I	Cologne	*Fagus*	1,864	1,626	0,237	0,331	0,013
30	I	Salzburg	*Fagus*	1,874	1,722	0,152	0,321	0,008
31	I	Kalmar	*Acer*	1,882	1,470	0,411	0,283	0,026
32	I	Lund	*Acer*	1,849	1,384	0,464	0,308	0,027
33	I	Kiel	*Acer*	1,853	1,513	0,340	0,282	0,022
34	I	Cologne	*Acer*	1,860	1,559	0,301	0,301	0,018
35	I	Salzburg	*Acer*	1,848	1,509	0,338	0,267	0,023
36	I	Kalmar	*Fraxinus*	1,868	1,068	0,800	0,298	0,048
37	I	Lund	*Fraxinus*	1,887	1,127	0,759	0,304	0,045
38	I	Kiel	*Fraxinus*	1,883	1,277	0,607	0,245	0,044
39	I	Cologne	*Fraxinus*	1,891	1,261	0,630	0,308	0,037
40	I	Salzburg	*Fraxinus*	1,873	0,936	0,936	0,288	0,058
41	II	Kalmar	*Pinus, Sorbus*	1,905	1,097	0,808	0,305	0,047
42	II	Lund	*Pinus, Sorbus*	1,886	1,227	0,659	0,216	0,055
43	II	Kiel	*Pinus, Sorbus*	1,895	1,371	0,524	0,246	0,038
44	II	Cologne	*Pinus, Sorbus*	1,888	1,081	0,808	0,306	0,047
45	II	Salzburg	*Pinus, Sorbus*	1,869	0,930	0,939	0,227	0,074
46	II	Kalmar	*Quercus, Pinus*	1,888	1,531	0,358	0,276	0,023
47	II	Lund	*Quercus, Pinus*	1,867	1,129	0,738	0,332	0,040
48	II	Kiel	*Quercus, Pinus*	1,879	1,698	0,181	0,301	0,011
49	II	Cologne	*Quercus, Pinus*	1,855	1,182	0,673	0,317	0,038
50	II	Salzburg	*Quercus, Pinus*	1,869	1,270	0,599	0,218	0,049
51	II	Kalmar	*Fagus, Acer*	1,870	1,533	0,337	0,268	0,022
52	II	Lund	*Fagus, Acer*	1,851	1,561	0,290	0,260	0,020
53	II	Kiel	*Fagus, Acer*	1,852	1,566	0,286	0,281	0,018
54	II	Cologne	*Fagus, Acer*	1,862	1,569	0,293	0,316	0,017
55	II	Salzburg	*Fagus, Acer*	1,860	1,486	0,374	0,173	0,039

56	II	Kalmar	*Fagus, Quercus*	1,867	1,658	0,209	0,263	0,014
57	II	Lund	*Fagus, Quercus*	1,846	1,647	0,200	0,277	0,013
58	II	Kiel	*Fagus, Quercus*	1,850	1,694	0,156	0,312	0,009
59	II	Cologne	*Fagus, Quercus*	1,853	1,558	0,295	0,279	0,019
60	II	Salzburg	*Fagus, Quercus*	1,868	1,650	0,218	0,236	0,017
61	II	Kalmar	*Fraxinus, Acer*	1,882	1,075	0,807	0,295	0,049
62	II	Lund	*Fraxinus, Acer*	1,869	1,057	0,812	0,282	0,051
63	II	Kiel	*Fraxinus, Acer*	1,871	1,226	0,645	0,269	0,043
64	II	Cologne	*Fraxinus, Acer*	1,877	1,232	0,645	0,290	0,040
65	II	Salzburg	*Fraxinus, Acer*	1,861	0,924	0,937	0,337	0,050
66	II	Kalmar	*Betula, Picea*	1,900	1,078	0,821	0,241	0,061
67	II	Lund	*Betula, Picea*	1,865	1,328	0,537	0,297	0,032
68	II	Kiel	*Betula, Picea*	1,882	1,301	0,582	0,237	0,044
69	II	Cologne	*Betula, Picea*	1,887	1,247	0,640	0,295	0,039
70	II	Salzburg	*Betula, Picea*	1,871	1,276	0,596	0,213	0,050
71	II	Kalmar	*Betula, Fraxinus*	1,892	1,225	0,667	0,313	0,038
72	II	Lund	*Betula, Fraxinus*	1,886	1,132	0,755	0,387	0,035
73	II	Kiel	*Betula, Fraxinus*	1,895	0,925	0,970	0,289	0,060
74	II	Cologne	*Betula, Fraxinus*	1,904	1,267	0,637	0,316	0,036
75	II	Salzburg	*Betula, Fraxinus*	1,883	1,045	0,838	0,211	0,071
76	II	Kalmar	*Sorbus, Picea*	1,887	1,104	0,784	0,348	0,040
77	II	Lund	*Sorbus, Picea*	1,857	1,287	0,571	0,269	0,038
78	II	Kiel	*Sorbus, Picea*	1,875	1,259	0,616	0,243	0,045
79	II	Cologne	*Sorbus, Picea*	1,880	1,230	0,649	0,280	0,041
80	II	Salzburg	*Sorbus, Picea*	1,860	1,166	0,694	0,248	0,050
81	III	Kalmar	*Pinus, Sorbus, Betula, Picea*	1,893	1,217	0,676	0,368	0,033
82	III	Lund	*Pinus, Sorbus, Betula, Picea*	1,867	1,173	0,694	0,317	0,039
83	III	Kiel	*Pinus, Sorbus, Betula, Picea*	1,888	1,231	0,657	0,274	0,043
84	III	Cologne	*Pinus, Sorbus, Betula, Picea*	1,885	1,194	0,690	0,286	0,043
85	III	Salzburg	*Pinus, Sorbus, Betula, Picea*	1,870	1,048	0,822	0,287	0,051
86	III	Kalmar	*Pinus, Quercus, Betula, Picea*	1,891	1,241	0,649	0,342	0,034
87	III	Lund	*Pinus, Quercus, Betula, Picea*	1,861	1,381	0,479	0,324	0,026
88	III	Kiel	*Pinus, Quercus, Betula, Picea*	1,882	1,462	0,420	0,279	0,027
89	III	Cologne	*Pinus, Quercus, Betula, Picea*	1,882	1,166	0,716	0,276	0,046
90	III	Salzburg	*Pinus, Quercus, Betula, Picea*	1,867	1,279	0,589	0,170	0,062
91	III	Kalmar	*Fagus, Acer, Fraxinus, Sorbus*	1,879	1,056	0,822	0,347	0,042
92	III	Lund	*Fagus, Acer, Fraxinus, Sorbus*	1,871	1,102	0,770	0,246	0,056
93	III	Kiel	*Fagus, Acer, Fraxinus, Sorbus*	1,862	1,268	0,594	0,309	0,034
94	III	Cologne	*Fagus, Acer, Fraxinus, Sorbus*	1,879	1,327	0,552	0,311	0,032
95	III	Salzburg	*Fagus, Acer, Fraxinus, Sorbus*	1,869	1,013	0,856	0,239	0,064
96	III	Kalmar	*Fagus, Acer, Quercus, Sorbus*	1,884	1,069	0,815	0,354	0,041
97	III	Lund	*Fagus, Acer, Quercus, Sorbus*	1,855	1,067	0,788	0,361	0,039
98	III	Kiel	*Fagus, Acer, Quercus, Sorbus*	1,856	1,382	0,474	0,283	0,030
99	III	Cologne	*Fagus, Acer, Quercus, Sorbus*	1,867	1,398	0,469	0,294	0,029
100	III	Salzburg	*Fagus, Acer, Quercus, Sorbus*	1,863	1,421	0,442	0,323	0,024
101	III	Kalmar	*Fagus, Acer, Fraxinus, Quercus*	1,874	1,309	0,565	0,324	0,031
102	III	Lund	*Fagus, Acer, Fraxinus, Quercus*	1,857	1,339	0,519	0,300	0,031
103	III	Kiel	*Fagus, Acer, Fraxinus, Quercus*	1,863	1,479	0,384	0,312	0,022
104	III	Cologne	*Fagus, Acer, Fraxinus, Quercus*	1,872	1,344	0,528	0,277	0,034
105	III	Salzburg	*Fagus, Acer, Fraxinus, Quercus*	1,866	1,255	0,611	0,304	0,036
106	III	Kalmar	*Pinus, Acer, Betula, Picea*	1,889	1,236	0,653	0,338	0,034
107	III	Lund	*Pinus, Acer, Betula, Picea*	1,863	1,283	0,580	0,227	0,046
108	III	Kiel	*Pinus, Acer, Betula, Picea*	1,876	1,259	0,618	0,296	0,037
109	III	Cologne	*Pinus, Acer, Betula, Picea*	1,874	1,226	0,649	0,273	0,042
110	III	Salzburg	*Pinus, Acer, Betula, Picea*	1,866	0,990	0,876	0,242	0,065
111	III	Kalmar	*Pinus, Fagus, Betula, Fraxinus*	1,888	1,281	0,607	0,262	0,041
112	III	Lund	*Pinus, Fagus, Betula, Fraxinus*	1,876	1,290	0,585	0,262	0,040
113	III	Kiel	*Pinus, Fagus, Betula, Fraxinus*	1,886	1,341	0,545	0,276	0,035
114	III	Cologne	*Pinus, Fagus, Betula, Fraxinus*	1,891	1,130	0,762	0,282	0,048
115	III	Salzburg	*Pinus, Fagus, Betula, Fraxinus*	1,883	1,073	0,810	0,225	0,064
116	III	Kalmar	*Quercus, Picea, Fraxinus, Sorbus*	1,873	1,122	0,752	0,341	0,039
117	III	Lund	*Quercus, Picea, Fraxinus, Sorbus*	1,868	1,191	0,678	0,314	0,039
118	III	Kiel	*Quercus, Picea, Fraxinus, Sorbus*	1,861	1,133	0,728	0,309	0,042
119	III	Cologne	*Quercus, Picea, Fraxinus, Sorbus*	1,869	1,268	0,600	0,300	0,036
120	III	Salzburg	*Quercus, Picea, Fraxinus, Sorbus*	1,865	1,185	0,680	0,254	0,048
121	IV	Kalmar, Kiel	*Pinus*	1,902	1,573	0,329	0,267	0,022
122	IV	Lund, Cologne	*Pinus*	1,880	1,136	0,744	0,280	0,047
123	IV	Salzburg, Kiel	*Pinus*	1,886	1,308	0,578	0,290	0,036
124	IV	Kalmar, Kiel	*Picea*	1,870	1,281	0,589	0,284	0,037
125	IV	Lund, Cologne	*Picea*	1,850	1,329	0,521	0,324	0,029
126	IV	Salzburg, Kiel	*Picea*	1,857	1,417	0,441	0,245	0,032

127	IV	Kalmar, Kiel	*Sorbus*	1,889	1,104	0,784	0,300	0,047
128	IV	Lund, Cologne	*Sorbus*	1,886	1,294	0,592	0,297	0,036
129	IV	Salzburg, Kiel	*Sorbus*	1,872	1,211	0,661	0,260	0,045
130	IV	Kalmar, Kiel	*Betula*	1,906	1,217	0,689	0,294	0,042
131	IV	Lund, Cologne	*Betula*	1,902	1,278	0,624	0,315	0,035
132	IV	Salzburg, Kiel	*Betula*	1,897	1,198	0,699	0,276	0,045
133	IV	Kalmar, Kiel	*Quercus*	1,857	1,642	0,215	0,294	0,013
134	IV	Lund, Cologne	*Quercus*	1,851	1,597	0,254	0,294	0,015
135	IV	Salzburg, Kiel	*Quercus*	1,857	1,701	0,156	0,213	0,013
136	IV	Kalmar, Kiel	*Fagus*	1,855	1,570	0,286	0,318	0,016
137	IV	Lund, Cologne	*Fagus*	1,845	1,650	0,194	0,332	0,010
138	IV	Salzburg, Kiel	*Fagus*	1,863	1,599	0,265	0,245	0,019
139	IV	Kalmar, Kiel	*Acer*	1,866	1,384	0,483	0,289	0,030
140	IV	Lund, Cologne	*Acer*	1,854	1,455	0,400	0,287	0,025
141	IV	Salzburg, Kiel	*Acer*	1,851	1,482	0,369	0,307	0,021
142	IV	Kalmar, Kiel	*Fraxinus*	1,879	1,114	0,766	0,323	0,042
143	IV	Lund, Cologne	*Fraxinus*	1,886	1,094	0,792	0,265	0,053
144	IV	Salzburg, Kiel	*Fraxinus*	1,877	1,017	0,860	0,245	0,063
145	V	Kalmar, Kiel	*Pinus, Sorbus*	1,888	1,237	0,652	0,290	0,040
146	V	Lund, Cologne	*Quercus, Pinus*	1,867	1,280	0,588	0,368	0,029
147	V	Salzburg, Kiel	*Fagus, Acer*	1,858	1,559	0,299	0,243	0,022
148	V	Kalmar, Kiel	*Fagus, Quercus*	1,861	1,691	0,170	0,289	0,011
149	V	Lund, Cologne	*Fraxinus, Acer*	1,872	1,098	0,775	0,276	0,050
150	V	Salzburg, Kiel	*Betula, Picea*	1,877	1,137	0,740	0,289	0,046
151	V	Kalmar, Kiel	*Betula, Fraxinus*	1,892	1,132	0,760	0,309	0,044
152	V	Lund, Cologne	*Sorbus, Picea*	1,872	1,247	0,625	0,296	0,038
153	VI	Kalmar, Kiel	*Pinus, Sorbus, Betula, Picea*	1,886	1,316	0,570	0,304	0,033
154	VI	Lund, Cologne	*Pinus, Quercus, Betula, Picea*	1,874	1,113	0,761	0,308	0,044
155	VI	Salzburg, Kiel	*Fagus, Acer, Fraxinus, Sorbus*	1,865	1,168	0,698	0,308	0,041
156	VI	Kalmar, Kiel	*Fagus, Acer, Quercus, Sorbus*	1,857	1,300	0,558	0,278	0,036
157	VI	Lund, Cologne	*Fagus, Acer, Fraxinus, Quercus*	1,870	1,381	0,489	0,283	0,031
158	VI	Salzburg, Kiel	*Pinus, Acer, Betula, Picea*	1,868	1,198	0,670	0,263	0,045
159	VI	Kalmar, Kiel	*Pinus, Fagus, Betula, Fraxinus*	1,895	1,421	0,474	0,243	0,035
160	VI	Lund, Cologne	*Quercus, Picea, Fraxinus, Sorbus*	1,864	1,159	0,705	0,322	0,039
161	VII	Kalmar, Kiel, Salzburg, Cologne	*Pinus*	1,884	1,183	0,701	0,300	0,042
162	VII	Lund, Cologne, Salzburg, Kiel	*Pinus*	1,889	1,205	0,684	0,282	0,043
163	VII	Kalmar, Kiel, Salzburg, Cologne	*Picea*	1,866	1,296	0,570	0,285	0,036
164	VII	Lund, Cologne, Salzburg, Kiel	*Picea*	1,852	1,326	0,526	0,302	0,031
165	VII	Kalmar, Kiel, Salzburg, Cologne	*Sorbus*	1,880	1,140	0,740	0,272	0,049
166	VII	Lund, Cologne, Salzburg, Kiel	*Sorbus*	1,879	1,160	0,720	0,287	0,045
167	VII	Kalmar, Kiel, Salzburg, Cologne	*Betula*	1,905	1,456	0,450	0,254	0,032
168	VII	Lund, Cologne, Salzburg, Kiel	*Betula*	1,904	1,289	0,615	0,284	0,039
169	VII	Kalmar, Kiel, Salzburg, Cologne	*Quercus*	1,857	1,625	0,232	0,297	0,014
170	VII	Lund, Cologne, Salzburg, Kiel	*Quercus*	1,845	1,483	0,362	0,280	0,023
171	VII	Kalmar, Kiel, Salzburg, Cologne	*Fagus*	1,863	1,644	0,220	0,257	0,015
172	VII	Lund, Cologne, Salzburg, Kiel	*Fagus*	1,851	1,605	0,246	0,331	0,013
173	VII	Kalmar, Kiel, Salzburg, Cologne	*Acer*	1,857	1,325	0,532	0,376	0,025
174	VII	Lund, Cologne, Salzburg, Kiel	*Acer*	1,855	1,439	0,416	0,292	0,025
175	VII	Kalmar, Kiel, Salzburg, Cologne	*Fraxinus*	1,883	1,088	0,795	0,299	0,048
176	VII	Lund, Cologne, Salzburg, Kiel	*Fraxinus*	1,883	0,943	0,940	0,322	0,052
177	VIII	Kalmar, Kiel, Salzburg, Cologne	*Pinus, Sorbus*	1,891	1,120	0,771	0,312	0,044
178	VIII	Lund, Cologne, Salzburg, Kiel	*Quercus, Pinus*	1,865	1,251	0,614	0,352	0,031
179	VIII	Kalmar, Kiel, Salzburg, Cologne	*Fagus, Acer*	1,865	1,575	0,290	0,299	0,017
180	VIII	Lund, Cologne, Salzburg, Kiel	*Fagus, Quercus*	1,853	1,608	0,246	0,281	0,016
181	VIII	Kalmar, Kiel, Salzburg, Cologne	*Fraxinus, Acer*	1,881	1,078	0,803	0,306	0,047
182	VIII	Lund, Cologne, Salzburg, Kiel	*Betula, Picea*	1,869	1,394	0,475	0,272	0,031
183	VIII	Kalmar, Kiel, Salzburg, Cologne	*Betula, Fraxinus*	1,888	0,905	0,982	0,329	0,053
184	VIII	Lund, Cologne, Salzburg, Kiel	*Sorbus, Picea*	1,870	1,279	0,591	0,303	0,035
185	IX	Kalmar, Kiel, Salzburg, Cologne	*Pinus, Sorbus, Betula, Picea*	1,887	1,018	0,869	0,290	0,054
186	IX	Lund, Cologne, Salzburg, Kiel	*Pinus, Quercus, Betula, Picea*	1,873	1,209	0,665	0,282	0,042
187	IX	Kalmar, Kiel, Salzburg, Cologne	*Fagus, Acer, Fraxinus, Sorbus*	1,873	1,123	0,750	0,301	0,045
188	IX	Lund, Cologne, Salzburg, Kiel	*Fagus, Acer, Quercus, Sorbus*	1,863	1,432	0,431	0,308	0,025
189	IX	Kalmar, Kiel, Salzburg, Cologne	*Fagus, Acer, Fraxinus, Quercus*	1,868	1,430	0,438	0,281	0,028
190	IX	Lund, Cologne, Salzburg, Kiel	*Pinus, Acer, Betua, Picea*	1,876	1,245	0,631	0,275	0,041
191	IX	Kalmar, Kiel, Salzburg, Cologne	*Pinus, Fagus, Betual, Fraxinus*	1,887	1,237	0,649	0,282	0,041
192	IX	Lund, Cologne, Salzburg, Kiel	*Quercus, Picea, Fraxinus, Sorbus*	1,868	1,114	0,754	0,283	0,048

Appendix Chapter 4

Table 12: Data of feeding experiment of chapter 4. LD = Litter diversity (number of litter species in this treatment), L.c.sw = Litter - corrected start weight, L.fw = Litter - final weight , wl = weight loss, IL = Isopod weight (average), RCR = relative consumption rate. The genus-names of "litter species" refer to the species described in chapter 4.

Box	LD	Litter species	Group	L. c.sw	L. fw	wl	IW	RCR
BT 01	1	*Fagus*	mountain	1,853	1,725	0,128	0,291	0,010
BT 02	1	*Betula*	mountain	1,846	1,318	0,528	0,316	0,040
BT 05	1	*Picea*	mountain	1,848	1,442	0,406	0,295	0,033
BT 08	1	*Quercus*	mountain	1,876	1,665	0,211	0,300	0,017
BT 09	1	*Fraxinus*	mountain	1,846	1,182	0,663	0,328	0,048
BT 22	1	*Sorbus*	mountain	1,844	1,492	0,352	0,281	0,030
BT 27	1	*Acer*	mountain	1,845	1,543	0,303	0,247	0,029
BT 37	1	*Pinus*	mountain	1,843	1,161	0,682	0,290	0,056
BT 03	2	*Sorbus, Picea*	mountain	1,847	1,422	0,425	0,286	0,035
BT 07	2	*Fagus, Quercus*	mountain	1,862	1,702	0,160	0,273	0,014
BT 13	2	*Fagus, Acer*	mountain	1,850	1,627	0,223	0,337	0,016
BT 17	2	*Betula, Fraxinus*	mountain	1,845	1,149	0,697	0,328	0,051
BT 23	2	*Sorbus, Pinus*	mountain	1,845	1,086	0,758	0,343	0,053
BT 41	2	*Quercus, Pinus*	mountain	1,861	1,250	0,611	0,251	0,058
BT 42	2	*Betula, Picea*	mountain	1,849	1,328	0,521	0,287	0,043
BT 48	2	*Fraxinus, Acer*	mountain	1,847	1,312	0,536	0,292	0,044
BT 14	4	*Fagus, Acer, Fraxinus, Quercus*	mountain	1,855	1,389	0,466	0,271	0,041
BT 16	4	*Fagus, Acer, Quercus, Sorbus*	mountain	1,857	1,609	0,248	0,271	0,022
BT 25	4	*Pinus, Sorbus, Betula, Picea*	mountain	1,846	1,152	0,694	0,325	0,051
BT 30	4	*Pinus, Quercus, Betula, Picea*	mountain	1,855	1,296	0,558	0,339	0,039
BT 40	4	*Pinus, Acer, Betula, Fraxinus*	mountain	1,847	1,146	0,701	0,253	0,066
BT 43	4	*Quercus, Picea, Fraxinus, Sorbus*	mountain	1,852	1,336	0,516	0,255	0,048
BT 44	4	*Pinus, Fagus, Betula, Picea*	mountain	1,849	1,313	0,536	0,272	0,047
BT 47	4	*Fagus, Acer, Fraxinus, Sorbus*	mountain	1,849	1,292	0,557	0,327	0,041
BT 04	1	*Picea*	valley	1,846	1,487	0,359	0,352	0,024
BT 12	1	*Fraxinus*	valley	1,846	1,244	0,602	0,381	0,038
BT 18	1	*Sorbus*	valley	1,846	1,465	0,381	0,406	0,022
BT 20	1	*Fagus*	valley	1,852	1,713	0,138	0,380	0,009
BT 24	1	*Quercus*	valley	1,875	1,630	0,245	0,398	0,015
BT 31	1	*Betula*	valley	1,847	1,240	0,607	0,366	0,039
BT 34	1	*Acer*	valley	1,849	1,622	0,227	0,410	0,013
BT 36	1	*Pinus*	valley	1,844	1,098	0,745	0,417	0,043
BT 06	2	*Sorbus, Pinus*	valley	1,845	1,083	0,762	0,439	0,041
BT 11	2	*Betula, Picea*	valley	1,846	1,276	0,569	0,371	0,037
BT 15	2	*Betula, Fraxinus*	valley	1,847	1,113	0,734	0,366	0,048
BT 29	2	*Fraxinus, Acer*	valley	1,846	1,685	0,161	0,426	0,009
BT 35	2	*Fagus, Quercus*	valley	1,862	1,702	0,160	0,420	0,009
BT 38	2	*Fagus, Acer*	valley	1,850	1,665	0,184	0,326	0,013
BT 39	2	*Quercus, Pinus*	valley	1,861	1,275	0,586	0,330	0,042
BT 46	2	*Sorbus, Picea*	valley	1,845	1,359	0,486	0,384	0,030
BT 10	4	*Fagus, Acer, Fraxinus, Quercus*	valley	1,857	1,299	0,557	0,413	0,032
BT 19	4	*Pinus, Fagus, Betula, Picea*	valley	1,847	1,122	0,725	0,423	0,041
BT 21	4	*Pinus, Quercus, Betula, Picea*	valley	1,852	1,206	0,646	0,424	0,036
BT 26	4	*Quercus, Picea, Fraxinus, Sorbus*	valley	1,855	1,373	0,482	0,392	0,029
BT 28	4	*Fagus, Acer, Fraxinus, Sorbus*	valley	1,847	1,491	0,356	0,436	0,019
BT 32	4	*Pinus, Acer, Betula. Fraxinus*	valley	1,846	1,231	0,615	0,428	0,034
BT 33	4	*Pinus, Sorbus, Betula, Picea*	valley	1,844	1,137	0,707	0,420	0,040
BT 45	4	*Fagus, Acer, Quercus, Sorbus*	valley	1,855	1,568	0,287	0,402	0,017