

How animal-microbe interactions affect decomposition processes



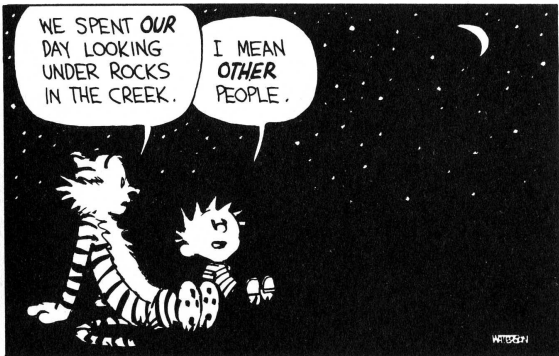
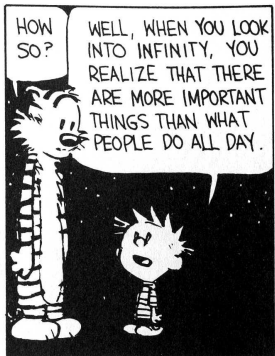
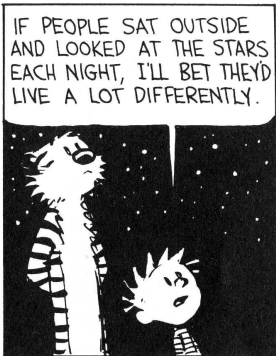
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Summary

Decomposition of dead biomass is one of the fundamental ecological processes in nature. Both, invertebrate detritivores and microbial decomposers exhibit great influence on decomposition processes. From terrestrial systems it has been learned that animal-microbe interactions drive nutrient and matter turn-over. However, to what extent this is true for aquatic systems remains largely unknown. It is not clear whether knowledge from terrestrial systems is applicable to aquatic animal-microbe interactions and subsequently to aquatic decomposition. If this was not the case, what would be the forces driving differences in detritus processing between the aquatic and the terrestrial environment? It has been suggested that differences in detritus quality, deriving from differences in primary producers, cause different detritus food web properties in aquatic and terrestrial systems. With the present thesis, I aimed at shedding light on these differences in decomposition and the particular role of animal-microbe interactions.

Investigations were carried out in the laboratory and in two field locations. Results from terrestrial and aquatic microcosm experiments under similar detritivore regimes using the same terrestrial leaf litter detritus were compared to results from a salt marsh, where aquatic and terrestrial conditions alternate, and to results from a cobble beach, receiving detritus of aquatic origin.

I found invertebrate detritivores to exert significant impacts on the microbial community and its performance in the aquatic environment. Detritus processing and animal-microbe interactions were significantly different compared to the terrestrial environment. Traits of detritivores and detritus were less pronounced in the aquatic than in the terrestrial environment. Decomposition in the salt marsh system was dominated by terrestrial patterns of decomposition, while detritus of aquatic origin accelerated decomposition on the cobble beach. Compared to the decomposition of terrestrial detritus, detritus of aquatic origin decomposed at high rates both, with and without detritivores, but detritivores significantly accelerated decomposition. Additionally, I found effects of detritivore and litter species-richness to be weak or fleeting in aquatic, terrestrial and salt marsh decomposition processes.

I conclude that decomposition and the role of invertebrate and microbial decomposers in decomposition is basically different in systems although they receive the same energy source in the form of deciduous leaf litter. Decomposition in early stages is

mediated through DOM in the aquatic and through POM in the terrestrial environment. The aquatic environment shapes the detritivorous feeding mode. Hence, differences in decomposition processes in aquatic and terrestrial systems can not exclusively be attributed to detritus quality: the aquatic environment itself shapes the process. Further, animal-microbial interactions appear to loose importance, when aquatic detritus of high nutritional value decomposes semi-terrestrially, since, due to the high quality, decomposition rates by detritivores and decay by leaching and microorganisms are by magnitudes faster than of terrestrial detritus in both, the aquatic and the terrestrial environment.

Species-mixture effects in general appear to play a minor role in decomposition processes. However, I found litter-mixture effects to be mediated by detritivores and to influence microbial activity.

Zusammenfassung

Die Dekomposition toter Biomasse ist einer der fundamentalen ökologischen Prozesse in der Natur. Invertebrate Detritivore und mikrobielle Destruenten üben einen starken Einfluss auf diesen Prozess aus. Studien an terrestrischen Systemen haben gezeigt, dass tierlich-mikrobielle Interaktionen eine zentrale Rolle im Nährstoff- und Materieumsatz des Detritus-Nahrungsnetzes spielen. Es ist jedoch nur wenig bekannt darüber, ob und inwieweit die aus den terrestrischen Untersuchungen gewonnenen Daten auf aquatische Verhältnisse übertragbar sind. Sollten die tierlich-mikrobiellen Interaktionen in aquatischen Systemen anderen Gesetzmäßigkeiten unterliegen, so stellt sich die Frage nach dem zentralen Unterschied im Detritus-Nahrungsnetz zwischen aquatischen und terrestrischen Systemen. Es wurde vermutet, dass dieser Unterschied auf die unterschiedliche Nahrungsqualität der jeweiligen Primärproduzenten und damit des Detritus zurückgeht.

Ich habe Experimente im Labor und in zwei Feldstationen durchgeführt. Die Ergebnisse aus einem Mikrokosmen-Versuch unter aquatischen und terrestrischen Bedingungen, bei gleichem Detritus-Regime und gleicher Laubstreu, wurden mit Ergebnissen aus Freilandversuchen in einem Salzwiesen- und einem Kiesstrand-Habitat verglichen.

In meinen Untersuchungen zeigten die Detritivoren einen signifikanten Einfluss auf die aquatische mikrobielle Gemeinschaft und deren Aktivität. Die Abbauraten des Detritus und die tierlich-mikrobiellen Interaktionen waren signifikant verschieden im Vergleich zum terrestrischen Habitat. Charakteristika der Detritivoren und des Detritus waren im aquatischen Habitat schwächer ausgeprägt als im terrestrischen. Im Salzwiesen-Habitat zeigte der Dekompositionsprozess trotz häufiger Überflutung ein deutlich terrestrisches Muster. Qualitativ hochwertiger Detritus am hochdynamischen Kiesstrand wurde extrem schnell umgesetzt. Verglichen mit der Dekomposition terrestrischen Detritus, wurde Detritus aquatischen Ursprungs wesentlich schneller abgebaut; mit und auch ohne Einfluss detritivorer Tiere, die die Umsatzrate aber noch einmal signifikant steigerten. Bei diesen Untersuchungen fand ich außerdem im aquatischen, terrestrischen und im Salzwiesen-Habitat schwach ausgeprägte und kurzweilige Effekte der Artenzusammensetzung der Detritivoren und des Detritus.

Ich schließe aus meinen Untersuchungen, dass die Dekomposition und die Rolle invertebrater und mikrobieller Destruenten in den Habitaten sich selbst bei Speisung aus der gleichen Energiequelle (Laubstreu) signifikant unterscheiden. Dekomposition im frühen Stadium wird im aquatischen System bevorzugt über den Weg gelöster organischer Materie verwirklicht, während im terrestrischen System die partikuläre organische Substanz direkt umgesetzt wird. Folglich können Unterschiede in aquatischer und terrestrischer Dekomposition nicht ausschließlich auf die Qualität des Detritus zurückgeführt werden; das Habitat selbst beeinflusst den Prozess. Des Weiteren scheinen tierlich-mikrobielle Interaktionen an Bedeutung zu verlieren, wenn hochwertiger aquatischer Detritus unter terrestrischen Bedingungen zersetzt wird. Der hohe Nährwert beschleunigt die Umsatzraten durch Detritivore und die Zersetzung durch physikalische Prozesse und mikrobielle Destruenten um ein Vielfaches, verglichen mit der Dekomposition terrestrischen Detritus in aquatischen und terrestrischen Habitaten.

Effekte der Artenzusammensetzung scheinen eine untergeordnete Rolle im gesamten Prozess zu spielen. Ich konnte jedoch zeigen, dass solche, wenn auch geringen, Effekte in Streu-Gemischen durch den Einfluss detritivorer Tiere hervorgerufen werden.

General Introduction

General remarks

In a biological context, the term “decomposition” comprises the many different and complex pathways from dead organic material to the complete release of its elemental constituents into the environment. These processes are essential to life, since they return the building-stones for the next generations of organisms to come. Without decomposition, nutrients would be fixed in a form unavailable to primary producers and life would quickly drown in a thick layer of organic matter. The by far largest contribution to the mass of decomposing material is made by photosynthetically active tissue: leaves, needles and thalli (Swift et al. 1979, Berg & Laskowski 2006, Zimmer in press). The term “detritus” comprises all dead organic material of no matter what origin (Zimmer in press). Here, due to its significance, I solely consider decomposition of litter material and refer to it as “detritus”.

All along the process from the senescing leaf to the release of its last carbon atom, a variety of organisms are directly or indirectly involved. As we know from soil ecology research, invertebrate animals can play an important role within this process, and in many cases faunal indirect influence on nutrient fluxes is several-fold higher than through obvious and visible ingestion of detritus (see below) (Anderson & Ineson 1984, Bardgett & Chan 1999, Berg & Laskowsky 2006, Huhta 2006). These mechanisms are not so well studied in aquatic systems and aquatic-terrestrial transition zones. The influence of (macro-)invertebrate animals on microbial decomposers in these systems is the subject of the present thesis.

General patterns in decomposition

Each ecosystem has specific detritus-processing properties. However, some aspects of plant material decomposition are universal. This is in particular true for microbial succession and action. Even before the deposition of detritus, invasion by microorganisms starts, in detail depending on many factors, but generally turning from fast-reproducing r-strategy organisms metabolizing labile low-molecular carbohydrates to more energy-efficient K-strategy organisms in the later stages of decomposition (Zvyagintsev 1994, Theenhaus & Scheu 1996, Dilly et al. 2001, Berg & Laskowsky 2006).

Thus, the microbial community changes during the course of decomposition adapting to the changing substrate condition and to the decline in detritus quality. In the initial phase, referred to as the “conditioning phase”, microbial biomass increases rapidly, and detritus becomes more attractive and valuable to invertebrate detritivores (Bärlocher & Kendrick 1975, Findlay & Arsuffi 1989). When easily degradable detrital compounds diminish, the microbial community shifts to species more specialized on degrading high-molecular structural compounds like hydrolyzing cellulose or oxidizing lignins. This succession is reflected by a co-succession of the corresponding enzymes produced by the microbiota and excreted into the surrounding biofilm matrix (Linkins et al. 1990, Dilly & Munch 1996, Dilly et al. 2001, Berg & Laskowsky 2006).

Most of our knowledge derived from research on terrestrial systems, where decomposition is closely associated to the soil systems. Since these are essential for agriculture, soil processes, in particular remineralization of plant material, are well studied (Bardgett & McAlister 1999, Berg & Laskowski 2006). Nevertheless, only since the 1980s, researchers have directed their attention to microorganisms involved in decomposition processes. Earlier, the major role in decomposition processes has been ascribed to soil-dwelling macroinvertebrates. We now know that in boreal forest, for instance, 95 % of plant litter carbon turnover is the work of fungi and bacteria (Berg & Laskowski 2006). The potential impact of microorganisms on litter and soil processes can in a first approach be elucidated simply by their huge numbers: some 10 billion bacterial cells are present on one gram of dry decaying birch litter (see Chapter II), and in one liter of humus the total fungal mycelium was estimated to reach a length of up to 2000 km (Berg & Laskowski 2006). These numbers justify the restriction of the term “microorganisms” in this thesis to bacteria and fungi, neglecting protists and algae.

It is only within the bacterial and fungal kingdom that we find organisms that are able to entirely degrade all litter structural polymers in plant cell walls. These polymers can be combined to fibers of cellulose and hemicellulose embedded in a matrix of lignin molecules forming complex and very stable compounds (Berg & Laskowski 2006). The ability of microorganisms to degrade these substances is crucial for plant material decomposition and hence for the circulation of nutrients.

The size relation of plant fibers and microorganisms demands extracellular digestion by excretion of a hydrolytic enzyme matrix onto the substratum. While hyphae-

producing fungi may penetrate plant cell walls and grow their hyphae into the cell lumen, most bacteria are surface-active through enzymatic lysis of the substratum (Berg & Laskowski 2006). The enzymatic pathways of plant matter degradation are much better studied for fungi than for bacteria; often only the outward appearance like tunneling, erosion, and cavitation of plant matter of bacterial degradation has been studied (Berg & Laskowski 2006). Bacterial decomposition is often closely associated with fungal decomposition when bacteria benefit from fungal intermediate products (Suberkropp & Klug 1976, Berg & Laskowski 2006).

Microbial activity is very sensitive to temperature and moisture changes - on a small scale due to mosaics of microclimates or on a larger scale due to the weather - resulting in high variation in decomposition (Chauvet & Suberkropp 1998, Kuehn et al. 2000, Berg & Laskowski 2006). Consequently, in cross-continental transect studies, the local annual evapotranspiration turned out to be the best predictor of local decomposition rates followed by the local average annual temperature (several studies summarized in Berg & Laskowski 2006). Thus, temperature and the availability of water determine the basic differences between decomposition processes in different ecosystems.

Terrestrial systems

More than 80 % of plant biomass directly enter the detritus chain in (almost) every terrestrial system; most of it as leaves from deciduous or coniferous trees (Zimmer in press). Consequently, decomposition of leaf litter is of greatest importance for ecosystem functioning. The availability of water and the ambient temperature constitute the frame in which differences in decomposition processes of boreal, temperate, and tropical systems are determined (see above). Terrestrial studies on decomposition processes have focused on forest and grassland ecosystems with strong emphasis on soil communities (reviewed in Bardgett & Chan 1999, Bradford et al. 2002, Coleman et al. 2004, Zimmer 2004, and Wardle 2006). This research gains a strong impulse from its importance for agricultural management and crop enhancement and includes the investigation of the influence of soil organisms on nutrient dynamics and decomposition. Our knowledge of animal-microbe interactions in decomposition processes to a large extent derives from these studies on soil ecology (see below).

Freshwater systems

Decomposition of allochthonous plant material plays a key role in the energy budgets of forest and grassland streams (Cummins 1974, Vannote et al. 1980). Terrestrial litter breakdown rates and how they depend on stream order, macroinvertebrates, fungi, litter input, eutrophication, and pollution regime has been studied extensively and in detail (Peterson & Cummins 1974, Gessner & Chauvet 1994, Baldy et al. 1995, Suberkropp & Chauvet 1995, Wallace & Webster 1996, Pereira et al. 1998, Graça 2001, Hieber & Gessner 2002, Gulis et al. 2006). The major part in aquatic decomposition is brought about by the fungal guild of hyphomycetes that are abundant and well adapted to breakdown of recalcitrant plant litter (Findlay & Arsuffi 1989, Bärlocher 1992, Baldy et al. 1995, Carter & Suberkropp 2004). Interactions with bacteria (Bengtsson 1992, Møller et al. 1999, Wohl & McArthur 2001, Gulis & Suberkropp 2003, Mille-Lindblom 2005) and fungal succession (Suberkropp & Klug 1976, Wardle 1993, Nikolcheva et al. 2004) give a clear picture of microbial impact on decomposition processes in the aquatic environment. The role of macroinvertebrates like insect larvae, gastropods, isopods, and oligochaets in litter breakdown has been the subject of many studies (reviewed in Anderson & Ineson 1984, Wallace & Webster 1996, Graça 2001). Despite this vast literature and knowledge, little is known about the influence of macroinvertebrates on aquatic microbial communities. Attention has been paid for litter mass loss or disappearance rates, but, unlike in terrestrial systems where remineralization is of great interest, nutrient release and, in particular, the role of macroinvertebrates therein are not well understood. Release of nutrients appears to be of little interest in running waters, since nutrients can not accumulate and exhibit direct influence at the site of their release. Whether results from terrestrial studies are applicable to aquatic macrofauna-microbiota interactions is questionable (see below).

Salt marsh systems

The salt marshes of the world are among the most productive ecosystems (Mitsch & Gosselink 1993). Much of this production is consumed by herbivores, but most of it enters the detritus cycle (Teal 1962, Nixon & Oviatt 1973, Valiela & Teal 1979). High salt concentrations in soil and detritus challenge the decomposer community. Additionally, breakdown is influenced by frequent cycles of inundation and drying in tidally exposed

salt marsh systems, exhibiting high physical stress on the deposited detritus. Under these specific conditions, efficient decomposition processes can be expected. The cord grass-fungi-periwinkle decomposition system in salt marshes of the southeastern coast of North-America, for instance (see Chapter III), is the consequence of adaptations to saltmarsh-specific conditions and has been the subject of several studies (Bärlocher et al. 1989, Graça et al. 2000, Silliman & Newell 2003). Fungi, in general, play an important role in salt marsh decomposition (Newell 2001, 2002). Beyond this, the influence of the macrofauna on decomposition processes in these highly productive systems is still not well defined (but see: Lillebø et al. 1999, Zimmer et al. 2004).

Beaches (marine-terrestrial ecotone)

Sandy and rocky beaches lack primary production above the high-water line. Nevertheless, beaches are transition zones for nutrient fluxes from marine to terrestrial systems. (Ochieng & Erfemeijer 1999, Piriz et al. 2003, Orr et al. 2005). Like low-order streams, they are recipients of allochthonous phyto-biomass. High amounts of algal and sea grass detritus can be washed ashore and build up hot spots of high decomposition activity attracting large quantities of invertebrate decomposers (Griffiths et al. 1983, Wildish 1988, Piriz et al. 2003, Orr et al. 2005). A large part of the released nutrients is washed back into the sublittoral area such that decomposition of sublittoral primary production can be regarded as “sourced out” onto the supralittoral beach. Algal tissue in the beach-cast material contains much lower amounts of high-molecular, slowly decaying structural compounds (lower C:N ratio) than vascular plant detritus and, thus, is more valuable to invertebrate decomposers, irrespective of potential repellent secondary compounds (Pennings et al. 2000). Thus, unlike low-order streams or forest ponds, where allochthonous material is of low nutritional quality, beaches receive at least in part highly valuable phytodetritus.

Animal-microbe interactions

Soil ecology has a long tradition, probably starting with Charles Darwin’s observations on earthworms (Darwin 1881). In this long run the macrofauna has always been regarded having a major impact on decomposition due to the amount of litter it ingested. Until the 1960s, when ecologists started using the litterbag method to study faunal influence on

decomposition, macroinvertebrates other than earthworms have rarely been included in investigations (Huhta 2006). The cognition of the importance of microorganisms in soil and litter processes came along with the advances in microbiology. Based on respiration data, this led to the suggestion, that macrofaunal assimilation of soil organic matter is hardly ever more than 10 % of the total decomposer system (Petersen & Luxton 1982). Later studies corrected this value including faunal ingestion rates in more detailed investigations to about 23 % (Seastedt 1984) and estimates of nitrogen immobilization to about 30 % (Verhoef & Brussaard 1990), 37 % (Hunt et al. 1987), and 50 % (Panikov et al. 1985).

In a comprehensive review, Anderson & Ineson (1984) differentiated between direct and indirect effects of fauna in decomposition processes. Direct effects involve the transfer between pools of nutrients mediated directly by animals, i.e. through feeding on plant or microbial biomass and subsequent excretion of nutrients that are readily available. These effects can not exceed a certain level, assuming that the fauna does not have the digestive ability to hydrolyze plant structural polymers (but compare Zimmer & Topp 1998). Indirect effects involve a feedback on lower trophic levels, and by this the fauna indirectly (meaning not through the body of the animal) alters fluxes between nutrient pools. This occurs, among others, when through feeding on microbial communities, microbial respiration and activity is increased, which results in higher nutrient mobilization (Kautz & Topp 1997, Zimmer 2004). Such an effect was reported, for instance, by Hanlon & Anderson (1979) for isopods and millipedes and by Zimmer (2004) for isopods. The presence of the macrofauna induces higher microbial respiration on leaf litter and subsequently higher nutrient mobilization in the system. Anderson & Ineson (1984) conclude that indirect effects of fauna are of much greater importance than obvious direct effects through feeding and excretion.

For ecosystems other than forest and grassland soils, little is known about faunal influence on microbial communities and decomposition processes. Studies on detritivores in aquatic systems usually take the perspective of litter processing and faunal nutrition supported by microbiota (Anderson & Ineson 1984, Wallace & Webster 1996, Graça 2001). A process-orientated perspective with emphasis on nutrient fluxes or detritivore impact on microbial communities is scarce.

However, it is beyond doubt that interactions of invertebrate and microbial decomposer, at least in part can, be considered as truly mutualistic (Zimmer in press). While invertebrate detritivores generally prefer microbially preprocessed detritus and assimilate microbially derived nitrogen (Bärlocher & Kendrick 1975), they also facilitate microbial spread by fragmentizing detritus and distributing propagules.

Species diversity in ecosystem processes

Each species exhibits specific influences on its environment. Life on earth is pervaded by interactions of species and, thus, interactions of influences. Onto an ecosystem process these influences can operate additive or non-additive. Non-additive influences can operate synergistically, when promoting the particular process more than the sum of the single influences or antagonistically, when promotion is less than the sum.

Non-additive influences are the basis for species richness effects, which has become a widely accepted focus of ecological research since the 1990s (Cardinale et al. 2006). The influence of species diversity on decomposition processes has been studied intensively in terrestrial (reviewed in Wardle et al. 2006) and aquatic systems (reviewed in Covich et al. 2004). There is no typical pattern of diversity effects across ecosystem processes. As Cardinale et al. (2006) report from a meta-analysis of 111 diversity studies, species diversity generally does influence ecosystem functions in both terrestrial and aquatic systems. However, the mechanisms that cause diversity effects are manifold and cryptic. Smith & Bradford (2003) even state that any mechanistic interpretation of litter mixing effects is ultimately confounded by the plethora of possible ways of interaction.

Approaches to diversity effects often include the stepwise increase of species numbers and the monitoring of a single parameter such as litter breakdown rate. Since ecological impact is never distributed evenly among the species, the probability of adding a dominant and highly influential species into the study is increasing with the number of species included in the study. This “sampling effect” (Huston 1997, Wardle 1999) leads to a correlation of the observed parameter and species number that is due to the dominance of single species rather than to species richness per se.

Furthermore, a systematic challenge in diversity studies is the simulation and manipulation of natural species composition. Many investigations simulate diversity effects by mixing several species equally, disregarding that natural communities, as

involved in ecosystem processes, are mostly dominated by few common species, while the remainder is rare (Dangles & Malmqvist 2004).

The human ideal of high species numbers in ecosystems does not account for the “needs” of ecosystem functioning. When functional redundancy among species is high, ecosystem process rates may not be promoted. From the process-point-of-view, low functional redundancy, thus species identity, is more important.

When studying the underlying mechanisms of diversity effects, it is therefore more promising to investigate several parameters as influenced by few non-redundant species, than to investigate a single ecosystem function under the influence of many species. In the present studies I establish a model system allowing for this investigation in the aquatic and terrestrial environment.

Differences in aquatic and terrestrial decomposition

As stated above, the general pattern of decomposition processes is valid for all ecosystems. Studies on particular habitats revealed detailed differences, refining the general pattern. However, the conditions under which decomposition takes place can be extremely divergent, as they are in aquatic compared to terrestrial systems. The basic differences between aquatic and terrestrial food- and detritus-webs have been suggested by Shurin et al. (2006) to be due to the difference in quality of primary production: aquatic phytobiomass, in particular phytoplankton, lacks carbon-rich high-molecular structural compounds that reduce decomposition process and efficiency in terrestrial systems. (Detritus-)Food web properties like food chain length, size hierarchy, and number of trophic levels depend on the quality of the primary production. This is true when aquatic-originated detritus decomposes in an aquatic system and terrestrial-originated detritus in a terrestrial system. However, low-order streams or forest ponds, receiving allochthonous terrestrial leaf litter, share detritus of the same source (deciduous or coniferous litter). The most separating variable, according to Shurin et al. (2006), is thus eliminated. Consequently, decomposition processes in both habitats receiving the same detritus should be much more alike.

Based on these facts and considerations, the following questions arose and are to be answered in the present thesis:

1. Does the fauna exert not only direct, but also indirect effects on aquatic decomposition (by feeding on litter and by altering the microbial community, respectively) like it does in the terrestrial environment?
2. What role does species identity of the fauna and the detritus play in the systems?
3. What role does species composition play in the systems?
4. What are the conditions for diversity effects to occur?
5. Are decomposition processes in aquatic and terrestrial systems similar, when fed by the same energy source?
6. Do the emerging patterns exist in other detritus-based ecosystems?
7. Considering that the system (aquatic versus terrestrial) affects detritus quality, how does the process change, when aquatic detritus decomposes terrestrially?

Outline

The present thesis focuses on the role of animal-microbe interactions in decomposition processes of different ecosystems. This includes the affection of decomposition variables by animal-microbe interaction, but to the same degree the influence of the environment on these interactions. Since knowledge of the influence of aquatic detritivores on microbial parameters is vague, I created a microcosm system, that was first, appropriate for the investigation of aquatic decomposition (**Chapter I**) and second, appropriate for the comparison of aquatic and terrestrial decomposition (**Chapter II**) under the same detritus, detritivore, and species richness regimes. For this I chose leaf litter of deciduous trees as typical energy source for both, terrestrial and aquatic detritus-based systems. As detritivores in the microcosm experiment I chose isopods and gastropods, since both can be regarded as model decomposers inhabiting nearly all ecosystems of the world.

Among these ecosystems, salt marshes stand out as areas of very high productivity and thus, high biomass throughput in decomposition. The study reported on in **Chapter III** applies the results of Chapter I and II to this fascinating ecosystem, which is rendered by the alternation of terrestrial and aquatic conditions. I performed the experiments for this study in a salt marsh on Sapelo Island, Georgia, USA, benefiting from the vast research background of this particular location.

As stated before, the influence of detritus quality is regarded as the basic driver in detritus food webs. How decomposition and the role of detritivores and microorganism change in a (semi-)terrestrial system based on high-quality detritus is elucidated in **Chapter IV**. Such conditions can be found on ocean shorelines receiving detached and senescent macroalgae. In Chapter IV, I present the results of a short-term investigation on the cobble beaches of Vancouver Island, Canada. Here, large amounts of high quality detritus accumulate and attract very high numbers of talitrid amphipods. I discuss the findings of Chapter IV considering results from a parallel study on detritus dynamics, not separately reported in this thesis (Orr, Jelinski, Zimmer, and Mews, 2005: “*Wreck decomposition on different beach types: spatial and temporal variation in the pattern of subsidy*” - Ecology, vol. 86).

Chapter IV has been published in 2006 in volume 328 of “Marine Ecology Progress Series”.

For the Chapters I-IV I chose to report from the “we” point of view, appreciating not just the support by my supervisor, but also by the people, who gave a helping hand in the field and the lab.

Animal-microbe interactions in aquatic leaf litter
decomposition

Chapter I

Introduction

The decomposition of allochthonous plant litter of terrestrial origin, henceforth detritus, is of great importance for the nutrient supply of aquatic systems, especially when they lack autochthonous macrophytes like many low order streams (Vannote et al. 1980). The chemical processing during decomposition is almost entirely brought about by microorganisms (Cummins 1974) next to leaching of water-soluble compounds. Assemblages of detritus material in aquatic systems are inhabited by a variety of invertebrate species as refuge and nutritional substratum. Among these species are insect larvae, nematodes, amphipods, snails and isopods, which represent different living and feeding types with diverse functional contributions to decomposition (Graça 1993, 2001, Wallace & Webster 1996). The impact of this fauna on litter breakdown rates is well studied including effects of species richness. In contrast, little is known about the influence of the fauna on microbial communities and biomass and hence, on microbially mediated nutrient release in aquatic environments (but see Bärlocher 1992, Rossi 1985, Sabetta et al. 2000, Liess & Haglund 2007). In a comprehensive review on the role of macroinvertebrates in stream ecosystems, Wallace & Webster (1996) leave the impact of fauna on microbial parameters rather unmentioned, except for the co-ingestion of bacteria colonizing fine particulate organic matter (FPOM) by gatherers and filter-feeders.

Since it is well documented in studies on terrestrial systems that soil- and litter-dwelling microorganisms can strongly be influenced in their abundance and performance by micro- and macroinvertebrates, similar effects of invertebrates can be expected for aquatic systems (Anderson & Ineson 1984, Swift & Boddy 1984, Bardgett & Chan 1999, Cole et al. 2004, Huhta 2006). Here, earlier studies focused on litter breakdown rates, in particular in streams, mostly neglecting the possibly strong direct and indirect impacts of the fauna on microbial parameters and, further, on nutrient dynamics (detritivores: e.g. Peterson & Cummins 1974, Wallace & Webster 1996, Pereira et al. 1998, Graça 2001, Hieber & Gessner 2002; fungi: e.g. Findlay & Arsuffi 1989, Bärlocher 1992, Gessner & Chauvet 1994, Baldy et al. 1995, Carter & Suberkropp 2004).

As reviewed by Graça (2001), results from studies on how invertebrates influence litter breakdown in the aquatic environment are contradictory. Authors reported either high or no differences between litterbag experiments with fine and coarse mesh size, always depending on the specific circumstances of the particular study, not to forget the

strong dependence on invertebrate density (Steward 1992). It is well accepted that aquatic invertebrates prefer conditioned (i.e. colonized by microorganisms) over unconditioned litter as food source, benefiting from the high nutritional value of microbial biomass plus enzymatic and mechanical pre-processing of litter structure and compounds (Bärlocher & Kendrick 1975). This suggests a close relationship between the fauna and microorganisms that can not remain without consequences for the microorganismic partner. This relationship was described by Bärlocher (1992) such, that shredding invertebrates in early stages of decomposition prey on common fungal species reducing their sporulation, and in later stages compete with rare species by consuming leaf substratum that is subsequently not available for fungal colonization anymore. From terrestrial studies, it is further known that invertebrate feeding activity can have a growth-stimulating influence on microbial organisms (Hanlon 1981) next to shifts in microbial community composition by selective feeding (Cole et al. 2002, De Mesel et al. 2004, Mummey et al. 2006). Whether this is true for aquatic habitats as well, where the medium can be expected to have a much higher influence on propagule transportation and nutrient fluxes, remains largely unknown.

The influence of biodiversity on ecosystem processes, in particular in litter decomposition, is extensively investigated and controversially discussed. Numerous studies, mostly in terrestrial systems, found either effects or no effects of invertebrate or litter diversity (e.g. Blair et al. 1990, Wardle et al. 1997, Bradford et al. 2002, Zimmer 2002, Smith & Bradford 2003, Covich et al. 2004, Zimmer et al. 2004, Hättenschwiler & Gasser 2005, Wardle 2006). Effects of species richness per se are not easy to investigate as most studies state that it is not species number but species identity or single species dominance that mainly affects decomposition (Jonsson & Malmqvist 2003, Dangles & Malmqvist 2004, Covich et al. 2004, Duarte et al. 2006, LeRoy & Marks 2006). The approach to diversity effects can be of experimental or monitoring character. In laboratory experiments including stream filter feeders, grazers and predators, Jonsson & Malmqvist (2003) found species identity to be the only significant factor. In litterbag experiments by Dangles & Malmqvist (2004), decomposition rates depended on identity of the dominant species, while in a microcosm experiment including 12 multispecies combinations, Bjelke & Herrmann (2005) did not find any positive or negative effect of species richness on litter breakdown. Additionally, in microcosm experiments, Schädler et

al. (2005) were able to predict decomposition and faunal and microbial variables of mixed litter treatments from the corresponding single-species treatments, indicating no non-additional diversity effects. Diversity effects seem to be easier to encounter by investigating natural habitats, like Jonsson et al. (2001) did when they compared 23 boreal streams and discovered shredder species richness as an important variable in stream litter dynamics. In contrast, no such effects were found in a comparable study by Larned et al. (2003). Correspondingly, LeRoy & Marks (2006) found diversity effects of litter on decomposition that depended on litter quality and stream characteristics rather than species richness. In the aforementioned studies only one side has been manipulated, either decomposers or litter. This does not account for indirect influences, like the effect of litter diversity on the influence of the fauna on the microbial community.

Non-additive effects of species mixtures occur because effects of single species on ecological processes add up to more (or less) than the sum of these single effects. Effects of species diversity are thus due to the concert action of species functions in the ecosystem, that is to say functional diversity. To get a clearer view of the conditions under which non-additive effects emerge from interactions of species, it might be helpful to concentrate on only two species that are not redundant in their influence on the ecosystem and a variety of process parameters under different conditions (compare Zimmer et al. 2005). Since detritus decomposition is ultimately mediated by the microbial community, animal-microbe interactions appear to be most promising in the search for the cause of species-mixture effects.

Thus, we chose *Asellus aquaticus* LINNAEUS (Isopoda, Asellota) and *Bithynia tentaculata* LINNAEUS (Gastropoda, Prosobranchia) as representatives of the macrofauna. Both are common and abundant in northern Germany, where they inhabit aquatic detritus patches. While *Asellus* is commonly attributed to the guild of shredders (Marcus et al. 1978, Graça et al. 1994), *Bithynia* grazes surface biofilms and can be filter-feeding under inappropriate conditions (Brendelberger & Jürgens 1993). The different feeding types can be expected to exhibit different influences on litter dwelling microbiota. Associated with north German forest ponds and creeks are birch (*Betula pendula* ROTH) and oak (*Quercus rubor* LINNAEUS) trees (Heydemann 1997). Oak and birch leaves used in this study are very different in both, tissue strength and chemical composition. Like for feeding type, contrasting litter properties, of which tissue strength is the most apparent

one, may increase the probability of species mixing effects by avoiding redundancy. To assess faunal impact, microbial biomass and activity were recorded.

We tested the following hypotheses:

1. Macroinvertebrates under natural (low) densities have strong impacts not only on litter mass loss as a direct effect of feeding activity, but also indirectly on microbial parameters. The term “microorganisms” is restricted, here, to bacteria and fungi, according to their high cell number of several billions per gram litter (compare results) and their dominance in biomass of more than 90 %, respectively (e.g. Graça 1993, Kuehn et al. 2000).
2. Distinct feeding types of detritivores exhibit non-additive effects on decomposition processes, even a at low diversity level. The combined effects of different feeding types (functional diversity) will depend on characteristics and diversity of the available leaf litter.

Material and Methods

Experimental setup

Experiments were carried out in microcosms made of 900 mL plastic boxes (Perga Plastic, Walldürn-Altheim, Germany) containing 200 g (wet weight) of washed and autoclaved sand (100-500 μm grain size; layer of ca. 1.5 cm). 2 g of air-dried litter (birch, *Betula pendula*; oak, *Quercus robur*), hand-collected in a public park (Forstbaumschule, Kiel). 500 mL of naturally inoculated water from a pond near the institute, filtered through a 40 μm mesh, were added. Isopods (*Asellus aquaticus*) and snails (*Bithynia tentaculata*) were collected in a forest pond near Neuwühren (Kreis Plön) and from Kuhfurtsau, a creek in Russee (Kiel), respectively. Animals were separated by size, and equal sets of different sizes were introduced into microcosms, which were then covered with a punctuated lid to reduce evaporation. Litter sets (birch litter, oak litter, and both 1:1) were added to detritivore sets (10 isopods (I), 10 snails (S), 20 isopods (2I), 20 snails (2S), 5 isopods + 5 snails (IS), no fauna(C)) in all possible combinations. These treatments were set up 4 times to be destructively sampled after 14, 42, 70 and 112 days, resulting in a total of 72 treatments, replicated 7 times. Temperature and light regime in the climate chamber were adjusted to simulate a winter-to-spring period: 42 days at 5°C, 14 days at 6°C, 14 days at 8°C, 14 days at 10°C, 14 days at 12°C, 14 days at 14°C. Light:darkness regime ranged from 14 h:10 h to 12 h:12 h after 56 days back to 14 h:10 h in the course of the experiment. Every two weeks, microcosms were screened for dead animals, which were removed and replaced by new ones of the same size. Setup was spread over a period of one month to give enough time for analyses later during the experiment.

At the end of the experiment, total remaining litter was weighed to the nearest mg. About 1/5 of litter was used for subsamples for litter analyses (see below), 1/5 was dried to constant weight (60°C) for calculation of fresh weight/dry weight ratio, and 3/5 was used for respiration measurement (see below).

Microbial respiration

Microbial respiration was measured immediately after the end of the experiment with the BSB/BOD-Sensomat (Aqualytic, Lagen, Germany). The device traps free CO₂ in KOH solution, while the decrease in atmospheric pressure resulting from microbial oxygen

consumption is recorded. Litter was given into 250 mL flasks containing 200 mL of autoclaved and oxygen-saturated pond water; 1 mL 45 % KOH was given into a rubber quiver inside the flasks, which were then closed with the pressure-measuring sensor and stored at 20°C for 24 h. During the measurement, water was kept in motion with a magnetic stirrer to facilitate oxygen transfer between the water and the atmosphere inside the flasks. The litter was separated from the magnetic stirrer by gauze, to avoid strong disturbance of the litter inhabiting microbial community. Results were given in $\text{mg O}_2 \cdot \text{L}^{-1} \cdot (24 \text{ h})^{-1}$ and then calculated to $\text{mg O}_2 \cdot (\text{kg litter})^{-1} \cdot (24 \text{ h})^{-1}$ using formula provided by the manufacturer.

Bacterial biomass

From litter of each microcosm, 10 disks of 10 mm diameter were cut from 10 different leaves, using a cork borer. Disks were stored in 15 mL plastic vials with 10 mL of 4 % Formaldehyde at 4°C until further processing. For detaching bacteria, 5 glass beads (5 mm) were added to the vials before shaking for 10 minutes on a horizontal shaker at 420 rpm. After letting stand for 10 min, 0.5 mL of the supernatant were mixed with 6 mL ddH₂O and filtered through a 0.2 μm polycarbonate filter (25 mm; DHI, Hørsholm, Denmark). Litter discs were dried at 60°C for 1 h and weighed. After staining for 10 min with DAPI-staining solution (1 $\mu\text{g}/\text{mL}$), the filter was washed first in ddH₂O then in 96 % ethanol and dried on lab tissue for one minute. It was then covered with one drop of CITIFLUOR solution (Citifluor Ltd., London/GB) to prevent quick fading of staining intensity. From each filter, photos of 3 different sections were taken with a digital camera using an epifluorescent microscope equipped with a DAPI-staining-specific optical filter set at 1000x magnification. Because of high background noise, the photos needed to be optimized before automated bacteria counting. For this purpose, a software routine was written (using freeware Dev-C++-compiler, Bloodshed-Software) that increased image-contrast of blue DAPI-stained bacterial cells. Stained cells were then counted using the VisualBasic-based macro language interpreter of SigmaScan image processing software (SigmaStat Software Inc., San Jose, USA). From a counted area of 80 μm x 80 μm , bacterial cell number in the sample was calculated as the arithmetic mean of counts of the 3 filter sections.

Fungal biomass estimation

From litter of each microcosm, 10 disks of 10 mm diameter were cut from 10 different leaves, using a cork borer, and stored at -20°C until further processing. The ergosterol content was measured as an estimate for fungal biomass of litter samples according to Davis & Lamar (1992), modified to be used with smaller samples.

Using ergosterol content as an indicator for fungal biomass is a standard method for soil analyses and has been used and modified in several studies (e.g. Newell et al. 1987, Gessner et al. 1991, Davis & Lamar 1992). It delivers only an estimate of fungal biomass since ergosterol content of fungal cell membranes differs among species, mycelium age and growth medium (Newell et al. 1987). Nevertheless, it is widely accepted as a reliable, robust and inexpensive biomarker (Young et al. 2006; but see Zhao et al. 2005).

Frozen litter disks were transferred to a 2 mL screw cap plastic vial together with 20 glass beads of 3 mm diameter. 200 µL methanol (HPLC-grade) containing 10 % (w/v) KOH were added. The sample was homogenized at 4800 rpm in a MiniBeadBeater (GlenMills, Clifton NJ, USA) for 1 minute. Another 600 µL KOH/MeOH and 200 µL cyclohexane were added and the sample was sonicated for 15 minutes in an ultrasonic bath at 80 W and room temperature, followed by a 90-minutes heating period at 70°C at permanent slow rotation. The vial was allowed to cool to room temperature before 200 µL ddH₂O and 600 µL cyclohexane were added. Subsequently, the sample was vortexed for 30 seconds and centrifuged with 1000 * g (room temperature) for 5 minutes. The cyclohexane phase was transferred into an extra 2 mL tube. The sample was extracted a second time with 400 µL cyclohexane, vortexed, and centrifuged. Cyclohexane phases were pooled. Cyclohexane was evaporated at 40°C under a gentle stream of air; the residue was dissolved in 1 mL methanol (HPLC-Grade) at 40°C for 15 min. The extract was passed through a 0.2 µm PTFE syringe filter (Roth, Karlsruhe, Germany) into a new 1.5 mL tube. HPLC was run on a Kontron HPLC system (BIO-TEK Kontron Instruments GmbH, Neufahrn, Germany) equipped with a 250 * 4 mm C18 column (Eurospher 100, 5 µm; Knauer, Berlin, Germany) applying 80 µL extract. At room temperature, using a flow rate of 1.5 mL min⁻¹, the ergosterol peak appeared after ~12 min ($\lambda = 282$ nm). As a standard, crystal ergosterol (Sigma-Aldrich #45480) dissolved in methanol was used. Fungal biomass was calculated according to Gessner & Chauvet (1993), assuming 5.5 mg ergosterol per gram fungal biomass.

Statistical analyses

Diagrams of data were created using PRISM 4.0 (GraphPad Software). Data were analysed using two-way-ANOVA to test for an influence of litter and detritivore combinations (Factors: “litter” with levels *birch*, *oak*, and *mixed*; and “fauna” with levels *I* (low density of isopods), *2I* (high density of isopods), *S* (low density of snails), *2S* (high density of snails), *IS* (isopods + snails), and *C* (no fauna). Raw data were either ln- or square root-transformed to meet ANOVA requirements of normal distribution and homogenous variances. In case of a statistically significant difference, a Holm-Sidak post-hoc test with multiple pair wise comparisons was performed on the data set to identify the source of difference.

To clarify whether mixing of litter influenced decomposition parameters, expected values (“E”) for mixed litter were calculated from values of single-litter type treatments and compared with the observed values (“O”) of mixed-litter treatments. Replicates of birch and oak litter were randomly assigned and for expected litter mass loss the arithmetic mean was calculated:

$$E_e = \frac{B_e + O_e}{2} \quad \text{with } e \in [1..7]$$

B_e : litter mass loss in replicate of birch treatment

O_e : litter mass loss in replicate of oak treatment

Pairs of B_e and O_e were randomly assigned.

For the rest of the parameters (bacterial cell numbers, fungal biomass, litter respiration), according to Wardle et al. (2006), the expected values were calculated with respect to litter mass loss of each of the litter types to take into account the different litter composition at the end of the experiment:

$$E_e = \frac{rB_e \cdot PB_e}{rB_e + O_e} + \frac{rO_e \cdot PO_e}{rB_e + O_e} \quad \text{with } e \in [1..7]$$

rB_e : remaining birch litter mass

rO_e : remaining oak litter mass

PB_e : parameter replicate of birch treatment

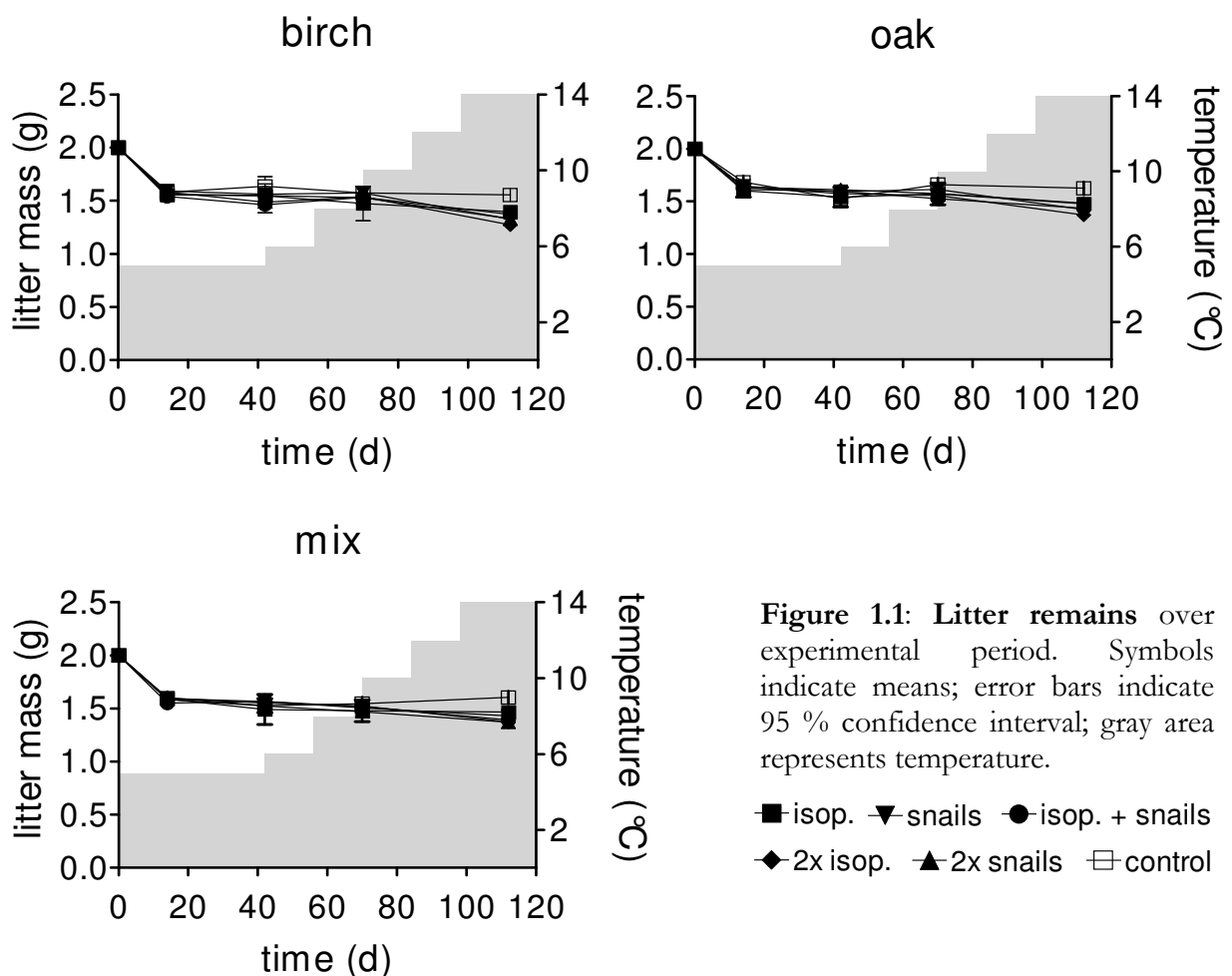
PO_e : parameter replicate of oak treatment

Data sets of expected and observed values were statistically compared using the Mann-Whitney U test for non-parametric data.

Results

Litter mass loss

Over 112 days of incubation the greatest impact on litter mass loss can be ascribed to leaching processes during the first 14 days (Fig. 1.1). Due to low temperature until the 56th day, faunal and microbial activity can be considered low and consequently litter mass did not change significantly. After temperature increase, litter mass began to decrease in the microcosms including fauna until after 112 days litter amount was significantly lower than in the control (Fig 1.4).



Since little variability was observed until day 70, we will henceforth present results for the last two sampling dates only. Figure 1.2 shows litter mass loss until day 70 in more detail. Litter identity had significant influence on mass loss (Tab. 1.1), although the difference between litter types was low. Faunal influence on litter mass loss was present, but not statistically significant ($p = 0.07$; Tab. 1.1); even at high detritivore densities. The effect of

fauna did not depend on the identity of litter (no statistical interaction; $p = 0.8$). On mixed litter, the observed litter mass loss induced by the presence of a low abundance of snails and a high abundance of isopods was significantly higher than calculated from the single litter species treatments (Fig. 1.3).

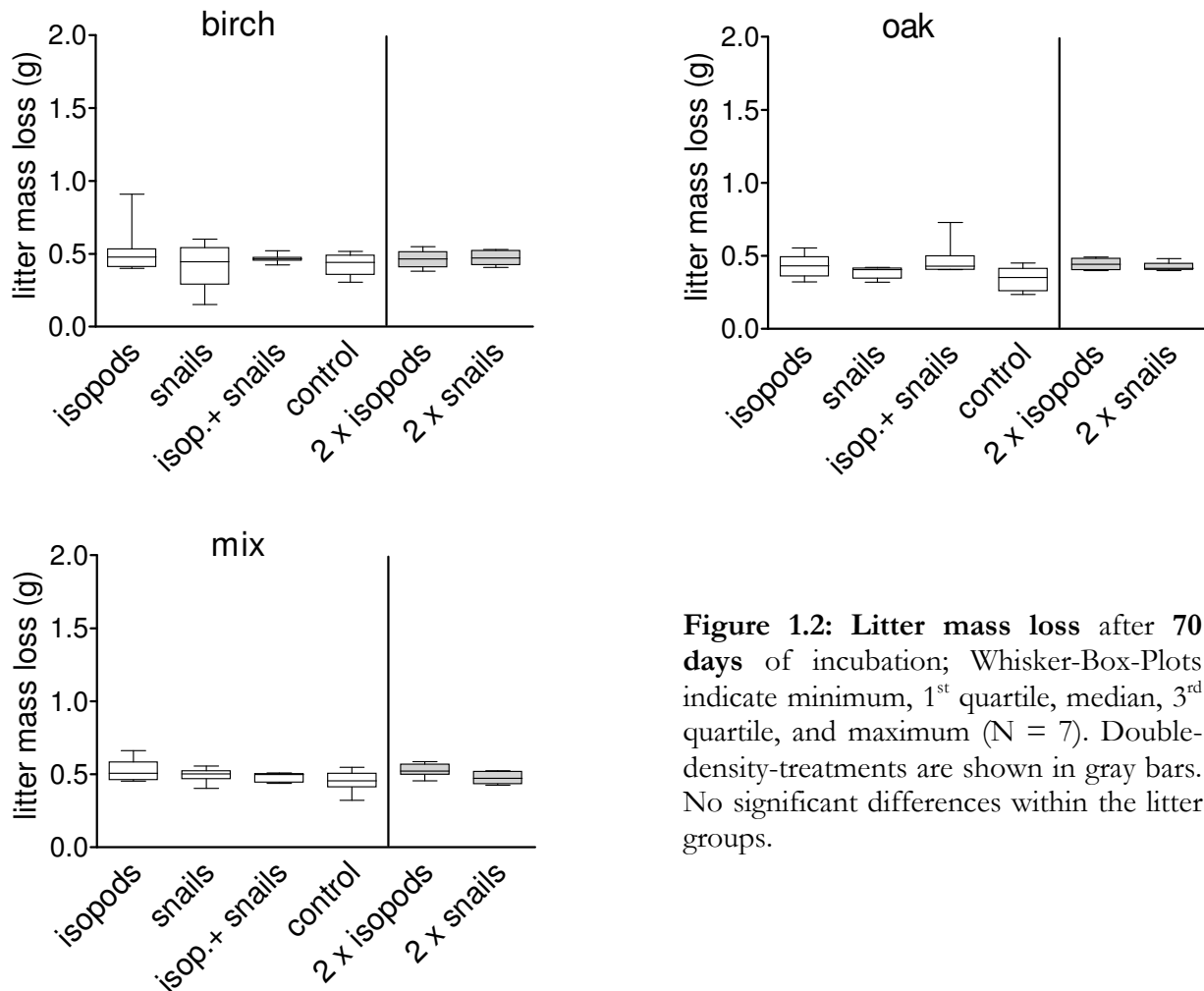


Figure 1.2: Litter mass loss after 70 days of incubation; Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum ($N = 7$). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

Table 1.1: Results from Two-Way-Analysis-of-Variance for **litter mass loss** after 70 days

source of variation	df	ss	ms	f	p
litter	2	0.0372	0.0186	5.585	0.005
fauna	5	0.0352	0.0071	2.119	0.071
litter x fauna	10	0.0197	0.002	0.593	0.815
Residual	85	0.283	0.0033		
Total	102	0.376	0.0037		

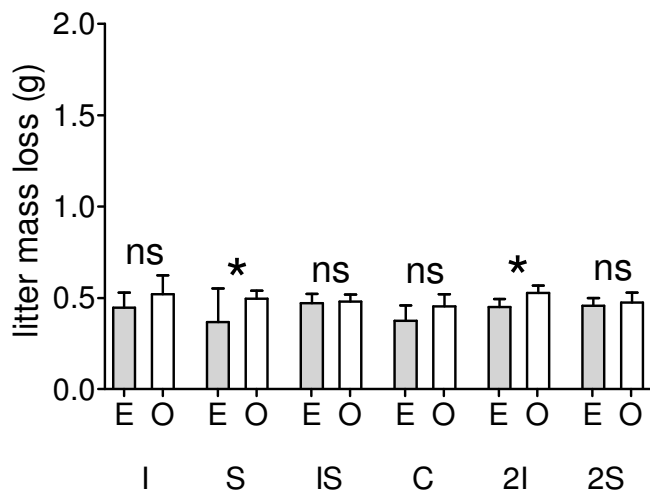


Figure 1.3: Expected (E) and observed (O) litter mass loss of mixed litter after 70 days of incubation (mean \pm 95 % CI, N = 7). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails. ** : 0.001 < p \leq 0.01; * : 0.01 < p \leq 0.05; (*) : 0.05 < p \leq 0.1; ns: p > 0.1

With increasing temperature, the influence of fauna on litter mass loss increased strongly (Fig. 1.4). All detritivore-treatments lost significantly more litter mass than the corresponding controls. The influence of litter identity remained significant (Tab. 1.2). However, no difference between observed and expected mass loss on mixed litter was detectable any more (Fig. 1.5).

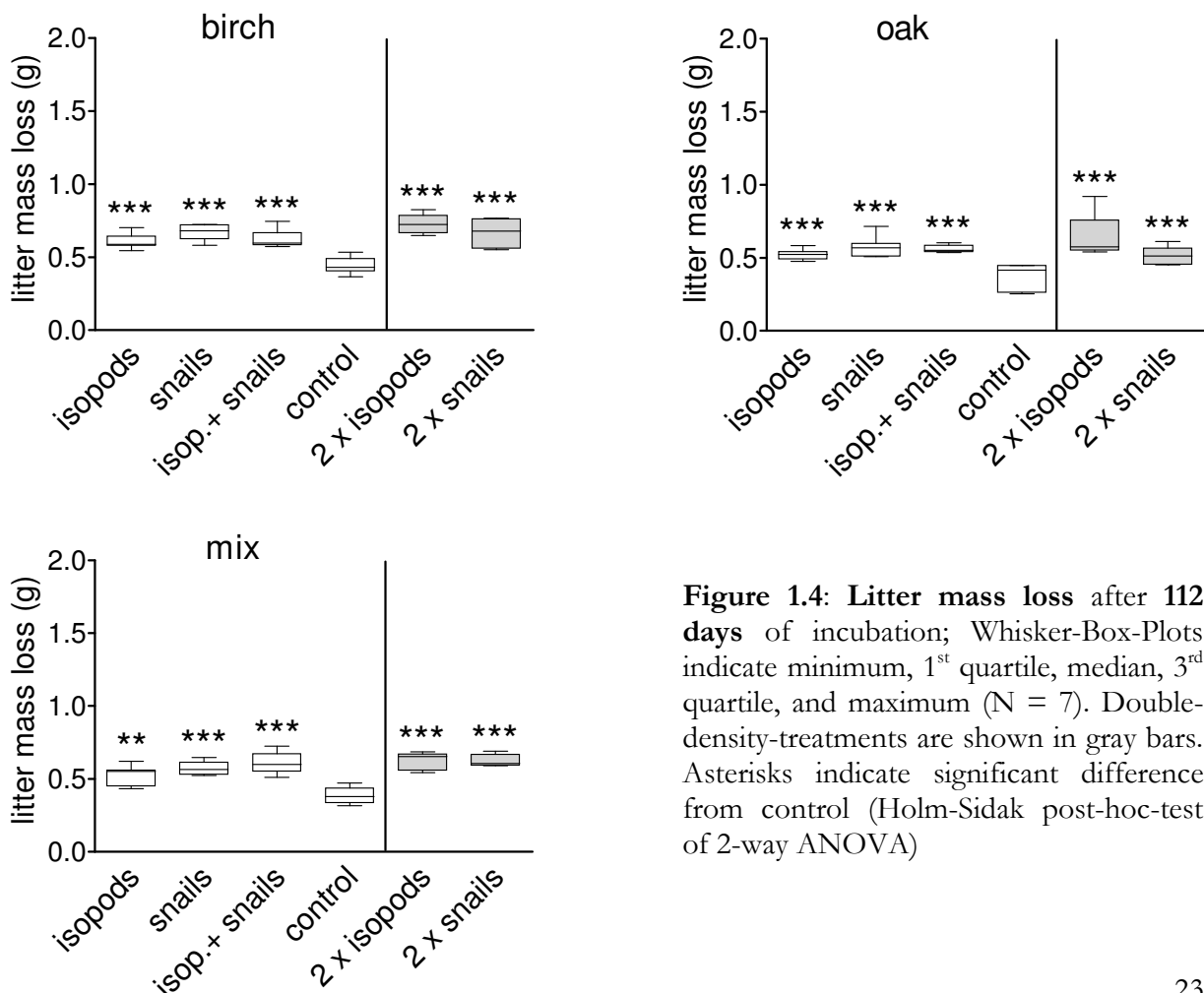


Figure 1.4: Litter mass loss after 112 days of incubation; Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum (N = 7). Double-density-treatments are shown in gray bars. Asterisks indicate significant difference from control (Holm-Sidak post-hoc-test of 2-way ANOVA)

Table 1.2: Results from Two-Way-Analysis-of-Variance for **litter mass loss** after **112 days**

source of variation	df	ss	ms	f	p
litter	2	0.0966	0.0483	25.187	< 0.001
fauna	5	0.28	0.0561	29.229	< 0.001
litter x fauna	10	0.024	0.0024	1.25	0.27
Residual	97	0.186	0.0019		
Total	114	0.589	0.0052		

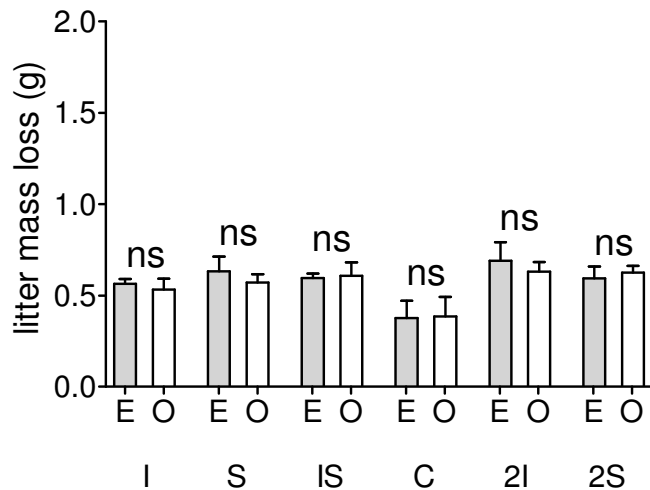


Figure 1.5: Expected (E) and observed (O) litter mass loss of mixed litter after 112 days of incubation (mean \pm 95 % CI, N = 7). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails. ** : $0.001 < p \leq 0.01$; * : $0.01 < p \leq 0.05$; (*) : $0.05 < p \leq 0.1$; ns: $p > 0.1$

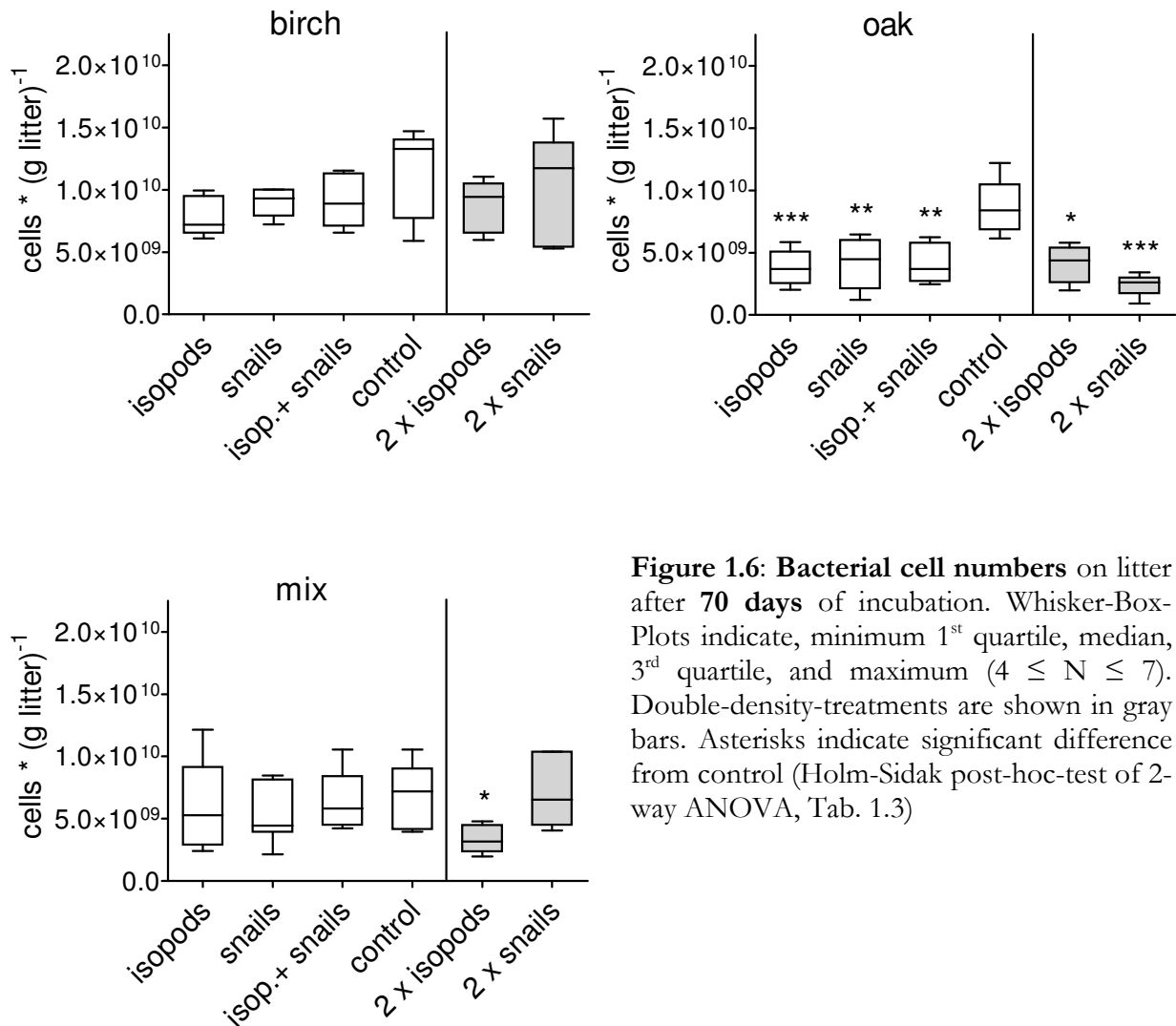
Bacterial biomass

Bacterial biomass is given as number of bacterial cells per gram of litter after an incubation of 70 and 112 days, hence after the end of the simulated winter temperature and light regime.

After 70 days, both, litter identity and presence of macrofauna had a significant overall influence on bacterial numbers (Tab. 1.3). On birch litter (Fig. 1.6), about $1 * 10^{10}$ bacterial cells per gram litter were found. The median cell number in the control group was increased relative to the animal treatments, though in no case significantly. This pattern was also found on oak litter, but here, in contrast, a strong influence of fauna presence per se was observed. Bacterial number was significantly reduced by macroinvertebrate presence, resulting in a reduction by about 50 %. Mixing birch and oak litter did result in intermediate bacterial cell numbers with high variability (Fig. 1.7). Only a high abundance of isopods resulted in higher bacterial reduction than expected.

Table 1.3: Results from Two-Way-Analysis-of-Variance for **bacterial cell numbers** after **70 days**

source of variation	df	ss	ms	f	p
litter	2	5.236	2.618	16.987	< 0.001
fauna	3	2.397	0.799	5.184	0.003
litter x fauna	6	1.187	0.198	1.284	0.279
residual	57	8.785	0.154		
total	68	17.614	0.259		

**Figure 1.6: Bacterial cell numbers** on litter after **70 days** of incubation. Whisker-Box-Plots indicate, minimum 1st quartile, median, 3rd quartile, and maximum ($4 \leq N \leq 7$). Double-density-treatments are shown in gray bars. Asterisks indicate significant difference from control (Holm-Sidak post-hoc-test of 2-way ANOVA, Tab. 1.3)

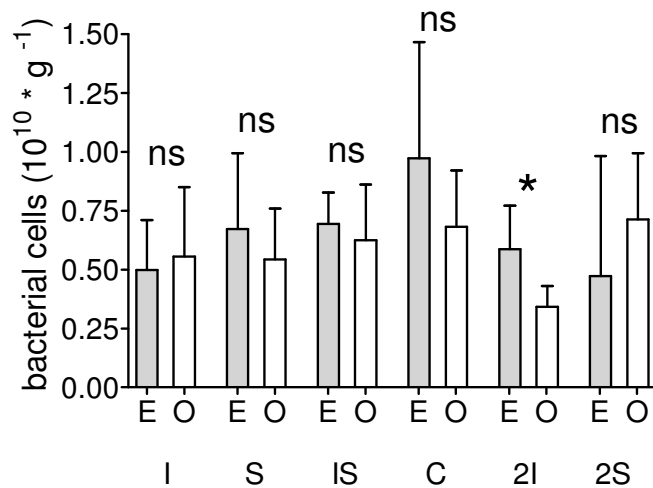


Figure 1.7: Expected (E) and observed (O) bacterial cell number of mixed litter after 70 days of incubation (mean \pm 95 % CI, $4 \leq N \leq 7$). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails.
 **: $0.001 < p \leq 0.01$; *: $0.01 < p \leq 0.05$;
 (*): $0.05 < p \leq 0.1$; ns: $p > 0.1$

Doubling the density of macroinvertebrates resulted in two effects: on birch litter the variability of bacterial density increased in the presence of snails, and on mixed litter, a higher density of isopods significantly reduced bacterial cells compared to the control.

After 112 days of incubation the over-all pattern of bacterial cell numbers did not change with respect to the 70-days treatments (Fig. 1.8). Significant over-all influence of litter identity and macrofauna presence remained (Tab. 1.4). Minor changes could be observed on mixed litter, where the median cell number of the control increased from ca. 7.2×10^9 cells per gram after 70 days of incubation to 9.5×10^9 cells per gram after 112 days, while the treatment groups remained unchanged. In the presence of a higher density of isopods, however, bacterial number increased from 3.4 to 7.2×10^9 cells per gram and was no longer significantly different from the control. In contrast to the 70-days results, after 112 days a high abundance of snails reduced bacterial biomass stronger than expected (Fig. 1.9).

Table 1.4: Results from Two-Way-Analysis-of-Variance for bacterial cell numbers after 112 days

source of variation	df	ss	ms	f	p
litter	2	9.604	4.802	42.296	< 0.001
fauna	3	3.774	1.258	11.080	< 0.001
litter x fauna	6	0.791	0.132	1.160	0.338
residual	69	7.834	0.114		
Total	80	22.057	0.276		

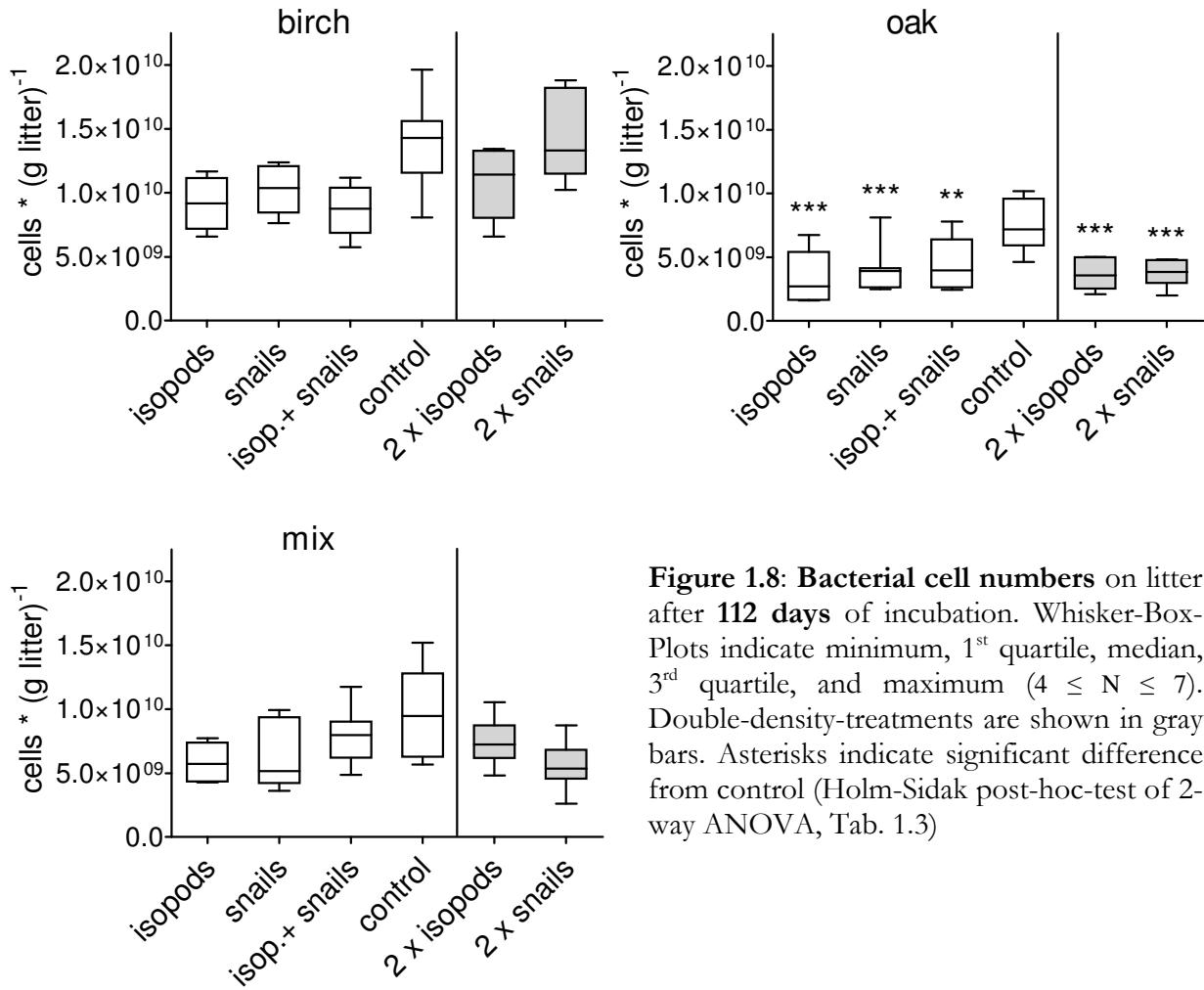


Figure 1.8: Bacterial cell numbers on litter after **112 days** of incubation. Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum ($4 \leq N \leq 7$). Double-density-treatments are shown in gray bars. Asterisks indicate significant difference from control (Holm-Sidak post-hoc-test of 2-way ANOVA, Tab. 1.3)

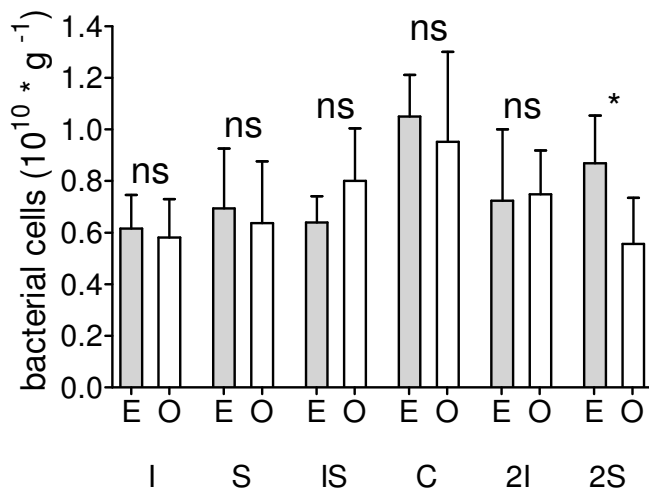


Figure 1.9: Expected (E) and observed (O) bacterial cell number of mixed litter after **112 days** of incubation (mean \pm 95 % CI, $4 \leq N \leq 7$). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails.
 ** : $0.001 < p \leq 0.01$; * : $0.01 < p \leq 0.05$;
 (*) : $0.05 < p \leq 0.1$; ns: $p > 0.1$

Fungal biomass

As Table 1.5 shows, no significant influence of litter nor fauna on fungal biomass could be found after 70 days of litter incubation. Three observations are remarkable (Fig. 1.10): the presence of snails increased the median fungal biomass on birch litter like isopod presence did on oak, while the presence of isopods lowered the median fungal biomass on mixed litter, though all insignificantly. Adding double density of macrofauna, neither had any significant influence. Next to an increase of the median fungal biomass of oak litter, double density of isopods in this case increased variability of results on oak and on. Due to too many missing values, expected versus observed fungal biomass on mixed litter could not be calculated.

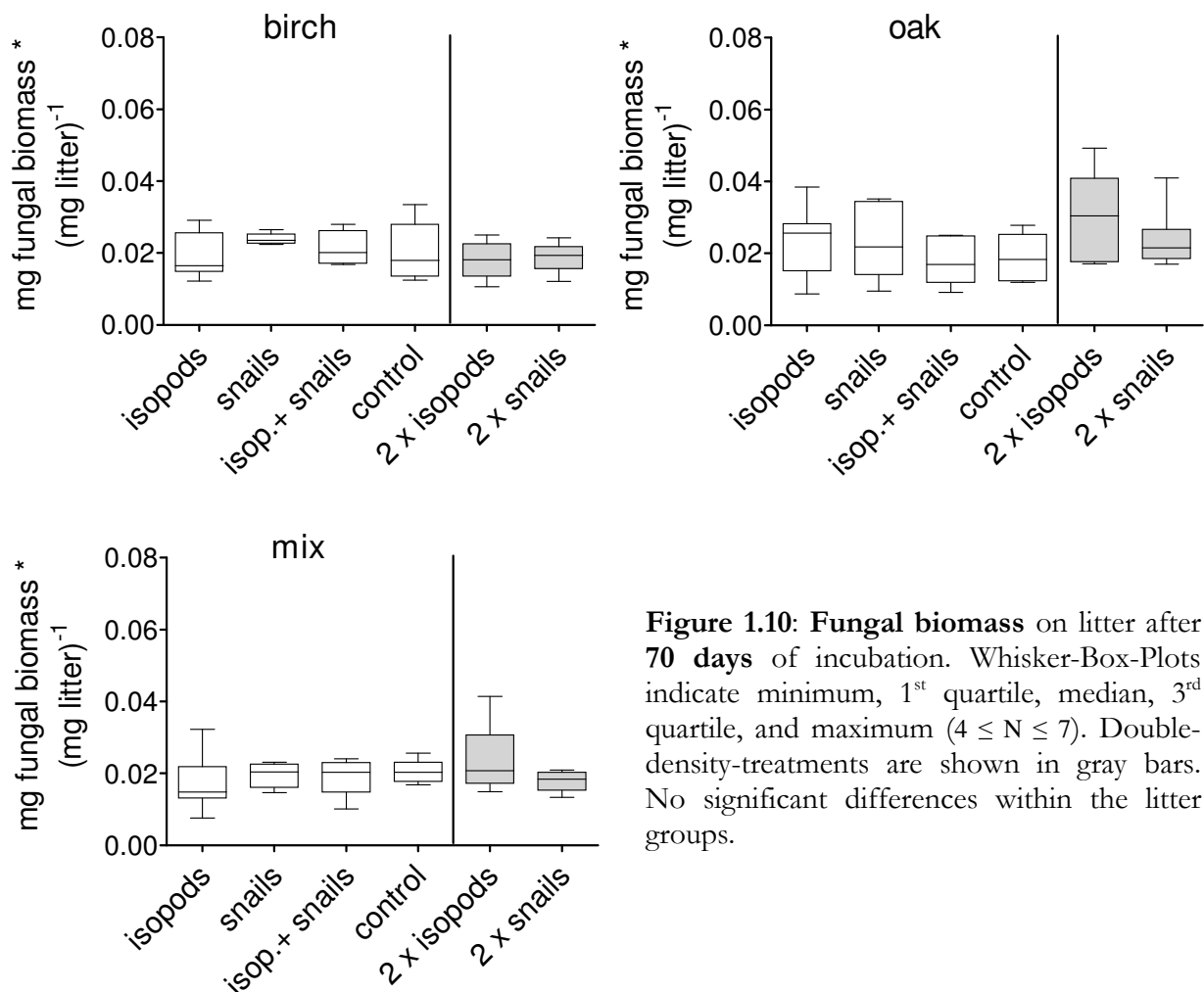
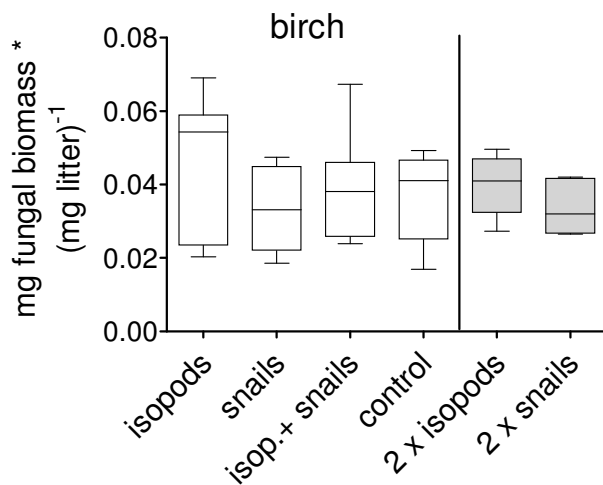


Figure 1.10: Fungal biomass on litter after **70 days** of incubation. Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum ($4 \leq N \leq 7$). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

Table 1.5: Results from Two-Way-Analysis-of-Variance for **fungal biomass** on litter after 70 days

source of variation	df	ss	ms	f	p
litter	2	0.0000630	0.0000315	0.734	0.485
fauna	3	0.000108	0.0000359	0.836	0.480
litter x fauna	6	0.000310	0.0000517	1.205	0.317
residual	57	0.00245	0.0000429		
total	68	0.00290	0.0000426		

From 10 to 16 weeks, where due to technical breakdown only birch litter was analysed, overall fungal biomass in the microcosms increased by 2-3 times (Fig. 1.11). No significant differences induced by the macrofauna were found (Tab. 1.7). Variability of results is higher than after 10 weeks, and covers an increase of median fungal biomass in lower-density isopod treatments.

**Figure 1.11:** Fungal biomass on litter after 112 days of incubation. Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum ($4 \leq N \leq 7$). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.**Table 1.6:** Results from One-Way-Analysis-of-Variance for **fungal biomass** on birch litter after 112 days

source of variation	df	ss	ms	f	p
fauna	5	0.0008468	0.0001694	1.085	0.386
residual	35	0.005463	0.0001561		
total	40	0.006310			

Microbial respiration

Only little influence of the macrofauna on total microbial respiration could be found (Fig. 1.12 and Fig. 1.14). After 70 days of incubation, there was a significant influence of litter identity, but none of fauna (Tab. 1.7). No differences in the microbial respiration on birch litter were found (Fig. 1.12). The same is true for oak litter, but with higher variability within the groups. The only influence of macrofauna was found on mixed litter in the presence of isopods at higher density. Here, the microbial respiration was increased with respect to the control. Microbial respiration was not influenced by litter mixing; expected and observed oxygen uptake did not differ (Fig. 1.13).

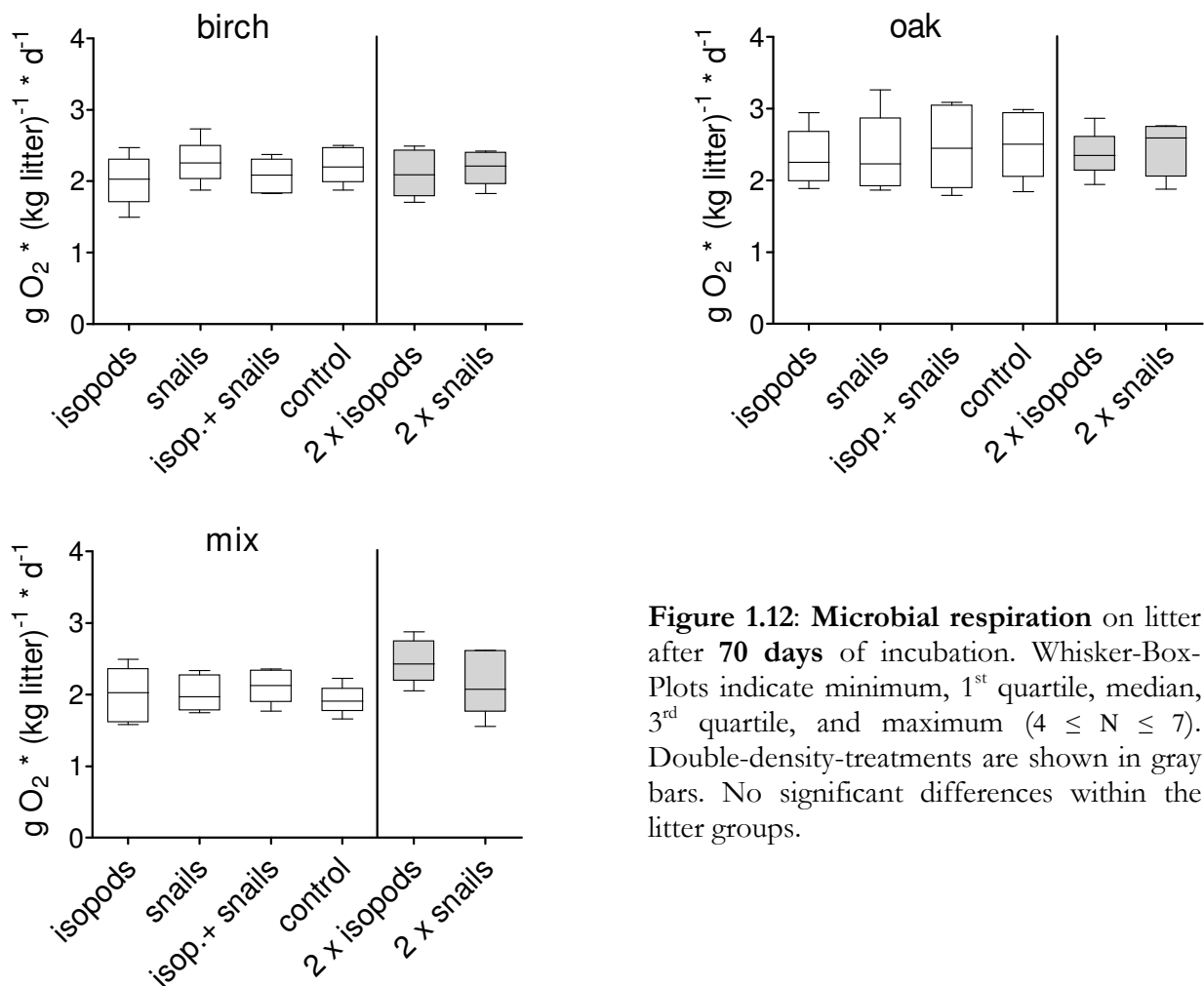


Figure 1.12: Microbial respiration on litter after **70 days** of incubation. Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum ($4 \leq N \leq 7$). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

Table 1.7: Results from Two-Way-Analysis-of-Variance for **microbial respiration on litter** after 70 days

source of variation	df	ss	ms	f	p
litter	2	1757438.221	878719.110	7.154	0.002
fauna	3	112367.095	37455.698	0.305	0.822
litter x fauna	6	353615.244	58935.874	0.480	0.821
residual	56	6878076.134	122822.788		
total	67	9157956.229	136685.914		

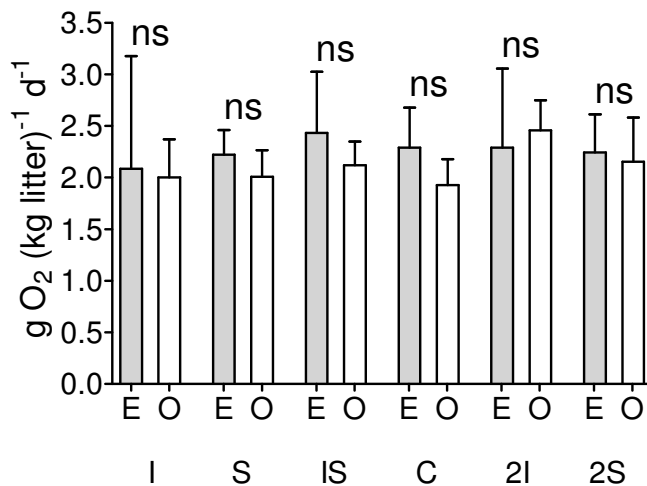


Figure 1.13: Expected (E) and observed (O) **microbial respiration** of mixed litter after 70 days of incubation (mean \pm 95 % CI, $4 \leq N \leq 7$). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails.
 ** : $0.001 < p \leq 0.01$; * : $0.01 < p \leq 0.05$;
 (*) : $0.05 < p \leq 0.1$; ns: $p > 0.1$

112 days after the start of the experiment, the over-all variability in microbial respiration increased (Fig. 1.14). While the influence of litter identity decreased compared to day 70 and was no longer significant, influence of detritivores increased ($p = 0.09$; Tab.1.8). In general, the median microbial respiration, hence, activity on birch and mixed litter was higher than after 70 days. In the case of snails dwelling on mixed litter, the median microbial activity increased from 1970 to 2580 mg O₂ kg⁻¹ d⁻¹. None of the differences after 112 days was significant, although, like after 70 days of incubation, the treatments of higher isopod density exhibited increased oxygen uptake. Again, like at day 70, none of the observed respiration values of mixed litter differed from the expected ones, despite the increased values induced by a high isopod abundance.

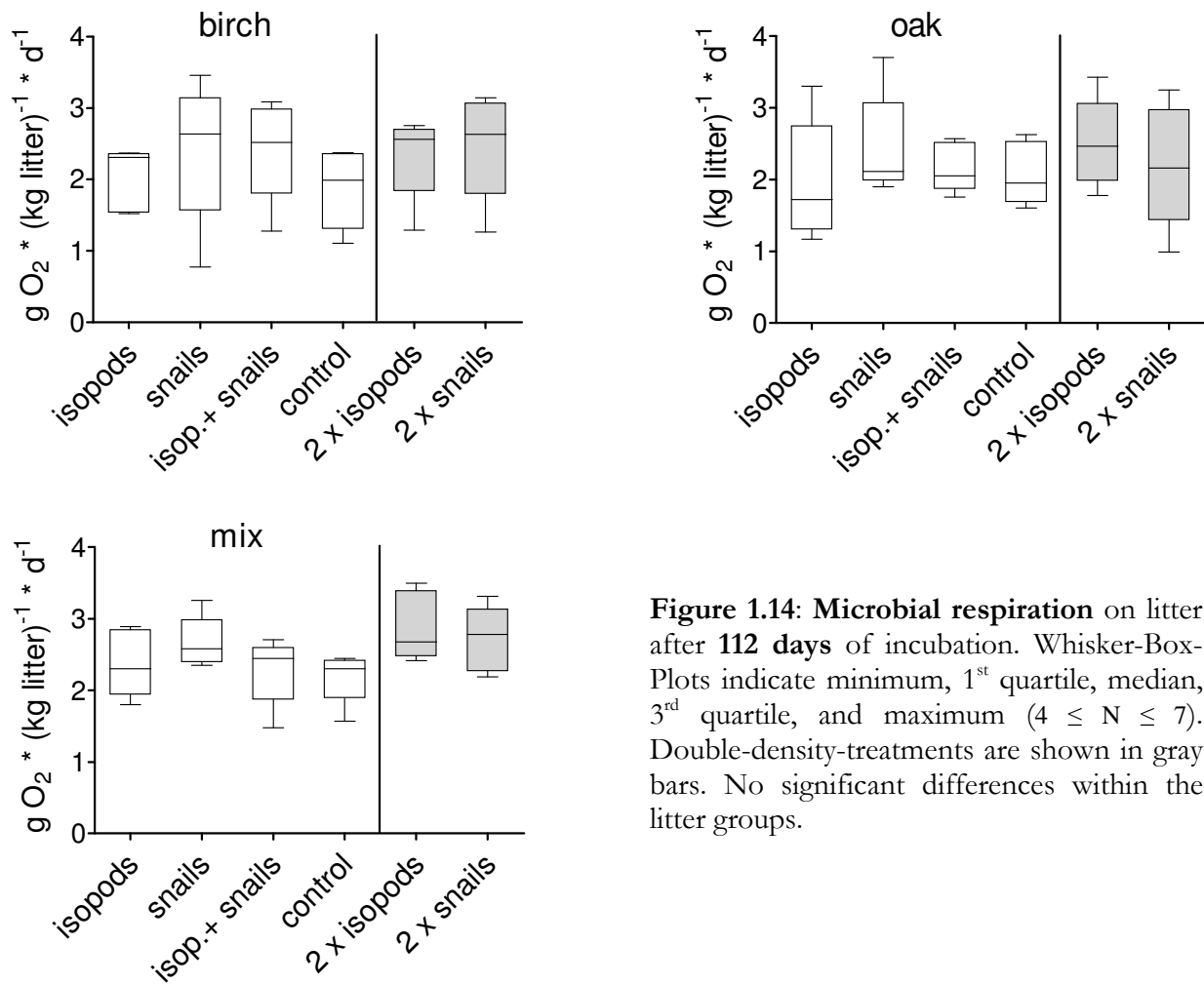


Figure 1.14: Microbial respiration on litter after **112 days** of incubation. Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum ($4 \leq N \leq 7$). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

Table 1.8: Results from Two-Way-Analysis-of-Variance for **microbial respiration** on litter after **112 days**

source of variation	df	ss	ms	f	p
litter	2	528109.108	264054.554	0.802	0.454
fauna	3	2240945.508	746981.836	2.268	0.091
litter x fauna	6	624015.494	104002.582	0.316	0.926
residual	52	17123310.527	329294.433		
total	63	20565377.952	326434.571		

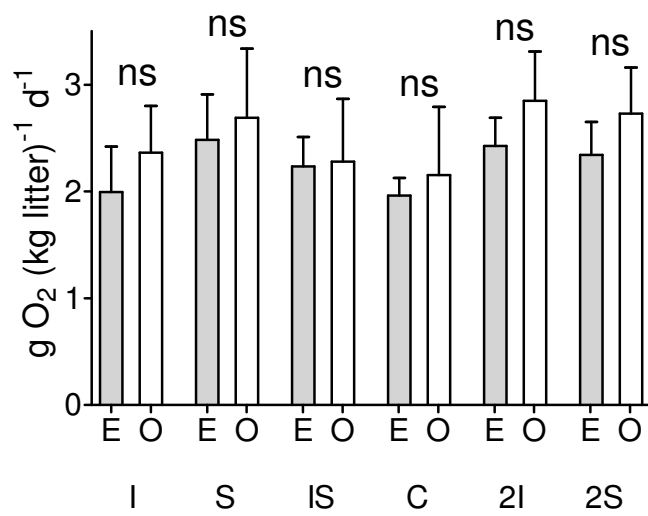


Figure 1.15: Expected (E) and observed (O) **microbial respiration** of mixed litter after **112 days** of incubation (mean \pm 95 % CI, $4 \leq N \leq 7$). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails.

** : $0.001 < p \leq 0.01$; * : $0.01 < p \leq 0.05$;

(*) : $0.05 < p \leq 0.1$; ns: $p > 0.1$

Discussion

Summary of the results

The present results clearly support our hypothesis that macroinvertebrates at natural densities have a strong direct influence on litter mass loss through feeding. Additionally, we found low indirect effects on microbial respiration. Besides significant litter mass loss caused by litter consumption, there was a significant reduction of bacterial biomass by both detritivore species. However, no reduction of fungal biomass was observed in any of the treatments. We did not find distinct effects of distinct feeding types (grazers versus shredders), nor became consistent effects of combining species of litter or detritivores obvious. Combinations of detritivores did not influence any of the tested parameters, but combining the functionally distinct litter species promoted microbial respiration markedly, albeit insignificantly, in the presence of twice the natural detritivore, in particular isopod, abundance.

Litter mass loss

The impact of shredding macroinvertebrates on leaf litter is well documented (see introduction). Here, like in many of these studies, we found significant mass loss induced by litter-processing macrofauna. Despite the different initial tissue strength, which is one of the main markers for potential detritivore consumption (Pennings et al. 1998), the impact of fauna on litter mass loss was the same on oak and on birch litter. At the end of the experiment, both leaf types were soft and much more alike in tissue toughness. The macerating effect of water on leaf structural integrity equaled litter palatability for both detritivore types (compare Chapter II).

Bacterial biomass

Our knowledge of direct effects of the macrofauna on the abundance of microbial litter colonizers and of indirect effects on microbial performance, like nutrient release, is profound for terrestrial decomposition processes (summarized by Anderson & Ineson 1984, Swift & Boddy 1984, Zimmer 2004, Hassel et al. 2006, Huhta 2006), but little is known for aquatic environments. For terrestrial decomposition processes in soil and litter layers of forest floors, it is well documented that microbial biomass can be significantly

increased by feeding activities of earthworms, Dipteran larvae, millipedes, mites, and isopods (reviewed in Anderson & Ineson 1984). Furthermore, Huhta (2006) and Anderson & Ineson (1984) report enhanced microbial respiration rates and nutrient mobilization in several studies including litter-feeding macrofauna.

Here, in an aquatic environment, we found contrary results. Bacterial biomass was reduced by either detritivore species by removing microbial biofilm. Bacteria exudate, and are embedded in, a gelatinous matrix of extracellular enzymes covering submerged surfaces. This matrix also contains algae, fungi, and particulate organic and inorganic matter (Golladay & Sinsabaugh 1991, Hax & Golladay 1993, Costerton et al. 1995). This nutritionally valuable biofilm is consumed by surface-scraping organisms, in particular grazing macroinvertebrates like in the present case (McNamara & Leff 2004).

At the end of the experiment, both, birch and oak leaves were soft, but with different surface texture. While birch leaf surface texture was heterogeneous, oak leaves showed plain and homogeneous surface. It is likely that microbial biofilm can more easily be removed from plain oak leaves than from birch leaves. As a consequence we observed higher removal of bacterial biomass on oak than on birch litter (Fig. 1.6 + 1.8) and conclude that leaf surface properties governed invertebrate impact on microbial biomass (compare Yadav 2005). A remarkable low reduction of bacterial biomass was observed on birch litter in the presence of a high snail density (Fig. 1.6 + 1.8). Liess & Haglund (2007) found the aquatic snail *Theodoxus fluviatilis* to have different effects on nutrient release and bacterial production in submersed biofilm, but suggest a supportive effect on bacteria under nutrient limited conditions (compare Chapter 3). Such mechanisms may have been present here, counteracting gastropod feeding on bacterial biomass.

Fungal biomass

Fungal biomass, in contrast, was not reduced by detritivores. This is remarkable, since reduction of fungal biomass has been reported (Bärlocher & Kendrick 1975, Graça 2001) However, since hyphomycete fungi grow their hyphae into the leaf mesophyll, grazing on the surface and biting off leaf fragments might not reduce the fungal biomass per litter weight (Canhoto & Graça 1996). Additionally, high fungal growth rates may have compensated for biomass reduction through consumption. Kuehn et al. (2000) determined a fungal production rate of up to 2.8 mg fungal C (g AFDW)⁻¹ d⁻¹ in a stream,

which equals ~ 6.6 mg fungal biomass $(\text{g AFDW})^{-1} \text{d}^{-1}$ assuming a fungal carbon content of 43 % (Baldy et al. 1995). Using ingestion rates provided by Constantini (2005), length-to-AFDW-ratios for *Asellus aquaticus* by Mews (2002), and the average fungal biomass per mg litter in this study, we calculated an average ingestion of fungal biomass by isopods of roughly $0.1 \text{ mg g}^{-1} \text{d}^{-1}$ with a standing stock of fungal biomass of about $20 \text{ mg (g litter)}^{-1}$. Though fungal production rate in this study was probably lower than under natural conditions as given in Kuehn et al. (2000), it is still likely that loss to detritivore feeding activity was counterbalanced by fungal production.

Microbial respiration

Fungi contribute more than 90 % to the total microbial biomass in aquatic environments (e.g. Findlay & Arsuffi 1989, Graça 1993, Kuehn et al. 2000, Mille-Lindblom & Tranvik 2003, Carter & Suberkropp 2004). As Figure 1.16 shows, microbial respiration in this study was strongly correlated with fungal, but less so with bacterial biomass. We hold that bacteria contribute little to microbial respiration in aquatic systems. Thus, the observed reduction of bacterial biomass by detritivores had only little influence on the total microbial oxygen uptake. Since in these treatments fungal biomass was not reduced and microbial respiration remained on the

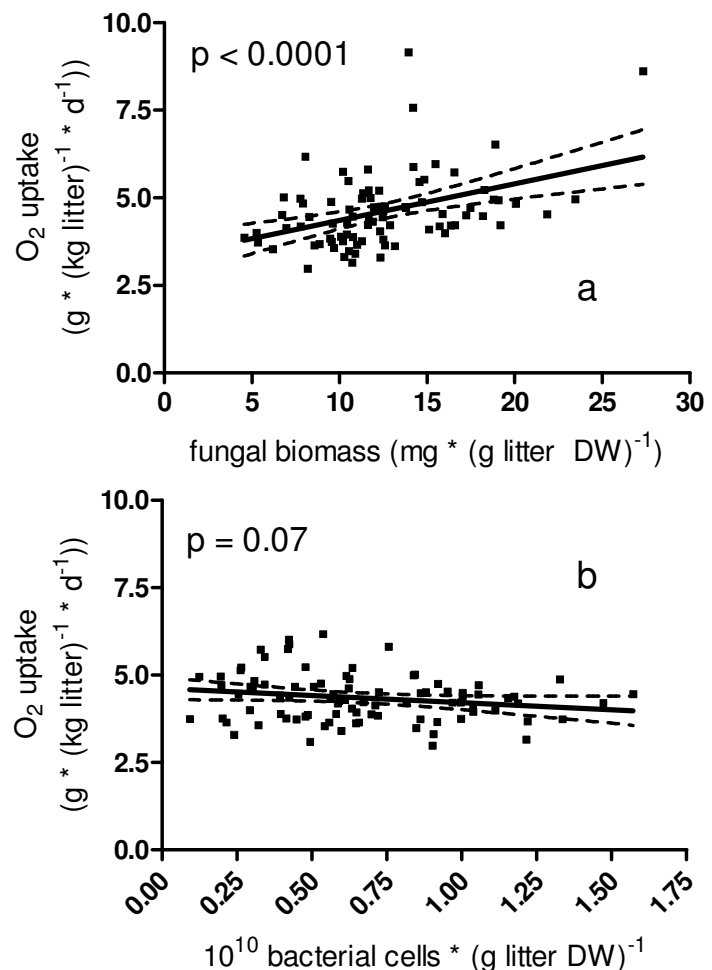


Figure 1.16: Regression of microbial respiration and fungal biomass (a) and bacterial cell numbers (b) after 70 days. p-value indicates significance of difference of regression line slope from zero. Broken lines: 95 % confidence interval.

control-level, the specific respiration (oxygen uptake per microbial biomass unit) was not affected by invertebrate feeding.

However, on mixed litter high abundance of isopods after 70 d and isopods and snails after 112 d increased microbial respiration compared to the control. These effects were low and not significant, but for isopods they appeared independently at both sampling dates and can therefore not be ignored. In these treatments no change in fungal biomass was observed. Hence, the presence of a dense macrofauna stimulated fungi to increase activity. Such an influence by the macrofauna on the microbial community, as described for terrestrial systems, can result from litter surface disruption that opens up new surfaces for bacteria and fungi to colonize on (Danell & Sjöberg 1979, Merritt et al. 1984), while grazing on bacterial colonies and fungal hyphae can induce microbial growth as a counteracting strategy. Hanlon (1981) describes doubling of respiration of fungal mycelium by grazing, which is attributed to removal of senescent parts of the colony and re-growth of new fungal mycelium. This increase in microbial activity can be measured as total litter oxygen uptake. The effect was higher in the presence of isopods and may thus be of importance in the field, where high abundances of *Asellus aquaticus* occurs in litter patches.

Effects of species composition

We tested for effects of species-mixing on different parameters and over an extended period of time using different densities of detritivores. It is known that species-mixing and biodiversity effects show idiosyncratic patterns, making Hättenschwiler (2005) doubt that there is a single, true correlation of biodiversity and ecosystem process rates. Species identity rather than species number is commonly accepted as driver for mixing effects (Jonsson & Malmqvist 2003, Covich et al. 2004, Dangles & Malmqvist 2004, Duarte et al. 2006, LeRoy & Marks 2006). In addition, the present study shows that species-mixing effects might only emerge under specific conditions. We found an effect of mixed litter to be apparent for only one parameter (microbial respiration) only in the presence of high detritivore densities and in no other litter-fauna combination. This reflects the contrasting results from other studies that investigated diversity effects in aquatic systems and did not show a clear pattern (e.g. Jonsson et al. 2001, Jonsson & Malmqvist 2003, Larned et al. 2003, Dangles & Malmqvist 2004, Bjelke & Herrmann 2005).

As possible mechanisms inducing diversity effects of litter mixtures, like observed here, translocation of nutrients (mainly nitrogen; positive effect) and inhibitory compounds (mainly phenols; negative effect) between leaves of different quality are suggested (Chapman et al. 1988, Blair et al. 1990, Wardle et al. 1997, Schimel & Hättenschwiler 2007). Further, an increase of micro-environmental heterogeneity, optimizing conditions for microorganisms and micro- and mesofauna, has been found to positively influence decomposition (Hector et al. 2000). In translocation of nitrogen from leaves of high to leaves of low nitrogen content a major role is ascribed to fungi (McTiernen et al. 1997), since the fungal mycelium pervades detritus assemblages connecting detrital subunits; the increase of nitrogen content enhances microbial performance (Suberkropp 1995, Abelho & Graça 2006; but compare Ferreira & Graça 2007). The most significant vector for translocation of chemical compounds, however, is water. Thus, in the present study it is likely that a combined effect of litter mixture that increased microbial respiration resulted from detritivore activity and translocation of nutrients through fungal mycelium and water.

Conclusion

We found different effects of the functionally distinct detritivores on bacterial and fungal biomass and activity. While bacterial biomass (biofilm) was reduced by detritivores, fungal biomass was not influenced by macroinvertebrate presence at all. In turn, fungal activity could be increased by high abundance of detritivores but only on mixed litter. Nevertheless, the faunal impact on decomposition process was surprisingly low and uniform. In particular, functional difference between the detritivore and litter types had little influence on litter mass loss.

These results help catching up with the investigations in terrestrial systems, where animal-microbe interactions have broadly been studied. Since water as a medium has significant impact on mediation of nutrient fluxes and physical and chemical reactions and pathways, different relationships than in terrestrial systems can be expected. Notwithstanding this assumption, we found indirect effects of fauna on the microbial community like known from terrestrial studies.

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An aquatic-terrestrial comparison of leaf litter
decomposition

Chapter II

Introduction

The main characteristic of aquatic habitats is the permanent and all-encompassing presence of a solvent. This solvent transports soluble substances and counteracts the impact of gravity. In a comprehensive work Shurin et al. (2006) worked out the basic differences of aquatic and terrestrial food webs and came to the conclusion that the differences are greatly driven by the difference in primary producer properties. In phytoplankton and aquatic macrophytes we find little structural components like cellulose or lignin that decrease the plant's nutritional value by decreasing its nitrogen content and palatability. This is due to the fact that gravity does not act as strongly on aquatic as it does on terrestrial producers, and aquatic producers do not have to adapt to it. Other food web characteristics like size hierarchy through the trophic pyramid and decomposition processes in aquatic systems can, according to the authors, be traced back to these fundamental plant properties. However, the conclusions from this principle fail for aquatic decomposition processes and the aquatic detritus web, when these are based on terrestrial plant material entering an aquatic environment. If, according to Shurin et al. (2006), all (detritus-) food web characteristics can be traced back to primary producer properties, then aquatic and terrestrial decomposition of terrestrially produced material should not differ significantly, since the source material is the same.

Terrestrially derived plant material like wood, twigs, roots, and leaves are an important allochthonous carbon source for forest and grassland aquatic systems like ponds, creeks, and streams (Vannote et al. 1980). In these habitats deciduous trees shed leaf litter into aquatic and terrestrial habitats in immediate vicinity. Research on direct comparison of detritus processing of the same origin under aquatic and terrestrial conditions is rare. Ribas et al. (2006), comparing litter breakdown of the same origin in both, a tropical aquatic and a terrestrial system, found no influence of macrofauna on litter breakdown rates. Apart from this recent study, we did not encounter any studies on direct ecosystem comparison concerning decomposition. For such a comparison two systems are needed, that are subject to the same environmental conditions apart from the presence and absence of surrounding water. This can for sure only be accomplished to a certain degree, in particular, when detritivore animals are to be included.

Isopods and gastropods are common and abundant detritivores in terrestrial and aquatic habitats of northern Germany. Occurring syntopically, *Porcellio scaber* LATREILLE

(Isopoda, Oniscidea) and *Discus rotundatus* MÜLLER (Gastropoda, Pulmonata). Both are involved in terrestrial decomposition processes as shredder and grazer in the conventional guild categorization, respectively (Mason 1970, Zimmer 2004). Concerning feeding type and size these species are comparable to *Asellus aquaticus* and *Bithynia tentaculata* used in the experiment of Chapter I, with *D. rotundatus* being about 2/3 the size of *B. tentaculata* (personal observation). *A. aquaticus* and *P. scaber* mainly live on microbial biomass ingested along with leaf litter material (Graça et al. 1993a+b, Zimmer 2002). Both have the ability to use microbially derived enzymes inside the gut lumen supporting digestion of cellulose (Zimmer 2002, Zimmer & Bartholmé 2003). The gastropods both ingest leaf surface associated biomass and epidermal cell layer by scraping it off; in the gut of *Discus rotundatus* Mason (1970) found large amounts of leaf material and fungal biomass. *Bithynia tentaculata*, in accordance, dwells on aquatic detritus patches and consumes detritus and associated microbial biomass (Brendelberger 1997). Both, terrestrial and aquatic species can be considered to contribute to ecological processes in a similar way and are thus regarded as comparable in this study.

When plant material is submerged, through osmotic processes low molecular substances are washed out of the tissue into the surrounding water column. Such substances are mainly amino acids, sugars, aliphatic acids, and phenolics (Nykqvist 1963). These substances accumulate in the water column as dissolved organic matter (DOM). Microorganisms – bacteria, fungi, microalgae – when growing on submerged surfaces form organic layers additionally composed of exoenzymes and detritus particles enclosed within a gelatinous matrix excreted by bacteria (Golladay & Sinsabaugh 1991, Hax & Golladay 1993, Costerton et al. 1995). Microorganisms in these biofilms can benefit from DOM in the water column (Sobczak 1996) by assimilating it. Heterotrophic bacteria, for example, are the dominant users of DOM in streams (McNamara & Leff 2004). Consequently, leachates from submerged litter have got the potential to promote biofilm production. Since biofilms are easily accessible, they form a valuable nutritional source for surface scraping macroinvertebrates (Bärlocher & Kendrick 1975, McNamara & Leff 2004). The leached detritus material, in contrast, is reduced in nutritional quality and its relative content of recalcitrant high-molecular or water-insoluble compounds like structural proteins and lignocellulose complexes is increased.

Plant tissues of different origin have dissimilar content of water-soluble substances and different nutritional value for herbi- or detritivore organisms. After extended submersion in water, when all water-soluble substances are washed out and mainly compounds of little nutritional value remain, the nutritional qualities of these tissues are probably not the same, but approach each other on a lower level.

After submersion cells and intercellular spaces soak up water. Through osmotic pressure cells in dead tissue may burst and tissue macerates and destabilizes. The surface of macerated tissue, dependent on the epicuticular wax layer, is heterogeneous and pervaded by cracks and consequently suitable for microbial colonization (Yadav et al. 2005). Structural qualities of different plant tissues may thus, after longer submersion, also approach the same level.

In summary, besides leaching of water-soluble substances a very important effect of litter submersion is potential diminishing of differences in litter structural properties.

Based on these assumptions and considerations, we hypothesize the following:

1. Early decomposition in aquatic environment is largely realized via DOM, while in terrestrial environment it is a matter of POM (particulate organic matter).
2. Due to equalized litter properties, less differences in microbial and detritivore parameters on different litter types occur in aquatic environment.
3. Accordingly less species mixing effects occur compared to the terrestrial environment.

To test these hypotheses an experiment, analogous to Chapter I, was conducted in terrestrial microcosms, using *Porcellio scaber* and *Discus rotundatus* on the same litter as in the aquatic part. The results are discussed in comparison to Chapter I.

Material and Methods

Experimental setup

Microcosms were made of 900 mL plastic boxes (Perga Plastic, Walldürn-Altheim/Germany) containing 100 g of 2 mm-sieved and air-dried forest soil (H-horizon). 2 g of air-dried litter (birch, *Betula pendula* Roth; oak, *Quercus robur* L.), hand-collected in a public park (Forstbaumschule, Kiel) were added. Isopods (*Porcellio scaber* Latreille) and snails (*Discus rotundatus* Müller) were collected in private gardens in Plön/Kreis Plön and in the graveyard of Kronshagen/Kiel under stones and decaying wood. Animals were separated by size. Equal sets of different sizes were introduced into the microcosms which were then covered with a lid equipped with a 1 mm mesh of 25 mm diameter to enable atmosphere exchange. Litter sets (birch litter, oak litter, or both 1:1) were added to detritivore sets (6 isopods (I), 12 isopods (2I), 10 snails (S), 20 snails (2S), 6 isopods + 10 snails (IS), and no fauna (C)) in all possible combinations. These treatments were set up 4 times to be destructively sampled after 14, 42, 70 and 112 days, resulting in a total of 72 treatments, replicated 7 times. Temperature and light regime in the climate chamber were adjusted to simulate a winter-to-spring period: 42 days at 5°C, 14 days at 6°C, 14 days at 8°C, 14 days at 10°C, 14 days at 12°C, 14 days at 14°C. Light:darkness regime ranged from 14 h:10 h to 12 h:12 h after 56 days back to 14 h:10 h in the course of the experiment. Every two weeks, microcosms were screened for dead animals, which were removed and replaced by new ones of the same size. 2.6 mL of distilled water were sprayed into each microcosm after 28, 47, 70, 77, 84, 91, 98, and 105 days to compensate for evaporation. The setup was spread over a period of one month to give enough time for analyses later during the experiment.

At the end of the experiment live animals were counted and subsamples of soil were taken and stored at -20°C. Total remaining litter was weighed to the nearest mg. About 1/5 of litter was used for subsamples for litter analyses (see below), 1/5 was dried to constant weight (60 °C) for calculation of fresh weight/dry weight ratio, and 3/5 was used for respiration measurement (see below).

Microbial respiration

Microbial respiration was measured immediately after the end of the experiment with the BSB/BOD-Sensomat (Aqualytic, Lagen/Germany). The device traps free CO₂ in KOH solution, while the decrease in atmospheric pressure resulting from microbial oxygen consumption is recorded. Litter was given into 250 mL flasks. 1 mL 45 % KOH was given into a rubber quiver inside the flasks which were then closed with the pressure-measuring sensor and stored at 20°C for 24 h. Results were given in mg O₂ * L⁻¹ * (24 h)⁻¹ and then calculated to mg O₂ * (kg litter)⁻¹ * (24 h)⁻¹ using formula provided by the manufacturer.

Bacterial biomass

From litter of each microcosm, 10 disks of 10 mm diameter were cut from 10 different leaves, using a cork borer. Disks were stored in 15 mL plastic vials with 10 mL of 4 % Formaldehyde at 4°C until further processing. For detaching of bacteria, 5 glass beads (5 mm) were added to the vials before shaking for 10 minutes on a horizontal shaker at 420 rpm. After letting stand for 10 min, 0.5 mL of the supernatant were mixed with 6 mL ddH₂O and filtered through a 0.2 µm polycarbonate filter (25 mm; DHI, Hørsholm, Denmark). Litter discs were dried at 60°C for 1 h and weighed. After staining for 10 min with DAPI-staining solution (1 µg/mL), the filter was washed first in ddH₂O then in 96 % ethanol and dried on lab tissue for one minute. It was then covered with one drop of CITIFLUOR solution (Citifluor Ltd., London/GB) to prevent quick fading of staining intensity. From each filter, photos of 3 different sections were taken with a digital camera using an epifluorescent microscope equipped with a DAPI-staining-specific optical filter set at 1000x magnification. Because of high background noise, the photos needed to be optimized before automated bacteria counting. For this purpose, a software routine was written (using freeware Dev-C++-compiler, Bloodshed-Software) that increased image-contrast of blue DAPI-stained bacterial cells. Stained cells were then counted using the VisualBasic-based macro language interpreter of SigmaScan image processing software (SigmaStat Software Inc., San Jose, USA). From a counted area of 80 µm x 80 µm, bacterial cell number in the sample was calculated as the arithmetic mean of counts of the 3 filter sections.

Fungal biomass estimation

From litter of each microcosm, 10 disks of 10 mm diameter were cut from 10 different leaves, using a cork borer, and stored at -20°C until further processing. The ergosterol content was measured as an estimate for fungal biomass of litter samples according to Davis & Lamar (1992), modified to be used with smaller samples. For discussion of ergosterol as fungal biomass indicator see Chapter I.

Frozen litter disks were transferred to a 2 mL screw cap plastic vial together with 20 glass beads of 3 mm diameter, and 200 µL methanol (HPLC-grade) containing 10 % (w/v) KOH were added. The sample was homogenized at 4800 rpm in a MiniBeadBeater (GlenMills, Clifton NJ, USA) for 1 minute. Another 600 µL KOH/MeOH and 200 µL cyclohexane were added and the sample was sonicated for 15 minutes in an ultrasonic bath at 80 W and room temperature, followed by a 90-minutes heating period at 70°C at permanent slow rotation. The vial was allowed to cool to room temperature before 200 µL ddH₂O and 600 µL cyclohexane were added. Subsequently, the sample was vortexed for 30 seconds and centrifuged with 1000 * g (room temperature) for 5 minutes. The cyclohexane phase was transferred into an extra 2 mL tube. The sample was extracted a second time with 400 µL cyclohexane, vortexed, and centrifuged. Cyclohexane phases were pooled. Cyclohexane was evaporated at 40°C under a gentle stream of air; the residue was dissolved in 1 mL methanol (HPLC-Grade) at 40°C for 15 min. The extract was passed through a 0.2 µm PTFE syringe filter (Roth, Karlsruhe, Germany) into a new 1.5 mL tube. HPLC was run on a Kontron HPLC system (BIO-TEK Kontron Instruments GmbH, Neufahrn, Germany) equipped with a 250 * 4 mm C18 column (Eurospher 100, 5 µm; Knauer, Berlin, Germany) applying 80 µL extract. At room temperature, using a flow rate of 1.5 mL min⁻¹, the ergosterol peak appeared after ~12 min ($\lambda = 282$ nm). As a standard, crystal ergosterol (Sigma-Aldrich #45480) dissolved in methanol was used. Fungal biomass was calculated according to Gessner & Chauvet (1993), assuming 5.5 mg ergosterol per gram fungal biomass.

Statistics

Diagrams of data were created using PRISM 4 (GraphPad Software). Data were analysed using two-way-ANOVA to test for influence of litter and detritivore combinations. Factors were “litter” with levels “birch”, “oak”, and “mixed” and “fauna” with levels “I”

(low density of isopods), “2I” (high density of isopods), “S” (low density of snails), “2S” (high density of snails), “IS” (isopods + snails) and “C” (no fauna). Raw data were either ln- or square root-transformed to meet ANOVA requirements of normal distribution and homogenous variances. In case of a statistically significant difference, a Holm-Sidak post-hoc test with multiple pairwise comparisons was performed on the data set to identify the source of difference.

To clarify whether mixing of litter influenced decomposition parameters, expected values (“E”) for mixed litter were calculated from values of single-litter type treatments and compared with the observed values (“O”) of mixed-litter treatments. Replicates of birch and oak litter were randomly assigned and for expected litter mass loss the arithmetic mean was calculated:

$$E_e = \frac{B_e + O_e}{2} \quad \text{with } e \in [1..7]$$

B_e : litter mass loss in replicate of birch treatment

O_e : litter mass loss in replicate of oak treatment

Pairs of B_e and O_e were randomly assigned.

For the rest of the parameters (bacterial cell numbers, fungal biomass, litter respiration), according to Wardle et al. (2006), the expected values were calculated with respect to litter mass loss of each of the litter types to take into account the different litter composition at the end of the experiment:

$$E_e = \frac{rB_e \cdot PB_e}{rB_e + O_e} + \frac{rO_e \cdot PO_e}{rB_e + O_e} \quad \text{with } e \in [1..7]$$

rB_e : remaining birch litter mass

rO_e : remaining oak litter mass

PB_e : parameter replicate of birch treatment

PO_e : parameter replicate of oak treatment

Data sets of expected and observed values were statistically compared using the Mann-Whitney U test for non-parametric data.

Results

Litter mass loss

The course of litter mass loss differed among the three substrates (Fig. 2.1). Birch litter mass decreased slowly during the first 70 days, before mass loss accelerated during the last sampling interval. Oak litter remained constant in mass until temperature reached 10°C. Oak litter mass then quickly dropped to the level of birch litter. Mixed litter, in contrast, appeared to gain mass during the first 42 days and then rapidly lost mass; again with an accelerated rate after 70 days, finally resulting in the same average mass loss as birch and oak litter in the single species treatments. On all substrates, mass loss in the control was highest at the end. Over all, variation within treatments (given as confidence interval) decreased over time (Fig. 2.1).

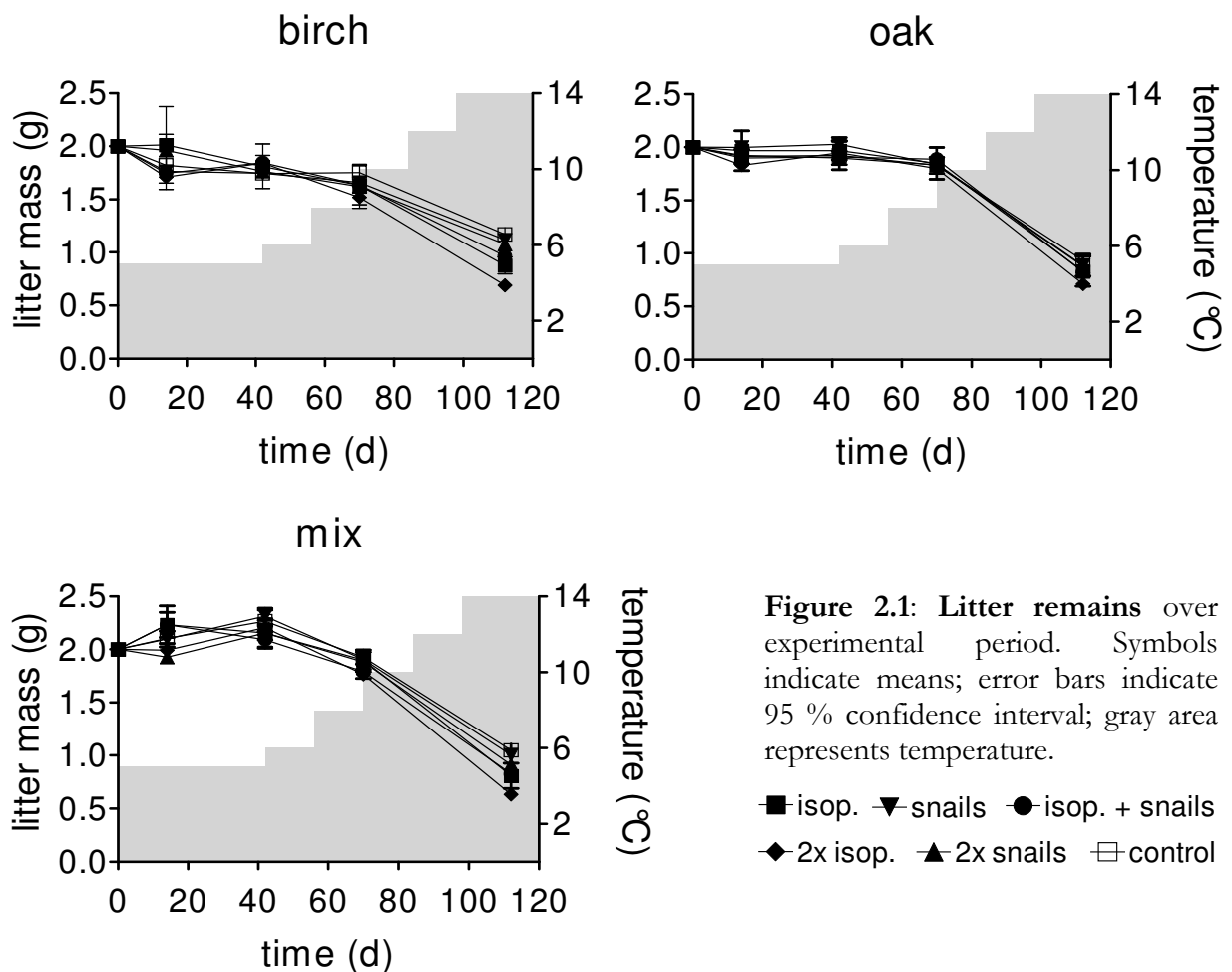


Figure 2.1: Litter remains over experimental period. Symbols indicate means; error bars indicate 95 % confidence interval; gray area represents temperature.

■ isop. ▼ snails ● isop. + snails
◆ 2x isop. ▲ 2x snails □ control

In more detail (Fig. 2.2), after 70 days, for birch litter, an insignificant influence of the fauna was apparent: the control exhibited the lowest median mass loss. Accordingly, mixed litter was reduced most by a high abundance of isopods. Over-all faunal influence was low (Tab. 2.1).

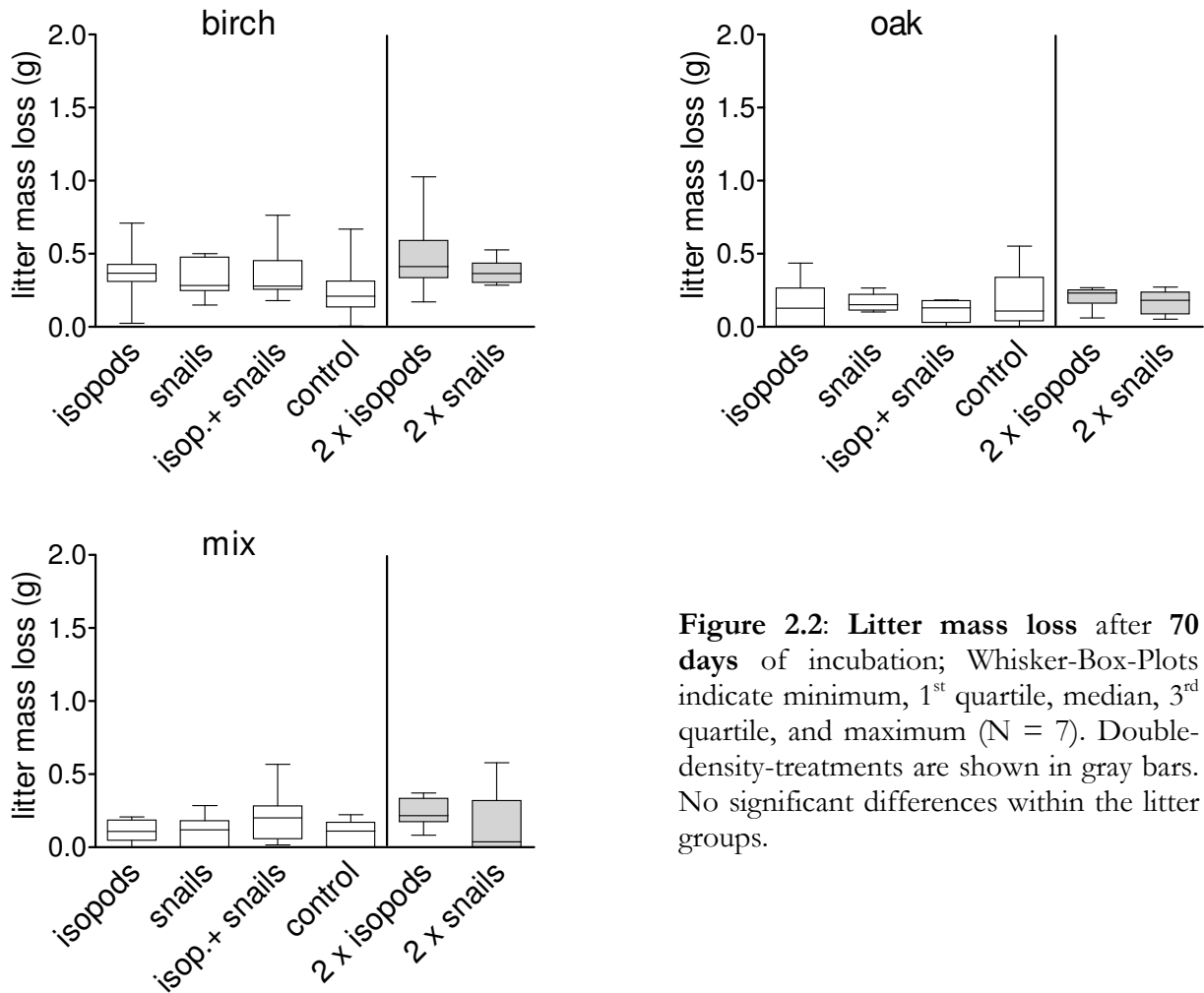


Figure 2.2: Litter mass loss after 70 days of incubation; Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum (N = 7). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

Table 2.1: Results from Two-Way-Analysis-of-Variance for litter mass loss after 70 days

source of variation	df	ss	ms	f	p
litter	2	0.983	0.491	24.374	< 0.001
fauna	5	0.133	0.0265	1.315	0.265
litter x fauna	10	0.0835	0.00835	0.414	0.937
residual	90	1.814	0.0202		
total	107	3.009	0.0281		

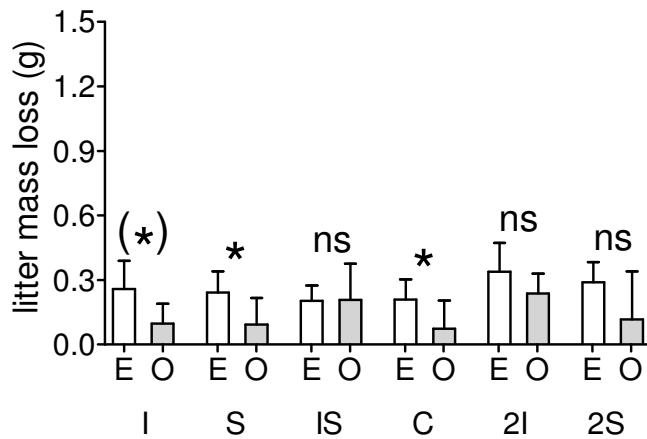


Figure 2.3: Expected (E) and observed (O) litter mass loss of mixed litter after 70 days of incubation (mean \pm 95 % CI, N = 7). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails. ** : $0.001 < p \leq 0.01$; * : $0.01 < p \leq 0.05$; (*) : $0.05 < p \leq 0.1$; ns: $p > 0.1$

For mixed litter, both, the control and the treatment with low snail density exhibited significantly lower mass loss than could be expected based on single-species treatments (Fig. 2.3).

After 112 days, both litter and fauna exhibit significant influence (Tab. 2.2) on litter mass loss. On birch and mixed litter, all treatments containing isopods exhibited significantly higher mass loss than the control (Fig. 2.4). Snails, in contrast, did not reduce litter significantly. Variation of oak litter mass loss within the treatments was increased in comparison to birch and mixed litter.

Table 2.2: Results from Two-Way-Analysis-of-Variance for litter mass loss after 112 days

source of variation	df	ss	ms	f	p
litter	2	0.0962	0.0481	15.229	< 0.001
fauna	5	0.374	0.0748	23.657	< 0.001
litter x fauna	10	0.0492	0.00492	1.558	0.131
residual	95	0.3	0.00316		
total	112	0.802	0.00716		

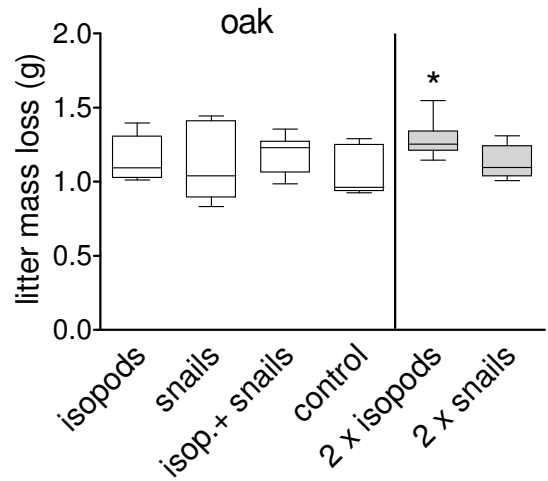
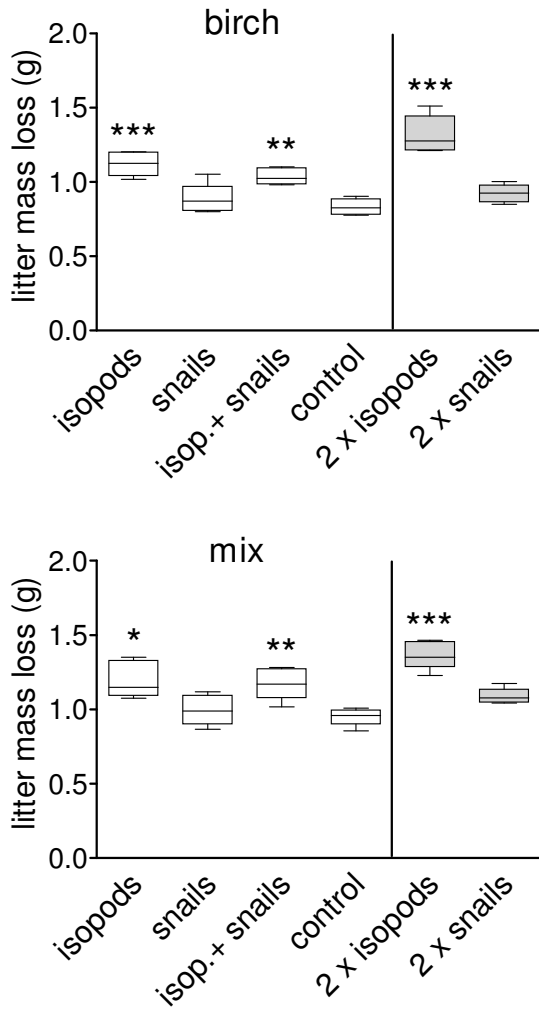


Figure 2.4: Litter mass loss after 112 days of incubation; Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum (N = 7). Double-density-treatments are shown in gray bars. Asterisks indicate significant difference from control (Holm-Sidak post-hoc-test of 2-way ANOVA)

In contrast to the results after 70 days, an effect of litter mixing (Fig. 2.5) was detectable only for high snail abundance after 112 days; here litter mass loss was significantly higher than could be expected from the single-species treatments.

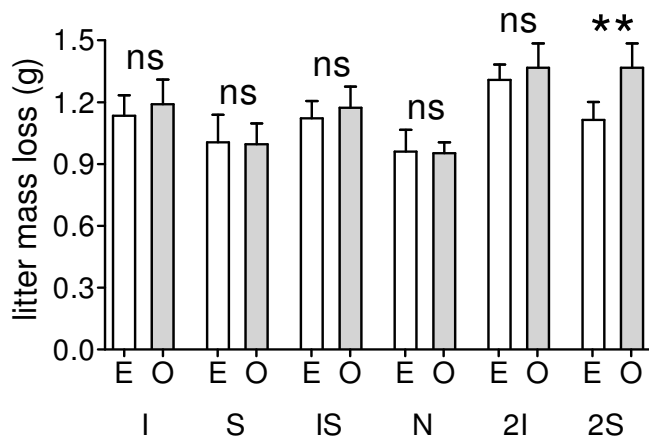


Figure 2.5: Expected (E) and observed (O) litter mass loss of mixed litter after **112 days** of incubation (mean \pm 95 % CI, N = 7). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails. ** : 0.001 < p \leq 0.01; * : 0.01 < p \leq 0.05; (*) : 0.05 < p \leq 0.1; ns: p > 0.1

Bacterial biomass

Little influence of the fauna on bacterial cell numbers was observed after 70 days (Fig. 2.6). The presence of small numbers of detritivores increased the median cell density on birch litter insignificantly. Bacterial numbers on oak litter were about 10 times lower than on birch litter. When mixing the litter, in all cases the observed bacterial cell numbers were below the calculated values (Fig. 2.7). This difference was significant in the combined presence of isopods and snails.

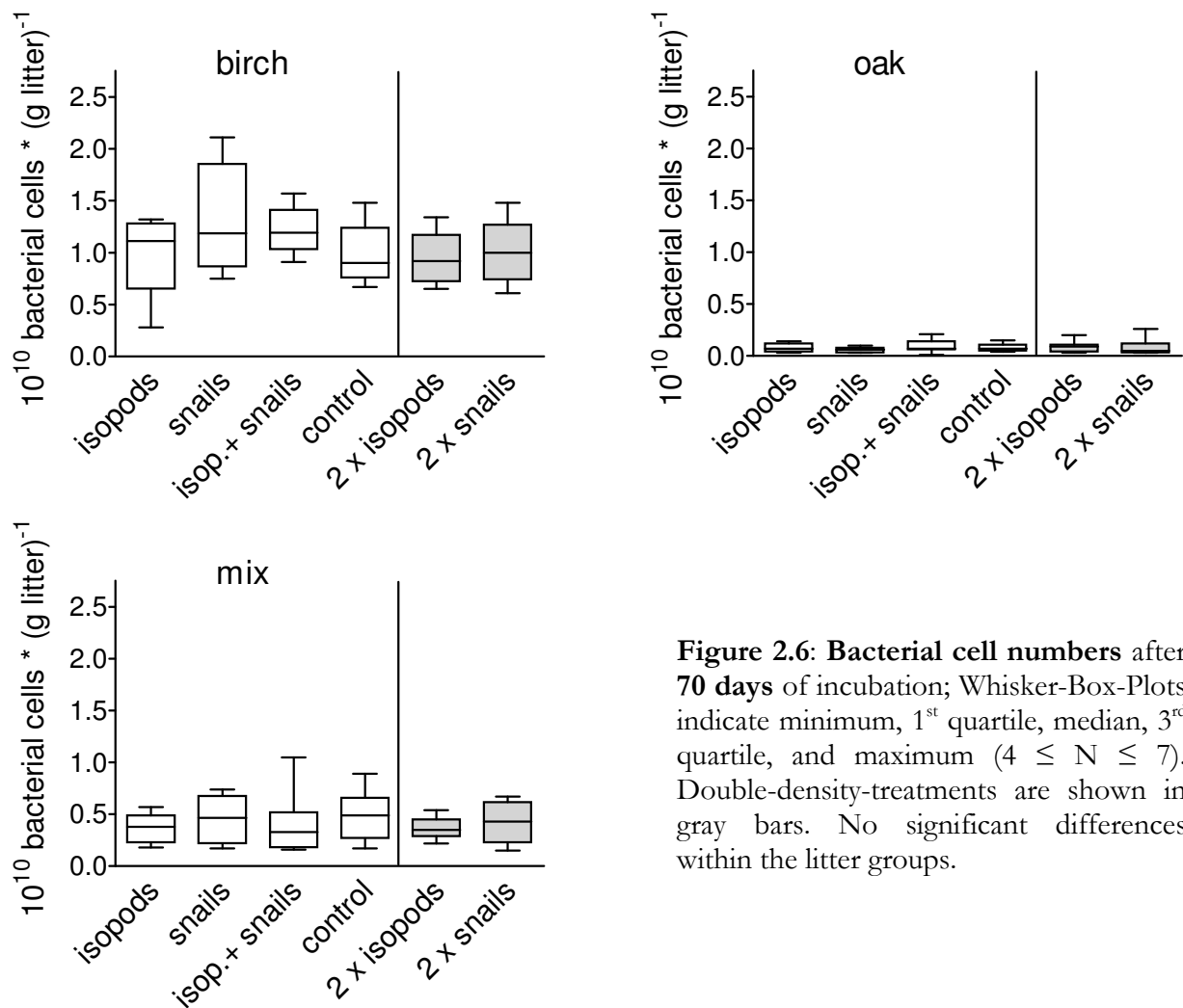
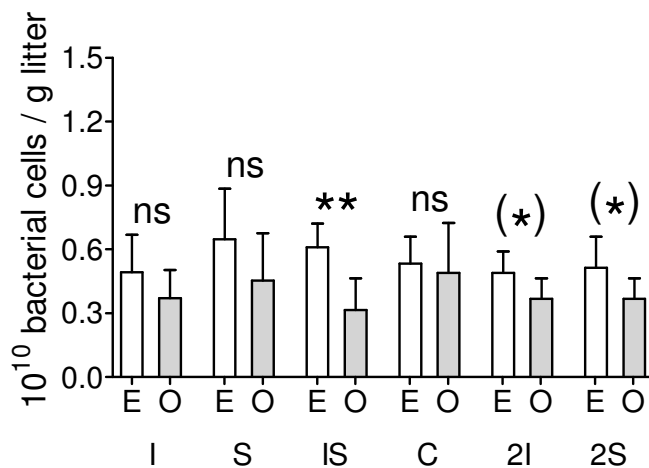


Figure 2.6: Bacterial cell numbers after 70 days of incubation; Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum (4 \leq N \leq 7). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

Table 2.3: Results from Two-Way-Analysis-of-Variance for **bacterial cell numbers** after **70 days**

source of variation	df	ss	ms	f	p
litter	2	11.739	5.87	289.386	< 0.001
fauna	5	0.0677	0.0135	0.667	0.649
litter x fauna	10	0.139	0.0139	0.687	0.735
residual	105	2.13	0.0203		
total	122	14.017	0.115		

**Figure 2.7:** Expected (E) and observed (O) **bacterial cell numbers** of mixed litter after **70 days** of incubation (mean \pm 95 % CI, $4 \leq N \leq 7$). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails.
** : $0.001 < p \leq 0.01$; * : $0.01 < p \leq 0.05$; (*) : $0.05 < p \leq 0.1$; ns: $p > 0.1$

After 112 days of incubation, the faunal impact was still insignificant (Tab. 2.4). Oak litter exhibited a low level of bacterial cells, like after 70 days (Fig. 2.8). On mixed litter, all fauna insignificantly decreased bacterial density compared to the control. All treatments containing mixed litter, including the control, showed lower bacterial numbers than would be expected from the single-species treatments (Fig. 2.9). For four out of six comparisons the difference was significant.

Table 2.4: Results from Two-Way-Analysis-of-Variance for **bacterial cell numbers** after **112 days**

source of variation	df	ss	ms	f	p
litter	2	0.187	0.0934	4.019	0.024
fauna	1	0.0299	0.0299	1.286	0.262
litter x fauna	2	0.00618	0.00309	0.133	0.876
residual	51	1.185	0.0232		
total	56	1.423	0.0254		

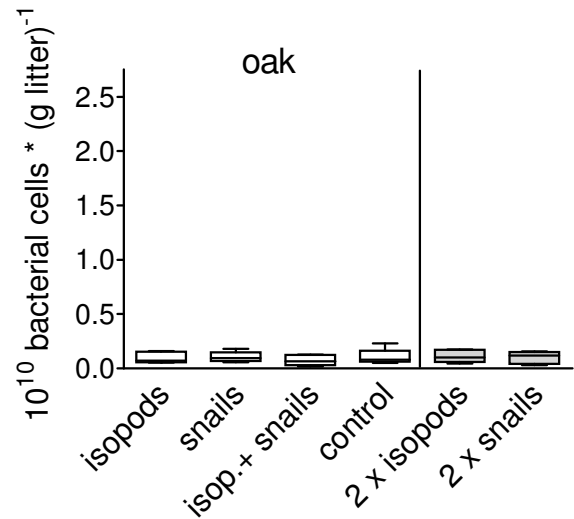
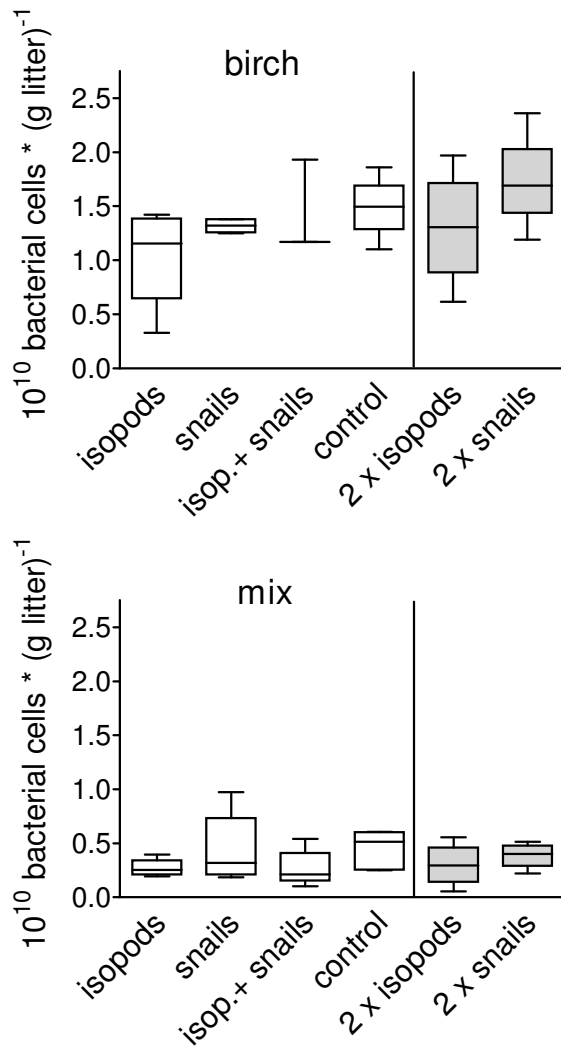


Figure 2.8: Bacterial cell numbers after 112 days of incubation; Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum ($4 \leq N \leq 7$). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

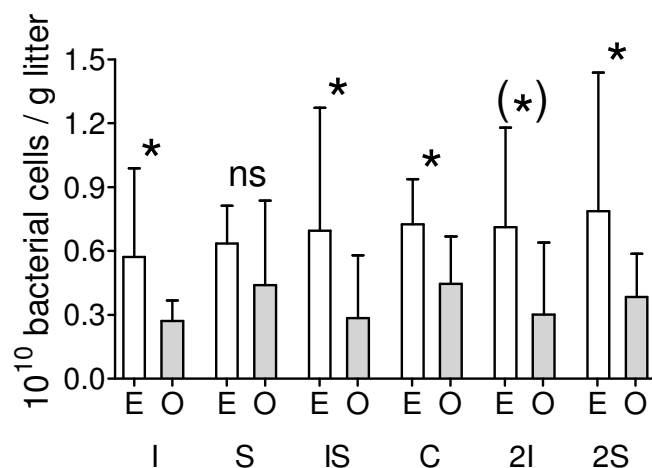


Figure 2.9: Expected (E) and observed (O) bacterial cell numbers of mixed litter after 112 days of incubation (mean \pm 95 % CI, $4 \leq N \leq 7$). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails.
 **: $0.001 < p \leq 0.01$; *: $0.01 < p \leq 0.05$;
 (*): $0.05 < p \leq 0.1$; ns: $p > 0.1$

Fungal biomass

Like bacterial biomass, fungal biomass was higher on birch than on oak litter (Fig. 2.10 + 2.12); the over-all influence of litter identity on faunal biomass was significant (Tab. 2.5). After 70 days of incubation, only a high abundance of snails on birch litter led to

remarkable decrease in fungal biomass. However, the difference to the control was not significant. Neither on oak nor on mixed litter was any impact of fauna apparent (Tab. 2.5). The observed fungal biomass on mixed litter in the presence of mixed detritivores was significantly lower than expected from single-species treatments (Fig. 2.11).

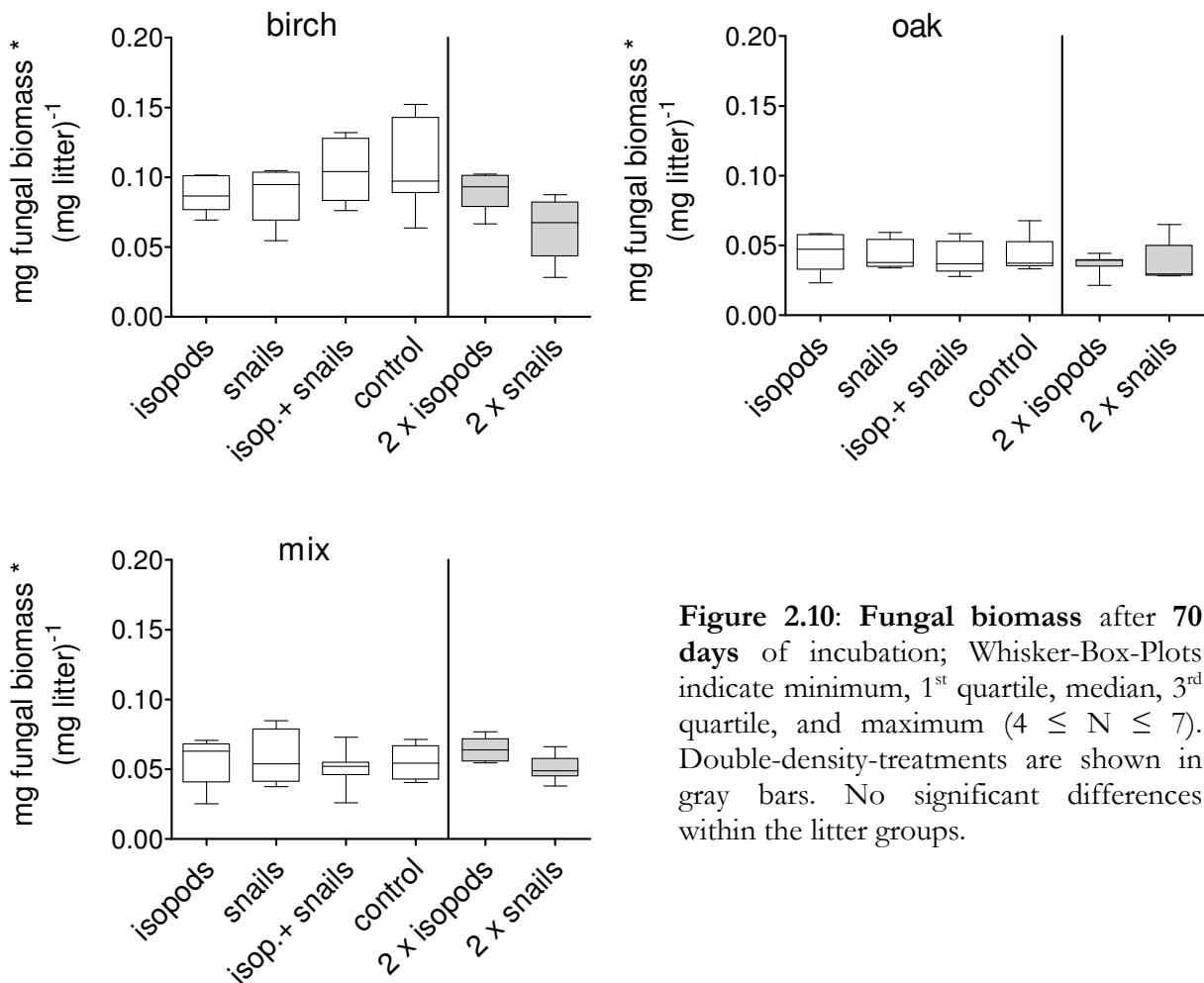


Figure 2.10: Fungal biomass after 70 days of incubation; Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum ($4 \leq N \leq 7$). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

Table 2.5: Results from Two-Way-Analysis-of-Variance for **fungal biomass** after 70 days

source of variation	df	ss	ms	f	p
Litter	2	0.12	0.0602	67.018	< 0.001
Fauna	5	0.00882	0.00176	1.963	0.093
litter x fauna	10	0.00693	0.000693	0.771	0.656
Residual	82	0.0737	0.000899		
Total	99	0.209	0.00212		

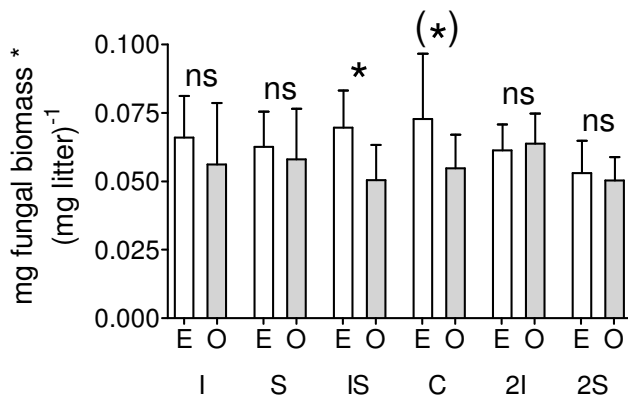


Figure 2.11: Expected (E) and observed (O) fungal biomass of mixed litter after 70 days of incubation (mean \pm 95 % CI, $4 \leq N \leq 7$). I: isopods; S: snails; IS: isopods + snails; C: control; 2I: 2x isopods; 2S: 2x snails.

** : $0.001 < p \leq 0.01$; * : $0.01 < p \leq 0.05$; (*) : $0.05 < p \leq 0.1$; ns: $p > 0.1$

While from 70 to 112 days of incubation, fungal biomass on birch and mixed litter increased, it remained on the same level on oak litter (Fig. 2.12). All fauna influence on birch and mixed litter disappeared during this incubation interval, despite decreased overall p-value (Tab. 2.6). All fauna combinations, in turn, decreased fungal biomass insignificantly on oak litter. After 112 days, expected and observed fungal biomass on mixed litter differed significantly only in the control (Fig. 2.13).

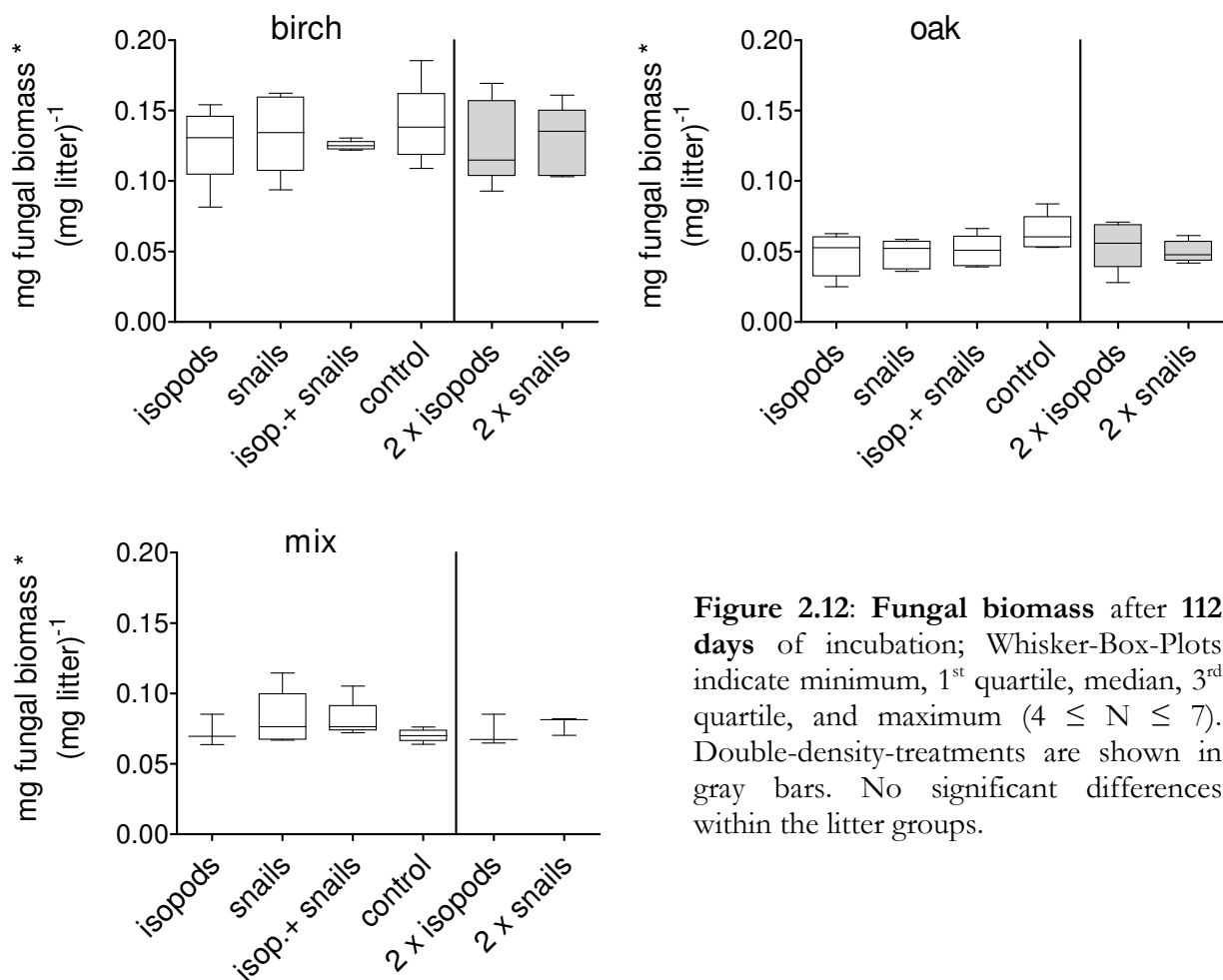
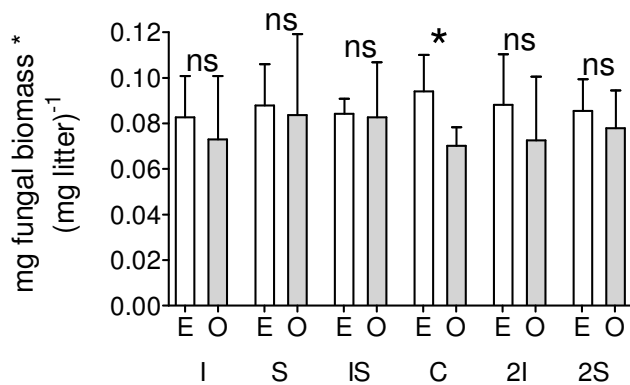


Figure 2.12: Fungal biomass after 112 days of incubation; Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum ($4 \leq N \leq 7$). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

Table 2.6: Results from Two-Way-Analysis-of-Variance for **fungal biomass** after **112 days**

source of variation	df	ss	ms	f	p
litter	2	0.0261	0.013	75.568	< 0.001
fauna	5	0.00189	0.000377	2.188	0.063
litter x fauna	10	0.00153	0.000153	0.89	0.546
residual	82	0.0141	0.000172		
total	99	0.0435	0.00044		

**Figure 2.13:** Expected (E) and observed (O) **fungal biomass** of mixed litter after **112 days** of incubation (mean \pm 95 % CI, $4 \leq N \leq 7$). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails.

** : $0.001 < p \leq 0.01$; * : $0.01 < p \leq 0.05$;
 (*) : $0.05 < p \leq 0.1$; ns: $p > 0.1$

Microbial respiration

Microbial respiration after 70 days exhibited high variability (Fig. 2.14). In general, respiration of birch litter was higher than that of oak litter. The observed pattern to some extent resembled the fungal biomass results; e.g., a high abundance of snails reduced microbial respiration on birch litter. Besides this, no faunal effects were observed (Tab. 2.7).

Table 2.7: Results from Two-Way-Analysis-of-Variance for **microbial respiration** after **70 days**

source of variation	df	ss	ms	f	p
litter	2	1783.875	891.937	34.982	< 0.001
fauna	5	110.052	22.01	0.863	0.509
litter x fauna	10	232.821	23.282	0.913	0.524
residual	99	2524.238	25.497		
total	116	4676.776	40.317		

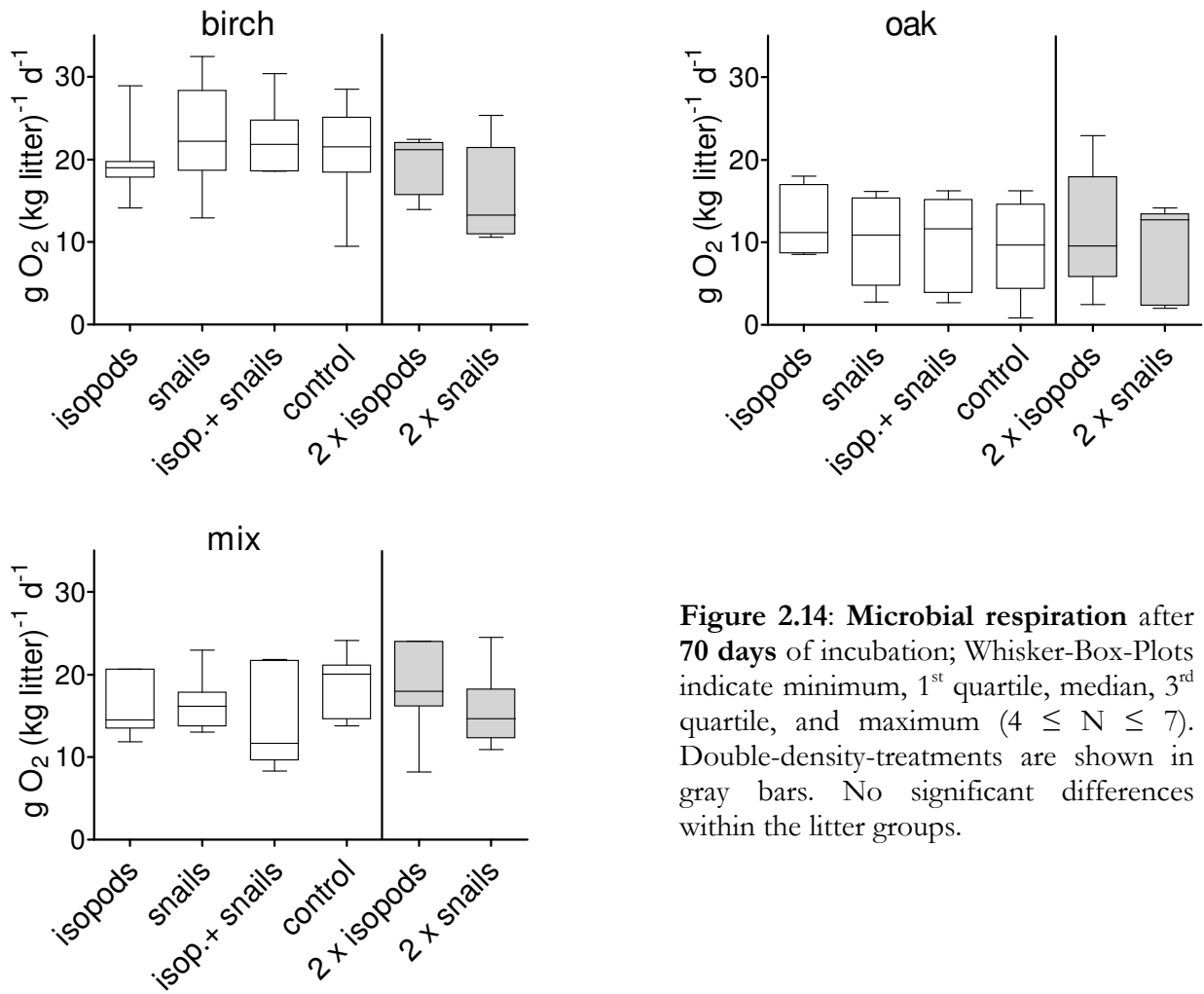
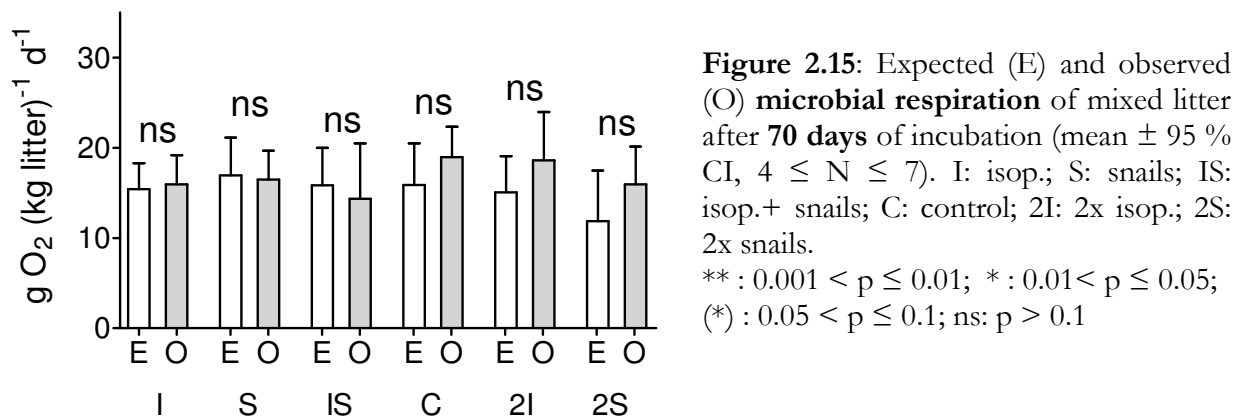


Figure 2.14: Microbial respiration after 70 days of incubation; Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum (4 ≤ N ≤ 7). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

Mixing birch and oak litter did not produce unexpected values of microbial respiration (Fig. 2.15).



Patterns changed from 70 to 112 days of incubation: respiration in general was lower than after 70 days. The same was true for the variability within treatments (Fig. 2.16). The

influence of litter identity was reduced and was not significant anymore (Tab. 2.8). On all substrates, high isopod abundance increased microbial respiration leading to over-all significant faunal influence (Tab. 2.8), but only insignificant pair-wise Post-Hoc comparisons. In general, no significant effects of mixing litter were observed (Fig. 2.17).

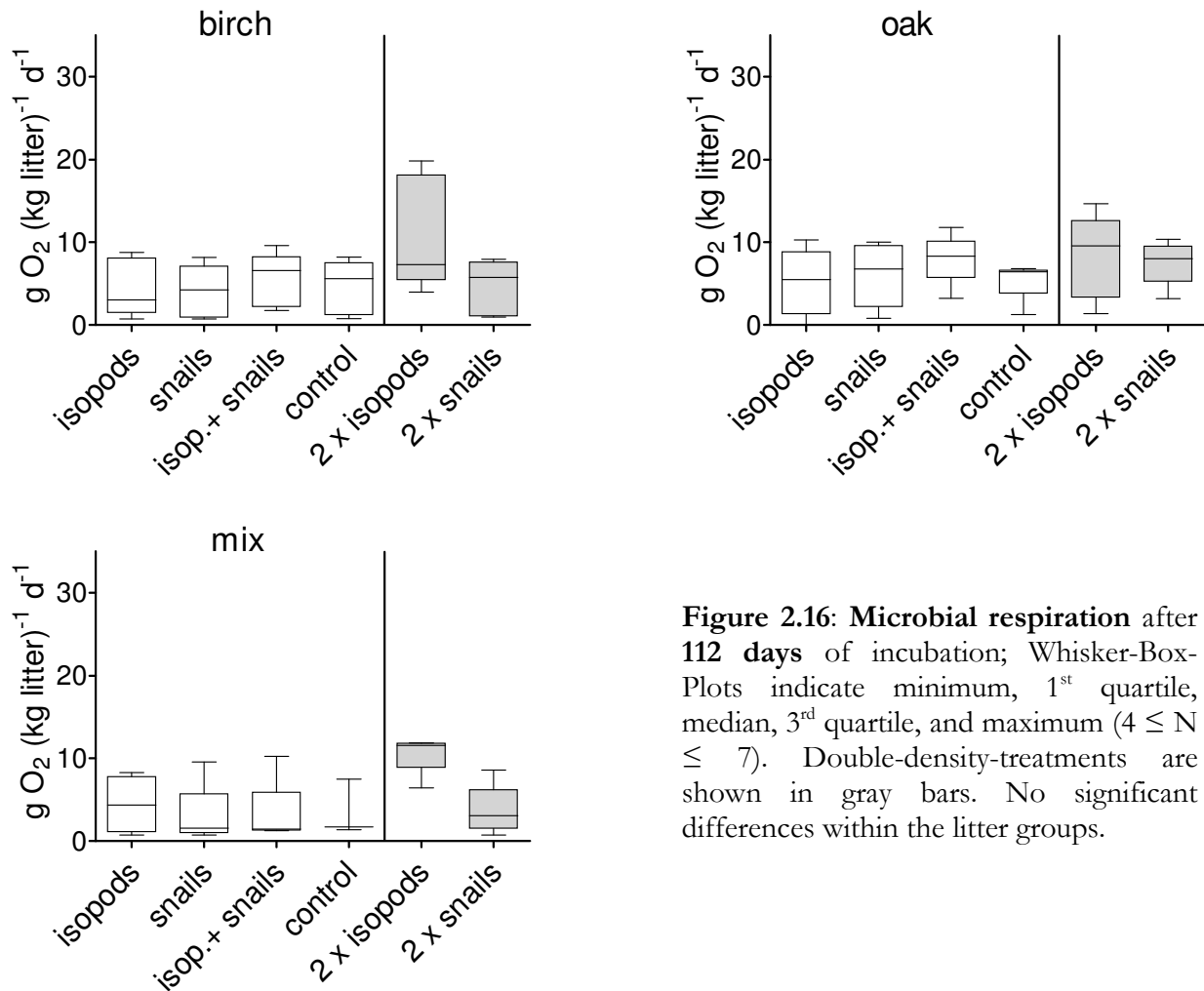


Figure 2.16: Microbial respiration after 112 days of incubation; Whisker-Box-Plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum ($4 \leq N \leq 7$). Double-density-treatments are shown in gray bars. No significant differences within the litter groups.

Table 2.8: Results from Two-Way-Analysis-of-Variance for **microbial respiration** after 112 days

source of variation	df	ss	ms	f	p
litter	2	3.342	1.671	1.955	0.15
fauna	5	10.348	2.07	2.422	0.045
litter x fauna	10	4.713	0.471	0.552	0.847
residual	64	54.691	0.855		
total	81	72.534	0.895		

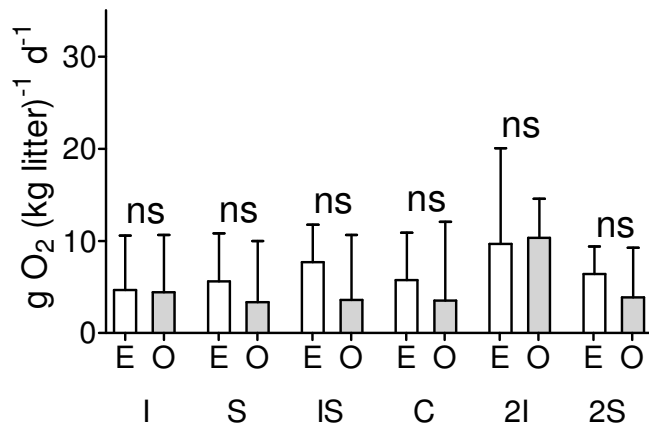


Figure 2.17: Expected (E) and observed (O) **microbial respiration** of mixed litter after **112 days** of incubation (mean \pm 95 % CI, $4 \leq N \leq 7$). I: isop.; S: snails; IS: isop.+ snails; C: control; 2I: 2x isop.; 2S: 2x snails.
 ** : $0.001 < p \leq 0.01$; * : $0.01 < p \leq 0.05$;
 (*) : $0.05 < p \leq 0.1$; ns: $p > 0.1$

Discussion

Summary of the results

Repeating the experiment of Chapter I in the terrestrial environment resulted in distinct effects of the two feeding types on litter mass loss. However, detritivores exhibited little influence on microbial biomass. Indirect effects on the respiration of the microbial community were found under high detritivore density pressure on birch and mixed litter and to less extent on oak litter, after 112 days of incubation (Fig. 2.16). Beyond this, effects of species mixture were inconsistent in all of observed parameters.

Time course of litter mass loss

Litter mass over time in the terrestrial environment of this study was characterized by little change during the first 70 days (Fig. 2.1) followed by rapid decline with rising temperature: after temperature reached 10°C, litter mass decreased dramatically in all treatments, with or without fauna. In the aquatic environment, in contrast, a rapid initial drop of litter mass occurred (Fig. 1.1); followed by a phase of stagnation and only little decline as temperature increased after 70 days. This initial loss of mass can be ascribed to loss of water-soluble substances. Leaching acts instantly on litter – Nykvist (1963) reports a loss of 33 % of all soluble inorganic substances due to leaching within a day. In the terrestrial environment leaves were not soaked and litter was covered only by a thin water film. Thus, leaching of leaf compounds was of little magnitude and, additionally, potentially leached substances remained attached to the surface – the total leaf mass did not change and a leaching impact was thus not detected.

Leaching effect on litter quality

When after about 70 days temperature in the microcosms rose and micro- and macrobiota increased their activity, the quality of the unleached litter in the terrestrial environment was still comparatively high, and biological activity began to decrease litter mass rapidly. In the aquatic environment, in contrast, litter quality at that point in time has already been reduced by leaching of valuable substances and thus, no abrupt mass loss through decomposer consumption (microbial and invertebrate) occurred. Micro- and macro-decomposers did not start reducing litter intensively like they did in the terrestrial

part, because of lower litter quality. At day 112 we found much more litter remains in treatments and controls of the aquatic than of the terrestrial environment. It is likely that micro-decomposers compensated for lower litter quality in the aquatic environment by assimilating DOM, while detritivores consumed the litter-covering biofilm and less litter material, as suggested above (see introduction).

DOM effect on biofilm and biofilm consumption

Leaching of water-soluble substances (DOM) was visible as a brown coloring of the water in the microcosms within a day. After a few days, a smeary biofilm covered the leaves, that was not present to the same extent on leaves in the terrestrial environment. Biofilms in aquatic habitats are not exposed to evaporation and can therefore become structurally and spatially more complex and thicker than biofilms on terrestrial surfaces (Costerton et al. 1995). Consequently, aquatic biofilms are easier for invertebrates to consume. We found a remarkable reduction of surface-attached bacteria in the aquatic treatments by detritivores (due to high deviation in the control, reduction on birch and mixed litter is not significant, but obvious; Fig. 1.6 + 1.8), while this was not the case in the terrestrial environment (Fig 2.6 + 2.8). Consumption of biofilm on submerged surfaces through macrofaunal grazing is common in aquatic systems (e.g. Ward et al. 1998). In the controls of the aquatic and the terrestrial environment about the same amount of bacterial cells were present. This indicates that aquatic detritivores fed on the nutritionally valuable biofilm, while the terrestrial biofilm is obviously of minor importance for detritivores. It is likely that due to the biofilm on terrestrial surfaces being thinner, detritivores can not scrape it off the litter surface as easily as they do on aquatic surfaces. Thus, grazing played a more important role in the aquatic environment.

Litter-specific bacterial biomass

The two litter types differ significantly in litter structural properties. Other than birch, oak litter is stiff with a plain wax-covered epicuticular (Prasad et al. 1990), which is sub-optimal for microbial colonization (Yadav et al. 2005). Consequently, in the terrestrial environment bacterial biomass on oak litter is only about one 10th of that on birch litter. In the aquatic environment much more bacteria colonized on oak litter and the birch:oak bacteria ratio is only 2:1. Water-treated oak litter is soft and thus more suitable for

microorganisms to colonize on than the terrestrial counterpart. Hence, microbial biofilm assemblages on the two leaf types in the aquatic environment are much more alike in terms of bacterial density than in the terrestrial environment. Here, the influence of water balances litter properties as hypothesized above and, consequently, aligns microbial parameters and may thus also align detritivores' feeding habits (see below).

Fungal biomass

Although Golladay & Sinsabaugh (1991) found fungi to be very important structuring elements of biofilms, we found fungal biomass in both environments to be little affected by faunal presence. This can either be due to fast regrowth of fungal biomass or some general difference in fungal and bacterial presence (see Chapter I). The major part of fungal biomass is present as hyphae inside the leaves and only exposes fruiting bodies on the leaf surface (Suberkropp & Klug 1974). Consequently, fungi, in our case, were not consumed along with the biofilm to measurable extent when detritivores grazed the litter surface. When detritivores consumed litter mass, along with it they consumed the litter-pervading fungal mycelium. The ratio of fungi to litter mass remained unchanged. Consequently, we did not measure any fungal reduction even though litter and biofilm was consumed and fungal biomass probably had great impact on detritivore nutrition (Bärlocher & Kendrick 1973, 1975, Arsuffi & Suberkropp 1985, Graça et al. 1993a+b).

Like bacterial biomass the difference in fungal biomass between birch and oak litter appears to be influenced by water. In the terrestrial environment fungal biomass on birch litter is about 2-3 times higher than on oak litter (Fig. 2.10 + 2.12). In the aquatic environments, in contrast, fungal biomass on birch and on oak litter is on the same level (Fig. 1.10). Leaf characteristics that may govern fungal processing like phenolic compounds, nitrogen, carbon-to-nitrogen-ratio, and leaf toughness were found to only weakly predict aquatic decomposition (Ostrofsky 1997), since, as we conclude, these properties are equalized in the aquatic environment. In addition, the fungal species compositions of different litter types in aquatic habitats have been found to be very similar (Tiwari et al. 1994). Again, the presence of water balances decomposition parameters – in this case fungal biomass. These findings in combination with our results make us conclude, that the aquatic environment balances fungal biomass like suggested for bacterial biomass before.

Fungi and bacteria interaction and succession

While bacterial biomass in the controls was roughly the same in the terrestrial and the aquatic environments (Fig. 1.6 + 1.8; Fig. 2.6 + 2.8), fungal biomass was 2-3 times higher in the terrestrial environment (Fig. 1.10 + 1.11; Fig. 2.10 + 2.12). The colonization of plant litter by fungi and bacteria and the subsequent symbiosis in decomposing systems is well researched. In the beginning of the experiment litter was inoculated with terrestrial fungi and bacteria that had started colonizing the leaves, while these were still attached to the tree. Submersion in pond water exposed the leaves to a different propagule spectrum. Aquatic and terrestrial fungi and bacteria started competing for settling space on the leaf surface (Nikolcheva et al. 2004). Terrestrial microorganisms remain alive after submersion, but are impaired in reproduction (Kuehn et al. 2000). Due to the spatial density and the complex substratum, in combination with water as a vector for interspecific pathways, many interactions between aquatic fungi and bacteria were described. These interactions range from very simple to rather complex scenarios over different developmental stages. Little competition, for instance, is suggested by Wohl & McArthur (2001), stating that fungi utilize particulate organic matter (POM), while bacterial nutrition to larger extent depends on dissolved organic carbon (DOC). Gulis & Suberkropp (2003), additionally, found bacteria in the close vicinity of fungal hyphae to benefit from fungal extra-cellular enzymes, while fungi, at certain decomposition stages, excrete antibacterial substances to protect nutritional resources. In earlier stages of colonization Mille-Lindblom & Tranvik (2003) found bacteria to inhibit fungal colonization and growth through the extra-cellular matrix more than vice versa. A synergistic relationship with fungi and bacteria - each growing better in the presence of the other - was observed by Bengtsson (1992). Similar competitive interactions exist in terrestrial decomposition (de Boer et al. 2005), but do, in general, lead to fungal dominance (de Boer et al. 2005). These findings are in accordance with our results of different fungal and similar bacterial biomass in aquatic and terrestrial environments, in particular when fungi are more dependent on POM (detritus), which is reduced in quality in the aquatic environment (see above).

Notwithstanding this diversity of interaction patterns, the general succession of microorganisms in decomposition processes is well accepted. In the beginning easily accessible compounds are present in the litter to large extent. These substances are

consumed by fast-growing microorganisms (Bengtsson 1992, Berg & Laskowsky 2006). In later stages of decomposition, when easily accessible compounds decrease and refractory compounds remain, microbial community changes to slow-growing species specialized on degrading recalcitrant high-molecular compounds like lignocellulose complexes (Bengtsson 1992, Berg & Laskowsky 2006). Since degradation of these structural compounds under ambient conditions is slow, oxygen consumption of microbiota is low. When in the last stages of decomposition structural integrity of detritus is lost, planar substratum for fungal colonization declines and bacterial decomposers come to dominance (Suberkropp & Klug 1976, Bengtsson 1992).

Comparison of decomposition stage

In the present study the first phase of litter decomposition in the aquatic environment was short, since easily accessible substances were quickly leached out of the leaves into the water column. We can assume that the microbial community in the aquatic environment very soon changed to slow-growing fungal species inside the leaves consuming little oxygen and, as stated above, to a massive bacterial biofilm, consuming DOM from the water column. In the terrestrial environment, decomposition at the end of the experiment was in a much earlier stage, where fungal activity was still high due to higher litter quality. Our data showing low fungal biomass and low respiration in the aquatic and high fungal biomass and high respiration in the terrestrial environment support these assumptions. Oxygen consumption by bacteria is in general negligible, since bacteria represent less than 10 % of the microbial biomass in aquatic and terrestrial decomposition (Findlay & Arsuffi 1989, Mille-Lindblom & Tranvik 2003, Carter & Suberkropp 2004, de Boer et al. 2005). From the fungal and bacterial data we conclude that leaching shortens the initial phase of litter decomposition and dislocates the degradation of easily accessible compounds in the form of DOM out of the leaves into the water column.

Detritivore feeding mode

At the end of the experiment litter mass loss through non-faunal activity (control) was significantly different between birch and oak in the terrestrial environment ($p = 0.001$, Mann-Whitney test; Fig. 2.4). Surprisingly, loss of oak litter was higher. However, in the

aquatic environment no difference was found ($p = 0.18$, Mann-Whitney test; Fig. 1.4). Here, the impact of water neutralized differences in litter properties that were responsible for dissimilar mass loss in the terrestrial environment. These litter properties are likely to be equalized by maceration and leaching, making both substrata similar in their ability to support microbial colonization and in their low nutritional value for microbial consumers. Although mass loss of oak litter through microbial activity and leaching in the controls of the terrestrial environment was higher than of birch litter, detritivores had significant impact on mass loss of birch litter and less so of oak litter (Fig.2.4). While terrestrial isopods reduced birch litter mass significantly, snails did not. In the aquatic environment there was no difference in litter consumption by snails and isopods (Fig. 1.4). Additionally, in the terrestrial environment isopods reduce litter mass remarkably only on birch litter, while in the aquatic environment isopods reduced mass of both litter types. Strength and toughness of food item were found by Pennings et al. (1998) to be an important marker predicting invertebrate litter consumption rates. These characteristics, contrasting in birch and oak litter, were leveled through litter submersion as we state earlier. A small grazing gastropod's radula is optimized for scraping off epibionts rather than comminuting tough substratum. It is conceivable that snails do acquire the ability to comminute an initially tough substratum, if this has been macerated through water treatment. Accordingly, while isopods in the terrestrial environment feed by shredding leaf tissue, in the aquatic environment they additionally feed by grazing the litter covering biofilm. Consequently, we hold that the specificity of feeding types in terrestrial environment is neutralized by the presence of water.

Species mixtures

The influence of a concert impact of different species on ecosystem processes has been in the scientific focus for some time. What emerged was the opinion that it is rather species identity than species number that is responsible for altered mixture performance (Cragg & Bardgett 2001, Jonsson & Malmqvist 2003, Covich et al. 2004, Dangles & Malmqvist 2004, Hättenschwiler 2005, Duarte et al. 2006, LeRoy & Marks 2006, Wardle 2006). Here, species identity is represented by the instance of feeding type and leaf litter characteristics and their implications. We found different feeding and litter types to affect decomposition processes more (terrestrial) or less (aquatic) differently. It can be expected that different

processing pathways add to, subtract from, or influence each other in a mixture of litter or detritivores. For all possible interaction effects there are reports in literature (see introduction). Here, we found increased microbial activity induced by the presence of isopods on mixed litter in aquatic environments compared to the corresponding control after 70 days of incubation. After 112 days this effect was less pronounced and respiration was also increased in snails-treatments, but the effect was still highest in the presence of a high abundance of isopods on mixed litter. In the terrestrial environment, in contrast, this increased microbial respiration did not occur after 70 days. After 112 days, however, respiration was increased by a high isopod abundance on birch and to very small extent on oak litter but most clearly on mixed litter (Fig. 2.16, all comparisons not significant). The fact that this effect occurred in the aquatic environment after 70 days and in the terrestrial environment after 112 days is another support for the afore-mentioned suggestion, that the initial decomposition phase is shortened in the aquatic habitat. Furthermore, the occurrence of this effect in both environments is a strong confirmation that we chose two comparable model systems. More important, however, is that we see here the fleeting character of species mixture effects. Upon rising temperature microbial activity first increased in the isopod treatments on mixed litter before it could also be observed in the other treatments. The mixture of litter appears to be favorable for increased microbial activity by isopod presence only at a certain stage in decomposition. Later in decomposition process the species-mixture-specific character gets lost. We can not state that this succession was similar in the terrestrial environment: we observed an isopod-induced increase of microbial respiration after 112 days on all litter substrates. Hättenschwiler (2005) elaborates three possible mechanisms leading to litter diversity effects for terrestrial interactions that are in the same way applicable to aquatic environments. First, higher litter species richness leads to higher micro-habitat richness favorable for microinvertebrate colonization. Second, specific litter compounds, like some polyphenoles, present in virtually all litter species, may be involved in antagonistic or synergistic effects on decomposition of litter mixtures by influencing processes. And third, the presence of fast-decomposing high-quality litter may stimulate the decomposition of low-quality litter by nutrient transfer from low- to high-quality litter. In our case the microbial (most likely fungal) respiration was influenced. When isopods stimulate fungi by mycelium consumption, fungi might find better growth conditions in a

higher surface heterogeneity (micro-habitats) in litter mixture. Further, phenolics and nutrient transfer mediated by isopods may act stimulatingly on the fungal community resulting in the observed pattern. Hättenschwiler & Gasser (2005) found soil fauna to determine the magnitude and direction of litter diversity effects. They argue that litter mixture might change faunal consumption rate leading to shifted impact on microbial biomass. However, Hättenschwiler (2005) also states that interactions between litter diversity and macrofauna are very poorly studied, yet, and mechanisms are poorly understood.

Other than this stimulating effect of isopods on mixed litter no pattern in species mixing could be observed. Neither in comparison to the control nor in the comparison of expected and observed values. Supporting this inconsistency, Covich et al. (2004) found indications that effects of biodiversity change are highly variable over space and time. This is probably due to the inference of synergistic, antagonistic, and additive effects acting at the same time (Hättenschwiler 2005). However, we found more significant differences in expected versus observed values in the terrestrial than in the aquatic environment. As stated before, in the aquatic environment differences in several aspects of decomposition are dampened by water influence. This surely also results in less mixing effects, which we observed in the aquatic environment.

Conclusions

The chosen model systems appear to be well suited for comparisons of aquatic and terrestrial decomposition process parameters. Our results show fundamental differences in litter processing that can very well be attributed to water impact on detrital food source. Two processes induced by the aquatic environment are casual for the observed differences: leaching of water-soluble compounds and maceration or softening of leaf tissue. These processes alter the basic properties of leaf litter: nutrient content and leaf tissue toughness. The aquatic environment equalized these properties, reducing litter species specificity, while in the terrestrial environment differences in litter quality persist. Consequently, differences in litter mass loss, fungal biomass, bacterial biomass, and consumption of litter and bacterial biomass by detritivores are higher between litter species in the terrestrial than in the aquatic environment. This implies a shift in the

feeding mode of detritivores towards a more grazing feeding habit in the aquatic environment.

Indirect effects of macrofaunal presence, which emerged as increased microbial respiration in the aquatic environment were also found under the same conditions in the terrestrial environment, indicating that the underlying mechanisms are system-independent. This is in particular interesting, since these effects were well pronounced on mixed litter substratum in both systems.

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Effects of detritivores and species composition on
salt marsh litter degradation

Chapter III

Introduction

In the aquatic environment, strong leaching, litter maceration by water and microbial load govern detritivore feeding activity (Chapter I). In the terrestrial environment, by contrast, higher nutritional value of litter and less well accessible biofilm determine consumption rates of detritivores (Chapter II). In salt marshes, other than in purely aquatic or terrestrial systems, we find aquatic and terrestrial conditions alternating, and detritus to be exposed to frequent and periodic wetting and drying. These cycles exert strong physical impact on the detritus, especially in areas where temperature and solar radiation during summer are high. Leaching of water-soluble substances from detritus under these conditions is enhanced compared to the terrestrial environment (Newell et al. 1996, Bouchard & Lefeuvre 2000, Anderson & Smith 2002), reducing the nutritional value of litter for potential consumers like in the aquatic environment (Chapter I). However, the physical disruption of detritus and the frequent moistening support fast microbial colonization (Newell et al. 1996), rendering conditions different from the aquatic environment. Detritus with high microbial load, in turn, is of high nutritional value for detritivorous consumers (Bärlocher et al. 1989, Newell 2001, Buchan et al. 2003). Thus, we encounter two counteracting processes that determine detritus quality as food source for detritivores and justify a closer look at decomposition of salt marsh litter under these aspects.

Most of the experiments performed to elucidate consumption and feeding preference of salt marsh detritivores were done in the laboratory or in litterbag experiments. These results are not always directly applicable to field conditions since they were produced in a simplified environment (compare Samiaji & Bärlocher 1996, Castor & Freitas 2000). In particular, litter substratum in laboratory experiments is not exposed to the harsh field conditions with the above-mentioned consequences for litter leaching. With the present experimental setup, we investigated detritivore consumption of salt marsh litter under near-natural conditions using enclosure cages in the field that provide more natural conditions than litterbags, in which detritus and detritivores are usually packed densely.

Information on the influence of species richness on decomposition processes in salt marsh systems is rare (compare Zimmer et al. 2004), despite the fact that salt marsh food webs are regarded detritus-based (Teal 1962, Nixon & Oviatt 1973, Valiela & Teal

1979, Silliman & Zieman 2001). A lack of knowledge exists for influence of species richness of detritivores, but also for species richness of detritus. Zimmer et al. (2004) provided valuable information from laboratory experiments, suggesting that salt marsh detritivores are not functionally redundant. Shedding light on the interaction of detritivores and plant litter of different origin in decomposition in the field is one objective of the present study.

Much of our knowledge on trophic interactions in salt marsh systems has been gained from experiments in salt marsh areas of the Atlantic coast of North America. In particular, the trophic interactions in salt marshes on Sapelo Island, Georgia, are well investigated which makes this place a model system for salt marsh research (e.g. Silliman & Zieman 2001, Newell 2002, Silliman & Bertness 2002, Zimmer et al. 2002, 2004, Sharitz & Pennings 2006). The most abundant detritivore here is the Periwinkle *Littoraria irrorata*, which feeds on standing senescent and fungally pervaded leaves of the smooth cordgrass *Spartina alterniflora* (Bärlocher et al. 1989). In the upper marsh the coffee-bean snail *Melampus bidentatus* feeds on detritus-covering biofilms (Lee & Silliman 2006), while on the upper marsh fringe we find the omnivorous warf crab *Armases cinereum* (Zimmer et al. 2004). Due to high production of plant biomass, resulting in a high detritus load, the influence of these potential detritivores in the field is of interest (Pennings et al. 1998, Buck et al. 2003, Graça et al. 2000, Newell 2001).

Decomposition of leaves of the marsh-dominating cordgrass is well understood (e.g. Newell et al. 1989, Samiaji & Bärlocher 1996). Senescent parts get quickly pervaded by mycelial fungi and are consumed by *Littoraria* while the leaves are still attached to the plant. The stems of *Spartina*, however, decompose slowly and can build up vast mats in the marsh when detached and accumulated by tidal and storm activity. Little is known about the role of salt marsh detritivores in degradation of these recalcitrant plant parts.

Against the background of the salt marsh-specific environmental regime acting on both litter and its consumers, we aim at answering the following questions:

1. Does the presence of detritivores in natural densities alter salt marsh plant detritus mass loss or bacterial numbers under natural field conditions?
2. Does mixing of three abundant litter types in the presence of the dominant detritivore have non-additive effects on litter mass loss or bacterial numbers compared to litter monocultures?

3. Does mixing of three common salt marsh detritivore species have non-additive effects on *Spartina alterniflora* stem mass loss or bacterial numbers compared to single detritivore cultures?

To ensure near-natural conditions we conducted the experimental series in enclosure cages in the high marsh. This allowed for direct contact of the enclosed litter and detritivores to marsh soil as the source for microbial infection. The target parameters were litter mass loss as most important marker for litter decomposition and bacterial load of litter surface as marker for grazing on litter surface biofilm by detritivores.

Material and Methods

Experimental setup

The experiment was conducted on Sapelo Island in Georgia/USA (31.3900°N, 81.2883°W). The experimental field site was located at Dean's Creek, a shallow slope salt-marsh of about 0.7 km². The cages were constructed of plastic garden fencing, a 1.5 m plastic tube of 2 cm diameter, and black rubber-coated fiber insect gaze of 0.5 mm mesh size. The plastic fencing was formed into a ring of ~35 cm diameter. The gaze was sewed into a tube fitting tightly onto the plastic ring and was wrapped around it at the bottom. The ring was completely dug into the soil. In the center of the ring, the plastic tube was stuck into the soil 80 cm deep and the net was wrapped above the top of the tube with a cable binder to form a tent-like cage of 70 cm height.

As litter substrate, senescent leaves of *Juncus roemerianus* SCHEELE, *Quercus virginiana* MILLER, and dead stems of *Spartina alterniflora* LOISEL were used. Litter was collected from plant stands of the adjacent area, air dried and weighed to single- and two-species sets of 20 g in every possible combination. Animals were collected from the same area and kept in the lab until use in the experiment. These were two snails and a decapod crab: *Melampus bidentatus* SAY, *Littoraria irrorata* SAY, and *Armases cinereum* BOSC, respectively. A ratio of fresh weight to soft body water content was determined for each species. Animal biomass was equalized according to these ratios based on the common density of *L. irrorata* at Dean's creek of 100 snails per m². The soft body water content of 9 medium sized *L. irrorata* equaled that of 45 medium sized *M. bidentata*, and that of 2 medium sized *A. cinereum*. All possible one- and two-species litter sets were combined with 9 *L. irrorata*, and all zero-, one-, and two-species detritivore combinations were added to a single litter species (20 g of *Spartina alterniflora*, as the dominant salt-marsh plant at the site). The design was replicated 7 times making 91 setups.

The cages were placed in the upper salt-marsh in a *Batis maritima* stand ~2.1 m ± 0.05 m above mean low water level (leveled with a theodolite). All vegetation under the cages was removed and cages were placed in a rectangular design with 50 cm distance each. During the experimental run of 3 months the site was inundated about 50 times with a maximum level of 45 cm above the soil level.

After 3 months, cages were sampled for detritivore numbers and litter mass. Sub-samples of litter were taken for bacterial cell counting after DAPI-staining and for

freshweight:dryweight ratio. Residual litter was oven-dried to constant weight to obtain total dry weight. Sub-sample dryweight was calculated from freshweight:dryweight ratios. Due to strong weather and tidal regime, some cages were destroyed or animals missing. These cages were not included into the analyses.

Bacterial biomass

For determination of bacterial cell numbers, litter sub-samples of about 0.1 g dryweight were stored in 1.5 mL of 4 % formaldehyde. For bead-beating, sample plus storage medium were transferred into a 15 mL plastic tube together with 8.5 mL of 4 % formaldehyde. For detaching bacteria, 5 glass beads (5 mm) were added to the vials before shaking for 10 minutes on a horizontal shaker at 420 rpm. After letting stand for 10 min, 0.5 mL of the supernatant were mixed with 6 mL ddH₂O and filtered through a 0.2 µm polycarbonate filter (25 mm; DHI, Hørsholm, Denmark). Litter discs were dried at 60°C for 1 h and weighed. After staining for 10 min with DAPI-staining solution (1 µg/mL), the filter was washed first in ddH₂O then in 96 % ethanol and dried on lab tissue for one minute. It was then covered with one drop of CITIFLUOR solution (Citifluor Ltd., London/GB) to prevent quick fading of staining intensity. From each filter, photos of 3 different sections were taken with a digital camera using an epifluorescent microscope equipped with a DAPI-staining-specific optical filter set at 1000x magnification. Because of high background noise, the photos needed to be optimized before automated bacteria counting. For this purpose, a software routine was written (using freeware Dev-C++-compiler, Bloodshed-Software) that increased image-contrast of blue DAPI-stained bacterial cells. Stained cells were then counted using the VisualBasic-based macro language interpreter of SigmaScan image processing software (SigmaStat Software Inc., San Jose, USA). From a counted area of 80 µm x 80 µm, bacterial cell number in the sample was calculated as the arithmetic mean of counts of the 3 filter sections.

Statistical analyses

Diagrams of data were created using PRISM 4.0 (GraphPad Software). Data were analysed using one-way-ANOVA (PRISM 4.0; GraphPad Software) to test for an influence of litter and detritivore identity. Raw data were ln-transformed to meet ANOVA requirements of normal distribution and homogenous variances. In case of a

statistically significant difference, a Tukey's multiple comparison post-hoc test with pair wise comparisons was performed on the data set to identify the source of difference.

To clarify whether mixing of litter or detritivores influenced decomposition parameters, expected values ("E") were calculated from values of single-species type treatments and compared with the observed values ("O") of mixed-species treatments. Replicates of the treatments were randomly assigned and for expected litter mass loss the arithmetic mean was calculated:

$$E_e = \frac{S1_e + S2_e}{2} \quad \text{with } e \in [1..7]$$

$S1_e$: litter mass loss in replicate of species 1 treatment
 $S2_e$: litter mass loss in replicate of species 2 treatment
 Pairs of $S1_e$ and $S2_e$ were randomly assigned.

For bacterial cell numbers on mixed litter, according to Wardle et al. (2006), the expected values were calculated with respect to litter mass loss of each of the litter types to take into account the different litter composition at the end of the experiment:

$$E_e = \frac{rS1_e \cdot PS1_e}{rS1_e + S2_e} + \frac{rS2_e \cdot PS2_e}{rS1_e + S2_e} \quad \text{with } e \in [1..7]$$

$rS1_e$: remaining litter mass species 1
 $rS2_e$: remaining litter mass species 2
 $PS1_e$: bacterial biomass replicate of species 1
 $PS2_e$: bacterial biomass replicate of species 2

Data sets of expected and observed values were statistically compared using the Mann-Whitney U test for non-parametric data.

Results

Mass loss

Litter mass loss after 3 months of incubation was highest in the case of *Juncus roemerianus* (Fig. 3.1). In the single-species treatments, *Juncus* litter lost about 62 % of its initial weight. *Spartina alterniflora* and *Quercus virginiana* lost ~26 % and ~16 %, respectively.

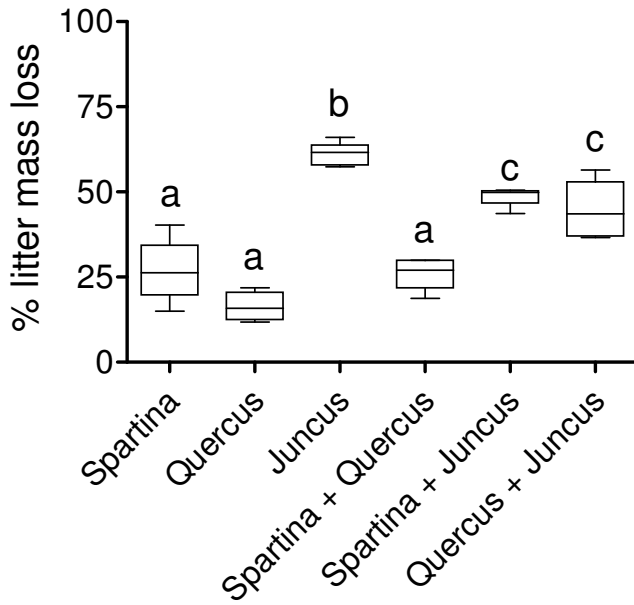


Figure 3.1: Percent **total litter mass loss** of one- and two-litter species treatments after 12 weeks of incubation in the presence of *Littoraria irrorata*. Different letters indicate significant difference (One-Way ANOVA, $p < 0.001$, $3 \leq N \leq 7$).

In the two-litter-species treatments, three groups can be identified according to ANOVA results (Fig. 3.1). All *Juncus* treatments exhibited significantly higher mass loss than non-*Juncus* treatments, with sole-*Juncus* treatment being still significantly higher in mass loss than *Spartina*-*Juncus*- and *Quercus*-*Juncus* treatments.

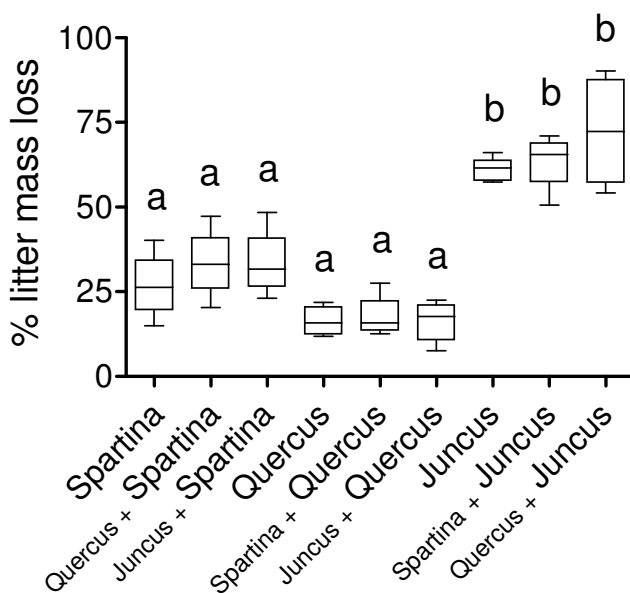


Figure 3.2: Percent **species specific litter mass loss** of one- and two-litter species treatments after 12 weeks of incubation in the presence of *Littoraria irrorata*. Different letters indicate significant difference (One-Way ANOVA, $p < 0.0001$, $3 \leq N \leq 7$).

Figure 3.2 shows litter mass loss specifically for each species. Both *Spartina*- and *Juncus*-litter mass loss was increased in the presence of other litter types, though not significantly. *Quercus*-litter mass loss was not influenced by any of the litter combinations. Thus, species-specific litter mass loss was never decreased in two-litter treatments for any of the litter species.

Figure 3.3 compares the observed litter mass loss of the two-species treatments with the expected mass loss estimated from the single-species treatments. Litter mass loss was in all cases higher than expected, albeit none of the differences were significant. However, there was non-additive effect of all of the litter species combinations.

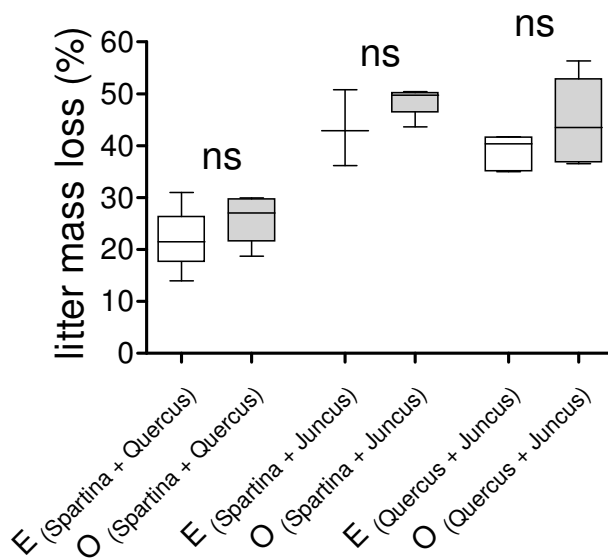


Figure 3.3: Calculated, expected (E) litter mass loss versus observed (O) in the two-litter species experiment. No significant difference found (non-parametric Mann-Whitney U-test)

In the second experimental series, the faunal influence on *Spartina alterniflora* litter mass loss was investigated. We did not find any significant influence of detritivores on the litter mass loss at all (Fig. 3.4), although a clear trend is visible ($p = 0.063$). Median mass loss under the influence of *Littoraria irrorata* was 1.5 times higher (38 % versus 25 %; Fig. 3.4) than in the control with no fauna included. In contrast, the median mass loss in the sole presence of *Armasas cinereum* was 20 % below the control (Fig. 3.4).

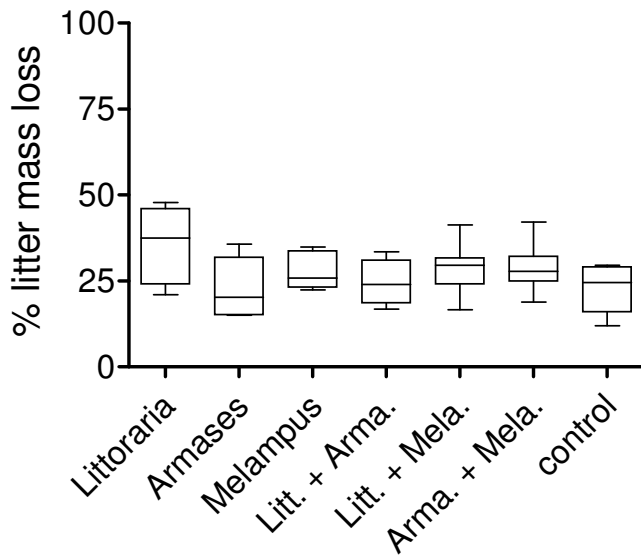


Figure 3.4: Percent litter mass loss of *Spartina alterniflora* after 12 weeks of incubation in one- and two-detrivore species treatments. No significant differences found (One-Way ANOVA, $p = 0.063$, $3 \leq N \leq 7$).

Like for the two-litter species experiments, no significant difference in the two-detrivores species treatments were found between expected and observed litter mass loss (Fig. 3.5). There was no non-additive effect of any of the detrivore species combinations.

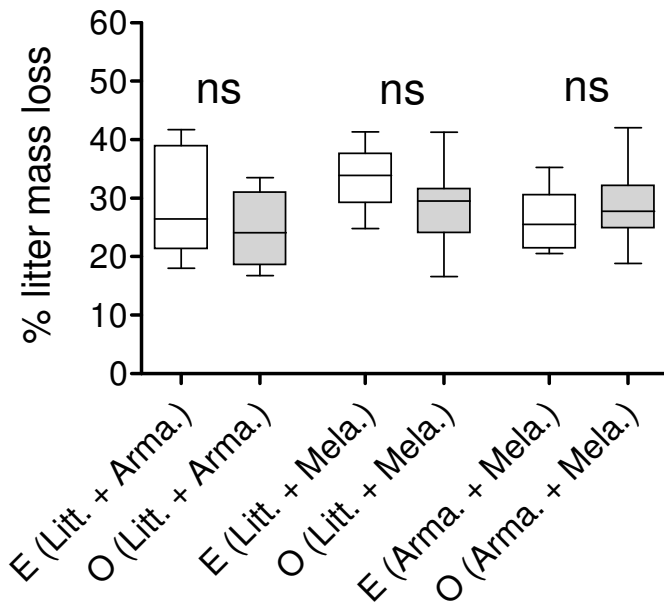


Figure 3.5: Calculated, expected (E) litter mass loss versus observed (O) in the two-detrivores species experiment. No significant difference found (non-parametric Mann-Whitney U-test)

Bacterial biomass

After 3 months of incubation, bacterial biomass on the litter surface showed high variability within the treatments (Fig 3.6). Bacterial density was highest on *Juncus* litter. Mixtures containing *Quercus* were strongly reduced in bacterial density on *Quercus* leaves compared to sole-*Quercus* treatments.

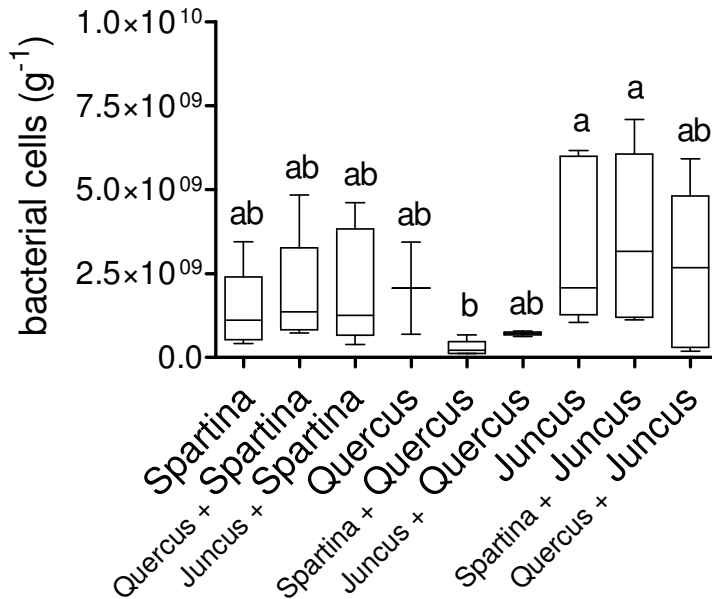


Figure 3.6: Bacterial density of one- and two-litter species treatments after 12 weeks of incubation in the presence of *Littoraria irrorata*. Different letters indicate significant difference (One-Way ANOVA, $p = 0.02$, $3 \leq N \leq 7$).

Due to the high variability within groups, calculation of expected versus observed value was not reasonable.

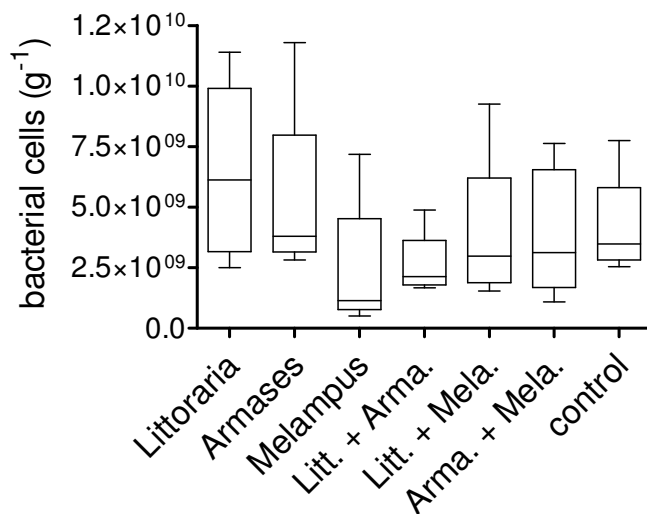


Figure 3.7: Bacterial density on *Spartina alterniflora* after 12 weeks of incubation in one- and two-detritivore species treatments. No significant differences found (One-Way ANOVA, $p = 0.42$).

Bacterial density on *Spartina* stems was reduced by *Melampus bidentatus* and increased by *Littoraria irrorata* compared to the control, though insignificantly in both cases. The presence of *Armasas cinereum* did not influence bacterial numbers (Fig. 3.7).

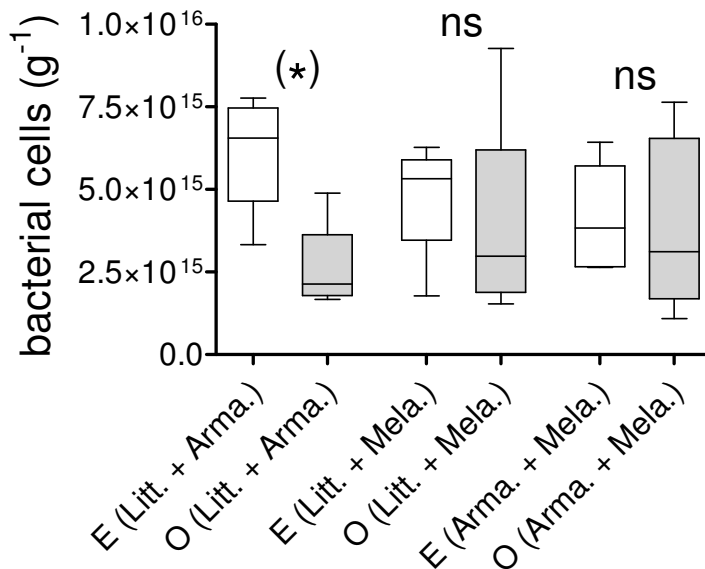


Figure 3.8: Calculated, expected (E) **bacterial density** versus observed (O) in the two-detritivores species experiment. (non-parametric Mann-Whitney U-test)

All mixtures of detritivores caused lower bacterial density than would be expected from the single-species treatments (Fig. 3.8). *Littoraria* and *Armasas* did not reduce bacterial density in the single-species treatments compared to the control (Fig. 3.7); but when applied together, bacterial biomass suffered high reduction compared to the expected density.

Discussion

General findings

We found surprisingly low influence of the fauna on litter mass loss of cord grass stems. In contrast, the influence of litter species on litter mass loss was high. Mixing litter species had an insignificant but conservative influence on litter mass loss. Additionally, despite high variability in bacterial biomass within the treatments, all mixtures of detritivores caused lower bacterial density on litter surface than expected. We discuss these aspects in turn.

Litter properties

In the lab experiments of Chapter I and II, we found an effect of the aquatic environment to be the main source of differences in decomposition of leaf litter in an aquatic-terrestrial comparison. In the present experiment, the decomposing plant material differed remarkably in constitution. Stems of the cord grass *Spartina alterniflora* are stiff and of tight structure with a heterogeneous surface that becomes soft when moistened and subsequently easy-to-colonize by microbiota. Leaves of live oak, *Quercus virginiana*, are rigid with a thick wax layer (personal observation). Unlike *Quercus rubor* (Chapter I + II), they remain stiff after long immersion in water. The black needle rush, *Juncus roemerianus*, has long, thin, needle-like leaves that become soft and flexible when soaked in water; the surface is not textured. At the beginning of the experiment stems of *Spartina* have been detached for presumably several months and leached by salt water before collection. Leaves of *Quercus* and *Juncus* were senescent and standing dead stock, respectively. They were not leached in salt water before collection. During the experiment the site was inundated about 50 times. The marsh soil and the sheltered experimental setup supported longer water retention. We can assume the detritus to have been moist throughout the experiment. This strongly influenced litter palatability for detritivores. Pennings et al. (1998) found litter toughness to be the most important marker for predicting feeding choice of *Armadillos*. While wet *Juncus* litter is soft and can easily be fed on, *Quercus* litter is stiff and recalcitrant; wet *Spartina* stems are moderately soft. Feeding traces were well visible after the experiment on *Spartina* stems but not on *Quercus*.

Detritivores

General impact

Detritus mass loss in the course of the experiment was in accordance with these structural properties (Fig. 3.1). Mass loss in the presence of *Littoraria* was highest on soft *Juncus* litter and lowest on tough *Quercus* litter. To what extent this loss was due to consumption by *Littoraria* is not known. However, the influence of the fauna on *Spartina* mass loss was in no case significant (Fig. 3.4). Zimmer et al. (2004), on the basis of laboratory feeding experiments, calculated potential annual litter consumption of all three detritivores (Tab. 3.1). They performed their study in the same location using the same litter types and detritivore species. When calculating annual consumption rates from the present study based on detritivore densities given in Zimmer et al. (2004) we found consumption of *Littoraria* to be of the same magnitude ($500 \text{ g} \cdot (\text{m}^2 \cdot \text{a})^{-1}$ versus $352 \text{ g} \cdot (\text{m}^2 \cdot \text{a})^{-1}$ in this study). Thus, results from laboratory experiments under artificial conditions are well applicable to the field for this periwinkle.

Table 3.1: consumption rates of salt marsh detritivores after Zimmer et al. (2004); bold: results from the present study.

	Detritivores per m^2	<i>Spartina</i>	<i>Juncus</i>	<i>Quercus</i>	Unit
<i>Littoraria</i>	300	500 / 352	800	100	$\text{g} \cdot (\text{m}^2 \cdot \text{a})^{-1}$
<i>Armasas</i>	10	20 / 0	40	20	$\text{g} \cdot (\text{m}^2 \cdot \text{a})^{-1}$
<i>Melampus</i>	100	25 / 2.4	50	20	$\text{g} \cdot (\text{m}^2 \cdot \text{a})^{-1}$

In contrast, we found much lower and no consumption by *Melampus* and *Armasas*, respectively. These rates were not significantly different from the control (Fig. 3.4). Although detritus mass loss in the presence of *Littoraria* were clearly increased compared to the control, ANOVA did not indicate any significance. Still, we hold that the impact of *Littoraria* on the decomposition of *Spartina* stems is of some ecological significance, while the impact of *Armasas* and *Melampus* is negligible in the field compared to decay due to microbial activity under these circumstances.

Littoraria

The conditions of the present study are, like in every experiment, to some extent artificial, such that the used detritivores and litter types do only occasionally occur syntopically. *Littoraria* is found in very high densities of up to 600 individuals per m^2 on standing dead

or senescent *Spartina* leaves (Teal 1962, Fell et al. 1991; Bärlocher & Newell 1994; Zimmer et al. 2002, personal observation). A close interaction between *Littoraria* and leaf-pervading fungi on *Spartina alterniflora* is known. Silliman & Newell (2003) were able to show that *Littoraria* farms fungi by destroying the surface layer of living leaves and later feeds on fungi that are growing on the injured leaf-spot. Dead and detached stems of *Spartina* accumulate in vast mats, which cover several hundred m² over the year (personal observation). *Littoraria* typically encounters this detritus form, and its ability to exclusively feed on *Spartina* stems has been shown previously (Zimmer et al. 2002). Bacterial biomass was increased in the presence of *Littoraria* (Fig. 3.7), indicating some *Littoraria*-induced mechanism that supported bacterial growth and outweighed feeding impact. Zimmer et al. (2004) report increased microbial respiration, among others, on *Spartina* in the presence of *Littoraria*. This may have led to the higher microbial production we observed. Alternatively, a positive influence of snail mucus on bacterial growth is possible, as observed in intertidal limpet trails, that support microalgae growth (Connor & Quinn 1984). The higher mass loss of *Spartina* (Fig. 3.4) in the presence of *Littoraria* can hence be ascribed to both, higher bacterial number and *Littoraria* feeding activity (feeding-tracks were visible).

Melampus

In the upper salt marsh fringe, we found *Melampus* in high densities of several hundred per m² at the base of *Juncus roemerianus* stems (compare Lee & Silliman 2006). *Melampus* mainly feeds on microbial and algal biomass (Lee & Silliman 2006) and encounters all detrital material used in this study in the field frequently. Its ability to feed on detritus has been proven (Zimmer et al. 2004). *Melampus* exhibited the highest reduction of bacterial biomass in this study (Fig. 3.7). Thus, microbivory in concert with little feeding on the detritus resulted in weak litter mass loss in the presence of *Melampus* (Fig 3.4). In contrast, Zimmer et al. (2004) report high comminution of litter and increased microbial respiration through feeding by *Melampus* in the laboratory. We can not make a statement on the microbial activity, but do – from the background of our data – doubt a major influence of *Melampus* on *Spartina* stem decomposition.

Armases

Armases cinereum dwells as an omnivore in the upper fringe of the salt marsh up to the adjacent coastal forest. Here, it typically encounters *Quercus* litter. In laboratory experiments, *Armases* fed on all detritus types used in this study, but only very little on *Quercus* (Zimmer et al. 2004). According to Buck et al. (2003), *Armases* also feeds on salt marsh mud. Due to its omnivorous (including predacious) behavior, Zimmer et al. (2004) describe its ecological role as a mediator of energy fluxes between trophic layers and adjacent habitats. Thus, *Armases* may act on decomposition directly through consuming detritus and indirectly through controlling smaller detritivores such as snails and crustaceans. We did not find *Armases* contribute to salt marsh litter mass loss or bacterial biomass alteration. An application of laboratory results (compare Zimmer et al. 2004) like in the case of *Littoraria* is not supported here. In the field, the omnivorous feeding strategy of *Armases* leaves enough space for alternative nutritional sources that were not present in lab experiments; under field conditions, the offered plant material is obviously of little attraction to the decapod crab. As alternative food sources for *Armases*, micro- and meiofauna, which was not excluded from the cages, mud, and potentially also small *Melampus* snails (Buck et al. 2003) can be considered, although praying on snails was not observed here.

Species composition

Litter

Litter types used in this study differed from each other in texture, structure, and toughness (see above). Synergistic effects of litter mixing on nutrient fluxes and litter decomposition have been described frequently (reviewed in Hättenschwiler 2005). Here, we found such effects (on litter mass loss) in all three two-litter combinations, although these were not significant. Mixing effects have been suggested to result from higher spatial heterogeneity creating microhabitats that support microorganisms and the microfauna, and from transfer of nutrients, in particular nitrogen, from high-quality litter species to low-quality species, which increases microbial activity on low-quality litter (Kaneko & Salamanca 1999, Schimel & Hättenschwiler 2007). Both mechanisms are likely to have influenced processes in the present study.

A mixture of different litter types is packed less densely than a monoculture, in particular, when litter types are very different in shape like here; aeration is increased, supporting microbial colonization. However, each tidal flush reassembles the mixture, destroys established nutrient flux links (e.g. by fungal mycelium), and dislocates free nutrients, rendering detrital accumulations dynamic. While nitrogen contents of *Spartina*, *Quercus*, and *Juncus* do not differ remarkably (Zimmer, unpublished data), nitrogen release from litter tissue is likely to differ, owing to the different toughness. Consequently, we consider nutrient translocation mechanisms between litter types of contrasting quality to of decreasing importance with increasing inundation frequency (lower marsh). Hence, non-additive effects of litter diversity are likely to occur only at the higher marsh, where detritus assemblage is less frequently disturbed.

Detritivores

On *Spartina* litter, bacterial biomass was increased in the presence of *Littoraria* compared to the control without detritivores (Fig. 3.7). In mixed-litter treatments this increase through *Littoraria* can affect nutrient mobilization and transfer between litter types and thus, increase total decomposition as observed in the mixed litter treatments (Fig. 3.5).

While litter decomposition pathways are forced to interact, since litter stays in place, as long as not moved by the tides, animals may mutually exclude each other and thus, decrease interaction probability. Detritivores used here differed greatly in size, behavior, and feeding type. With a comparably big radula, *Littoraria* can ingest fragments of plant material with the associated microbial biomass. *Melampus*, with a much smaller radula, rather scrapes epibionts off the substratum (as seen in Figure 3.7: *Melampus* reduced bacteria strongly), while, equipped with claws, *Armasas* can bite off large fragments. In addition to this, Zimmer et al. (2004) found great differences in digestion of litter material. Besides, snails excrete a mucus track that might influence microbial colonization (see above). Thus, all three species exhibit specific impact on the environment and thus on decomposition (Zimmer et al. 2004).

Likely, the probability of encountering effects of mixing detritivores increases with decreasing functional redundancy, because the spectrum of potential impacts on the environment is broadened. As Figure 3.8 shows, the presence of two detritivore species produced lower bacterial densities than expected, in all combinations. Species mixing

appeared to have a negative effect on bacterial biomass. This effect was pronounced when *Littoraria* and *Armas* were combined. Bacterial cells were reduced to 1/3 of the expected number, although in the single-species treatments both detritivores did not reduce bacteria at all compared to the control (Fig. 3.7). A facilitating mechanism in utilizing the substrate between the two species or an overgrazing effect and subsequent breakdown of the bacterial community are possible explanations for the combined reduction of bacteria.

Dangles & Malmqvist (2004) suggested that low species evenness of a community supports non-additive effects of mixing species on ecosystem processes due to strong influential traits of single species. They summarized the requirements for invertebrate decomposers to skew community evenness and exhibit particularly strong impact on ecosystem processes as follows: strong interspecific interactions, high density, year-round presence, and high motility.

Littoraria is highly abundant throughout the salt marsh and is present at all seasons, though dormant in winter (Vaughn & Fisher 1992). Its seasonal range of activity appears to be no more than a circle of 2 m (Vaughn & Fisher 1992), but *Littoraria* shows extended vertical tidal migration alternating between *Spartina* leaves and marsh soil always staying just above the water line (Vaughn & Fisher 1992, Hovel et al. 2001). A strong regulating impact of *Littoraria* on *Spartina* production has been proven (Silliman & Zieman 2001). Further, *Littoraria* is competitively superior to *Melampus* and has the ability to displace the smaller snail (Lee & Silliman 2006).

Melampus and *Armas* show high densities in the marsh fringe. Motility of *Armas* is high around its burrows (personal observation), while *Melampus* is reduced in its activity range and degree (Hutchens & Walters 2006, Lee & Silliman 2006). Additionally, *Armas* exhibits high interspecific interactions by preying on the micro- and mesofauna (Zimmer et al. 2004). Applying the concept of Dangles & Malmqvist (2004), we expect *Littoraria* and *Armas* to be superior to *Melampus* in affecting ecosystem processes due to presence, abundance, motility, and interspecific interactions. When aggregated in our experimental setup, these properties are likely to play important roles. We argue that the strong non-additive effect on bacterial biomass (Fig.3.8) we observed is founded in the species' interacting properties of *Littoraria* and *Armas* as two strong players in the salt marsh ecosystem. By contrast, *Melampus* seems to play a minor role in the system studied herein.

Conclusions

The over-all impact of salt-marsh detritivores on *Spartina* stem decomposition is surprisingly low. However, *Littoraria irrorata* increases litter mass loss on average by ~50%, confirming its dominant role in salt marsh ecology. Concert impact of detritivores does not reveal synergistic contributions to litter mass loss, but manifests in non-additive influence on microbial biomass and subsequently on nutrient fluxes by two dominant and most influential species. Detritus shows strong species-specific differences in mass loss in the presence of the dominant decomposer, reflecting specific structural traits. These differences likely drive non-additive shifts in decomposition rates in mixed-litter patches. We conclude that diversity effects on decomposition processes through the non-additive concerted action of different species can be encountered in transition zones, such as the upper marsh fringe, where the presence of dominant invertebrate decomposer species and the occurrence of mixed detritus types overlap.

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Species-specific decomposition rates of beach-
cast wrack in Barkley Sound, British Columbia,
Canada

Chapter IV

Introduction

Living seaweed function as nutrient sinks in coastal waters. However, following senescence, storms or other heavy weather, they become detrital material that is often deposited ashore where it decomposes, and hence ends up as a nutrient source (Hanisak 1993). Thus, marine phytodetritus (henceforth termed “wrack”) can result in large net fluxes of nutrients from the subtidal/intertidal areas to beaches and other near-shore terrestrial systems (Ochieng & Erftemeijer 1999, Piriz et al. 2003, Orr et al. 2005). The role of macrophyte-derived wrack as an input for organic carbon and nitrogen has been extensively examined for subtidal consumers (Seiderer & Newell 1985, Mann 1988, Fielding & Davis 1989). By contrast, relatively little attention has been given to the consequences for food web dynamics of the intertidal zone (Bustamante & Branch 1996). The degree to which this “spatial subsidy” (sensu Polis et al. 1997) has consequences for intertidal and supralittoral macro- and meiofauna depends on both the quantity and the species composition of beach-cast wrack.

In a previous study, Orr et al. (2005) observed both overall mass loss of wrack and changes in its species composition during the course of wrack line aging. The factors contributing to this observation include mechanical break down of wrack as a consequence of repeated drying-rewetting events brought about through solar radiation, morning dew and rain (Newell et al. 1996, Vahatalo et al. 1998), flocculation and sedimentation (Harrison 1989), removal of wrack through tidal currents and wave action (Orr et al. 2005), as well as leaching and microbial decomposition of detritus and its fragmentation through feeding by detritivores (Newell & Bärlocher 1993, Zimmer et al. 2002, 2004). Tidal events render the input of wrack to, and removal from, the wrackline a dynamic process that allows for in situ decomposition through leaching and microbial and faunal beach dwellers for no more than one day in some instances (Orr et al. 2005). Thus, we were interested in short-term (= 1d) mass loss of freshly deposited wrack patches. The present study aims at evaluating short-term wrack mass loss in terms of food preferences of detritivores that are found to forage on this allochthonous material. We here differentiate between mass loss solely owing to leaching of water-soluble compounds and microbial activity (henceforth decay) and mass loss by decomposition, i.e. by decay plus feeding by detritivorous macroinvertebrates.

We hypothesize that the species composition of aged wracklines (~2 weeks) can be

explained by (1) differential input and removal of wrack species (Orr et al. 2005), (2) differential decay rates of initially present wrack species and (3) the activity of supratidal detritivores colonizing beach-cast wrack.

Material & Methods

The main study site is Barkley Sound, a large embayment ($\gg 550 \text{ km}^2$) on the west coast of Vancouver Island, British Columbia, Canada (Fig. 1). Four beaches on Edward King Island with different exposure to wave action but similar sediment characteristics (for details, see Orr et al. 2005) were chosen for field studies on in situ decomposition, namely EKN (exposed): N $48^\circ 50' 01.1''$ W $125^\circ 12' 46.2''$, EKW (exposed): N $48^\circ 49' 44.9''$ W $125^\circ 12' 57.8''$, EKE (sheltered): N $48^\circ 49' 45.7''$ W $125^\circ 12' 18.8''$, EKS (sheltered): N $48^\circ 49' 39.9''$ W $125^\circ 12' 47.0''$). The first two beaches had west facing aspects, the latter two south-east facing. All beaches were lunate-shaped with cobbles on the surface underlain by a gravel mixture. Field studies were performed in August 2003.

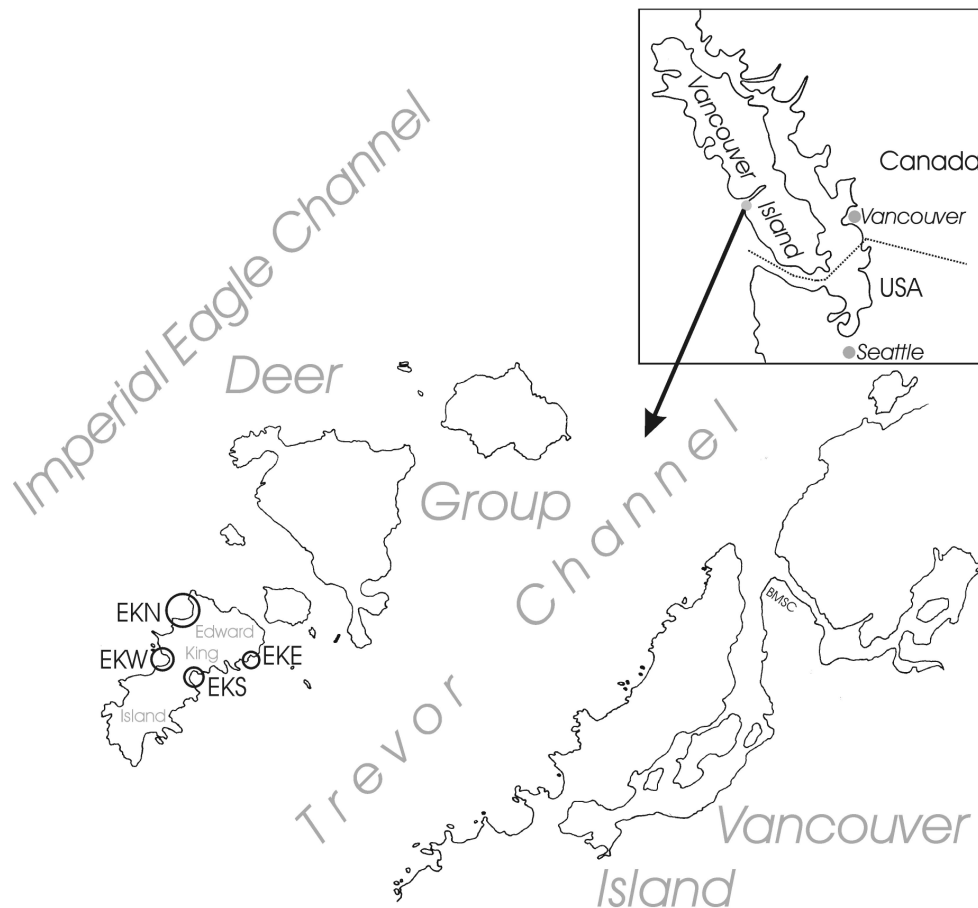


Figure 4.1. Study sites on Edward King Island, Barkley Sound.

In situ decay of freshly deposited wrack was studied using nylon mesh bags ($100 \times 100 \text{ mm}^2$) with a mesh size of $350 \mu\text{m}$ ($n = 7$ for each beach), which prevented the intrusion of meso- and macroinvertebrate detritivores (mostly amphipods). Detritivore-

aided decomposition was studied using nylon bags (150 x 100 mm²) with a mesh size of 10 mm (n = 7 for each beach). We collected near-shore floating debris of five algal species common to subtidal catchments in the area (*Nereocystis luetkeana*, *Macrocystis integrifolia*, *Fucus* sp., *Ulva* sp. and *Phyllospadix* sp.). This material, which would serve as drift wrack, was blotted dry in the laboratory, weighed and placed in mesh bags after re-wetting with sea water. Later that same day, mesh bags with ca. 0.6 g dry weight wrack, which is well within the range of daily wrack input observed by Orr et al. (2005) at the same site, were buried in the most recent fresh wet drift line, and then collected approximately 24 h later. In the laboratory, wrack remnants were rinsed with deionized water to remove inorganic material, oven-dried (24 h at 60 °C) and weighed. Samples (n = 7) of each fresh wrack species were oven-dried (24 h at 60 °C) and weighed to obtain a fresh:dry mass ratio of freshly deposited wrack. Differences between dry mass before (calculated) and after the experiment served as measure of wrack mass loss through decay and detritivore activity.

For feeding preference tests with one of the most abundant amphipods, *Megalorchestia pugettensis*, we collected the five most common species of algae (*Fucus* sp., *Ulva* sp., *Macrocystis integrifolia*, *Chondracanthus corymbiferus*, and *Phyllospadix* sp.) as well as leaf litter of salal (*Gaultheria shallon*), a terrestrial shrub species that accumulates in wrack lines higher on the beach platform (cf. Orr et al. 2005). In the laboratory, detritus was thoroughly washed with deionized water, oven dried at 30 °C for 48 h to simulate air-drying in the deposited drift line, then cut into pieces and weighed to determine the initial dry mass. After re-wetting by spraying with sea water, pieces of all different types of detritus were offered simultaneously to 10 amphipods (n = 9) in Petri dishes that had 5 mm of wet sand. After two days, the remaining detritus was removed, washed with deionized water, dried and weighed again. Consumption was determined as mg wrack consumed by 10 amphipods during 2 d. These data were used for statistical analyses through resampling statistics after Bärlocher (1998) using 9999 iterations for each comparison, followed by pairwise-comparisons subjected to Bonferroni corrections. For graphical presentation these data were transformed to percent of total amount consumed.

Results

Our mesh bag decomposition experiment revealed that freshly deposited *Nereocystis luetkeana* decomposed rapidly through decay (350 μm mesh size: no access for meso- and macro-invertebrate decomposers) with mass loss rates of 60-90 % d^{-1} . Decay of *Macrocystis integrifolia* and *Phyllospadix* spp. ranged from 10 to 40 % d^{-1} , *Ulva* spp. and *Fucus* spp. degraded with rates of less than 30 and 15 % d^{-1} , respectively (Fig. 2a). Access of meso- and macro-invertebrates (10 mm mesh size) increased mass loss of *Nereocystis*, *Macrocystis* and *Ulva* more than 1.2-fold, 2.8-fold, and 3.7-fold, respectively (Fig. 2b). Mass loss of *Fucus* was essentially not affected by the presence of detritivores. *Nereocystis*, *Macrocystis* and *Ulva* (except for EKS) were removed completely from the mesh bags during 24h incubation. Thus, actual mass loss may be expected to be much higher in the field, with the present data showing only the minimum of decay. These patterns were similar among the four beaches irrespective of their aspect or exposure.

Feeding preference tests using the amphipod *Megalorchestia pugettensis* revealed feeding rates (Fig. 3) that to some degree corroborated the overall decomposition rates we deduced from the comparison of fresh and aged drift lines (Fig. 4). Among six available food sources, *Macrocystis integrifolia* and *Fucus* sp. were clearly preferred over *Chondracanthus* sp., *Ulva* sp., *Phyllospadix* sp. and *Gaultheria shallon* (salal) leaf litter, the latter being little consumed.

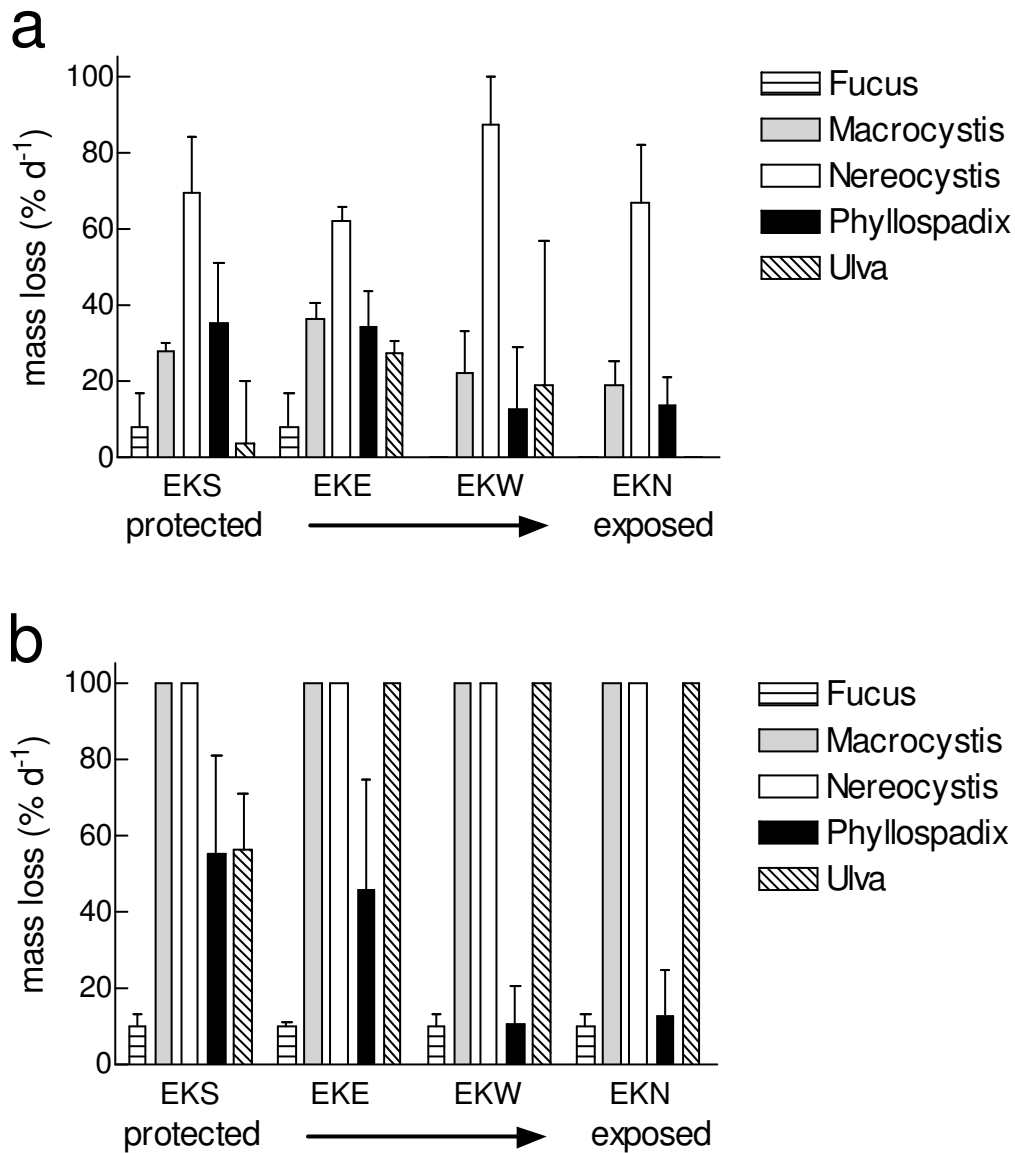


Figure 4.2: Mass loss of selected wrack species in mesh bags due to (a) decay through leaching and microbial activity and (b) decomposition (decay plus and detritivore feeding: for details see text) at different beaches on Edward King Island (see Fig. 1) – Data are mean \pm standard deviation ($n=7$ for each of four beaches) – *Macrocystis*, *Nereocystis* and *Ulva* in (b) where degraded completely at all beaches (except for *Ulva* at EKS).

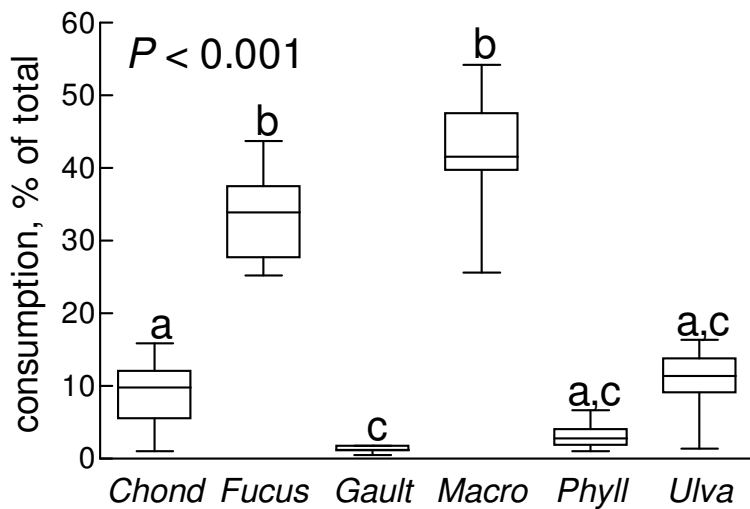


Figure 4.3: Feeding preference of the amphipod, *Megalorchestia pugettensis*, simultaneously offered selected wrack (*Chond*: *Chondracanthus*; *Fucus*: *Fucus*; *Gault*: *Gaultberia*; *Macro*: *Macrocytis*; *Phyll*: *Phyllospadix*; *Ulva*: *Ulva*) – Box plots represent median, 25 % percentiles, and minimum and maximum ($n=7$). Different lower case letters designate significant differences ($\alpha \leq 0.05$).

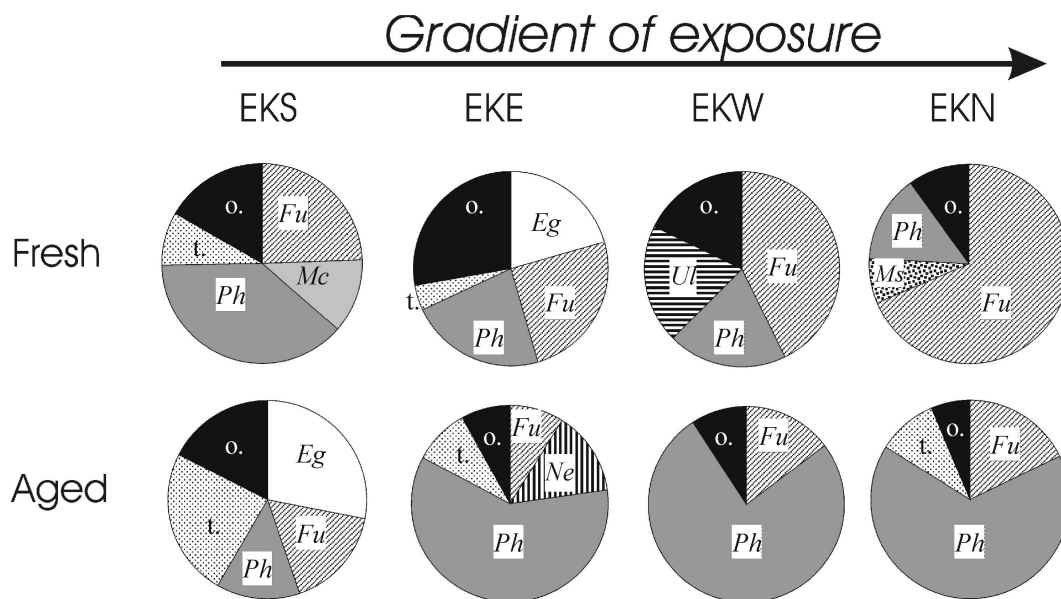


Figure 4.4: Species composition of fresh (most recent) and aged (~2 weeks) wrack lines at beaches with different wave exposures on Edward King Island in July–August 2003. Data are median percent values of three collections each at two beaches per substratum type. Species abbreviations: *Eg*, *Egregia*; *Fu*, *Fucus*; *Mc*, *Macrocytis*; *Ms*, *Mastocarpus*; *Ne*, *Nereocystis*; *Ph*, *Phyllospadix*; *Ul*, *Ulva*; t, terrestrial; o., other. (from Orr et al. 2005)

Discussion

Our findings clearly indicate differential mass loss of different wrack species due to decay and faunal decomposition processes. This coincides with reported differences in species composition between fresh and aged wrack lines (Orr et al. 2005; Fig. 4).

While *Nereocystis luetkeana* wrack was characterized by rapid mass loss through decay, *Fucus* sp. and *Ulva* sp. lost mass more slowly, with *Phyllospadix* sp. and *Macrocystis* exhibiting intermediate decay rates. These differences can be explained in part by variation in the chemical composition of the wrack. *Fucus* is high in phenolic compounds (Targett et al. 1992, Zimmer et al. 2001) that are known to slow down decay of detritus. This might shift the microbial preference to other brown algae with lower phenol content like *Nereocystis* and *Macrocystis* (Pennings et al. 2000). In contrast to *Fucus* spp., wrack of *Nereocystis luetkeana* and *Macrocystis integrifolia* is extensively covered by mucus soon after deposition ashore. This mucus mainly consists of alginate the major structural component of brown algae cell walls. Leaching of this component highly promotes mass loss. *Phyllospadix* is also high in phenols (Agostini et al. 1998, Bianchi et al. 1999) and in addition does contain lignin and cellulose. Nevertheless decay of *Phyllospadix* was comparable to *Macrocystis*, indicating that factors other than phenolic or lignocellulose contents must be considered here. There are essentially no phenolics in *Ulva* spp. which are also characterized by a low C:N ratio favorable for foraging (Pennings et al. 2000). Notwithstanding these two features, *Ulva* did not decompose as fast as *Nereocystis*. Afzal Rizvi & Shameel (2005) found remarkable amounts of phytotoxins in *Ulva intestinalis* that might also be active against microbes, inhibiting microbial colonization and decay of algal thalli.

In accordance with the rapid mass losses we observed in the field, *Macrocystis* was one of the preferred food sources for *Megalorchestia pugettensis*, a common detritivore feeding upon wrack on beaches in Barkley Sound, but *Fucus* was just as well accepted as was *Macrocystis*. A common talitrid amphipod at beaches in northern Europe, *Talitrus saltator*, like *Megalorchestia* preferentially feeds on *Fucus serratus* in Brittany (France) (Adin & Riera 2003). Both drift and wrack of *Macrocystis* exhibit a significantly lower C:N ratio than *Fucus* as well as a lower content of phenolic compounds (Pennings et al. 2000; Zimmer et al. 2001). However, *Traskorchestia traskiana*, another common beach hopper on cobble beaches at Barkley Sound, prefers freshly detached *Fucus* (and *Ulva*) over *Macrocystis* and

Nereocystis; wrack is preferred over drift, and consumption of *Macrocystis*, *Fucus* and *Ulva* increases by a factor 6-8, while *Nereocystis* wrack is consumed at only twice the rate as *Nereocystis* drift (Pennings et al. 2000). Thus, factors other than mucus and phenolics or C:N ratio must be responsible for amphipod feeding preferences. In the case of *T. traskiana*, the content of organic carbon rather than the often mentioned nitrogen determines feeding preferences (Pennings et al. 2000). Although the availability of nitrogen is one of the most important limiting factors to detritivores, the quality of the available carbon source, i.e. its digestibility has been suggested as a main contributing factor (Hobbie 2000). Detritivorous amphipods may use wrack-colonizing microbiota as a source of easily accessible nitrogen, while an important carbon source is obtained from wrack plant material (Wildish 1988). Detailed comparative studies about tensile strength of the plant material, lipid content or secondary compounds other than phenolics, as well as microbial biomass on beach-cast wrack and its significance for beach detritivores are needed at this place.

Jędrzejczak (2002) and Feike (2004) observed insignificant contributions of macrofaunal decomposers to the breakdown of beach-cast seagrass on sandy beaches in Poland and Germany, respectively. By contrast, Griffiths et al. (1983) found that on South African sand beaches half of the kelp wrack input is consumed by talitrid amphipods. When meso- and macro-detritivores were permitted access in our wrack decomposition experiments (10 mm mesh size, Fig. 2b) these two contradicting findings were corroborated: detritivores did not contribute to the decay of *Phyllospadix*, but did increase mass loss of *Fucus* at those two exposed beaches that, according to Orr et al. (2005; cf. Fig. 4) accommodate large amounts of this wrack, while the effects of detritivores were insignificant at those two sheltered beaches that, according to Orr et al. (2005; cf. Fig. 4) accommodate only relatively small amounts of *Fucus* (Fig. 2a+b). These findings are, at least in part, in accordance with the feeding preferences of *Megalorchestia pugettensis*, ingesting only small amounts of *Phyllospadix* but preferring *Fucus* over most other wrack types. By contrast, the faunal impact on degradation of *Macrocystis*, *Nereocystis* and *Ulva* was high, resulting in complete removal of the algal tissue. This means an up to 4-fold acceleration of decomposition processes in mesh bags that provided access for detritivorous invertebrates; taking into account the complete removal of *Nereocystis*, *Macrocystis* and *Ulva* from large-meshed bags, we may even assume a much higher increase

in wrack mass loss if more material is provided in the field. Considering our experimental design to reflect the average daily input of wrack into the upper intertidal zone (cf. Orr et al. 2005), we hold that all of the daily deposited wrack of *Nereocystis*, *Macrocystis* and *Ulva* will be removed from the wrack line through the activity of detritivores within one day, while no more than 15 % of the daily input of *Fucus* wrack is degraded on a daily basis (Orr et al. 2005). We obtained similar values for *Phyllospadix* on exposed beaches, but comparably high mass losses of roughly 50 % of the daily input at sheltered sites. Overall, our data explain the persistence of these wrack types in aging wrack lines that has been described by Orr et al. (2005; cf. Fig. 4). By comparison, communities of kelp flies (Diptera: Helcomyzidae) in sub-Antarctic South Georgia, consumed 100–120 g kelp m⁻² d⁻¹, reducing the wrack dry mass by 70 % in 7 days (Chown 1996). Williams (1984) observed decomposition rates of 50 % of a freshly detached chlorophyte, *Caulerpa cupressoides*, after 7 d in the tropical water column as well as in oxic beach sediments and anoxic seagrass bed sediments. In an artificial tank connected to a subtropical lagoon in Florida, submerged wrack of the green *Ulva lactuca* and the red *Gracilaria verrucosa* was reduced by 50 % after 5 and 3 weeks, respectively, at 25–30 °C (Hanisak 1993). However, decomposition rates varied greatly over time in Hanisak's (1993) study, being highest after 5 and 3 weeks, respectively. Our findings suggest that decomposition of beach-cast wrack proceeds much faster in drift lines on Barkley Sound beaches: Considering the potential decomposing activity of invertebrate detritivores, as in Williams' (1984) and Hanisak's (1993) studies, the entire amount of daily deposited *Nereocystis*, *Macrocystis* and *Ulva* will be decomposed within one day, i.e. before the next higher of two succeeding unequal high tides can remove the wrack from the beach into subtidal wrack pools (cf. Orr et al. 2005). By contrast, based on the daily input estimated by Orr et al. (2005) and an extrapolation of the present results on mass loss to a per meter square basis, wrack of *Fucus* and *Phyllospadix* would last for about 30 d, if it stayed in place for long enough. However, this will only be the case for wrack lines deposited by spring high tides, since beach-cast material is prone to highly dynamic removal through tidal events (Orr et al. 2005). This observation of rapid decomposition of some (but not all) types of wrack may explain why Orr et al. (2005) did not find significant amounts of *Nereocystis*, *Macrocystis* and *Ulva* in most aged wrack deposits of cobble beaches, despite these algae being common in subtidal waters in Barkley Sound (authors' unpubl. observation), while others, such as

Fucus and *Phyllospadix*, persist in the wrack upon aging (cf. Fig. 4).

We were surprised to see extremely low mass loss rates of *Fucus* through decay at exposed sites where this species, owing to its predominant contribution to living macrophyte biomass, contributes significantly to the total wrack input (cf. Fig. 4). However, in accordance with the feeding preference of *Megalorchestia*, detritivore access to the wrack significantly increased mass loss of *Fucus* at these sites, while this was not the case at the sheltered sites with relatively insignificant contribution of *Fucus* to the wrack line. We suggest that these between-site differences are due to adaptations of the detritivorous beach fauna to the prevailing food source, be it in terms of different species composition or through individual physiological response within the same species. On the opposite, mass loss of *Phyllospadix* through decay tended to be higher at those (sheltered) sites where this species, owing to its predominant contribution to subtidal macrophyte biomass, contributes significantly to the total wrack input than at those (exposed) sites with relatively little *Phyllospadix* input to the wrack line (cf. Fig. 4). In coincidence with the feeding preferences of *Megalorchestia*, detritivores did not affect mass loss of *Phyllospadix* in the field.

With the present results, we can explain the nature of some transient patterns of beach-cast wrack at Barkley Sound and its underlying mechanisms. Detailed studies on the nutritive ecology of both wrack-dwelling amphipods and microbial decomposers are needed to strengthen our understanding of decomposition patterns of beach-cast wrack, and thus, nutrient fluxes within the marine-terrestrial ecotone.

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General Discussion

Synthesis

The subjects of this thesis were in the General Introduction condensed to a set of questions. I will first answer these questions recapitulatorily and later refer to them in the two larger contexts of aquatic-terrestrial differences and species composition influence.

1. *Does the fauna exert not only direct, but also indirect effects on aquatic decomposition (by feeding on litter and by altering the microbial community, respectively) like it does in the terrestrial environment?*

In this series of experiments, a broad spectrum of animal and microbial actions and interactions of different intensities revealed. We found faunal effects on litter mass loss, reduction or enhancement of bacterial biomass, and enhancement of microbial respiration. In turn microbiota appeared to influence faunal feeding mode.

2. *What role does species identity of the fauna and the detritus play?*

The identity of macroinvertebrates played a major role in the terrestrial and, to a smaller extent, in the salt-marsh environment, while differences in function were negligible in the aquatic system. In all systems, in turn, the impact of microbial decomposers was indisputable. Additionally, a strong influence of the bacterial biofilm on faunal nutrition should be considered.

3. *What role does species composition play?*

Concert influence of macroinvertebrates was observed only in the salt-marsh system, where grapsid crabs and periwinkle snails together decreased bacterial biomass. However, positive non-additive effects of mixing litter species prevailed in all accordingly tested systems, though weakly in the field.

4. *What are the conditions for diversity effects to occur?*

A high impact of the fauna appears to support the emergence of species-mixing effects. In the laboratory experiments, non-additive effects of litter mixing were only detectable in the presence of detritivores in high abundances; in the salt-marsh experiment, an effect of detritivore mixing emerged in the presence of two species with high ecosystem impact and high motility. Litter mixing effects were found in the presence of the ecosystem-structuring periwinkle.

5. *Are decomposition processes in aquatic and terrestrial systems similar, when fed by the same energy source?*

Decomposition and animal-microbe interactions differed significantly in aquatic and terrestrial systems. When decomposing the same plant material, the decomposition system is strongly influenced by the presence of water. This influence pervades all aspects of litter breakdown and animal-microbe interactions, reducing differences in species-specific traits of litter and detritivores. These effects can basically be explained by the change of detritus quality as mediated through water.

6. *Do the emerging patterns exist in other detritus-based ecosystems?*

In the salt-marsh system at intermediate inundation regime, terrestrial patterns prevail, as can be deduced from the high differences in detritus and, to a smaller extent, from differing detritivore species influence on litter mass loss and bacterial biomass.

7. *Considering that the system (aquatic versus terrestrial) affects detritus quality, how does the process change, when aquatic detritus decomposes terrestrially?*

Decomposition process changes dramatically when detritus of aquatic origin is decomposing under terrestrial conditions. Very fast decomposition rates through microbial and faunal activity appear to be the consequences of the high nutritional value of carbon-poor macroalgal tissue. As adaptations to the high detritus quality and temporal and spatial dynamics, detritivores occur in very high densities.

The influence of the aquatic environment

An essential learning from the present work is the all-encompassing relevance of water as a variable that influences decomposition and animal-microbe interactions in the different systems. Water acts on different scales, from the osmotic transportation of nutrients, as we observed in the leaching of litter, to the influence on phytobiomass structure on the evolutionary scale, such that aquatic phytobiomass has lower levels of structural compounds, producing nutritionally highly valuable aquatic detritus as allochthonous subsidy for beaches (Shurin et al. 2006).

Thus, when comparing aquatic and terrestrial systems, water influenced the observed parameters through support of a prominent biofilm on litter surfaces that macroinvertebrate detritivores fed on, and through the levelling of detritus quality. By this, the presence of water reduced the species-specificity of both, detritus and

detritivores as far as the decomposition process is concerned, while in the terrestrial environment distinct differences between detritivore species and litter types were present.

Water is a solvent for chemical substances and fine particulate matter. Further, it is a buffer and a vector for chemical and physical reactions. The buffering and dissolving capacity of air, in contrast, is by magnitudes lower. As a consequence, the need to create structural barriers against the loss of water does not exist under water or, on a larger scale, detritus is less densely packed, than on land. In a terrestrial system, in the absence of a surrounding water body, I thus expected more distinct properties and interactions and consequently more clearly distinguished ecosystem processes. Our results confirm this assumption. We found more distinct litter, detritivore and microbial properties and interactions in the terrestrial than in the aquatic environment.

Notwithstanding the significant differences in processes in both systems, we also found similarities. Indirect effects of detritivores increasing microbial activity appeared in both environments. Interestingly, these effects emerged under the same specific conditions (mixed-litter, high detritivore abundance), suggesting system-independent mechanisms. The increase in microbial respiration was ascribed to an increase in fungal activity. Fungal biomass density was largely unaffected by faunal presence. Since a great part of the fungal biomass was “protected” inside the leaf body, its exposure to faunal impact was unaffected by the aquatic environment, unlike bacterial biomass. Obviously, the stimulating impulse of the fauna was similar in both environments, resulting in similar patterns of animal-fungi interaction (see Chapter 2).

Considering leaching of litter into the surrounding water and the support of a valuable biofilm being important effects of water on decomposition, it is not surprising that we found patterns in the salt marsh experiment to be rather similar to those from the terrestrial laboratory experiment. Leachates from detritus are taken away by tidal activity and do thus not support microbial growth like we observed in the laboratory aquatic environment. Further, strong water movement with every tide creates friction with other detritus and soil particles, such that growth of a thick biofilm is hindered. Hence, salt marsh detritus is largely reduced to its structural properties, governing detritivore feeding, as we observed.

The decomposition rate of detritus of aquatic origin is significantly faster than that of terrestrial detritus. we did not assess the interactions of detritivores and microbial

colonizers, but due to the very fast degradation that leaves little time for a community of microbial decomposers to establish, and the strong dynamics in the supralittoral system we investigated, I conclude that these interactions are reduced to co-ingestion of microorganisms by detritivores along with detrital biomass. This is for sure only true for the experimental runtime of one day. Once detritus is deposited above the high water line, more complex interactions will establish that were not considered here.

This experiment drastically showed the impact of detritus quality on the decomposition process. The impact of the aquatic environment in this example is not of direct nature like in the case of leaching, but it indirectly influences decomposition by rendering the evolution of low structural compounds unnecessary, resulting in carbon-poor and nitrogen-rich primary producers. However, when aquatic detritus is leached out and dry, like in the old upper wracklines we encountered on the beaches of Vancouver Island, decomposition slows down dramatically.

According to Shurin et al. (2006), differences between aquatic and terrestrial systems are driven by characteristics of the primary producer community and the quality of detritus that it produces. With high nitrogen and phosphorus and low carbon contents of primary producers, aquatic systems support more herbivory and less detritus accumulation. This suggestion is supported by our findings on the cobble beaches of Vancouver Island, where aquatic detritus induces decomposition rates that are by magnitudes higher than typically found in terrestrial or semi-terrestrial systems like wetlands.

However, when we go more into detail, the picture changes. We exposed litter of the same species and detritivores of similar ecological function to both, terrestrial and aquatic environment, reducing system differences as far as possible to the identity of the medium. When regarding animal-microbe interactions in, and the faunal influence on, decomposition, I found that decomposition processes are not exclusively depending on detritus quality, but that, as stated before, the medium itself has a strong influence on detritus processing.

The influence of species richness

This work can not finally answer the questions on the importance of species richness effects in ecology, but some valuable additions to the present knowledge can be made. Species richness of litter has in the past been proven to exhibit non-additional effects on ecosystem functioning (e.g. Zimmer 2002 and literature reviewed in Hättenschwiler 2005). We found system-independent mechanisms driving such litter-mixture effects. In accordance with Hättenschwiler & Gasser (2005) and Dangles & Malmquist (2004), I suggest that the fauna determines the magnitude of litter-mixture effects (see Chapter 2) and that, further, animals exhibiting a strong ecosystem impact drive non-additive fauna-mixture effects (see Chapter 3).

Over all, mixing-effects were present in all studies, but their character was fleeting (Chapter 1 + 2) or not well pronounced, particularly in the field (Chapter 3). In no case did we find an effect on the observed ecosystem function (decomposition, i.e. mass loss of litter). Bradford et al. (2002) found a strong influence of faunal composition on several ecosystem parameters including decomposition in a model grassland system. Despite these strong effects, two key ecosystem processes, aboveground net primary productivity and net ecosystem productivity, remained unaffected. Bradford et al. (2002) hypothesized that positive and negative fauna-mediated effects cancel each other out. Linking to the present studies, I deduce that, despite the impact on single parameters, such as microbial activity in the laboratory experiments and bacterial biomass and, to smaller extent, litter mass loss in the field study, species richness effects do not alter ecosystem functions, since these are buffered by the multiple pathways in the complexity of decomposition processes.

Conclusions

Not many of the results obtained in this thesis were significant on the commonly accepted biological scale of 5 % probability of error. However, I am confident with the following statements:

1. Invertebrate detritivores exert significant impacts on the microbial community and its performance in aquatic decomposition processes.
2. Decomposition processes in aquatic and terrestrial systems receiving the same energy source in the form of deciduous leaf litter are basically different.

3. The patterns of animal-microbe interactions strongly depend on the ecological system.
4. Traits of detritivores and detritus are less pronounced in the aquatic than in the terrestrial environment.

Further, our results support the following suggestions:

5. Decomposition in early stages is mediated through DOM in the aquatic and POM in the terrestrial environment.
6. Effects of species-richness are abundant but weak or fleeting in decomposition processes.
7. Influences of invertebrate detritivores mediate litter-mixture effects.
8. Detritivores that exhibit strong effects on ecosystems drive non-additive effects, when acting in concert.

I was able to show up some basic differences in decomposition in different systems. The laboratory system we established proved to be suitable for direct comparison of animal-microbe-detritus interaction between aquatic and terrestrial environments. However, generalization from this study need to be tested and future research should take a broader spectrum of detritivores and detritus into account. The laboratory model system, beyond this, would allow for a closer look on, and direct comparison of, nutrient fluxes providing valuable information on the essential process of decomposition in these contrasting ecological systems.

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Auch wenn ich dies alles selbst niedergeschrieben habe, wäre hier trotzdem nichts zu lesen ohne die Mithilfe vieler netter Menschen.

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Lebenslauf

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Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation nach den Regeln guter wissenschaftlicher Praxis selbst verfasst habe.

Dabei habe ich keine Hilfe, außer der wissenschaftlichen Beratung durch meinen Doktorvater PD Dr. Martin Zimmer und der nicht-wissenschaftlichen Unterstützung der in der Dissertationsschrift angegebenen Personen, in Anspruch genommen.

Des Weiteren erkläre ich, dass ich noch keinen Promotionsversuch unternommen habe.

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