Interactions between warming, nutrient enrichment and detritivores on litter decomposition and associated microbial decomposers

Dissertation

Zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Christian-Albrechts-Universität zu kiel

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Der Dekan

Dedicated to My family, with love

Content

Summary

Leaf litter decomposition constitutes an important source of energy in many aquatic environments that is controlled by the joint action of microbial decomposers such as bacteria and fungi and also animal detritivores. In view of current scenarios of global environmental change, it is predicted that rapid temperature increases could directly affect most ecosystems including freshwaters. Additionally, human activities and *industrial development* have impacted water quality of many streams and rivers. In freshwater systems, eutrophication is a process, whereby excessive receive of inorganic nutrients, especially N and P, that may effect on leaf litter processing.

In the present study, I investigated how warming, nutrient-addition (N and P) and detritivores, interact to affect multiple parameters associated with leaf decomposition. Investigations were carried out in the laboratory in two sets. For the studies presented here leaf litter *Betula pendula* (Birch) and the detritivore *Gammarus pulex* (Amphipoda) were chosen because of their numerical importance in northern temperate ecosystem.

In the first set of experiments (Chapter I), I investigated the synergistic effects of warming and nutrient-addition (N and P) on the impact of amphipods on density and community composition of leaf litter-colonizing bacteria. I found that warming significantly exhibit stronger effects on the composition of litter-associated bacterial communities, irrespective of nutrient load but amphipods mediated warming-effects on bacterial community composition by selective feeding. In addition, Short-term effects of nutrient-addition on bacterial biofilm density were stronger than warming-effects but less pronounced so at increased temperatures. Alongside, Long-term effects of nutrient-addition on bacterial density were strongest, irrespective of environmental temperature. Additionally, nutrient-addition effectively compensated for biofilm reduction upon grazing by amphipods.

In second set of experiments (chapter II), I proceeded to improve understanding of leaf litter decomposition process by comprehensive experiments to investigate how warming, nutrient-addition (N and P) and detritivores, interact to affect multiple parameters associated with leaf decomposition. These parameters included microbial (bacteria and fungi) biomass and community structure, decomposition rate and detritivore growth. I found that detritivores and nutrient-addition have strong effects on leaf litter decomposition rate but relative growth of detritivores does not increase with warming and nutrient addition. Additionally, bacterial biofilm density increases by both warming and nutrient-addition, but reduced by amphipod grazing-pressure and fungal biomass also appears to be stimulated by warming and nutrient-addition but also by amphipod presence. Moreover, litter-associated fungal composition were only slightly affected by warming or nutrient-addition, but strongly responded to selective feeding by amphipods and the community composition of bacterial colonizers on birch litter was also influenced by grazing pressure of amphipods and warming.

In summary, this study provides new insights into the effect of simultaneous change in temperature and nutrient-load on microbial decomposers and also helps in better understanding of selective role of detritivores on bacterial and fungal communities on litter surfaces in freshwaters.

Zusammenfassung

Die Dekomposition von Laubstreu stellt in zahlreichen aquatischen Lebensräumen eine wichtige Energiequelle dar, die unter gemeinsamer Kontrolle von mikrobiellen Mineralisierern –Pilze und Bakterien– und detritivoren Tieren steht. Derzeitige Szenarien bezüglich globaler Umweltveränderungen berücksichtigend, hohe Erwärmungsraten könnten die meisten Ökosysteme, so auch Süßwässer, direkt beeinträchtigen. Zudem haben die industrielle Entwicklung und weitere menschliche Aktivitäten die Wasserqualität in zahlreichen Süßgewässern negativ beeinflusst. Eutrophierung ist z.B. durch übermäßige Zufuhr anorganischer Nährstoffe –insbesondere N und P– verursacht und könnte Dekomposition beeinträchtigen.

In der vorliegenden Arbeit habe ich untersucht, wie Erwärmung und Nährstoffzufuhr (N und P) mit der Aktivität detritivorer Tiere interagieren, und wie dies sich auf verschiedene Parameter des Laubstreuabbaus auswirken. Diese Untersuchungen wurden im Rahmen zweier großvolumiger Laborstudien durchgeführt. Die Laubstreu von *Betula pendula* (Birke) und der Detritivor *Gammarus pulex* (Amphipoda) wurden hierzu aufgrund ihrer Bedeutung in nord-temperierten Ökosystemen gewählt.

Im ersten Experiment (Kapitel I) untersuchte ich die synergistischen Effekte von Erwärmung und Nährstoffzufuhr (N und P) auf den Einfluss von Amphipoden auf die Dichte und Gemeinschaftszusammensatzung von Laubstreu besiedelnden Bakterien. Erwärmung hatte signifikant stärkere Effekte auf die Zusammensatzung der bakteriellen Gemeinschaft –unabhängig von der Nährstoffzufuhr– aber Amphipoden modulierten die Konsequenzen einer Erwärmung auf die bakterielle Gemeinschaft durch selektiven Fraß. Außerdem waren kurzzeitige Effekte der Nährstoffzufuhr auf bakterielle Dichten ausgeprägter als die einer Erwärmung, wurden jedoch durch zunehmende Temperaturen abgemildert. Langfristige Konsequenzen einer Nährstoffzufuhr

auf bakterielle Dichten waren unabhängig von der Temperatur sehr ausgeprägt, und Nährstoffzufuhr kompensierte die Biofilmreduktion durch Amphipodenfraß.

Mit dem zweiten Experiment (Kapitel II) versuchte ich detaillierter zu verstehen, wie die Dekomposition von Laubstreu durch die Interaktion von Erwärmung, Nährstoffzufuhr und die Aktivität von Detritivoren beeinflusst wird. Die hierzu untersuchten Parameter waren mikrobielle (pilzliche und bakterielle) Biomasse und Gemeinschaftszusammensetzung, Streuabbauraten und Wachstum der Detritivoren. Sowohl Detritivore als auch Nährstoffzufuhr beeinflussten den Streuabbau signifikant, aber Wachstumsraten der Detritivoren waren von Erwärmung oder Nährstoffzufuhr unabhängig. Auch die bakterielle Dichte nahm mit Erwärmung und Nährstoffzufuhr zu, wurde aber durch den Fraßdruck durch Amphipoden reduziert. Pilzliche Biomasse wurde ebenfalls durch Erwärmung und Nährstoffzufuhr, aber auch durch die Anwesenheit von Amphipoden, gefördert. Die Zusammensetzung der pilzlichen Gemeinschaft wurde von Erwärmung oder Nährstoffzufuhr nur geringfügig beeinflusst, reagierte aber auf selektiven Fraß der Amphipoden sehr stark, so wie auch de bakterielle Gemeinschaft durch Fraß und Erwärmung signifikant verändert wurde.

Zusammenfassend liefert die vorliegende Arbeit neue Einblicke in gemeinsame Effekte gleichzeitiger Veränderungen in Temperatur und Nährstoffverfügbarkeit auf mikrobielle Mineralisierer und hilft dadurch, die Bedeutung detritivorer Tiere für bakterielle und pilzliche Gemeinschaften auf der Laubstreu in Süßwässern besser zu verstehen.

General Introduction

Importance of leaf litter decomposition in freshwaters

The non-living particulate organic material (detritus) is produced from living organisms. One of the vegetal detritus is dead plant material especially when deciduous trees shed their leaves that fall to the ground or into water (Lampert and Sommer 1997, Zimmer 2008). In small-forested streams, allochthonous plant litter input of terrestrial origin from the surrounding forest, is a primary source of nutrients and energy for detritus food webs and microorganisms associated with detrital substrate, can transfer energy from dead organic matter to upper trophic level as they enhance detritus palatability to invertebrate shredders (Bärlocher 2005, Suberkropp 1998)

In principle, nutrient turnover from plant litter in freshwaters proceeds in three phases, an initial rapid phase via leaching of soluble organic substances, followed by structural degradation , primarily due to fungal and bacterial activity, and fragmentation by physical break up or invertebrate shredding (Gessner *et al*. 1999, Graça 2001). The release of water-soluble substances from litter is an early process in decomposition of leaf litter. Leaching begins within the first 24 to 48 hours after leaves have entered to aquatic system (Bärlocher 1992, Abelho 2001, Treplin and Zimmer 2012), and soluble compounds in litter leachates include phenolics, hydrocarbons and glycerides (Berg and Mc Claugherty 2003).

Leaf litter processing is controlled by the joint action of microbial decomposers, such as bacteria and fungi, and animal detritivores, and depends on both environmental conditions and leaf litter quality (Gessner *et al.* 1999, Hieber and Gessner 2002, Gessner *et al.* 2007, Treplin and Zimmer 2012)

The role of microbial decomposers in leaf litter decomposition

The quality of leaf litter as food to detritivores is determined by the composition of both the leaf itself and its attached biofilm and the specific microbial composition of the biofilm modifies the nutritional quality of the material through providing polysaccharides, proteins and amino acids, polyunsaturated fatty acid (PUFA), sterols, vitamins to detritivorous consumers of leaf litter (Lock *et al.* 1984, Hax and Golladay 1993, Davey and O′Toole 2000, Thompson *et al.* 2002). Microorganisms, especially fungi and bacteria, can break down cells of leaf litter using biochemical reactions that convert the detrital matter into metabolically chemical products for their growth and development and also useful for stream detritivores (Findley *et al*. 2002, Bärlocher 2005, Gessner *et al*. 2007, Sridhar *et al*. 2009).

Microbial decomposers with their respective extracellular enzyme capabilities degrade structural leaf components such as cellulose, hemicelluloses and lignins (Bärlocher *et al.* 1992, Berg and McClaugherty 2003) and improve litter palatability to invertebrate shredders, utilize fine particulate organic matter used, and release inorganic nutrients to primary producers (Suberkropp 1998, Bärlocher 2005). Among microbial decomposers, fungi produce a wider range of plant cell wall degrading enzymes than bacteria such as lignin-degrading enzymes (Kirk and Farrell 1987). Fungal biomass extensively exceeds bacterial biomass and constitutes the major portion of total microbial biomass (Komínková *et al.* 2000, Kuehn *et al.* 2000, Findley *et al.* 2002, Duarte *et al.* 2010), and among fungi, aquatic hyphomycetes are known the key drivers of leaf litter decomposition in temperate forested streams (Bärlocher 1992). Moreover, bacteria complete decomposition processes of plant-derived organic matter, especially in late stages of leaf litter breakdown (Suberkropp and Klug, 1980).

The role of shredders in leaf litter decomposition

Presence of shredders in addition to microbial decomposers in streams can also effect on plant litter decomposition. Invertebrate shredders widely contribute to leaf litter breakdown (Wallace and Webster 1996, Gessner *et al*. 1999, Sponseller and Benfield 2001, Hieber and Gessner 2002, Treplin and Zimmer 2012). Detritivores both directly and indirectly affect decomposition processes. Direct contributions of detritivores result quantitatively in mass loss of detritus through feeding, digestion and conversion of detrital matter into faeces. Additionally, changes in chemical composition from detritus to detritivore faeces moderate subsequent decomposition processes. Indirect contributions are the result of detritivore/microbe interactions, such as selective consumption and digestion of microbes (e.g., Ihnen & Zimmer 2008), distribution of microbial propagules through a high-quality substrate (faeces) and subsequent inoculation of detritus (Cummins and Klug 1979). But it is mostly the interactions of detritivorous invertebrates and leaf litter microbes that mediate breakdown processes (Gessner *et al*. 1999, Hieber and Gessner 2002, Gessner *et al*., 2007, Treplin and Zimmer 2012).

Since microbial decomposers increase the food quality of submerged leaves, shredders feeding on allochthonous plant material prefer to consume leaves after establishment of microorganisms, especially aquatic hyphomycetes (Suberkropp 1992, 1998, Graça 2001, Lecerf *et al*. 2005, Canhoto and Graça 2008, Chung and Suberkropp 2009). Moreover, fungi enrich the nutritional quality of detritus that affects the life history characteristics of shredders such as growth, survivorship and reproduction (Chung and Suberkropp 2009).

Increase in agricultural input in some streams may limit the distribution of shredders and thus influence leaf breakdown in these streams. Similarly, Pascoal *et al.* (2005) attributed low leaf breakdown rates to low shredder densities in a polluted river and Robinson and Gessner (2000) showed no effect of euthrophication on invertebrates in an alpine stream However in many studies,

euthrophication effects on diversity, density and biomass of macro-invertebrates colonizing decomposing leaves are positive (Rosemond *et al.* 2002, Niyogi *et al.* 2003, Pascoal *et al.* 2003, Menéndez *et al.* 2003, Gulis *et al.* 2006).

Global change and its effect on leaf litter decomposition

Global change is among the multitude of factors that continue to threaten biodiversity, ecosystem functioning and their providing services (Dudgeon *et al.* 2006, Vörösmarty *et al.* 2010). Future climate change could result in rapid temperature increases that directly affect most ecosystems including streams and rivers (Stefan and Sinokrot 1993, Eaton and Scheller 1996).

The recent Intergovernmental Panel on Climate Change report (IPCC 2007) anticipated global temperature increases by 1.1 °C to 6.4 °C and the average annual temperature in Europe increases by 2.5 °C to 5.5 °C, and following changes in ecosystems are expected to include the same increase in freshwaters temperature (Langan *et al.* 2001).

Several studies have documented the effects of environmental variables (i.e.: temperature), as well as stream water nutrient concentrations on microbial decomposer activity (Suberkropp 1984, Gönczöl *et al*. 2003, Pascoal and Cássio 2004, Ferreira and Chauvet 2011). Based on the experimental findings of warming effect on the litter decomposition, many studies have indicated faster decomposition at higher temperatures (Dang *et al.* 2009, Fernandes *et al.* 2009, Ferreira and Chauvet 2011). Higher stream water temperature stimulate leaf decomposition by increasing either leaching of soluble compounds (Chergui and Pattee 1990) or growth and reproduction of aquatic hyphomycetes (Rajashekar and Kaveriappa 2000; Dang *et al*. 2009) and by promoting leaf consumption by invertebrate shredders (Gonzalez and Graça 2003, Azevedo-Pereira *et al*. 2006, Ferreira *et al*. 2010).

Additionally, it was hypothesized that human activities such as urbanization, industrial and agricultural development and water course

alterations, have jeopardized streams and rivers (Malmqvist and Rundle 2002, Galloway *et al*. 2008). In freshwater systems, eutrophication is a process, whereby enrichment of the ecosystem with inorganic nutrients, especially N and P, stimulate plant and algal growth.

Clearly, in some investigations faster leaf litter processing was observed in nutrient-rich systems *versus* nutrient-poor systems (Huryn *et al*. 2002, Gulis and Suberkropp 2003, 2004, Pascoal *et al.* 2003, Nikolcheva and Bärlocher 2005, Ferreira and Chauvet 2011, Tonin *et al*. 2011). However, some studies found no significant effects of nutrient-addition on leaf litter breakdown (Grattan and Suberkropp 2001, Royer and Minshall 2001, Abelho and Graça 2006, Abelho *et al*. 2010). Along the same line, in studies by Gulis and Suberkropp (2003a, b, c) positive responses of fungi to nutrient-addition was found in streams and in microcosm experiments. Similarly, litter-decomposing fungi in temperate streams were more than twice as productive in a nutrient-enriched as in a control stream (Gulis *et al*. 2008), and changes in aquatic hyphomycete diversity and sporulation was demonstrated in response to nutrient-enrichment (Pascoal *et al*. 2005).

Nutrient-addition also induced higher fungal species richness (Gulis and Suberkropp 2003a, 2004, Chung and Suberkropp 2008). Hence, on an ecosystem-scale, nutrient-enrichment resulted in less substrate for microbial colonization through increasing fungal biomass and production, bacterial production and total microbial respiration (but not bacterial biomass) (Suberkropp *et al*. 2010). Similarly, ecosystem respiration increased, and detritus-processing was accelerated, upon long-term nutrient-enrichment (Benstead *et al*. 2009).

Rising nutrient inputs and increasing temperatures tend to mutually intensify eutrophication symptoms (Kosten *et al*. 2009, Rustad *et al*. 2001, Brookshire *et al*. 2011), but rarely have the simultaneous effects of multiple environmental changes, e.g., warming and nutrient-enrichment, on ecosystem

processes been studied. Ferreira and Chauvet (2011) provided evidence for stream eutrophication to promote effects of warming on detritus processing, whereas warming might enhance fungal growth and reproduction in both oligotrophic and eutrophic streams.

Microbial decomposer community structures

Conventionally, most of microbial decomposer diversity studies are based on cultivation-dependent methods. Typically only a small fraction (< 1%) of microorganisms have been cultivated by standard techniques (Barns *et al*. 1996, Handelsman 2004).To decrease much of the inadequacies associated with the traditional techniques of microbiology and microscopy, other approaches have been employed that rely on DNA analysis of small-subunit RNA gene or internal transcribed spacer (ITS) regions and also rely on phospholipid fatty acid (PLFA) analysis or fluorescent in situ hybridization (FISH). One of the methods applicable to investigate microbial communities is DGGE (Denaturing Gradient Gel Electrophoresis) that has been used as a valuable molecular method for examination of leaf-associated microbial community structures and detection of shifts in fungal and bacterial assemblages (Nikolcheva *et al*. 2003, 2005 , Das *et al*. 2008, Duarte *et al*. 2008, 2010, Bärlocher 2010). To investigate community structure of microbial decomposers, 18S rRNA and 16S rRNA genes of fungi and bacteria, respectively, are amplified by PCR before the products are subjected to DGGE analysis (Muyzer and de Waal 1994).

Microbial studies examine the diversity of operational taxonomic units (OTUs) or phylotypes to determine the bacterial and fungal diversities. It has been stated that the assessment of microbial community diversity with nucleic acid-based methods has allowed the estimation of many previously undetected microbial decomposers. For example, in some investigation on bacterial diversity during litter decomposition in streams, only cultivable bacteria were taken in consideration (Hieber and Gessner 2002, Suberkropp and Klug 1976),

but DGGE allowed to assess some phylotypes belonging to actinomycetes group (Das *et al.* 2007). Additionally, DGGE proved useful for fungal conidia detection in freshwaters (Raviraja *et al*. 2005). By DGGE also has been shown low fungal diversity in circumneutral eutrophic streams (Pascoal *et al*. 2005) and high fungal diversity in initial stages of leaf decomposition (Nikolcheva *et al.* 2005).

Outlines

The major aim of this thesis is to assess the response of microbial decomposers and detritivores associated with decomposing leaf litter under the influence of warming and nutrient enrichment.

Our objectives in the present study were to:

i. Determine how warming, nutrient enrichment and presence of detritivores might affect the fungal and bacterial biomass on birch litter through time using traditional methods
ii. Examine bacterial and fungal community composition on birch litter under warming, nutrient enrichment and presence of detritivores conditions
iii. Test whether the decomposition rate of birch litter is affected by changes in environmental factors and detritivore presence
iv. Test whether detritivore dry mass is affected by changes in temperature and nutrient conditions.

The first objective was accomplished via laboratory experiments, microscopy and chemical estimation. The microbial biomass on birch leaf litter was examined during decomposition experiments carried out in a full-factorial designed microcosm experiment by increasing average water temperature to a target of 5 ºC above ambient and periodically fertilizing with a solution of nitrate and phosphate to a target of concentration 10 times above ambient. In this part of the study, the bacterial biomass on decomposing birch litter was

evaluated using the common procedure for the determination of total bacterial biomass based on staining of samples with a fluorescent dye such as 4, 6-diamidino-2-phenylindole (DAPI).Since ergosterol is an important membrane lipid in most fungi, ergosterol estimation method was used to quantify fungal biomass on decaying birch leaves.

The second objective of study focused on microbial community structure through DGGE (Denaturing Gradient Gel Electrophoresis) as a valuable molecular method that have been suggested in studies by Nikolcheva *et al*. (2003,2005) , Duarte *et al*.(2008,2010) and Bärlocher (2010).

The third objective of study estimates the birch (*Betula pendula*) leaves decomposition rate. Birch leaves were used because of their key presence in northern riparian forests and to determine the remaining dry mass, leaf disks were used in experiments.

The fourth objective of study focuses on changes in dry weight of amphipod, *Gammarus pulex*, over time in experiments.

References:

- Abelho, M. 2001. From litterfall to breakdown in streams: a review. *The Scientific World* 1, 656-680.
- Abelho, M. and M.A.S. Graça. 2006. Effects of nutrient enrichment on decomposition and fungal colonization of sweet chestnut leaves in an Iberian stream (Central Portugal). *Hydrobiologia* 560, 239-247.
- Azevedo-Pereira, H., González, J. and M. A. S. Graça. 2006. Life history of *Lepidostoma hirtum* in an Iberian stream and its role on organic matter processing. *Hydrobiologia* 559, 183-192.
- Barns, S.M., Delwiche, C.F., Palmer J.D. and N.R. Pace.1996. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. *Proceedings of the National Academy of Sciences of the United State of America* 93, 9188-9193.
- Bärlocher, F. 1992. Research on aquatic Hyphomycetes: historical background and overview.In: The ecology of aquatic Hyphomycetes (F. Barlocher, Ed.). Springer Verlag, Berlin, p.1-15.
- Bärlocher, F. 2005. Freshwater fungal communities, In: The fungal community: its organization and role in the ecosystem (3rd ed.), (Deighton, J.; Oudemans, P. and White, J. Eds.). Taylor and Francis, CRC Press, Boca Raton, Florida, p. 39-59.
- Bärlocher, F. 2010. Molecular approaches promise a deeper and broader understanding of the evolutionary ecology of aquatic hyphomycetes. *Journal of the North American Benthlological Society* 29, 1027-1041.
- Benstead, J.P., A.D. Rosemond, W.F. Cross, J.B Wallace, S.L. Eggert, K. Suberkropp, V. Gulis, J.L. Greenwood and C.J. Tant. 2009. Nutrient enrichment alters storage and fluxes of detritus in a headwater stream ecosystem. *Ecology* 90, 2556-2566.
- Berg, B. and C. McClaugherty. 2003. Plant Litter, Decomposition, Humus Formation, Carbon Sequestration. Springer Verlag, Berlin.
- Brookshire, E.N.J., S. Gerber, J.R. Webster, J.M. Nose and W.T. Swank. 2011. Direct effects of temperature on forest nitrogen cycling revealed through analysis of long-term watershed records. *Global Change Biology* 17, 297-308.

- Canhoto, C. and M. A. S. Graça. 2008. Interactions between fungi (aquatic Hyphomycetes) and invertebrates. In: Novel techniques and ideas in mycology (K. R. Sridhar, F. Bärlocher, and Hyde K. D. Eds.). Fungal Diversity Research Series, 20. Fungal Diversity Press, Chiang Rai, p. 205-325.

- Chergui, H. and E. Pattee. 1990. The influence of season on the breakdown of submerged leaves. *Archic für Hydrobiologie* 120, 1-12.
- Chung, N. and K. Suberkropp. 2008. Influence of shredder feeding and nutrients on fungal activity and community structure in headwater streams. *Fundamental and Applied Limnology Archiv für Hydrobiologie* 173, 35-46.
- Chung, N. and K. Suberkropp. 2009. Effects of aquatic fungi on feeding preferences and bioenergetics of *Pycnopsyche gentilis* (Trichoptera: Limniphilidae). *Hydrobiologia* 630, 257-269.

- Cummins, K. W. and M. J. Klug. 1979. Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* 10, 147-172.
- Dang, C.K., M. Schindler, E. Chauvet, and M.O. Gessner. 2009. Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. *Ecology* 90, 122-131.

- Das, M., T.V. Royer and L.G. Leff. 2007. Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. *Applied and Environmental Microbiology* 73, 756-767.

- Das, M., T. D. Royer and L. G. Leff. 2008. Fungal communities on decaying leaves in streams: a comparison of two leaf species. *Mycological Progress* 7, 267-275.
- Davey, M.E. and G.A. O'Toole. 2000. Microbial biofilms: from ecology to molecular genetics. *Microbiology and Molecular Biology Reviews* 64, 847-867.

- Duarte, S., C. Pascoal, A. Alves, A. Correia, and F. Cássio. 2008. Copper and zinc mixtures induce shifts in microbial communities and reduce leaf litter decomposition in streams. *Freshwater Biology* 53, 91-101.
- Duarte, S., C. Pascoal, A. Alves, A. Correia and F. Cássio. 2010. Assessing the dynamic of microbial communities during leaf decomposition in a low-order stream by microscopic and molecular techniques. *Microbiological Research* 165, 351-362.

- Dudgeon D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lèvêque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. Stiassny and C. A. Sullivan. 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews* 81, 163-182.
- Eaton, J. G., and R. M. Scheller. 1996. Effects of climate warming on fish thermal habitat in streams of the United States. *Limnology and Oceanography* 41, 1109-1115.

- Fernandes I., B.Uzun, C. Pascoal and F. Cássio. 2009. Responses of aquatic fungal communities on leaf litter to temperature-change events. *International Review of Hydrobiology* 94, 410-418.
- Ferreira, V., V. Gulis and M. A. S. Graça. 2006. Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. *Oecologia* 149, 718-729.
- Ferreira, V., A.L. Gonçalves, D.L. Godbold and C. Canhoto. 2010. Effect of increased atmospheric CO2 on the performance of an aquatic detritivore through changes in water temperature and litter quality. *Global Change Biology* 16, 3284-3296.

- Ferreira, V. and E. Chauvet. 2011. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology* 17, 551-564.

- Findlay, S., J. Tank, S. Dye, H.M. Valett, P.J. Mulholland, W.H. McDowell, S.L. Johnson, S.K. Hamilton, J. Edmonds, W.K. Dodds and W.B. Bowden. 2002. A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microbial Ecology* 43, 55-66.
- Flury, S. 2008. Carbon fluxes in a freshwater wetland under simulated global change: Litter decomposition, microbes and methane emission. PhD dissertation, ETH Zurich, Zurich.
- Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger and M. A. Sutton. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889-92.
- Gessner, M. O. and E. Chauvet. 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied and Environmental Microbiology* 59, 502-507.
- Gessner, M.O., E. Chauvet and M. Dobson. 1999. A perspective on leaf litter breakdown in streams. *Oikos* 85, 377-384.

- Gessner, M.O., V. Gulis, K.A. Kuehn, E. Chauvet and K. Suberkropp. 2007. Fungal decomposers of plant litter in aquatic ecosystems. In: The Mycota IV. Environmental and microbial relationships (2nd ed). (Kubicek C.P., Druzhinina I.S., Eds). Springer-Verlag, Berlin, p. 301-324.
- Gönczöl, J., P. Csontos, and Á. Révay. 2003. Catchment scale patterns of aquatic hyphomycetes. The role of physicochemical variables and substrate composition in structuring conidial communities. *Archiv für Hydrobiologie* 157, 249-266.
- González, J. M. and M. A. S. Graça. 2003. Conversion of leaf litter to secondary production by the shredder caddisfly *Sericostoma vittatum*. *Freshwater Biology* 48, 1578-1592.

- Graça, M. A. S. 2001. The role of invertebrates on leaf litter decomposition in streams – A review. *International Review of Hydrobiol*ogy 86, 383-393.
- Grattan, R. M. and K. Suberkropp. 2001. Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. *Journal of North American Benthological Society* 20, 33-43.
- Gulis, V. and K. Suberkropp. 2003 a. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology* 48, 123-134.

- Gulis, V. and K. Suberkropp. 2003 b. Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. *Microbial Ecology* 45, 11-19.

- Gulis V. and K. Suberkropp. 2003 c. Interactions between stream fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. *Aquatic Microbial Ecology* 30, 149-157.
- Gulis, V. and K. Suberkropp. 2004. Effects of whole-stream nutrient enrichment on the concentration and abundance of aquatic hyphomycete conidia in transport. *Mycologia* 96, 57-65.

- Gulis, V., K. A. Kuehn and K. Suberkropp. 2006. The role of fungi in carbon and nitrogen cycles in freshwater ecosystems. In: Fungi in biogeochemical cycles (G. M. Gadd Ed.). Cambridge University Press, Cambridge, p. 404-435
- Gulis, V., K. Suberkropp and A.D. Rosemond. 2008. Comparison of fungal activities on wood and leaf litter in unaltered and nutrient-enriched headwater streams. *Applied and Environmental Microbiology* 74, 1094-1101.

- Handelsman, J. 2004. Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews* 6, 669-85.
- Hax, C.L. and S.W. Golladay. 1993. Macroinvertebrate colonization and biofilm development on leaves and wood in a boreal river. *Freshwater Biology* 29, 79-87.

- Hieber, M. and M.O. Gessner. 2002. Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology* 83, 1026-1038.

- Huryn, A.D., V.M. Butz Huryn, C.J. Arbuckle and L. Tsomides. 2002. Catchment land use, macroinvertebrates and detritus processing in headwater streams: taxonomic richness versus function. *Freshwater Biology* 47, 401-415.
- Ihnen, K. and M. Zimmer. 2008. Selective consumption and digestion of litter microbes by *Porcellio scaber* (Isopoda: Oniscidea). *Pedobiologia* 51, 335-342.

- IPCC [International Panel on Climate Change]. 2007. Climate change 2007: the physical science basis. Cambridge University Press, Cambridge

- Kirk, T.K. and R.L. Farrell. 1987. Enzymatic "combustion": The microbial degradation of lignin. *Annual Review of Microbiology* 41, 465-501.

- Komínková, D., K.A. Kuehn, N. Bürsing, D. Steiner and M.O. Gessner. 2000. Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of large lake. *Aquatic Microbial Ecol*ogy 22, 271-282.

- Kosten, S., A. Kamarainen, E. Jeppesen, E.H. van Nes, E.T. H.M. Peeters, N. Mazzeo, L. Sass, J. Hauxwell, N. Hansel-Welch,T.L. Lauridsen, M. Søndergaard, R.W. Bachmann, G. Lacerot, and M. Scheffer. 2009. Climate-related differences in the dominance of submerged macrophytes in shallow lakes. *Global Change Biology*, 15, 2503-2517.

- Kuehn, K.A., M.J. Lemke, R.G. Wetzel and K. Suberkropp. 2000. Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effusus*. *Limnology and Oceanography* 45, 862-870
- Lampert, W. and U. Sommer. 1997. *Limnoecology*: *The Ecology of Lakes and Streams*. Oxford University Press, New York
- Langan, S.J., L. Johnston, M.J. Donaghy, A.F. Youngson, D.W. Hay, and C. Soulsby. 2001. Variation in river water temperatures in an upland stream over a 30-year period. *Science of the Total Environment* 265, 195-207.

- Lecerf, A., M. Dobson, C.K. Dang and E. Chauvet. 2005. Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. *Oecologia* 146, 432-442.
- Lock, M.A., R.R. Wallace, J.W. Costerton, R.M. Ventullo, and S.E.Charlton. 1984. River epilithon: toward a structural- functional model. *Oikos* 42, 10-22.

- MacNeil, C., J.T.A. Dick and R.W. Elwood. 1997. The trophic ecology of freshwater *Gammarus spp.* (Crustacea: Amphipoda): Problems and Persoectives concerning the functional feeding group concept. *Biological Reviews* 72, 349-364.

- Malmqvist, B., S. Rundle. 2002. Threats to the running water ecosystems of the world. *Environmental Conservation* 29, 134-153.

- Menendez, M. O. Hernandez and F.A. Comin. 2003. Seasonal comparisons of leaf processing rates in two Mediterranean rivers with different nutrient availability. *Hydrobiologia* 495, 159-169.

- Muyzer, G. and E.C. de Waal. 1994. Determination of the genetic diversity of microbial communities using DGGE analysis of PCRamplified 16S rRNA. *NATO ASI Series G*35, 207-214

- Nikolcheva, L.G. and F. Bärlocher. 2005. Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence. *Environmental Microbiology* 7, 270-280.

- Nikolcheva, L.G., A.M. Cockshutt, and F. Bärlocher. 2003. Determining diversity of freshwater fungi on decaying leaves: comparison of traditional and molecular approaches. *Applied and Environmental Microbiology* 69, 2548-2554.

- Nikolcheva, L.G., T. Bourque and F. Bärlocher. 2005. Fungal diversity during initial stage of leaf decomposition in a stream. *Mycological Research* 109, 246-253.

- Niyogi, D. K., K. S. Simon and C. R. Townsend. 2003. Breakdown of tussock grass in streams along a gradient of agricultural development in New Zealand. *Freshwater Biology* 48, 1698-1708.

- Pascoal, C., M. Pinho, F. Cássio, and P. Gomes. 2003. Assessing structural and functional ecosystem condition using leaf breakdown: studies on a polluted river. *Freshwater Biology* 48, 2033-2044.

- Pascoal, C. and F. Cássio. 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. *Applied and Environmental Microbiology* 70, 5266-5273.

- Pascoal, C., F. Cássio, and L. Marvanová. 2005. Anthropogenic stress may affect aquatic hyphomycete diversity more than leaf decomposition in a low-order stream. *Archiv für Hydrobiologie* 162, 481-496.

- Rajashekar, M. and K.M. Kaveriappa. 2000. Effects of temperature and light on growth and sporulation of aquatic hyphomycetes. *Hydrobiologia* 441, 149-153.

- Raviraja, N.S., L.G. Nikolcheva and F. Bärlocher. 2005. Diversity of conidia of aquatic hyphomycetes assessed by microscopy and by DGGE. *Microbial Ecology* 49, 1-7

- Robinson, C.T. and M.O. Gessner. 2000. Nutrient addition accelerates leaf breakdown in an alpine springbrook. *Oecologia* 122, 258-263.

- Rosemond, A. D., C. M. Pringle, A. Ramírez, M. J. Paul, and J. L. Meyer. 2002. Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. *Limnology and Oceanography* 47, 278-289.
- Royer, T. V. and G. W. Minshall. 2001. Effects of nutrient enrichment and leaf quality on the breakdown of leaves in a hardwater stream. *Freshwater Biology* 46, 603-610.
- Rustad, L.E., J.L.Campbell, G.M. Marion, R.J. Norby, M. Mitchell, A.E. Hartley, J.H.C. Cornellissen, and J. Gurevitch. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126, 543-562.

- Sridhar, K. R., S. Duarte, F. Cássio and C. Pascoal. 2009. The role of early fungal colonizers in leaf-litter decomposition in Portuguese streams impacted by agricultural runoff. *International Review of Hydrobiology* 94, 399-409.
- Sponseller, R. and E.F. Benfield. 2001. Influence of land use on leaf breakdown in southern Appalachian headwater streams: multiple scale analysis. *Journal of North American Benthological Society* 20, 44-59

- Stefan, H.G. and B. A. Sinokrot. 1993. Projected global climate change impact on water temperatures in five north central U.S. streams. *Climate Change* 24, 353-381.

- Suberkropp, K. 1984. Effect of temperature on seasonal occurrence of aquatic hyphomycetes. *Transactions of the British Mycological Society* 82, 53-62.
- Suberkropp, K. 1992. Interactions with invertebrates. In: The ecology of aquatic hyphomycetes (F. Bärlocher Ed.), Springer Verlag, Berlin. p. 118-134
- Suberkropp, K. 1998. Microorganisms and organic matter decomposition, In: River ecology and management: lessons from the Pacific coastal ecoregion (Naiman, R.J. and Bilby, R.E. Eds.), Springer, New York, p. 120-143
- Suberkropp, K. and M.J. Klug.1976. Fungi and bacteria associated with leaves during processing in a woodland stream. *Ecology* 57,707-719
- Suberkropp, K., M.O. Gessner and E. Chauvet. 1993. Comparison of ATP and ergosterol as indicators of fungal biomass associated with decomposing leaves in streams. *Applied and Environmental Microbiology* 59, 3367-3372.
- Suberkropp, K., V. Gulis, A. D. Rosemond, and J. P. Benstead. 2010. Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: results of a 5-year continuous enrichment. *Limnology and Oceanography* 55, 149-160.

- Tonin, A. M. and L. U. Hepp. 2011. Effects of nitrate enrichment on leaf litter decomposition. *Acta Limnologica Brasiliensia* 23, 86-94.
- Thompson, F.L., P.C. Abreu and W. Wasielesky. 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. *Aquaculture* 203, 263-278.

- Treplin, M. and M. Zimmer. 2012. Decomposition of autochthonous *versus* allochthonous detritus: a cross-habitat comparison. *Ecosystems* 15, 477-491.

- Vörösmarty, C.J., P.B. McIntyre, M.O. Gessner, D. Dudgeon, A. Prusevich, , P. Green, , S. Glidden, S.E. Bunn, C.A. Sullivan, C.A. Liermann, and P.M. Davies. 2010. Global threats to human water security and river biodiversity. *Nature* 467, 555-561.
- Wallace, J.B. and J.R. Webster .1996. The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology* 41, 115-139.

- Zimmer, M. 2008. Detritus, In: Encyclopedia of ecology (Jørgensen S.E., Fath B.D. Eds.).Elsevier, Oxford, p. 903-911.

Chapter I

Effects of predicted environmental changes in freshwater ecosystems on how grazing-pressure affects leaf litter-colonizing bacteria

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Abstract

In view of current scenarios of global environmental change, we investigated the effects of warming and nutrient-addition (N and P) on the impact of detritivores on density and community composition of leaf litter-colonizing bacteria in a freshwater environment. Within 10 d, detritivorous amphipods (*Gammarus pulex*) reduced bacterial numbers, both at low and, albeit to a lesser degree, at high temperature. However, the detritivore-induced decrease in bacterial numbers was compensated for by nutrient addition. In the long run (31 d), warming, did not compensate the reduction in bacterial counts when amphipods were present and nutrient addition did not counteract detritivore-effects. Similarly, changes in bacterial numbers in response to nutrient-addition were more pronounced at low temperature within 10 d, but nutrient-effects were stronger at high temperature in the long run. Thus, warming without detritivores did not have any effect on bacterial numbers under low-nutrient conditions (10 d). When detritivores were present, warming promoted bacterial density under low-nutrient conditions but negatively affected bacterial counts under high-nutrient conditions. In the long run (31 d), warming did not affect bacterial density in detritivore-free controls, irrespective of nutrient conditions and also did not affect bacterial density, irrespective of nutrient conditions, when detritivores were present. Warming exhibited strong effects on the composition of litter-associated bacterial communities, irrespective of nutrient load, whereas nutrients less consistently affected bacterial community composition.

Key words: Freshwater biofilm, Environmental changes, Detritivores, Nutrient-addition, Warming

Introduction

Upon submersion, leaf litter that enters freshwater bodies undergoes chemical change due to removal of soluble compounds through leaching (Nykvist, 1963; Schofield et al., 1998; Treplin and Zimmer, 2012) and becomes readily covered by biofilms composed of microorganisms (bacteria, algae, fungi, protists, microalgae and micrometazoa), exoenzymes, and detritus particles embedded in a gelatinous polysaccharide matrix (glycocalyx) of mostly bacterial origin (Geesey et al., 1978; Lock et al., 1984; Hall-Stoodley et al., 2004).

Leaf litter processing is controlled by the joint action of microbial decomposers, such as bacteria and fungi, and animal detritivores, and depends on both environmental conditions and leaf litter quality (Gessner et al., 1999; Hieber and Gessner, 2002; Gessner et al., 2007; Treplin and Zimmer, 2012). In turn, the quality of leaf litter as food to detritivores is determined by the composition of both the leaf itself and its attached biofilm (Lock et al., 1984; Hax and Golladay, 1993; Davey and O′Toole, 2000; Thompson et al., 2002). Recent studies stressed the substantial contribution of bacteria and fungi to leaf litter mass loss during decomposition processes (Baldy et al., 2002; Hieber and Gessner, 2002; Gulis and Suberkropp, 2003 b). Bacteria are capable to compete with fungi for leaf litter resources, and their contribution to leaf litter decay increases relative to fungal contributions upon nutrient enrichment (Gulis and Suberkropp, 2003 b; Pascoal and Cássio, 2004).

The main parameters controlling microbial communities and production are temperature and the availability of nutrients (Kirchman, 1994; Felip, 1996; Chauvet and Suberkropp, 1998; Rubin and Leff, 2007). The recent Intergovernmental Panel on Climate Change report (IPCC, 2007) predicts global temperature increases by 1.1 °C to 6.4 °C during the next decades, and freshwaters temperature is expected to follow the same trend (Langan et al. 2001). Within this range, an experimental 5 ˚C-increase in temperature changed

the community composition of aquatic hyphomycetes on leaf litter, but effects on decomposition depended on the identity of the dominant fungal species (Dang et al., 2009). By contrast, warming by 4 °C only slightly affected bacterial biomass on litter surfaces but caused a shift in bacterial community structure (Flury and Gessner, 2011); In some studies, warming effects on microbial responses were more relevant under high nutrient conditions (Ferreira and Chauvet, 2011; Villanueva et al.2011). The effects of warming on decomposition processes in freshwaters, however, particularly in combination with other environmental changes (e.g., nutrient-enrichment), are still not well understood.

Temperate headwaters in forested areas, being characterized by high input of detrital matter of terrestrial origin and a dense detritivorous macrofauna, are prone to anthropogenic nutrient-enrichment from agricultural land through surface-runoff and soil/ground water fluxes as well as atmospheric deposition. Ground waters contribute up to 42% of gross nitrate input into streams (Duff et al., 2008), and more than 70% of drinking water in Germany is supplied by groundwater (BGR, 2010). The Drinking Water Directive of the European Union (98/83/EC) sets a maximum allowable concentration of 50 mg NO_3 -N L^{-1}, but in some ground water sampling sites of Germany nitrate concentrations were above 50 mg NO3 –N L^{-1} (Petzoldt and Uhlmann, 2006).

Leaf litter biofilms in aquatic environments are often nutrient-limited (Francoeur, 2001; Tank and Dodds, 2003; Elser et al., 2007). Litter-decomposing fungi in temperate streams being more than twice as productive in a nutrient-enriched than in a control stream (Gulis et al., 2008). Nutrient-enrichment also increased the bacterial activity on leaf litter (Gulis and Suberkropp, 2003b; Pascoal and Cássio, 2004).

Invertebrate shredders contribute to leaf litter breakdown through feeding and digestion (Wallace and Webster, 1996; Gessner et al., 1999; Hieber and

Gessner, 2004; Treplin and Zimmer, 2012), but it is mostly the interactions of detritivorous invertebrates and leaf litter microbes that mediate breakdown processes (Gessner et al., 1999; Hieber and Gessner, 2004; Gessner et al., 2007; Treplin and Zimmer, 2012). The most direct effect that detritivorous invertebrates exert on microbial biofilms is its consumption, be it by shredders that consume entire leaf particles or by grazers that abrade biofilms from leaf surfaces. Upon grazing, cell density is reduced, but activity may increase through improved resource-availability to the remaining cells, and community composition may change upon selective feeding.

How invertebrate-microbe interactions during leaf litter decomposition are affected by environmental change, e.g., warming and eutrophication, is essentially unknown. Rising nutrient inputs and increasing temperatures tend to mutually intensify eutrophication symptoms (Kosten et al., 2009, Rustad et al., 2001, Brookshire et al., 2011), but rarely have the combined effects of these environmental variables on biotic interactions been studied.

Understanding the effect of multiple environmental changes on, interactions of detritivores and bacterial biofilm, is a prerequisite to predicting effects of environmental changes on decomposition processes in temperate freshwaters. With the present study, we aim at providing first insight into the complex effects that warming and nutrient-enrichment may have on biofilm-grazing by detritivorous invertebrates in temperate headwater streams which, in turn, controls microbe-mediated decomposition processes. We hypothesize that effects of grazing on bacterial density and community structure in biofilms are counteracted by warming and nutrient-enrichment. To test this hypothesis, we designed an orthogonal experimental setup that independently combined the three factors "grazers", "temperature" and "nutrients".

Materials and Methods

Experimental set-up

Experiments were carried out in microcosms (16 × 10 × 10 cm) which were supplied with 1 liter of aerated diluted creek water from Russee creek (10˚ 08′ E, 54˚ 29′ N) near Kiel (northern Germany), containing 1.5×10^7 bacteria mL^{-1}.

Leaf litter (Birch, *Betula pendula* Roth, a key component in northern riparian forests) was collected from Kiel soon after abscission, air-dried and conditioned in undiluted creek water for 2 weeks prior to introduction to the microcosms. Leaves were tested randomly for bacterial inoculation through quantitative bacterial measurement. Leaf disks (10 mm diameter) were cut from inoculated leaves, using a cork borer, and 9 disks were added to each microcosm.

Specimens of amphipods (*Gammarus pulex*, Linnaeus 1758) were collected from Russee creek. Nine individuals representing the natural size range observed in the stream were placed in half of the microcosms, simulating a natural amphipod density as observed in Russee creek. This species represents the major macro-detritivore in northern German woodland streams, where it occurs in densities ranging from 40 to 1,000 with a mean density of 506 ind. m^{-2}. Microcosms were examined daily for dead animals which were removed and replaced by animals of the same size. The other half of the microcosms served as detritivore-free control.

Ammonium nitrate and potassium phosphate were added to half of the microcosms to achieve nutrient (N, P) concentrations of 1 mM L^{-1} nitrate (as NH_4NO_3; Carl Roth, Karlsruhe, Germany; 90 mg L^{-1}, equivalent to 31.5 mg N L^{-1}) and 0.006 mM L^{-1} phosphate (equimolar quantities of KH_2PO_4 and K_2HPO_4; Carl Roth, Karlsruhe, Germany; 8.18 mg L^{-1}, equivalent to 1.5 mg P L^{-1}). Once a

week, half of the water was replaced by a fresh-made mixture of tap and creek water. The other half of microcosms served as ambient-nutrients controls.

Warming was mimicked by increasing the experimental temperature of 10 °C (N = 20 x 2 for two sampling dates) to 15 °C (N = 20 x 2) orthogonally to low (N = 20 x 2) versus high (N = 20 x 2) nutrient concentration. Therefore, the treatments for the two dates of sampling, each, were (a) control (ambient temperature, ambient nutrient load) (N = 10 x 2, for two sampling dates); (b) increased temperature, ambient nutrient load (N = 10 x 2); (c) ambient temperature, increased nutrient load (N = 10 x 2); (d) increased temperature, increased nutrient load (N =10 x 2); which were run either with (N = 5 for each of the above treatments and each sampling date) or without (N = 5) amphipods. Thus, in total 80 microcosms were set-up for being sampled after 10 d (40 microcosms) and 31 d (40 microcosms).

After 10 and 31 days, 5 microcosms, respectively, of each treatment were sacrificed. A subset of 4 randomly chosen leaf disks from each microcosm were stored in formaldehyde at 4 ˚C for the enumeration of bacteria; another subset of 4 leaf disks were kept frozen (-20˚C) for the extraction of bacterial DNA and subsequent genetic fingerprinting.

Bacterial Density and Microscopy

For detaching the bacteria from the leaves, two glass beads (5 mm) were added to a 2 mL-tube containing 4 leaf disks in formaldehyde solution (Carl Roth, Karlsruhe, Germany), and the tube was vortexed vigorously for 30 s. To determine bacterial cell counts, 0.5 ml aliquots of the bacterial suspensions were filtered through a polycarbonate membrane (0.22 µm pore size; Millipore) (Sartorius Stedim, Göttingen, Germany) placed on nitrocellulose support membrane (0.45 µm pore size; Millipore) by using a vacuum filtration unit. Bacteria attached to the polycarbonate membrane were stained with 4’,6-

Diamidino-2-phenylindole dihydrochloride (DAPI) (Invitrogen, Darmstadt, Germany) at a final concentration of 1µg mL^{-1} . The filters were rinsed with sterile water and mounted onto glass slides. The slides were observed under 1000x magnification with an epifluorescence microscope (Axio scope A1, Zeiss, Hamburg, Germany). For statistical evaluation, 12 microscopic fields (100 x 100 μm^2) were counted per slide.

Extraction of genomic DNA and 16S rRNA gene amplification

Total DNA was extracted from 4 frozen leaf disks using the QiaAmp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Amplification of bacterial 16S rRNA genes was performed using PureTaq Ready-To-Go PCR Beads (GE Healthcare, Munich, Germany) in a total PCR volume of 25 µL; 10 pmol of each bacterial primer 341F-GC (5'- [CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GC] CTA CGGGAG GCA GCA G-3') and 534R (5'-ATT ACC GCG GCT GCT GG-3') was used for amplification of suitable fragments for DGGE (Muyzer et al.,1993). A GC-clamp (sequence in square brackets) was attached to the 5' end of the forward primer. PCR conditions were as follows: initial denaturation at 94 °C for 2 min; 15 touchdown cycles starting with an annealing temperature of 65 °C for 40 s and an incremental reduction of 1 °C per cycle; elongation at 72 °C for 40 s; and denaturation at 94 °C for 30 s. The touchdown steps were followed by 35 cycles of annealing temperature at 50 °C for 40 s, elongation at 72 °C for 40 s and denaturation at 94 °C for 30 s; a final annealing step was performed at 42 °C for 60 s and a final elongation at 72 °C for 5 min. The correct size of the amplified DNA fragments was verified by electrophoresis of 10 % of the PCR reaction volume in 1.8 % agarose in 1 × TBE buffer.

Denaturing Gradient Gel Electrophoresis

Bacterial communities on leaf litter surfaces were characterized through DGGE using double gradient polyacrylamide gels (Petri and Imhoff, 2001). DGGE gels contained a denaturing gradient from 40 to 80 % (100 % defined as 7 M urea and 10 M formamide) and an acrylamide (Acrylamide-Bis:Rotipuran 37.5:1) gradient from 6 % to 8 %. 15 µL of PCR products mixed with 3 µL loading buffer (10 x) were loaded on the DGGE gel. Electrophoresis was run at 60°C and 80 V for 14 h in 0.5 × TAE buffer in a CBS Scientific DGGE-2001 system. After electrophoresis, the gel was stained for 45 min in SYBR Gold® (Invitrogen Gmbh, Darmstadt, Germany), rinsed for 30 min in 1 × TAE buffer and photographed under UV light.

Statistical analysis

The R statistical software environment (version 2.13.1, (http://www.r-project.org/)) was utilized to perform quantitative data analyses. A three-factorial analysis of variance (ANOVA) was used to test whether bacterial density was significantly affected by nutrient addition, temperature or fauna. Comparison of treatment effects was made using Tukey′s HSD test. Shapiro-Wilcoxon test was used for testing for normality, and Fligner-Killeen test was used for testing for homogeneity of variances.

DGGE gels were analyzed by the generation of a presence/absence matrix based on the band pattern. All visible bands in every gel lane were taken into account for further calculation using the Primer software v.6.1.9 (Primer-E) (Primer Ltd, Plymouth, UK). Bray-Curtis values without transformation were calculated. Sample similarities are shown by cluster analysis and non-metric multidimensional scaling (NMDS). Band positions were assigned to operational taxonomic units (OTUs). Differences among treatments were analyzed by Permutational multivariate analysis of variance (PERMANOVA, Anderson

2001) that compared the observed value of a test statistic (Pseudo F-ratio) against a recalculated test of random permutation of the data. Monte Carlo p-values were used as a test statistic for replicates.

Results

Bacterial density

In the short run (10 days of incubation: Fig. 1.1 A) all experimental factors proved significant in ANOVA (Tab. 1.1), but mutually affected each other (statistical pairwise interactions: Tab. 1.1). In comparison to detritivore-free controls, litter-colonizing bacteria were reduced almost two-fold when amphipods were present under low-temperature and low-nutrient conditions (Fig.1.1 A). Warming by 5 °C (15 °C-treatment) did not promote bacterial growth but reduced feeding pressure by amphipods, since the reduction in bacterial density upon amphipod presence was less pronounced, albeit still significant, whereas increasing temperature in detritivore-free controls did not affect bacterial counts. Nutrient-addition significantly changed the patterns observed in response to warming and grazing. In detritivore-free controls, nutrient addition more than doubled bacterial density at low temperature, but no effect of nutrients was observed at high temperature. Hence, nutrient-addition entirely compensated for loss of bacterial density through grazing by amphipods at low temperature, but did so only slightly at high temperature. Low-nutrient/low-temperature versus high-nutrient/high-temperature treatments differed significantly in the presence of detritivores, but bacterial densities were similar in detritivore-free treatments.

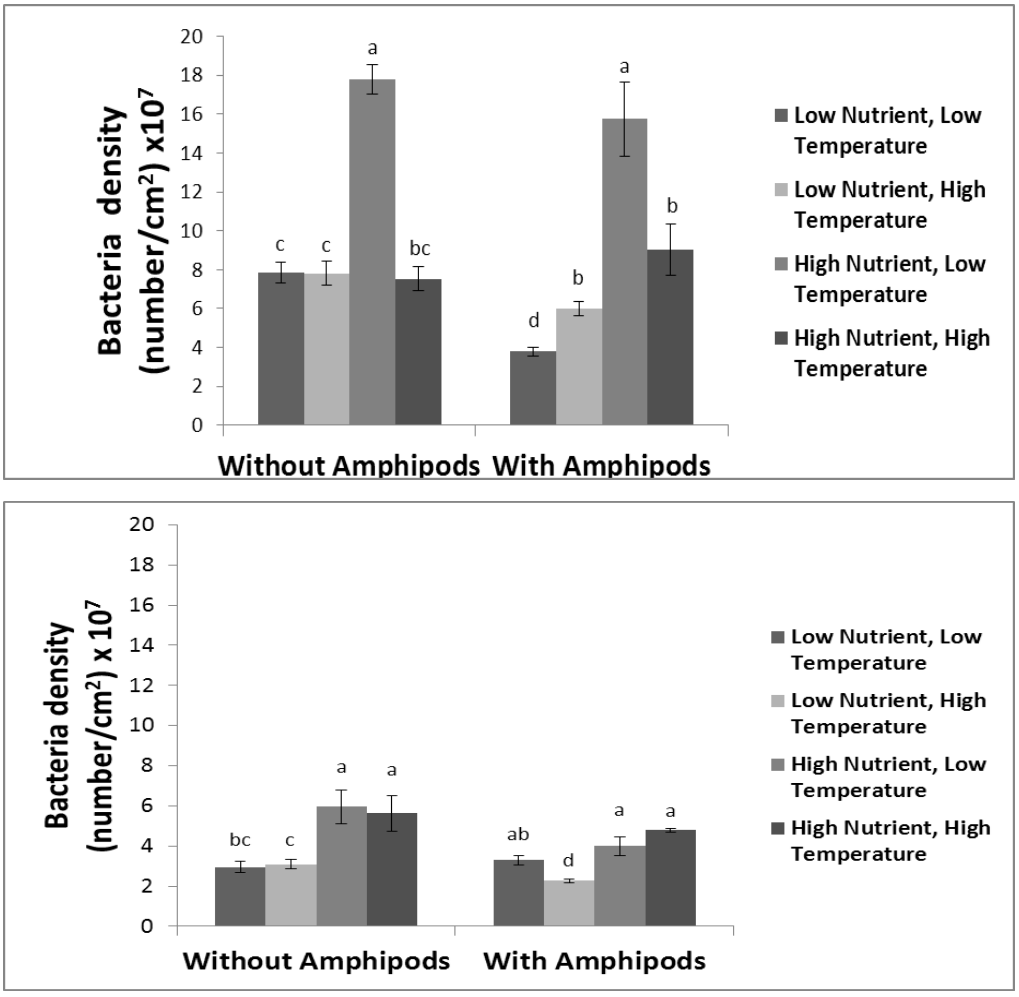
Beyond short-term responses of bacterial density to all experimental factors, only detritivores and nutrients affected bacterial density on the long run (31 d incubation: Fig. 1.1 B; ANOVA: Tab. 1.1). Feeding by amphipods did not affect bacterial density under low-temperature and low-nutrient conditions. However, increasing temperature resulted in a decrease in bacterial density by about one third through amphipods. Without amphipods, warming did not affect bacterial density, but nutrient-addition doubled bacterial counts. At high temperature, nutrient-addition almost compensated for grazing by amphipods,

but no such effect of nutrients was observed at low temperature. Low-nutrient/low-temperature versus high-nutrient/high temperature treatments differed significantly both with and without detritivores.

Table 1.1. ANOVA – comparison of **bacterial density** after 10 and 31 days of incubation.

Source of variation	df	F (10 d)	P (10 d)	F (31 d)	P (31 d)
Nutrients (N)	1	83.4929	<0.001	38.9303	<0.001
Temperature (T)	1	30.1075	<0.001	0.0866	0.770
Detritivores (D)	1	5.5402	0.025	5.4976	0.025
N × T	1	50.5080	<0.001	0.9624	0.334
N × D	1	3.8947	0.057	2.8143	0.103
T × D	1	4.6157	0.039	0.0024	0.961
N × T × D	1	0.2188	0.643	2.6842	0.111
Residuals	32				

Fig.1.1. **Bacterial density** on leaf litter **(A)** after 10 days of incubation, **(B)** after 31 days of incubation. Means ± standard errors are plotted; each bar represents N=5 for each treatment; Means that share the same lower-case letter do not differ significantly ($\alpha = 0.05$).



Bacterial community composition

DGGE analysis of bacterial DNA on birch leaves after 31 days of incubation allowed for discrimination of a maximum of 24 bacterial OTUs (Tab.1. 2).

Table 1.2. Number of **bacterial ribotypes** identified by DGGE on birch leaves after 31 days.

Treatment	Ribotype richness
Low temperature, Low NP level, with amphipods	15
Low temperature, Low NP level, without amphipods	13
High temperature, Low NP level, with amphipods	24
High temperature, Low NP level, without amphipods	19
Low temperature, High NP level, with amphipods	21
Low temperature, High NP level, without amphipods	14
High temperature, High NP level, with amphipods	24
High temperature, High NP level, without amphipods	20

According to PERMANOVA, bacterial community composition was affected by all experimental factors and their interactions (Tab. 1.3). However, visually inspecting cluster analysis and NMDS plot (Fig.1. 2 and Fig.1. 3) provides insight into the direction of how communities were affected. Biofilm communities can clearly be distinguished based on presence/absence of detritivores and low/high temperature, but less so by low versus high nutrient concentration. Without amphipods, bacterial communities were more similar when treated with the same temperature than when treated with the same nutrient-load. Bacterial communities under grazing pressure can be grouped

based on temperature, but low-temperature communities at high nutrient load were more similar to high-temperature communities than to low-temperature/low-nutrient communities. Hence, (selective) grazing by amphipods resulted in nutrient-effects resembling temperature effects on biofilm composition. Further, nutrient-enrichment stimulated bacterial growth (see above), but did not affect community composition (i.e., all bacteria were promoted equally). By contrast, temperature affected community composition but not bacterial growth (see above: 31 d).

Table1.3. PERMANOVA - comparison of **bacterial communities** derived from DGGE band pattern analysis after **31 days**. Analysis is based on Euclidean distance dissimilarities from untransformed data. Each term was tested using 999 random permutations of appropriate units.

Source of variation	df	Pseudo-F	P(PERM)	P(MC)
Nutrients (N)	1	10.786	0.001	0.001
Temperature (T)	1	4.2143	0.003	0.007
Detritivores (D)	1	8.0714	0.001	0.001
N × T	1	4.5	0.002	0.003
N × D	1	1.7857	0.048	0.107
T × D	1	2.6429	0.008	0.032
N × T × D	1	2.0714	0.025	0.057
Residuals	8			

Fig.1.2. DGGE gel and cluster analysis based on 16S rDNA amplified **biofilm bacterial community composition of Birch leaves after 31 days**. lNP: low nitrogen and phosphorus load; hNP: high nitrogen and phosphorous load; lT: low temperature; hT: high temperature; A: with amphipods. Cluster analysis of DGGE band patterns was performed using the Bray-Curtis similarity index; similarity values are given in %. n=2 for each treatment

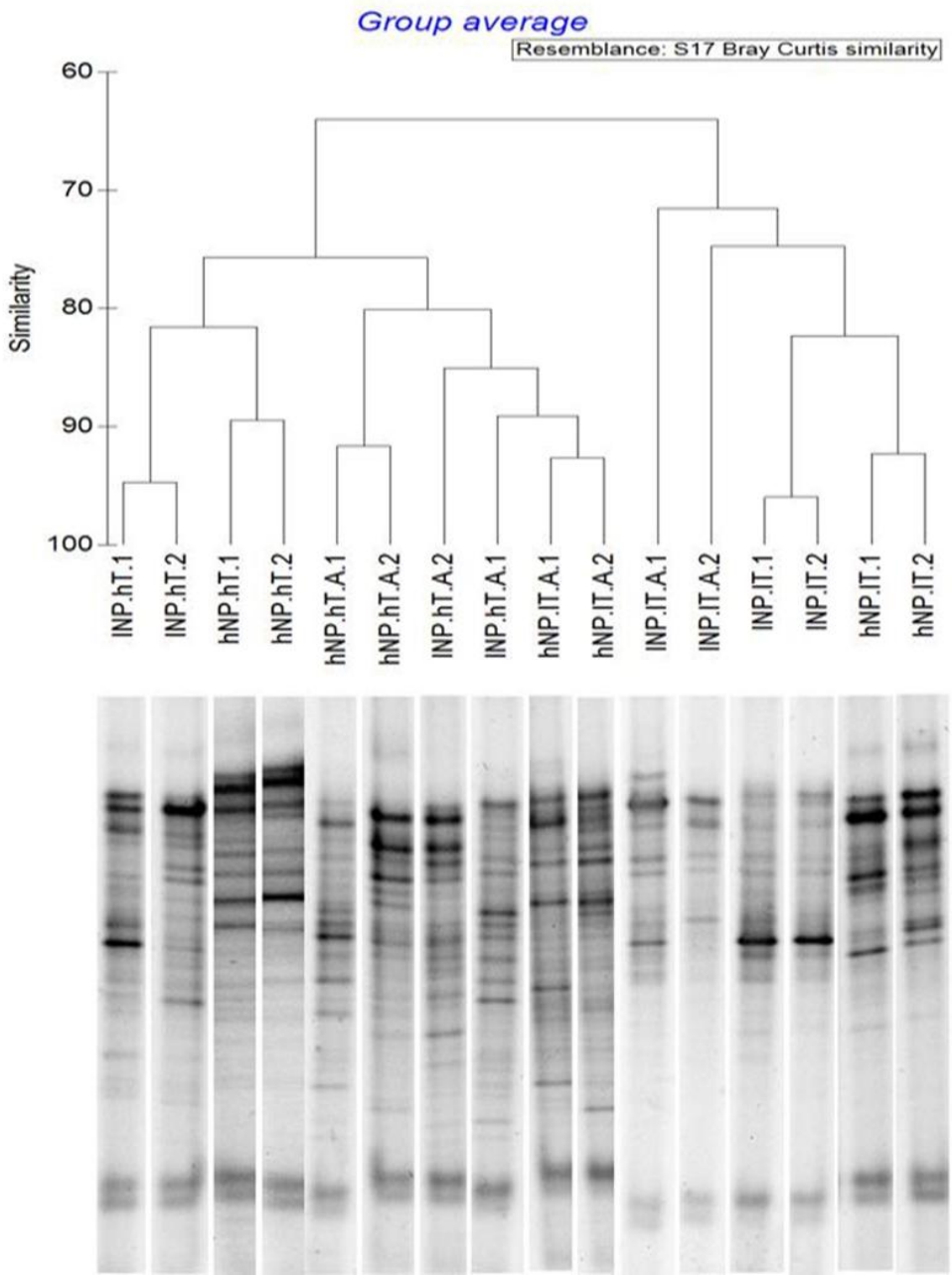
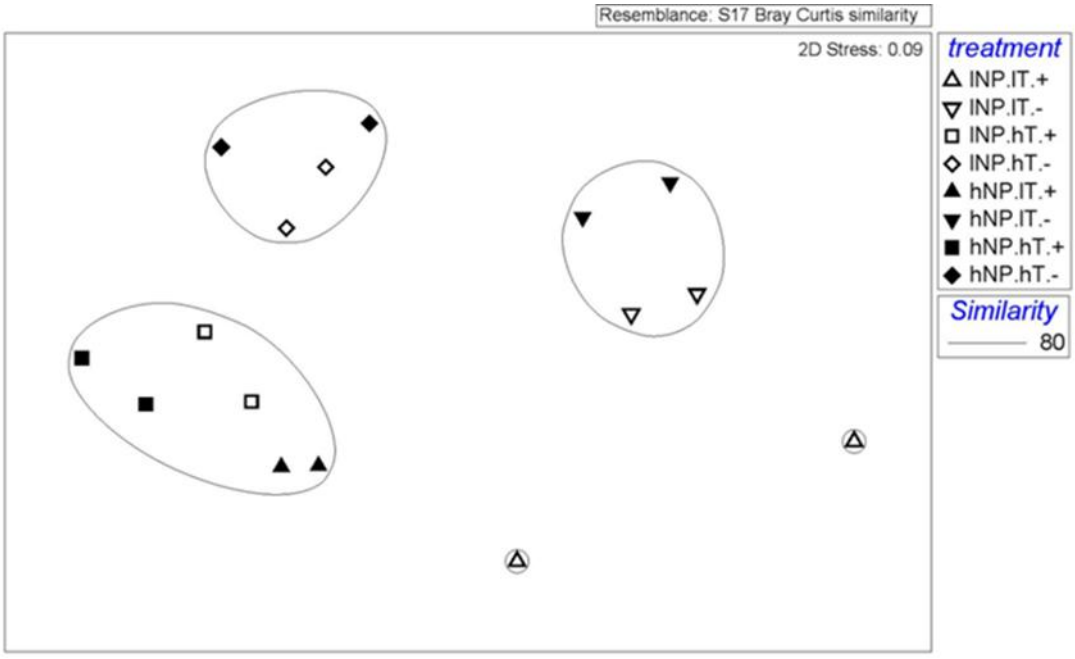


Fig.1.3. Non-metric multidimensional scaling (NMDS) of **litter-colonizing bacterial communities after 31 days**. lNP: low nitrogen and phosphorus load; hNP: high nitrogen and phosphorous load; lT: low temperature; hT: high temperature; +: with amphipods, –: without amphipods;

(----): 80 % similarity level. n=2 for each treatment



Discussion

With this study, we aimed at assessing the simultaneous effects of 5˚C-increased temperature, simulating a global warming scenario (IPCC, 2007), and nutrient-addition, simulating eutrophication in aquatic ecosystems (e.g., headwater streams) as caused by agricultural activities, atmospheric loading and human sewage input, on effects of detritivore grazing on bacterial communities on leaf litter surfaces. Short-term effects of nutrient-addition on bacterial biofilm density were stronger than warming-effects but less pronounced so at increased temperatures. Long-term effects of nutrient-addition on bacterial density were strongest, irrespective of environmental temperature. In particular, nutrients tend to interact with effects of detritivores and to promote warming effects in the presence of detritivores. The two way interactions effect of warming/nutrient and warming/detritivores on bacterial density in short run (10d) in comparison with no interactions effect in long run (31d) can imply the high dynamic of biofilm formation in early stages. In terms of biofilm composition, nutrient-addition exhibited significantly weaker effects than warming, but amphipods mediated warming-effects on community composition.

Microbial production is mainly controlled by temperature and nutrient availability (Kirchman, 1994; Felip, 1996; Chauvet and Suberkropp, 1998; Rubin and Leff, 2007), and leaf litter biofilms in aquatic environments are often nutrient-limited (Francoeur, 2001; Tank and Dodds, 2003; Elser et al., 2007). Their composition, in turn, determines their quality as food for detritivorous invertebrates (Lock et al., 1984; Hax and Golladay, 1993; Davey and O'Toole, 2000; Thompson et al., 2002). Since leaf litter decomposition is controlled by the joint action of microbial decomposers and animal detritivores and depends on both environmental conditions and leaf litter quality (Gessner et al., 1999; Gessner et al., 2007), we present evidence for indirect effects of warming and

nutrient-enrichment on decomposition processes in freshwaters through mediating detritivore-biofilm interactions.

As expected, grazing by amphipods reduced bacterial densities, but only under low-nutrient conditions. Nutrient-addition effectively compensated for biofilm reduction upon grazing. Warming, by contrast, only slightly counteracted the reduction in bacterial counts when amphipods were present (i.e., increased amphipod feeding activity). On the long run, bacterial density was little affected by grazing amphipods, and warming promoted density-reducing grazing-effects.

Along with bacterial density, the composition of bacterial communities differed in treatments with and those without detritivores. Grazing, hence, clearly affected the bacterial biofilm composition (lower versus upper part of NMDS plot), suggesting selective feeding by amphipods. Except for the control (low nutrients/low temperature), all amphipod treatments were highly similar in terms of biofilm composition (>80 % similarity), suggesting a mediating effect of environmental conditions on amphipod effects. However, nutrients had little effect on biofilm composition and did not interfere with community changes upon amphipod grazing. By contrast, bacterial community composition strongly depended on temperature (upper left versus lower right part of NMDS plot), and temperature clearly separated communities within amphipod treatments from each other.

Increased bacterial biomass, in favor of those bacteria that are preferentially eaten by grazing detritivores (c.f. Ihnen and Zimmer, 2008, for a terrestrial isopod), e.g. through warming, may provide additional food sources to grazing detritivores, such as amphipods, as the aquatic macro-fauna controls the development of bacterial biofilms on leaf litter (Liess and Hagulund, 2007; Treplin and Zimmer, 2012). However, effects of detritivores depend on the leaf litter (Treplin and Zimmer, 2012; here: birch) as well as on temperature and nutrient availability (this study). These factors also directly control the

composition of bacterial biofilms (Costerton et al.1995; this study), and hence, their quality as food for grazing detritivores (Lock et al., 1984; Hax and Golladay, 1993; Davey and O'Toole, 2000; Thompson et al., 2002). Since it is the interaction of microbes and detritivores that mediates decomposition processes in freshwaters (Gessner et al., 1999; Gessner et al., 2007; Treplin and Zimmer, 2012, and references therein), changes in these interactions may translate into changes in decomposition processes and, eventually, nutrient cycling. Numerous studies have focused on the effects of nutrients on litter decomposition as an indicator of the functional status of streams (e.g., Gessner and Chauvet, 2002; Woodward et al., 2012). However, only rarely have microbe-detritivore interactions been explicitly included. Our results demonstrate that, in contrast to short-term effects, the presence of Gammarus pulex does not affect bacterial density on leaf litter in the long run. However, whereas nutrient-enrichment but not warming promotes bacterial density in the absence of grazing-pressure, grazing counteracts nutrient-effects and warming promotes grazing-effects. Possibly, nutrient-induced promotion of bacterial biofilms increased their attractiveness and/or nutritive value for amphipods. In our study, changes in available autochthonous food source due to environmental changes are in agreement with previous studies about the combined effect of temporal changes on biofilm chemical composition in streams (Torres-Ruiz et al., 2007; Hill et al., 2011; Lyon and Ziegler, 2009). Along the same line, grazing-induced changes in biofilm composition are mediated by warming, possibly due to interacting effects of selective consumption of particular bacterial strains (c.f. Ihnen and Zimmer, 2008, for a terrestrial isopod) and strain-specific responses to warming.

Water temperature and nutrient concentrations will increase simultaneously in many streams (Murdoch et al., 2000; MEA, 2005). Although limited to a single detritivore species and a single leaf litter species, our findings may have important implications for assessing the effect of these changes in

freshwater systems due to changed bacterial biofilm density and composition on leaf litter, potentially resulting in changes in leaf litter decomposition. Based on our present findings, we hypothesize that simultaneous warming and nutrient-enrichment in temperate streams will interfere with grazing-pressure on bacterial biofilms by invertebrate detritivores. We predict warming and, to a lesser degree, nutrient-enrichment to predominantly affect those decomposition processes that depend on grazer-induced changes in bacterial biofilm composition. On the other hand, nutrient-enrichment will mostly interfere with those processes that depend on bacterial density, with warming potentially promoting the feeding activity of grazers.

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References

Anderson, M.J. 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Can. J. Fish. Aquat. Sci. 58:626-639.

Baldy, V., M.O. Gessner, and E. Chauvet. 1995. Bacteria, fungi and the breakdown of leaf litter in a large river. Oikos 74:93–102.

Baldy, V., E. Chauvet, J.Y. Charcosset, and M.O. Gessner. 2002. Microbial dynamics associated with leaves decomposing in the mainstem and floodplain pond of a large river. Aquat. Microb. Ecol. 28:25–36.

BGR . 2010. Grundwasseranteil an der öffentlichen Was¬serversorgung der Bundesländer in 2007. http://www. bgr.bund.de/nn_322854/DE/Themen/Wasser/grund-wasser_gewin__tab.html, Abruf 01.12.2010.

Brookshire, E.N.J., S. Gerber, J.R. Webster, J.M. Nose and W.T. Swank. 2011. Direct effects of temperature on forest nitrogen cycling revealed through analysis of long-term watershed records. Global Change Biol. 17:297–308.

Chauvet, E., and K.Suberkropp. 1998. The influence of temperature on sporulation of aquatic hyphomycetes. Appl. Environ. Microbiol. 64:1522-1525.

Costerton, J.W., Z. Lewandowski, D.E. Caldwell, D.R. Korber, and H.M. Lapin-Scott. 1995. Microbial biofilms. Annu. Rev. Microbiol. 49:711–745.

Dang, C.K., M. Schindler, E. Chauvet, and M.O. Gessner. 2009. Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. Ecology 90:122–131.

Davey, M.E., and G.A. O'Toole. 2000. Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Rev. 64:847-867.

Duff, J.H., A.J. Tesoriero , W.B. Richardson, E.A. Strauss, and M.D. Munn. 2008. Whole-stream response to nitrate loading in three streams draining agricultural landscapes. J. Environ. Qual. 37:1133 – 1144.

Elser, J.J., M.E.S. Bracken, E.E.Cleland, D.S. Gruner,W.S. Harploe,H. Hillebrand , J.T. Ngai, E.W. Seabloom, J.B. Shurin, and J.E.Smith. 2007. Global analysis of nitrogen and phosphorus

limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10:1135-1142.

Felip, M., M.L. Pace, and J.J. Cole. 1996. Regulation of planktonic bacterial growth rates: the effect of temperatures and resources. Microb. Ecol. 31:15-28.

Ferreira, V., and E. Chauvet. 2011. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Global Change Biol. 17:551–564.

Findlay, S.E., and T.L. Arsuffi.1989. Microbial growth and detritus transformations during decomposition of leaf litter in a stream. Freshw. Biol. 21:261–269.

Flury, S., and M.O. Gessner. 2011. Experimentally simulated global warming and nitrogen enrichment effects on microbial litter decomposers in a marsh. Appl. Environ. Microbiol.77: 803-9.

Francoeur, S.N. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. J. N. Am. Benthol. Soc. 20:358–368.

Geesey, G.G., R. Mutch, and J.W. Costerton. 1978. Sessile bacteria: an important component of the microbial population in small mountain streams. Limnol. Oceanogr. 23:1214-1223.

Gessner M.O., and E. Chauvet. 2002. A case for using litter breakdown to assess functional stream integrity. Ecol. Appl. 1: 498-510.

Gessner, M.O., E. Chauvet, and M. Dobson. 1999. A perspective on leaf litter breakdown in streams. Oikos 85:377-384.

Gessner, M.O., V. Gulis, K.A. Kuehn, E. Chauvet, and K. Suberkropp. 2007. Fungal decomposers of plant litter in aquatic habitats.p. 301-324. In: C.P. Kubicek and I.S. Druzhinina (ed.) The Mycota, Vol. IV: Environmental and microbial relationships, 2nd ed. Springer: Berlin.

Gulis, V., and K. Suberkropp. 2003 a. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshw. Biol. 48:123–134.

Gulis, V., and K. Suberkropp. 2003 b. Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. Microb. Ecol. 45:11-19.

Gulis, V., and K. Suberkropp. 2004. Effects of whole-stream nutrient enrichment on the concentration and abundance of aquatic hyphomycete conidia in transport. Mycologia 96: 57–65.

Gulis, V., K. Suberkropp, and A.D. Rosemond. 2008. Comparison of fungal activities on wood and leaf litter in unaltered and nutrient-enriched headwater streams. Appl. Environ. Microbiol. 74:1094-1101.

Hall-Stoodley, L., J.W. Costerton, and P. Stoodley. 2004. Bacterial biofilms: from the natural environment to infectious diseases. Nat. Rev. Microbiol. 2:95–108.

Hax, C.L., and S.W. Golladay. 1993. Macroinvertebrate colonization and biofilm development on leaves and wood in a boreal river. Freshw. Biol. 29:79-87.

Hieber, M., and M.O. Gessner. 2002. Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83: 1026-1038.

Hill W.R., J. Rinchard and S. Czesny. 2011. Light, nutrients and the fatty acid composition of stream periphyton. Freshw. Biol, 56:1825-1836.

IPCC [International Panel on Climate Change]. 2007. Climate change 2007: the physical science basis. Cambridge University Press, Cambridge, UK.

Ihnen, K., and M. Zimmer. 2008. Selective consumption and digestion of litter microbes by Porcellio scaber (Isopoda: Oniscidea). Pedobiologia 51:335-342.

Kirchman, D.L. 1994. The uptake of inorganic nutrients by heterotrophic bacteria. Microb. Ecol. 28:255–271.

Kosten, S., A. Kamarainen, E. Jeppesen, E.H. van Nes, E.T. H.M. Peeters, N. Mazzeo, L. Sass, J. Hauxwell, N. Hansel-Welch,T.L. Lauridsen, M. Søndergaard, R.W. Bachmann, G. Lacerot, and M. Scheffer. 2009. Climate-related differences in the dominance of submerged macrophytes in shallow lakes. Global Change Biol. 15:2503–2517.

Langan, S.J., L. Johnston, M.J. Donaghy, A.F. Youngson, D.W. Hay, and C. Soulsby . 2001. Variation in river water temperatures in an upland stream over a 30-year period. Sci. Total Environ. 265, 195–207.

Liess, A. and A.L. Haglund. 2007. Periphyton responds differentially to nutrients recycled by the snail grazer Theodoxus fluviatilis in dissolved or fecal pellet form. Freshw. Biol. 52:1997-2008.

Lock, M.A., R.R. Wallace, J.W. Costerton, R.M. Ventullo, and S.E.Charlton. 1984. River epilithon: toward a structural- functional model. Oikos 42:10-22.

Lyon, D. R. and S. E. Ziegler. 2009. Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. Limnol. Oceanogr. 54:439-449.

MEA (Millennium Ecosystem Assessment). 2005. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington, DC.

Murdoch, P.S., J.S. Baron, and T.L. Miller. 2000. Potential effects of climate change on surface water quality in North America. J. Am. Water. Resour. As. 36:347–366.

Muyzer, G., E.C. De Waal, and A.G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59:695-700.

Nykvist, N. 1963. Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. Stud. for. suec. 3:1-29.

Pascoal, C., and F.Cássio. 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Appl. Environ. Microbiol. 70: 5266-5273.

Petri, R., L. Podgorsek, and J.F. Imhoff. 2001. Phylogeny and distribution of the soxB gene among thiosulfate-oxidising bacteria. FEMS Microbiol. Rev. 197:171-178.

Petzoldt, T., and D. Uhlmann. 2006. Nitrogen emissions into freshwater ecosystems: is there a need for nitrate elimination in all wastewater treatment plants? Acta Hydrochim. Hydrobiol. 34:305–324.

Rubin, M.A., and L.G Leff. 2007. Nutrients and other abiotic factors affecting bacterial communities in an Ohio River (USA). Microb. Ecol. 54:374–383.

Rustad, L.E., J.L.Campbell, G.M. Marion, R.J. Norby, M. Mitchell, A.E. Hartley, J.H.C. Cornellissen, and J. Gurevitch. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126:543–562.

Schofield, J.A., A.E. Hagermann, and A. Harold. 1998. Loss of tannins and other phenolics from Willow leaf litter. J. Chem. Ecol. 24:1409-1421.

Sinsabaugh, R.L., S.W. Golladay, and A.E. Linkins. 1991. Comparison of epilithic and epixylic biofilm development in a boreal river. Freshw. Biol. 25:179-187.

Suberkropp, K., V. Gulis, A. D. Rosemond, and J. P.benstead. 2010. Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: results of a 5-year continuous enrichment. Limnol. Oceanogr. 55:149-160.

Tank, J., and W.K. Dodds. 2003. Responses of heterotrophic and autotrophic biofilms to nutrients in ten streams. Freshw. Biol. 48:1031-1049.

The Council of the European Union: Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Official Journal L 330, 05/12/1998, 32–54 (1998).

Thompson, F.L., P.C. Abreu, and W. Wasielesky. 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture 203: 263-278.

Torres-Ruiz, M., J. D. Wehr, and A. A. Perrone. 2007. Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. J. N. Am. Benthol. Soc. 26:509-522.

Treplin, M., and M. Zimmer. 2012. Decomposition of autochthonous versus allochthonous detritus: a cross-habitat comparison. Ecosystems 15:477-491.

Villanueva, V.D., J. Font, T. Schwartz, and A.M. Romani .2011. Biofilm formation at warming temperature: acceleration of microbial colonization and microbial interactive effects. Biofouling 27: 59−71.

Wallace, J.B., and J.R. Webster .1996. The role of macroinvertebrates in stream ecosystem function. Annu. Rev. Entomol. 41:115–139.

Weyers, H.S., and K. Suberkropp. 1996. Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. J. North. Am. Benthol. Soc. 15:408–420.

Woodward, G., M.O. Gessner, P.S. Giller, V. Gulis, S. Hladyz, A. Lecerf, B. Malmqvist, B.G. McKie, S.D. Tiegs, H. Cariss, M. Dobson, A. Elosegi, V. Ferreira, M.A.S. Graça, T. Fleituch, J.O. Lacoursière, M. Nistorescu, J. Pozo, G. Risnoveanu, M. Schindler, A. Vadineanu, L.B.-

M. Vought, and E. Chauvet. 2012. Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning. Science 336: 1438-1440.

Chapter II

Interactions between warming, nutrient enrichment and detritivores on litter decomposition and associated microbial decomposers

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Freshwater Biology (submitted)

Abstract

Leaf litter decomposition in aquatic environments constitutes a fundamental ecosystem process that is controlled by the joint action of microbial decomposers and animal detritivores. The structure and function of decomposer communities may be impaired by warming and eutrophication of the water body. We conducted a microcosm experiment to investigate how warming, nutrient-addition (N and P) and detritivores, interact to affect multiple parameters associated with leaf decomposition. These parameters included microbial (bacteria and fungi) biomass and community structure, decomposition rate and detritivore growth. Our analyses demonstrated increased bacterial and fungal biomass and changed community structure upon warming and nutrient addition. The presence of detritivores showed positive effect on fungi but negative effect on bacteria. Litter decomposition rates significantly increased with warming, nutrient addition and the presence of detritivores. Growth of detritivores did not significantly increase with warming and nutrient addition. The results of this study emphasize a general, pivotal role of microbial decomposers. However, their impact on leaf decomposition may be regulated by the interplay of detritivores.

Key words: Freshwater, Environmental changes, Leaf litter decomposition,

Microbial decomposers, Detritivores

Introduction

In many streams, allochthonous plant litter is an important source of energy (Bärlocher, 2005, Gessner *et al*., 2007). Aquatic detritivores mainly drive the process of leaf litter decomposition by fragmentation of the leaves (Wallace & Webster, 1996; Graça, 2001; Hieber & Gessner 2002), and microorganisms, especially fungi and bacteria, can break down leaf litter and convert the detrital matter into products metabolically useful for stream detritivores (Findley *et al*., 2002; Bärlocher, 2005; Gessner *et al*. 2007; Sridhar, Beaton & Bärlocher, 2011). The contribution of detritivores and microbial decomposers depends on both environmental conditions and leaf litter quality (Gessner, Chauvet & Dobson, 1999; Stelzer, Heffernan & Likens, 2003; Pascoal & Cássio, 2004; Gessner *et al*., 2007; Treplin & Zimmer, 2012).

The colonization of deciduous leaf litter by detritivores is determined by the composition of both the leaf itself and its attached biofilms (Lock *et al*., 1984; Hax & Golladay, 1993), and the specific microbial composition of the biofilm modifies the nutritional quality of the material for grazing microbivores through providing polysaccharides, proteins and amino acids, polyunsaturated fatty acid (PUFA), sterols and vitamins (Davey & O'Toole, 2000; Thompson, Abreu & Wasielesky, 2002). Since microbial decomposers increase the quality of submerged litter as food, shredders that feed on allochthonous plant material prefer to consume litter after establishment of microorganisms, especially aquatic hyphomycetes (Suberkropp, 1992, 1998; Graça, 2001, Sridhar *et al*., 2011).

It was hypothesized that human activities, such as urbanization, industrial and agricultural development and water course alterations, have jeopardized streams and rivers (Malmqvist & Rundle, 2002; Galloway *et al*., 2008). Several studies have documented the effects of environmental variables, such as temperature and stream water nutrient concentrations, on microbial decomposer

activity (Suberkropp, 1984; Gönczöl, Csontos & Révay, 2003; Pascoal & Cássio, 2004; Ferreira & Chauvet, 2011). Future climate change could result in rapid temperature increases that directly affect most ecosystems including streams and lakes. The recent Intergovernmental Panel on Climate Change report (IPCC, 2007) predicts temperature increases by 1.1 °C to 6.4 °C during the next decades. Many studies have indicated faster decomposition at higher temperatures (Dang *et al.*, 2009; Fernandes *et al*., 2009; Ferreira & Chauvet, 2011). Clearly, in some investigations, higher leaf litter breakdown rates were observed in nutrient-rich systems versus nutrient-poor systems (Huryn *et al*., 2002; Gulis & Suberkropp, 2003a, 2004; Pascoal *et al.*, 2003; Ferreira & Chauvet, 2011). Similarly, litter-decomposing fungi in temperate streams were more than twice as productive in a nutrient-enriched as in a control stream (Gulis, Suberkropp & Rosemond, 2008).

While much work has been published on the effect of environmental variables (i.e.: temperature, nutrient addition) on aquatic hyphomycetes, the simultaneous effects of these variables have received far less attention. Simultaneous effects of multiple environmental changes, e.g., warming and nutrient enrichment on microbial biofilm, have been studied by Ferreira & Chauvet (2011) who provided evidence for promoting effects of warming on fungal performance and decomposition rates in response to stream eutrophication. In another survey (Sanaei & Zimmer, unpubl. data), warming and nutrient enrichment exhibited strong effects on the density and composition of litter-associated bacterial communities.

Our objectives in the present study were to (i) determine how warming, nutrient enrichment and detritivore affect the fungal and bacterial biomass and their community composition, (ii) test whether the decomposition rate of birch litter will be affected by changes in these environmental factors and detritivore,

and (iii) test whether warming and nutrient enrichment will affect detritivore growth.

We predict that (i) fungal and bacterial biomass and species richness will increase with warming and nutrient enrichment and will decrease with detritivore presence, (ii) decomposition rates of birch litter will increase with warming, nutrient addition and detritivore presence, and (iii) warming and nutrient addition will increase detritivore growth rate due to increasing microbial decomposer biomass.

Materials and Methods

Experimental set-up

Experiments were carried out in microcosms (0.6 L) which were supplied with 0.5 liter of aerated diluted creek water. Control microcosms were filled with 0.5 L creek water (N and P concentrations <0.5 mg/ L and <0.02 mg/L, respectively) diluted with tap water. Nitrogen was added to a subset of microcosms as ammonium nitrate (NH_4NO_3; Carl Roth, Karlsruhe, Germany) at 4.5 mg L^{-1} (= 1.6 mg N/ L), in order to mimic a high nutrient load of headwater streams in agricultural land. Phosphorus was added to the same microcosms as phosphate (K_2HPO_4; Carl Roth, Karlsruhe, Germany) at 0.55 mg L^{-1} (= 0.1 mg P/ L) to attain a Redfield ratio of 16:1. Warming was mimicked by increasing the experimental temperature of 10 °C to 15 °C orthogonally to the nutrient concentration. Therefore, the treatments (N = 8 , each) were (a) control (ambient temperature, ambient nutrient load; (b) increased temperature, ambient nutrient load; (c) ambient temperature, increased nutrient load; (d) increased temperature, increased nutrient load; which were run either with (for each of the above treatments) or without amphipods (see below). After 0, 10, 20, 30 and 40 days, eight microcosms from each treatment were sacrificed and used for determination of fungal and bacterial biomass, leaf mass loss, amphipod dry mass remaining and DNA extraction.

Leaf litter (Birch, *Betula pendula* Roth, a key component in northern temperate forests) was collected near Kiel soon after abscission. Litter discs (10 mm diameter) were cut off the leaves and dried at 30 °C for 3 days to constant weight in sets of 20 leaf discs to determine initial litter dry mass. Leaf discs were conditioned in undiluted creek water for 2 weeks prior to introduction to the microcosms. Leaves were tested randomly for bacterial inoculation through quantitative bacterial measurement.

Specimens of amphipods (*Gammarus pulex*, Linnaeus 1758, a key

detritivore in northern temperate streams) were collected from Russee creek (10˚08′ E, 54˚29′ N) near Kiel (northern Germany), and seven individuals representing the natural size range observed in the stream were placed, after weighing, to half of microcosm, simulating a natural amphipod density in Russee creek, where it occurs in densities ranging from 40 to 1,000 with a mean density of 506 ind. m^{-2}. Microcosms were examined daily for dead animals which were removed and replaced by animals of the same size. Half of microcosms served as detritivore-free control for each treatment.

Bacterial abundance

For detaching the bacteria from the leaves, two glass beads (5 mm) were added to a 2 mL-tube containing 2 leaf discs in formaldehyde solution (Carl Roth, Karlsruhe, Germany), and the tube was vortexed vigorously for 30 s. To determine bacterial cell counts, 0.5 mL aliquots of the bacterial suspensions were filtered through a polycarbonate membrane (0.22 µm pore size; Millipore) (Sartorius Stedim, Goettingen, Germany) placed on nitrocellulose support membrane (0.45 µm pore size; Millipore) by using a vacuum filtration unit. Bacteria attached to the polycarbonate membrane were stained with 4', 6-Diamidino-2-phenylindole dihydrochloride (DAPI) (Invitrogen Gmbh, Darmstadt, Germany) at a final concentration of 1µg mL^{-1}. The filters were rinsed with sterile water and mounted onto glass slides. The slides were observed under 1000x magnification with an epifluorescence microscope (Axio scope A1, Zeiss, Hamburg, Germany). For statistical evaluation, 12 microscopic fields (100 x 100 μm^2) were counted per slide.

Fungal biomass

The estimation of fungal biomass associated with leaf discs was based on ergosterol concentration (Gessner & Newell, 2002). Eight leaf discs from each replicate were weighed (± 0.1 mg) and stored at -20 °C until used. Leaf discs

were ground with a tissue lyser (Retsch, Qiagen, Germany) for 10 minutes and heated (70 °C, 90 min) in KOH-Methanol (10 %). Extracted lipids were purified by solid-phase extraction according to Gessner (2005). The concentration of ergosterol in the samples was determined by high performance liquid chromatography (HPLC) run on a Varian 940-LC system using a Macherey-Nagel Nucleodor 100-5 C18 column (25 cm × 4 mm) (Macherey-Nagel, Düren, Germany). The system was run isocratically with HPLC-grade methanol at 1.4 ml/min and 25 °C. Ergosterol was detected at 282 nm and quantified based on a standard curve of standard ergosterol (Sigma-Aldrich, Hamburg, Germany) dissolved in methanol. Fungal biomass was estimated according to Gessner & Chauvet (1993), assuming 5.5 mg ergosterol per gram fungal biomass.

DNA extraction and amplification

Total DNA was extracted from 2 frozen leaf discs from each replicate, using the QiaAmp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Amplification of bacterial 16S rRNA genes was performed using PureTaq Ready-To-Go PCR Beads (GE Healthcare, Munich, Germany) in a total PCR volume of 25 µL; 10 pmol of each bacterial primer 341F-GC (5'-[CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GC]-CTA CGGGAG GCA GCA G-3') and 534R (5'-ATT ACC GCG GCT GCT GG-3') was used for amplification of suitable fragments for DGGE (Muyzer, De Waal &Uitterlinden, 1993). A GC-clamp (sequence in square brackets) was attached to the 5' end of the forward primer. PCR conditions were as follows: initial denaturation at 94 °C for 2 min; 15 touchdown cycles starting with an annealing temperature of 65 °C for 40 s and an incremental reduction of 1 °C per cycle; elongation at 72 °C for 40 s; and denaturation at 94 °C for 30 s. The touchdown steps were followed by 35 cycles of annealing temperature at 50 °C for 40 s, elongation at 72 °C for 40 s and denaturation at 94 °C for 30 s; a final annealing step was performed at 42 °C for

60 s and a final elongation at 72 °C for 5 min. The correct size of the amplified DNA fragments was verified by electrophoresis of 10 % of the PCR reaction volume in 1.8 % agarose in 1 × TBE buffer.

Amplification of fungal 18 S rRNA genes was performed using PureTaq Ready-To-Go PCR Beads (GE Healthcare, Munich, Germany) in a total PCR volume of 25 µL; 20 pmol of each fungal primer NS1 (5'-GTA GTC ATA TGC TTG TCT C -3') and GCfung (5'-[CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC]-ATT CCC CGT TAC CCG TTG-3'). The following cycling parameters were used: initial denaturation at 95 °C for 2 min; followed by 36 cycles of denaturation for 30 s at 95 °C, primer annealing at 55 °C for 30 s and elongation at 72 °C for 1 min. Final elongation was at 72 °C for 5 min (Nicholcheva & Bärlocher, 2005; Duarte *et al*. 2010).

Denaturing Gradient Gel Electrophoresis

Bacterial and fungal communities on leaf litter surfaces were characterized through DGGE using double gradient polyacrylamide gels (Petri and Imhoff, 2001). DGGE gels contained a denaturing gradient from 40 to 80 % (100 % defined as 7 M urea and 10 M formamide) and an acrylamide (Acrylamide-Bis:Rotipuran 37.5:1) gradient from 6 % to 8 %. Samples with 15 µL of bacterial PCR products mixed with 3 µL loading buffer (10 ×) were loaded on the DGGE gel and electrophoresis was run at 60 °C and 80 V for 14 h in 0.5 × TAE buffer in a CBS Scientific DGGE-2001 system. Samples with 15 µL of fungal PCR products mixed with 3 µL loading buffer (10 ×) were loaded on the DGGE gel and electrophoresis was run at 60°C and 40 V for 16 h in 0.5 × TAE buffer in a CBS Scientific DGGE-2001 system. After electrophoresis, the gel was stained for 45 min in SYBR Gold® (Invitrogen Gmbh, Darmstadt, Germany), rinsed for 30 min in 1 × TAE buffer and photographed under UV light.

Leaf mass loss

We used litter mass loss as an easily accessible proxy for decomposition. Remaining leaf discs from each replicate were dried at 30 °C for at least 3 days to constant weight, and dry mass was calculated for each sampling date. Dry mass remaining was calculated through subtraction of final from initial dry mass. Values were expressed as the percentage of remaining weight. Decomposition rate, k, for each treatment was estimated by linear regression of ln-transformed data.

Amphipods dry mass

Initial dry mass of amphipods from each replicate were calculated by correction factor, estimated from the ratio of dry/fresh weight at the end of experiment. After each sampling date, amphipods were dried at 60 °C for 2 days to constant weight. Relative growth rate (dry mass) of amphipods in each treatment was calculated using following linear growth rate formula:

$$r = \ln (w_f / w_i) / t$$

Where r is relative growth rate in day^{-1}; w_f is final weight; w_i is initial weight and t is time, and percentage of dry mass growth for each sampling date was calculated using following formula:

Dry mass growth percentage = $(w_f - w_i/w_i) \times 100$

Statistical analysis

The R statistical software environment (version 2.13.1, (http://www.r-project.org/)) was utilized to perform quantitative data analyses. Data of bacterial biomass were log (x+1)-transformed to achieve normality. A multi-factorial analysis of variance (ANOVA) was used to test whether bacterial and

fungal biomass was significantly affected by nutrient addition, temperature, detritivores and time. Comparison of treatment effects was made using Tukey′s HSD (Honest significant Differences) test. Shapiro-Wilcoxon test was used for testing for normality, and Fligner-Killeen test was used for testing for homogeneity of variances. Analysis of covariance (ANCOVA) with time as a covariate variable was used to compare the effect of nutrient addition, temperature and detritivores as categorical variables on leaf decomposition rate, followed by Tukey′s HSD test. For comparing the effects of nutrient addition and temperature as categorical variables on detritivore growth, ANCOVA was performed on final amphipod mass with initial mass as covariate.

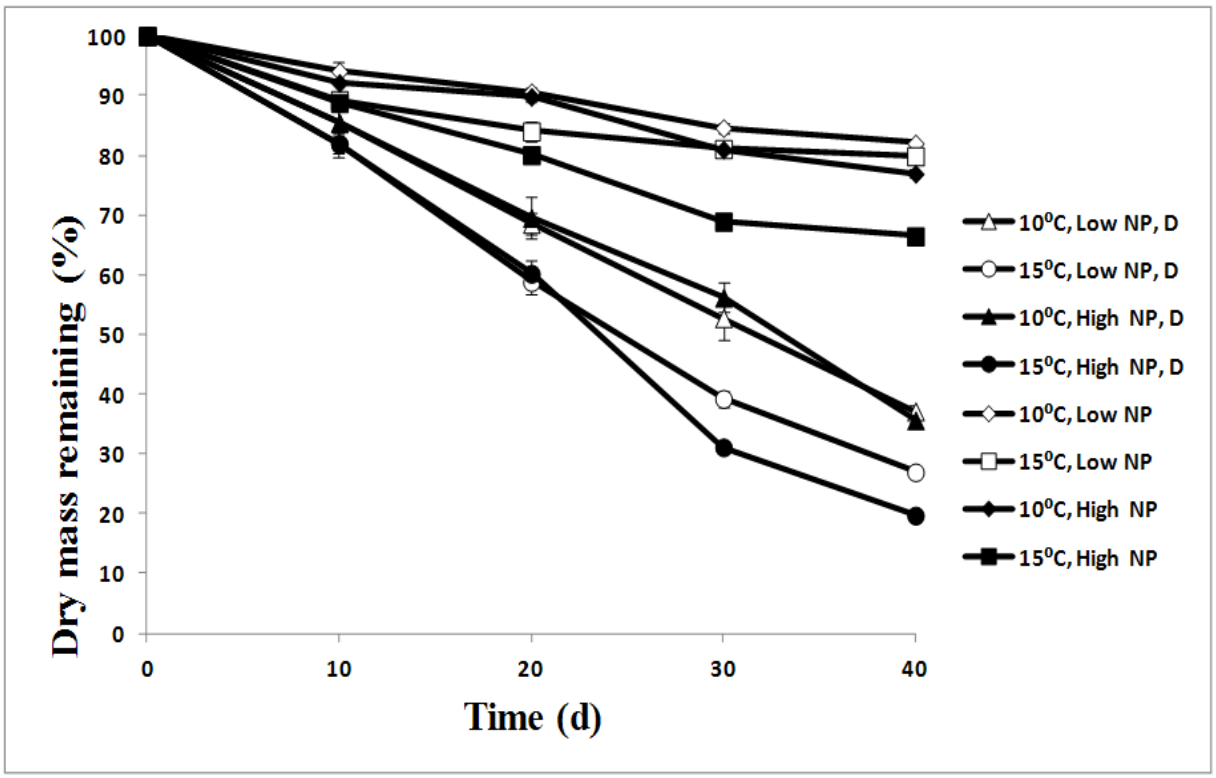
DGGE gels were analyzed by the generation of a presence–absence matrix based on the band pattern. All visible bands in every gel lane were taken into account for further calculation using the Primer software v.6.1.9 (Primer-E) (Primer Ltd, Plymouth, UK). The matrix was used to calculate a distance matrix using normalized Euclidean distances (root mean square differences). Sample similarities were shown by cluster analysis and non-metric multidimensional scaling (NMDS). Band positions were assigned to species (OTUs) and the differences between treatments were analyzed by Permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) that compared the observed value of a test statistic (Pseudo F-ratio) against a recalculated test of random permutation of the data. Monte Carlo p-values were used as a test statistic for replicates.

Results

Leaf mass loss

Mass loss of birch leaf discs for 40 days of incubation in simulated stream microcosms differed between 18 % (at low temperature, low nutrients and absence of detritivores) and 80 % (at high temperature, high nutrients and presence of detritivores) (Fig.2.1), which translated into leaf breakdown rates between 0.0050 and 0.0422 day^{-1} (linear model, Table 2.1).

Figure 2.1. Dry mass remaining of birch leaf discs incubated in microcosms with or without detritivores and at 10°C or 15°C and low or high NP levels for 40 days. D: with detritivores. Values are means ± SE



Leaf discs lost significantly more mass in the presence of detritivores than in detritivore-free controls, irrespective of temperature and nutrients (Tukey′s HSD, $p < 0.05$, Table 2.1). Additionally, when temperature and nutrients were increased simultaneously, mass loss rates also increased irrespective of detritivores presence (Tukey′s HSD, $p < 0.05$, Table 2.1). In detritivore-free controls, warming did not affect mass loss rate under high or low nutrient levels (Tukey′s HSD, $p > 0.05$, Table 2.1), but in the presence of detritivores, warming resulted in an increase in mass loss at both levels of nutrients (Tukey′s HSD, $p < 0.05$, Table 2.1).

Table 2.1. **Decomposition rates (day^{-1}) of birch leaf discs** incubated in microcosms with or without detritivores and at 10°C or 15°C and low or high NP levels for 40 days, and coefficient of determination of the regression

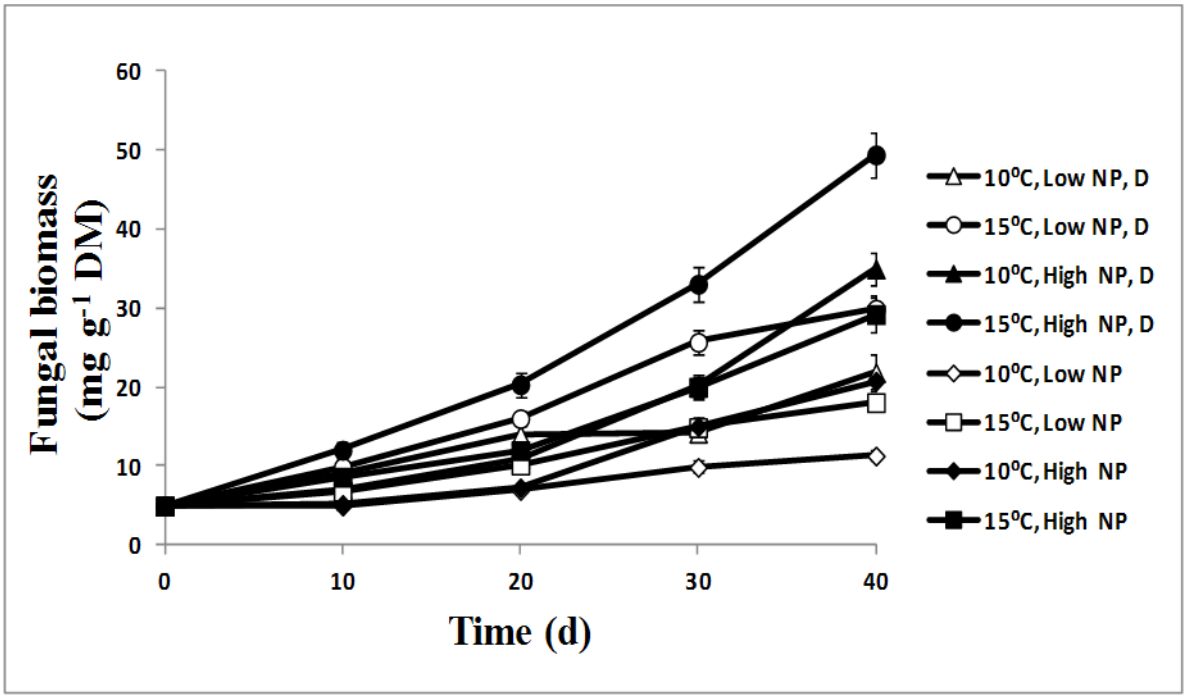
Detritivores	Temperature	NP level	k	R^2
with	10°C	Low	0.0248^{a}	0.90
		High	0.0249^{b}	0.90
	15°C	Low	0.0336^{c}	0.96
		High	0.0422^{d}	0.94
Without	10°C	Low	0.0050^{e}	0.89
		High	0.0065^{f}	0.88
	15°C	Low	0.0055^{e}	0.77
		High	0.0107^{f}	0.93

Treatments with the same letter are not significantly different (Tukey´s HSD), $P > 0.05$).

Fungal biomass

Fungal biomass associated with birch leaf discs increased over time for all treatments, and maximum values varied between 4.7 mg g^{-1} DM (at low temperature, low nutrients and absence of detritivores) and 39.8 mg g^{-1} DM (at high temperature, high nutrients and presence of detritivores) (Fig.2.2). Fungal biomass was stimulated by the presence of detritivores irrespective of temperature and nutrients (Tukey′s HSD, $p < 0.05$, Table 2.2) and increased when temperature and nutrients increased simultaneously with amphipods either absent or present (Tukey′s HSD, $p < 0.05$, Table 2.2). Fungal biomass increased with warming irrespective of detritivores and nutrients (Tukey′s HSD, $p < 0.05$, Table 2.2) and increased with nutrient addition irrespective of temperature and detritivores (Tukey′s HSD, $p < 0.05$, Table 2.2).

Figure 2.2. **Fungal biomass** associated with birch leaf discs incubated in microcosms with or without detritivores and at 10°C or 15°C and low or high NP levels for 40 days. D: with detritivores. Values are means ± SE



Bacterial abundance

Bacterial abundance associated with birch leaf discs increased over time for all treatments, and maximum values varied between 4.7×10^7 cm^{-2} (at low temperature, low nutrients and presence of detritivores) and 8.3×10^7 cm^{-2} (at high temperature, high nutrients and absence of detritivores) (Fig.2.3). Bacterial abundance decreased by the presence of detritivores irrespective of temperature and nutrients (Tukey′s HSD, $p < 0.05$, Table 2.2) and increased when temperature and nutrients increased simultaneously with amphipods either absent or present (Tukey′s HSD, $p < 0.05$, Table 2.2). Bacterial abundance increased with warming irrespective of detritivores and nutrients (Tukey′s HSD, $p < 0.05$, Table 2.2) and increased with nutrient addition irrespective of temperature and detritivores (Tukey′s HSD, $p < 0.05$, Table 2.2).

Figure 2.3. **Bacterial biomass** associated with birch leaf discs incubated in microcosms with or without detritivores and at 10°C or 15°C and low or high NP levels for 40 days. D: with detritivores. Values are means ± SE

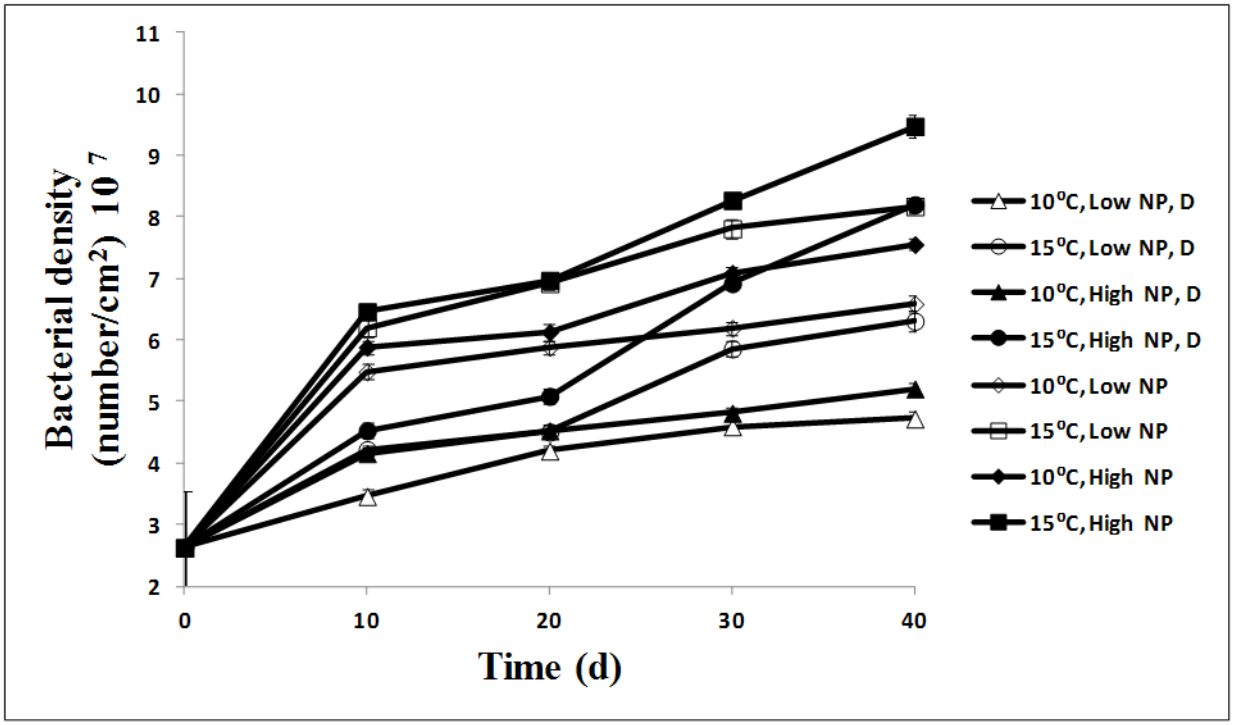


Table 2.2. summary table for multi-factorial ANOVAs performed on **bacterial biomass** (log(x+1) transformed) and **fungal biomass** associated with birch leaf discs incubated in microcosms with or without detritivores and at 10°C or 15°C and low or high NP levels for 40 days

		Bacterial biomass		**Fungal biomass**	
	df	F	P	F	P
Multi-factorial ANOVA					
Intercept	1	34513973	< 0.001	6428,673	< 0.001
Temperature	1	688	< 0.001	208,346	< 0.001
NP level	1	189	< 0.001	134,023	< 0.001
Detritivores	1	1851	< 0.001	319,805	< 0.001
Time	4	3159	< 0.001	496,070	< 0.001
Temperature × NP level	1	0	0,534	11,270	< 0.001
Temperature × detritivores	1	17	< 0.001	13,233	< 0.001
NP level × detritivores	1	10	0.001	3,017	0.083
Temperature × time	4	75	< 0.001	24,698	< 0.001
NP level × time	4	19	< 0.001	50,461	< 0.001
Detritivores × time	4	126	< 0.001	45,855	< 0.001
Temperature × NP level × detritivores	1	8	0.005	4,169	0.042
Temperature × NP level × time	4	5	0.001	1,507	0.200
Temperature × detritivores × time	4	9	< 0.001	3,534	0.007
NP level × detritivores × time	4	2	0.199	3,114	0.015
Temperature × NP level × detritivores × time	4	5	0.001	0,549	0.699
Error	280				
Tukey´s HSD					
10°C, Low NP, D			a		a
10°C, Low NP			b		b
15°C, Low NP, D			c		c
15°C, Low NP			d		a
10°C, High NP, D			e		cd
10°C, High NP			f		a
15°C, High NP, D			b		e
15°C, High NP			g		d

D; Detritivores, treatments with the same letter are not significantly different (Tukey´s HSD), P > 0.05).

Amphipods dry mass

Dry mass of amphipods after 40 days of incubation in simulated stream microcosms differed between 104% (at low temperature and low nutrients) and 110% (at high temperature and high nutrients) of initial mass (Fig.2.4) which translated into relative growth rates between 0.03 day^{-1} and 0.07 day^{-1}. Relative

growth rates of amphipods were not stimulated by warming or nutrient addition (Tukey′s HSD, $p > 0.05$, Table 2. 3)

Figure 2.4. **Amphipod dry weight** at 10°C or 15°C and low or high NP levels for 40 days.Values are means ± SE

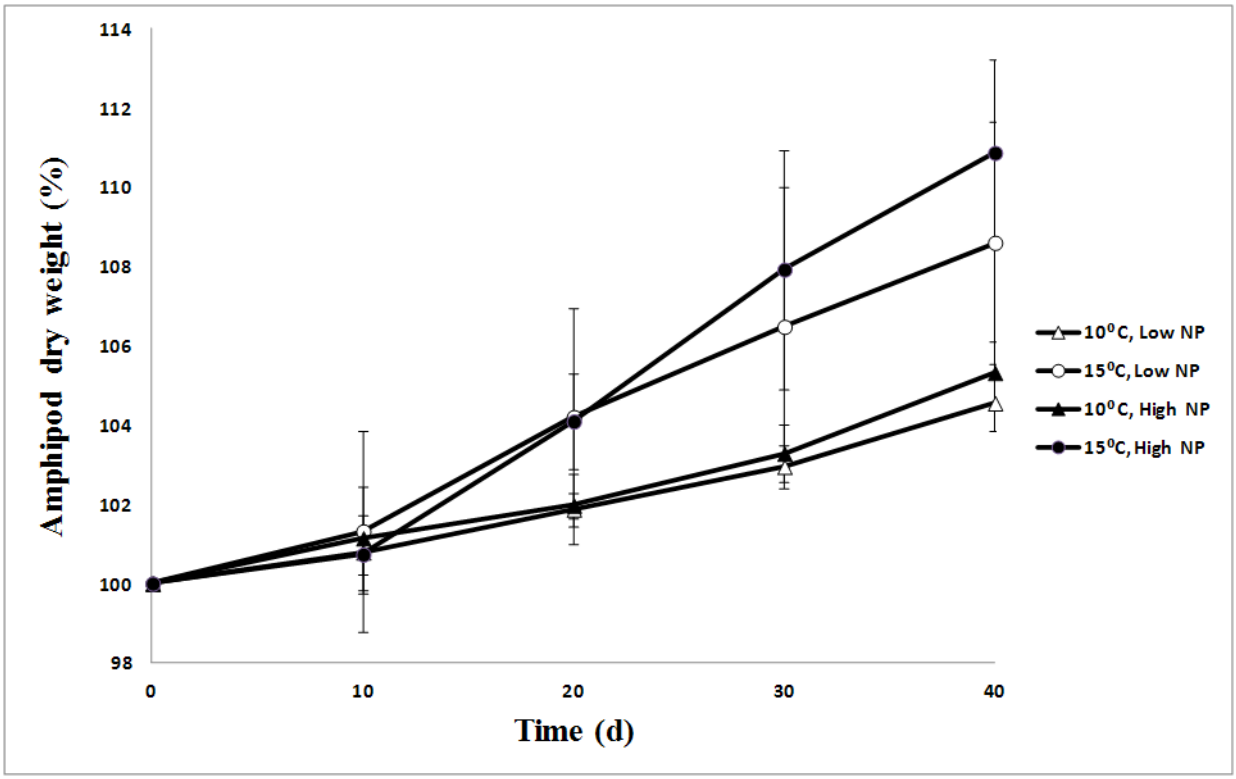


Table 2. 3. Relative growth rate (day^{-1}) of amphipods incubated in microcosms at 10°C or 15°C and low or high NP levels for 40 days, and coefficient of determination of the regression

Temperature	NP level	r	R^2
10°C	Low	0.030[a]	0.26
	High	0.034[a]	0.19
15°C	Low	0.056[a]	0.19
	high	0.077[a]	0.35

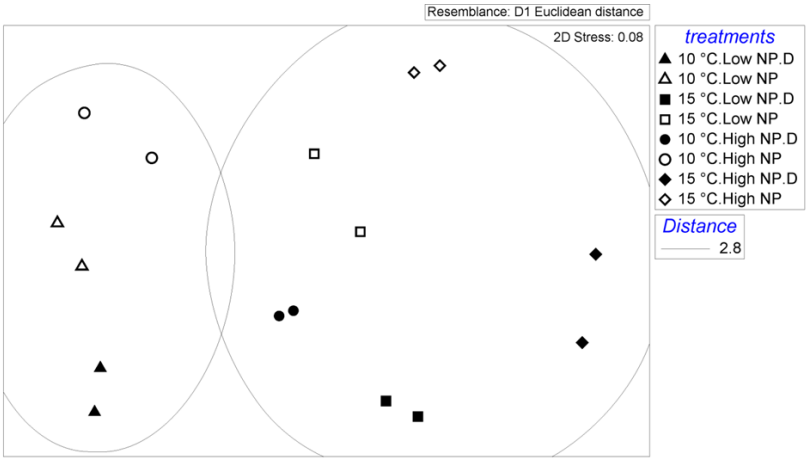
Treatments with the same letter are not significantly different (Tukey´s HSD), P > 0.05).

Bacterial and fungal community structure

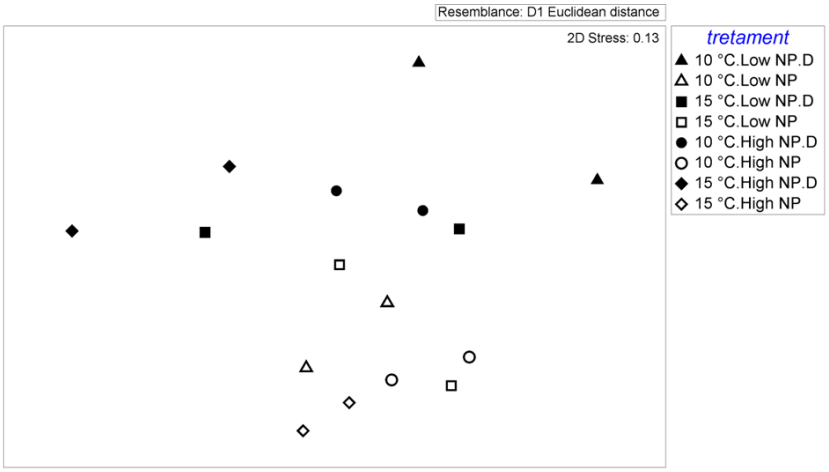
DGGE analysis of bacterial and fungal DNA on birch litter after 40 days of incubation allowed the discrimination of 25 bacterial and 22 fungal OTUs. According to MDS, bacterial communities can be grouped based on presence/absence of detritivores and low versus high temperature. A distinction by low *versus* high nutrient load was less consistent (Figure 2.5a).

Figure 2.5. Non-metric multidimensional scaling (NMDS) of litter-colonizing **bacterial (a), and fungal (b) communities** associated with birch leaf discs incubated in microcosms with or without detritivores and at 10°C or 15°C and low or high NP levels after 40 days. D: with detritivores

a)



b)



The community composition of fungal colonizers on birch litter was also influenced by grazing pressure of detritivores (MDS plot, Figure 2. 5b), whereas neither temperature nor nutrient-load had consistent effects. Overall, results of Permutational multivariate analysis of variance (PERMANOVA) indicated a significant effect of detritivore presence on bacterial (P = 0.002, Table 2. 4) and fungal community structure (P = 0.012, Table 2. 4). Moreover, warming showed a significant effect on bacterial community structure (P = 0.002, Table 2. 4) but no significant effect were observed in fungal OTUs band patterns (P = 0.082, Table 2. 4). Additionally, nutrient addition showed a significant effect on bacterial community structure (P = 0.001, Table2. 4) but no significant effect were observed in fungal OTUs band patterns (P = 0.07, Table 2. 4).

Table 2. 4. PERMANOVA - comparison of bacterial and fungal communities derived from DGGE band pattern analysis after 40 days. Analysis is based on Euclidean distance dissimilarities from untransformed data. Each term was tested using 999 random permutations of appropriate units.

		Bacterial community			Fungal community		
Source of variation	**df**	**Pseudo-F**	**P(PERM)**	**P(MC)**	**Pseudo-F**	**P(PERM)**	**P(MC)**
NP level	1	17.286	0.001	0.001	2.0286	0.035	0.075
Temperature	1	6.4286	0.002	0.002	2.0286	0.047	0.082
Detritivores	1	11.286	0.002	0.002	3.8571	0.007	0.012
NP level × temperature	1	7.2857	0.004	0.002	1.5714	0.18	0.185
NP level × detritivores	1	4.1429	0.005	0.006	1.5714	0.136	0.177
Temperature × detritivores	1	3.5714	0.007	0.014	2.2571	0.037	0.06
NP level × temperature × detritivores	1	2.1429	0.044	0.066	1.8	0.084	0.113
Residuals	8						

Discussion

Ecosystems are currently encountering unprecedented warming (IPCC7) including increase in temperature of freshwaters (Langan *et al.* 2001). Simultaneously, many freshwater bodies in agricultural areas are being enriched in nutrients (Murdoch, Baron & Miller, 2000; MEA, 2005). In the present study, mass loss of birch litter was stimulated by nutrient-enrichment (compare: Suberkropp & Chauvet,1995; Suberkropp ,1998 b; Sridhar & Bärlocher, 2000; Pascoal, Cássio & Gomes, 2001; Huryn *et al*. 2002; Gulis & Suberkropp 2003 b, 2004; Pascoal *et al*., 2003; Nikolcheva & Bärlocher, 2005; Ferreira & Chauvet 2011; Tonin *et al*., 2011) but not by warming, contrasting some previous studies (Dang *et al*., 2009; Fernandes *et al*., 2009; Ferreira & Chauvet, 2011; Ferreira, Encalada & Graça, 2012). Simultaneous change in temperature and nutrient-load stimulated the decomposition of birch litter, corroborating findings by Ferreira & Chauvet (2011) who provided evidence for stream eutrophication to promote effects of warming on detritus processing. Similarly, detritivores in the present study not only promoted litter mas loss but increased the effects of warming irrespective of nutrient concentrations.

Similar to overall litter mass loss, microbial growth was stimulated by warming and/or nutrient-addition. Increased microbial biomass may improve litter breakdown both directly and indirectly through improved conditions for detritivores: litter-colonizing microbes produce extracellular enzyme to degrade leaf litter substances (Bärlocher, 1992, Berg and McClaugherty, 2003) and increase litter palatability to invertebrate shredders (Suberkropp, 1998a, Bärlocher, 2005). Increased nutritional quality of detritus due to microbial (especially fungal) biomass improves litter consumption by detritivores (Suberkropp, 1992, 1998a; Graça, 2001; Lecerf *et al*., 2005; Bärlocher, 2005; Canhoto & Graça, 2008; Chung & Suberkropp, 2009; Sridhar *et al*., 2009, 2011). However, microbial responses to nutrient enrichment have shown a range

from positive effect (Gulis & Suberkropp, 2003a, 2004) to no effect at all (Ferreira, Gulis & Graça, 2006).

Typically, fungi account for a major portion of total microbial biomass (Komínková *et al.*, 2000, Kuehn *et al.*, 2000, Findley *et al.*, 2002, Duarte *et al.*, 2010), and among fungi aquatic hyphomycetes are key drivers of leaf litter processing in temperate forested streams (Bärlocher, 1992). Most field and laboratory studies have emphasized that aquatic hyphomycetes can obtain dissolved nutrients directly from stream water (Suberkropp & Chauvet, 1995; Suberkropp, 1998b; Grattan & Suberkropp, 2001) and strongly respond to temperature (Chauvet & Suberkropp, 1998; Rajashekar & Kaveriappa, 2000; Nikolcheva, Bourque & Bärlocher, 2005; Dang *et al*., 2009; Fernandes *et al.*, 2009: Ferreira & Chauvet, 2011; Duarte *et al*., 2013). In our experiments, stimulation of fungal biomass by nutrient-addition at high and low temperature levels suggests that eutrophication of freshwaters, as caused by sewage effluent and agricultural run-off, will enhance fungal growth and then leaf litter processing in eutrophic streams (c.f., Grattan & suberkropp, 2001; Rosemond *et al*., 2002; Gulis & Suberkropp, 2003a, b, and c; Pascoal & Cássio, 2004; Ferreira *et al*., 2006; Chung & Suberkropp, 2008; Gulis *et al*., 2008; Ferreira & Chauvet, 2011) Our findings further indicate that detritivores also positively affected fungal biomass on birch leaf litter in all treatments. This finding was not expected, since significant effects of detritivores on fungal biomass are rare (Robinson & Gessner, 2000; Chung & Suberkropp, 2008; Treplin & Zimmer, 2012). However, viable fungal spores in the feces of amphipods (Bärlocher, 1981; Sridhar *et al*., 2011) make indirect positive effects of amphipods on the distribution of fungal propagules likely.

Both warming and nutrient-addition increased bacterial abundance, corroborating the prediction that ecosystem response to nutrient addition will increase with increasing temperature (Blenckner, Malmaeus & Pettersson,

2006 ; Jeppesen *et al.*, 2009). By contrast, grazing by amphipods reduced bacterial abundance (c.f. Ward *et al*., 1998; Treplin & Zimmer, 2012; Sanaei & Zimmer, unpubl. data), supporting the direct consumption of litter-associated (bacterial) biofilms by detritivores in freshwaters. To this end, detritivores may bias the competitive relationship (Mille-Lindblom, 2006; Romaní *et al*., 2006) of litter-colonizing bacteria and fungi in favor of fungi.

Microbial species richness and community composition on submerged leaf litter depends on various environmental factors (Duarte et al., 2008, Duarte, Pascoal, & Cássio, 2009; Duarte *et al.* 2009; Nickolcheva & Bärlocher, 2005; Sridhar *et al*., 2009; Sanaei & Zimmer, unpubl. data). Initial stages of decomposition are characterized by high fungal diversity (Nikolcheva *et al.*, 2005). The present study provides evidence for at least 25 bacterial ribotypes (compared to 30 in a study by Das, Royer & Leff (2007), 33-35 in a study by Duarte *et al*. (2010) and 38 in a study by Duarte *et al*. (2012) and 22 fungal ribotypes (compared to 19 (Nikolcheva, Cockshutt & Bärlocher, 2003), 33 (Das *et al.*, 2007), 19-22 (Duarte *et al*., 2010) and 33 (Duarte *et al*., 2012). Among-treatment variation in bacterial ribotypes suggests selective effects of both warming and nutrient-addition along with selective feeding by amphipods (c.f. Ihnen & Zimmer, 2008, for isopods). By contrast, fungal communities were only slightly affected by temperature or nutrient concentration, but strongly responded to selective feeding by amphipods.

Microbial colonization of leaf litter increases its palatability for shredders (Suberkropp, 1992; Abelho, 2001; Maraun *et al.*, 2003; Zimmer, Kautz & Topp, 2003). Since microbial biomass, in turn, was promoted by experimentally changing the environment according to current predictions (see above), we were surprised to see that amphipod growth rates did not vary under different environmental regimes (temperature and nutrients). While we cannot exclude the possibility that the lack of response by *G. pulex* could be explained by the

relatively short duration (40 days) of our experiments, our results suggest little effects of environmental change on shredder growth rates in temperate streams.

In conclusion, we show strong effects of detritivores and nutrient-addition on leaf litter decomposition. Bacterial abundance on litter surfaces appears to be promoted by both warming and nutrient-addition, but reduced by grazing-pressure by detritivores. Moreover, fungal biomass also appears to be stimulated by warming and nutrient-addition but also by detritivores that, in turn, were not stimulated by warming and nutrient-enrichment. Therefore, we predict only little effect on life-history characteristics of detritivorous freshwater invertebrates. Hence, effects of environmental change on (microbe-driven) litter breakdown may be based on mechanisms other than increased detritivore populations. However, along with warming and nutrient-enrichment detritivores affected the composition of litter-biofilm communities. Thus, the result of this study provides strong support for the selective role of detritivores on bacterial and fungal communities on litter surfaces in freshwaters.

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References

Abelho M. (2001) From litterfall to breakdown in streams: a review. *The Scientific World,* **1**, 656-680.

Bärlocher F. (1981) Fungi on the food and in the faeces of *Gammarus pulex*. *Transactions of the British Mycological Society*, **76**, 160-165.

Bärlocher F. (1992) Research on aquatic Hyphomycetes: historical background and overview. In: *The ecology of aquatic Hyphomycetes* (Ed F. Bärlocher), pp. 1-15. Springer Verlag, Berlin.

Bärlocher F. (2005) Freshwater fungal communities, In: *The fungal community: its organization and role in the ecosystem* (3rd ed.), (Eds J. Deighton, P. Oudemans & J. White), pp. 39-59. Taylor and Francis, CRC Press, Florida.

Berg B. & McClaugherty C. (2003) *Plant Litter, Decomposition, Humus Formation, Carbon Sequestration*. Springer Verlag, Berlin.

Blenckner T., Malmaeus J.M. & Pettersson K. (2006) Climatic change and the risk of lake eutrophication. *International Association of Theoretical and Applied Limnology*, **29**, 1837-1840.

Canhoto C. & Graça M. A. S. (2008) Interactions between fungi (aquatic Hyphomycetes) and invertebrates. In: *Novel techniques and ideas in mycology* (Eds K. R. Sridhar, F. Bärlocher & K. D. Hyde). Fungal Diversity Research Series, 20, pp. 205–325. Fungal Diversity Press, Chiang Rai.

Chauvet E. & Suberkropp K. (1998) Temperature and sporulation of aquatic hyphomycetes. *Applied* and *Environmental Microbiology*, **64**, 1522-1525.

Chung N. & Suberkropp K. (2008) Influence of shredder feeding and nuturients on fungal activity and community structure in headwater streams. *Fundamental and Applied Limnology Archiv für Hydrobiologie*, **173**, 35-46.

Chung N. & Suberkropp K. (2009) Effects of aquatic fungi on feeding preferences and bioenergetics of *Pycnopsyche gentilis* (Trichoptera: Limniphilidae). *Hydrobiologia* , **630**, 257-269.

Dang C.K., Schindler M., Chauvet E. & Gessner M.O. (2009) Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. *Ecology*, **90**, 122-131.

Das M., Royer T.V. & Leff L.G. (2007) Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. *Applied and Environmental Microbiology*, **73**, 756-767.

Davey M.E. & O'Toole G.A. (2000) Microbial biofilms: from ecology to molecular genetics. *Microbiology and Molecular Biology Reviews*, **64**, 847-867.

Duarte S., Pascoal C., Alves A., Correia A. & Cássio F. (2008) Copper and zinc mixtures induce shifts in microbial communities and reduce leaf litter decomposition in streams. *Freshwater Biology*, **53**, 91-101.

Duarte S., Pascoal C., Garabétian F., Cássio F. & Charcosset J.Y. (2009) Microbial decomposer communities are mainly structured by the trophic status in circumneutral and alkaline streams. *Applied and Environmental Microbiology*, **75**, 6211-6221.

Duarte S., Pascoal C. & Cássio F. (2009) Functional stability of stream-dwelling microbial decomposers exposed to copper and zinc stress. *Freshwater Biology*, **54**, 1683-1691.

Duarte S., Fidalgo M.L., Pascoal C. & Cássio F. (2012) The role of the freshwater shrimp *Atyaephyra desmarestii* in leaf litter breakdown in streams. *Hydrobiologia*, **680**, 149-157.

Duarte S., Pascoal C., Alves A., Correia A. & Cássio F. (2010) Assessing the dynamic of microbial communities during leaf decomposition in a low-order stream by microscopic and molecular techniques. *Microbiological Research*, **165**, 351-362.

Duarte S., Fernandes I., Cássio F. & Pascoal C. (2013) Temperature alters interspecific relationships among aquatic fungi. *Fungal Ecology*, **6**, 187-191.

Fernandes I., Uzun B., Pascoal C. & Cássio F. (2009) Responses of aquatic fungal communities on leaf litter to temperature-change events. *International Review of Hydrobiology*, **94**, 410-418.

Ferreira V., Gulis V. & Graça M. A. S. (2006) Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. *Oecologia*, **149**, 718-729.

Ferreira V. & Chauvet E. (2011) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology*, **17**, 551-564.

FerreiraV., Encalada A.C. & Graça M.A.S. (2012) Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. *Freshwater Science*, **31**, 945-962.

Findlay S., Tank J., Dye S., Valett H.M., Mulholland P.J. & McDowell W.H. *et al.* (2002) A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microbial Ecology*, **43**, 55-66.

Galloway J. N., Townsend A. R., Erisman J. W., Bekunda M., Cai Z. & Freney J. R. *et al.* (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, **320**, 889-92.

Gessner M.O. (2005) Ergosterol as a measure of fungal biomass. In: *Methods to study litter decomposition: a practical guide* (Eds M.A.S. Graça, F. Bärlocher & M.O. Gessner), pp. 189-195. Springer, Dordrecht.

Gessner M. O. & Chauvet E. (1993). Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied* and *Environmental Microbiology*, **59**, 502-507.

Gessner M.O., Chauvet E. & Dobson M. (1999) A perspective on leaf litter breakdown in streams. *Oikos,* **85**, 377-384.

Gessner M. O. & Newell S. Y. (2002) Biomass, growth rate, and production of filamentous fungi in plant litter. In: *Manual of environmental microbiology. 2nd ed*. (Eds C. J. Hurst, R. L. Crawford, G. R. Knudsen, M. J. McInerney & L. D. Stetzenbach). pp. 390-408. American Society for Microbiology Press, Washington, D.C.

Gessner M.O., Gulis V., Kuehn K.A., Chauvet E. & Suberkropp K. (2007) Fungal decomposers of plant litter in aquatic ecosystems. In: *The Mycota IV. Environmental and microbial relationships. 2nd ed*. (Eds C.P. Kubicek , I.S. Druzhinina), pp. 301-324. Springer Verlag, Berlin.

Gönczöl J., Csontos P. & Révay Á. (2003) Catchment scale patterns of aquatic hyphomycetes. The role of physicochemical variables and substrate composition in structuring conidial communities. *Archiv für Hydrobiologie* , **157**, 249-266.

Graça M. A. S. (2001) The role of invertebrates on leaf litter decomposition in streams – A review. *International* Review of *Hydrobiology*, **86**, 383-393.

Grattan R. M. & Suberkropp K. (2001) Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. *Journal of the North American Benthological Society,* **20**, 33-43.

Gulis V. & Suberkropp K. (2003 a) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology*, **48**, 123-134.

Gulis V. & Suberkropp K. (2003 b) Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. *Microbial Ecology*, **45**, 11-19.

Gulis V. & Suberkropp K. (2003 c) Interactions between stream fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. *Aquatic Microbial Ecology*, **30**, 149-157.

Gulis, V. & Suberkropp K. (2004) Effects of whole-stream nutrient enrichment on the concentration and abundance of aquatic hyphomycete conidia in transport. *Mycologia*, **96**, 57-65.

Gulis V., Suberkropp K. & Rosemond A.D. (2008) Comparison of fungal activities on wood and leaf litter in unaltered and nutrient-enriched headwater streams. *Applied* and *Environmental Microbiology,* **74**, 1094-1101.

Hax C.L. & Golladay S.W. (1993) Macroinvertebrate colonization and biofilm development on leaves and wood in a boreal river. *Freshwater Biology*, **29**, 79-87.

Hieber M. & Gessner M.O. (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, 83, 1026-1038.

Huryn A.D., Butz Huryn V.M., Arbuckle C.J. & Tsomides L. (2002) Catchment land use, macroinvertebrates and detritus processing in headwater streams: taxonomic richness versus function. *Freshwater Biology,* **47**, 401-415.

IPCC (International Panel on Climate Change) (2007) *Climate change 2007: the physical science basis*. Cambridge University Press, Cambridge.

Jeppesen E., Kronvang B., Meerhoff M., Søndergaard M., Hansen K.M. & Andersen H. E. *et al.* (2009) Climate change effects on runoff, catchment phosphorus loading and lake ecological state, and potential adaptations. *Journal of Environmental Quality*, **38**, 1030-1041.

Komínková D., Kuehn K.A., Bürsing N., Steiner D. & Gessner M.O. (2000) Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of large lake. *Aquatic Microbial Ecol*ogy, **22**, 271-282.

Kuehn K.A., Lemke M.J., Wetzel R.G. & Suberkropp K. (2000) Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effusus*. *Limnology* and *Oceanography*, **45**, 862-870.

Langan S.J., Johnston L., Donaghy M.J., Youngson A.F., Hay D.W. & Soulsby C. (2001) Variation in river water temperatures in an upland stream over a 30-year period. *Science* of the *Total Environment,* **265**, 195-207.

Lecerf A., Dobson M., Dang C.K. & Chauvet E. (2005) Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. *Oecologia*, **146**, 432-442.
Lock M.A., Wallace R.R., Costerton J.W., Ventullo R.M. & Charlton S.E. (1984) River epilithon: toward a structural- functional model. *Oikos*, **42**, 10-22.

Malmqvist, B. & Rundle S. (2002) Threats to the running water ecosystems of the world. *Environmental Conservation*, **29**, 134-153.

Maraun M., Martens H., Migge S., Theenhaus A. & Scheu S. (2003) Adding to the enigma of soil animal diversity:fungal feeders and saprophagus soil invertebrates prefer similar food substrates. *European Journal* of *Soil Biology*, **39**, 85-95.

MEA (Millennium Ecosystem Assessment) (2005) *Ecosystems and Human Well-Being: Synthesis*. Island Press, Washington, DC.

Mille-Lindblom C., Fischer H. & Tranvik L. J. (2006) Antagonism between bacteria and fungi: substrate competition and a possible trade-off between fungal growth and tolerance towards bacteria. *Oikos*, **113**, 233–242.
Murdoch P.S., Baron J.S. & Miller T.L. (2000) Potential effects of climate change on surface water quality in North America. *Journal of the American Water Resources Association*, **36**, 347-366.

Muyzer G., De Waal E.C. & Uitterlinden A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied* and *Environmental Microbiology*, **59**, 695-700.

Nikolcheva L.G., Cockshutt A.M. & Bärlocher F. (2003) Determining diversity of freshwater fungi on decaying leaves: comparison of traditional and molecular approaches. *Applied* and *Environmental Microbiology*, **69**, 2548-2554.

Nikolcheva L.G. & Bärlocher F. (2005) Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence. *Environmental Microbiology*, **7**, 270-280.

Nikolcheva L.G., Bourque T. & Bärlocher F. (2005) Fungal diversity during initial stage of leaf decomposition in a stream. *Mycological Research*, **109**, 246-253.

Pascoal C., Cássio F. & Gomes P. (2001) Leaf breakdown rates: a measure of water quality? *International Review of Hydrobiology*, **86**, 407-416.
Pascoal C., Pinho M., Cássio F. & Gomes P. (2003) Assessing structural and functional ecosystem condition using leaf breakdown: studies on a polluted river. *Freshwater Biology*, **48**, 2033-2044.
Pascoal C. & Cássio F. (2004) Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. *Applied and Environmental Microbiology*, **70**, 5266-5273.

Petri R. & Imhoff J.F. (2001) Genetic analysis of sea-ice bacterial communities of the Western Baltic Sea using an improved double gradient method. *Polar Biology*, **24**, 252–257.

Rajashekar M. & Kaveriappa K.M. (2000) Effects of temperature and light on growth and sporulation of aquatic hyphomycetes. *Hydrobiologia*, **441**, 149-153.
Robinson C.T. & Gessner M.O. (2000) Nutrient addition accelerates leaf breakdown in an alpine springbrook. *Oecologia*, **122**, 258-263.
Romaní A.M., Fischer H., Mille-Lindblom C. & Tranvik L.J. (2006) Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. *Ecology*, **87**, 2559-2569.
Rosemond A. D.,Pringle C. M., Ramírez A., Paul M. J. & Meyer J. L. (2002) Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. *Limnology* and *Oceanography*, **47**, 278-289.
Sridhar K. R. & Bärlocher F. (2000) Initial colonization, nutrient supply and fungal activity on leaves decaying in streams. *Applied* and *Environmental Microbiology*, **66**, 1114-1119.
Sridhar K. R., Duarte S., Cássio F. & Pascoal C. (2009) The role of early fungal colonizers in leaf-litter decomposition in Portuguese streams impacted by agricultural runoff. *International Review of Hydrobiology*, **94**, 399-409.
Sridhar K.R., Beaton M. & Bärlocher F. (2011) Fungal propagules and DNA in feces of two detritus feeding amphipods. *Microbial Ecology*, **61**, 31-40
Stelzer R. S., Heffernan J. & Likens G. E. (2003) The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshwater Biology*, **48**, 1925-1937.
Suberkropp K. (1984) Effect of temperature on seasonal occurrence of aquatic hyphomycetes. *Transactions of the British Mycological Society*, **82**, 53-62.
Suberkropp K. (1992) Interactions with invertebrates. In: *The ecology of aquatic hyphomycetes* (Ed. F. Bärlocher), pp. 118-134. Springer Verlag, Berlin.

Suberkropp K. & Chauvet E. (1995) Regulation of leaf breakdown by fungi in streams: influences of water chemistry. *Ecology*, **76**, 1433-1445.

Suberkropp K. (1998a) Microorganisms and organic matter decomposition. In: *River ecology and management: lessons from the Pacific coastal ecoregion* (Eds R.J. Naiman & R.E. Bilby), pp. 120-143. Springer, New York.

Suberkropp K. (1998b) Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. *Mycological Research,* **102**, 998-1002.

Tonin A. M. & Hepp L. U. (2011) Effects of nitrate enrichment on leaf litter decomposition. *Acta Limnologica Brasiliensia,* **23**, 86-94.

Thompson F.L., Abreu P.C. & Wasielesky W. (2002) Importance of biofilm for water quality and nourishment in intensive shrimp culture. *Aquaculture* , **203**, 263-278.

Treplin M. & Zimmer M. (2012) Decomposition of autochthonous *versus* allochthonous detritus: a cross-habitat comparison. *Ecosystems*, **15**, 477-491.

Wallace J.B. & Webster J.R. (1996) The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology* , **41**, 115-139.

Ward J.V., Bretschko G., Brunke M., Danielopol D., Gibert J. & Gonser T. *et al.* (1998) The boundaries of river systems: the metazoan perspective. *Freshwater Biology*, **40**, 531-569.

Zimmer M, Kautz G. &Topp W. (2003) Leaf litter-colonized microbiota: supplementary food source or indicator of food quality for *Porcellio scaber* (Isopoda: Oniscidea)? *European Journal of Soil Biology*, **39**, 209-216.

General Discussion

Leaf mass loss

The allochthonous organic matter input to forested streams is derived from dissolved and particulate organic matter from water flow and terrestrial plant litter fall, which release nutrients and energy for detrital food webs (Bärlocher 2005, Suberkropp 1998). Organic matters such as leaves shed from riparian vegetation are rapidly colonized by microorganisms, mainly fungi and bacteria (Bärlocher 2005, Gessner *et al*. 2007, Suberkropp 1998), throughout a process known as microbial conditioning. In this process, microorganisms can convert dead organic matter to energy that transfer through the aquatic food chains (Bärlocher 2005, Suberkropp 1998).

Both microbial decomposers, such as bacteria and fungi, and animal detritivores are important players in leaf litter processing that depends on environmental factors and leaf litter quality (Gessner *et al.* 1999, Hieber and Gessner 2002, Gessner *et al.* 2007, Treplin and Zimmer 2012). Many studies have emphasized that the microbial decomposers have been affected by environmental variables such as temperature and nutrient concentration (Suberkropp 1984, Gönczöl *et al*. 2003, Pascoal and Cássio 2004, Ferreira and Chauvet 2011). The recent Intergovernmental Panel on Climate Change report (IPCC 2007) anticipated global temperature increases by 1.1 °C to 6.4 °C, and 2.5 °C to 5.5 °C in Europe, and the same warming trend is mirrored in freshwater ecosystems (Langan *et al.* 2001).Temperature is one of the essential environmental factors which determine the activity of organisms in ecosystems (Friberg *et al.* 2009), with higher temperatures stimulating chemical reactions and biological activities (Bergfur and Friberg 2012)

In our experiments, we tested the effect of warming on birch leaf decomposition rate by increasing average water temperature to a target of 5 ºC above ambient (according to IPCC 2007). Our study showed that the rate of

birch leaves breakdown was not stimulated by temperature increase in the treatment microcosms. The results of the present study are in contrast with previous studies' consequences that have shown notable faster decomposition in response to warming (Dang *et al.* 2009, Fernandes *et al.* 2009, Ferreira and Chauvet 2011, Ferreira *et al.* 2012).

Additionally, most human activities such as urbanization, industrial and agricultural development, and water course alterations are the main causes of ecosystem destruction that may lead to endanger waters such as streams and rivers (Malmqvist and Rundle 2002, Galloway *et al*. 2008). This happens throughout eutrophication process in which excessive amounts of nutrients for instance nitrate and phosphate enter an aquatic system that may affect leaf litter processing. Stimulation of birch leaves decomposition by nutrient addition to a target of concentration 10 times above ambient, as observed in our experiment, is in agreement with those reported in previous studies (Suberkropp and Chauvet 1995, Suberkropp 1998, Sridhar and Bärlocher 2000, Pascoal *et al.* 2001, Huryn *et al*. 2002, Gulis and Suberkropp 2003 a, 2004, Pascoal *et al.* 2003, Nikolcheva and Bärlocher 2005, Ferreira and Chauvet 2011, Tonin *et al*. 2011). However, our results contradicted those implying that addition of nutrients to streams with low ambient nutrient concentration might not necessarily affect leaf litter decomposition and activity of microbial decomposers (Grattan and Suberkropp 2001, Royer and Minshall 2001, Abelho *et al*. 2010).

Increase in nutrient input and rise in temperature level, will probably tend to heighten eutrophication evidences (Kosten *et al*. 2009, Rustad *et al*. 2001, Brookshire *et al*. 2011), but rarely have the combined effects of these environmental variables on ecosystem processes been studied. It was predicted that in many streams, with increase in global mean temperature in the near future, nutrient concentration will also increase concurrently (Murdoch et al. 2000; MEA, 2005). In our study, increasing average water temperature to a target of 5 ºC above ambient and periodically fertilizing with a solution of

nitrate and phosphate to a target of concentration 10 times above ambient, similar to results of Ferreira and Chauvet (2011), created the condition for warming and nutrient addition to stimulate the decomposition rate of birch leaves.

Fungal biomass

In streams, plant litter is usually colonized by microbial decomposers when fungi, typically, account for a major portion of total microbial biomass, degrading leaf litter substances by extracellular enzymes (Bärlocher 1992, Komínková *et al.* 2000, Kuehn *et al.*, 2000, Findley *et al.* 2002, Berg and McClaugherty 2003, Duarte *et al.*, 2010), and enhancing litter palatability to invertebrate shredders (Suberkropp 1992, 1998, Abelho 2001, Zimmer *et al.* 2003, Bärlocher 2005).

In temperate forested streams, aquatic hyphomycetes are known, among fungi, to be key drivers of leaf litter processing (Bärlocher 1992). In the current study, we assessed the effect of increased temperature by 5°C on fungal biomass associated with birch leaf litters. The positive response of fungal biomass to warming, in high and low nutrient levels, may support earlier evidences that one of the most important factors affecting the aquatic hyphomycetes is temperature (Chauvet and Suberkropp 1998, Rajashekar and Kaveriappa 2000, Nikolcheva *et al*. 2005, Dang *et al*. 2009, Fernandes *et al.* 2009, Ferreira and Chauvet 2011, Duarte *et al*., 2013).

Moreover, Microbial responses to nutrient enrichment experiments have shown positive effect (Gulis and Suberkropp 2003 a, 2004). In the present study, we compared the fungal biomass in high and low nutrient concentrations. Most studies have mentioned that aquatic hyphomycetes can use dissolved nutrients in stream water (Suberkropp and Chauvet 1995, Suberkropp 1998, Grattan and Suberkropp 2001). The results of the current study, which indicate a positive relationship between nutrient addition and fungal biomass, are in agreement

with those of several studies (Grattan and Suberkropp 2001, Rosemond *et al*. 2002, Gulis and Suberkropp 2003a, b, and c, Pascoal and Cássio 2004, Ferreira *et al*. 2006, Chung and Suberkropp 2008, Gulis *et al*. 2008). The fact that increase in fungal biomass occurs in response to nutrients addition at both high and low temperature levels, found out in this study, suggests that eutrophication of stream waters done by sewage effluent and agricultural run-off will promote fungal growth and then detritus processing in eutrophic streams. Furthermore, Ferreira and Chauvet (2011) suggested that stream eutrophication promoted effects of warming on leaf litter decomposition. Positive responses of fungal biomass to warming in synergy with nutrient addition in our results support this finding.

Leaf litter associated microbial decomposers, mainly fungi, with increase in nutritional value of detritus, probably, lead to enhance leaf litter consumption by detritivores (Suberkropp 1992, 1998, Graça 2001; Lecerf *et al*. 2005, Bärlocher 2005, Canhoto and Graça 2008, Chung and Suberkropp 2009, Sridhar *et al*. 2009, 2011). In the current study, contrary to our expectation, the presence of detritivores seemed to have positive effect on fungal biomass, associated with birch leaf litters in all treatments. In correlative studies by (Robinson and Gessner 2000, Chung and Suberkropp 2008, Treplin and Zimmer 2012), there were no significant effect of detritivores presence on fungal biomass associated with leaf litters. This unexpected result is likely to due to the indirect effect of detritivores on distribution of fungal propagules through their digestive tract or attached to their bodies. Such examples by (Bärlocher 1981, Sridhar *et al*. 2011) show the presence of viable fungal spores in the feces of amphipods incubated with sterile leaves.

Bacterial abundance

Organisms such as macroinvertebrates, fungi, and bacteria play a significant role in Leaf litter breakdown. However, the bacterial contribution to leaf decomposition is likely to be smaller than detritivores and fungi (Hieber and

Gessner 2002) but there is evidence that some bacteria secrete exoanzymes that break down plant material (Burns 1982) and play a significant role in the leaf litter decay process (Findlay and Arsuffi 1989, Baldy and Gessner 1997, Hieber and Gessner 2002) especially during later stages (Suberkropp and Klug 1980).

To have a better understanding of impacts of environmental factors on microbes mediating leaf litter decomposition in freshwaters, we assessed the effect of temperature increase nutrient addition, and detritivores presence on bacterial abundance on decaying birch leaves. As expected, warming stimulated bacterial abundance and bacterial abundance was higher at 15 °C, in contrast to 10 °C, in ambient nutrient concentration which might indicate the sensitivity of bacteria to temperature. Additionally, direct comparison of bacterial abundance in low nutrient versus high nutrient treatments indicated more bacterial abundance in high nutrient treatment, which supports the importance of nutrients in aquatic system (Meyers and Johnson 1983, Suberkropp and Chauvet 1995), especially in low temperature of fall season when the nitrate concentration is high in the agricultural catchment (Poor and McDonnell 2007).

In line with our expectations, grazing by amphipods reduced bacterial abundance in all temperature and nutrient level conditions which are in agreement with previous studies (Ward *et al*. 1998; Treplin and Zimmer 2012). Moreover, the high bacterial abundance in treatments without detritivores versus treatments with detritivores in high temperature and also in nutrient enriched conditions indicates compensatory effect of nutrient addition and warming for microbial decomposers abundance upon grazing. Correlations between detritivores presence and bacterial abundance reduction support importance of the nutritional role for bacterial biofilm in aquatic system.

Moreover, in our study, the synergistic effect of warming and nutrient enrichment on bacterial abundance demonstrate that ecosystem response to nutrient addition increases with temperature which is in line with observation of

correlative studies by Blenckner *et al*. 2006, Friberg *at al.* 2009, Jeppesen *et al*. 2009.

Amphipod dry mass

Detritivores by grazing (removing the biofilm from leaf surface) and shredding (consuming entire leaf) are able to convert nutrients from decaying leaf litter and associated microbes to animal tissue which play a major role in decomposition process of plant litter in freshwaters (Wallace and Webster 1996, Graça 2001, Hieber and Gessner 2002). Expected climate change due to global warming and also eutrophication will influence detritivores in both direct and indirect effects. Temperature increasing often accompanied directly by increasing animal activity and also higher metabolic rates that leads to higher consumption of detritus and associated microbes (Newell 1966, Halcrow and Boyd 1967, Newell and Northcroft 1967). Moreover, temperature influence on biofilm of leaf surface may, indirectly, lead to increased microbial food source of detritivores.

In our study, growth rate of amphipods showed no difference in response to 5°C increase in temperature but warming increased fungal biomass and bacterial abundance on birch leaf discs in our treatments. The lack of response in growth rate of amphipods to warming might be argued that ectothermic amphipods are incapable of controlling their body temperature significantly and thus their metabolic rate might vary in response to changes in ambient temperatures. Schmidt-Nielsen (1997) also reported the 20 to 25% rise in amphipod oxygen consumption in response to 3.2°C increase in temperature. Additionally, in our experiment nutrient addition also induced significant differences in total biomass of microbial biofilm on birch leaves while growth rate of amphipods showed no difference in response to nitrate and phosphate enrichment. It could be explained by the relatively short duration (40 days) of our experiment and also definite amount of leaf discs in each treatment.

Microbial community structure

To evaluate the structure of the microbial community attached to birch leaves and to understand how it is affected by warming, nutrient availability and detritivores presence, we chose the PCR-DGGE technique. In this method, 18S rRNA and 16S rRNA genes of fungi and bacteria, respectively, were PCR amplified by oligonucleotide primers designed for the specific amplification of these genes. Then, the products subjected to DGGE whereby, allows for the detection of individual fungal and bacterial species based on their operational taxonomic units (OTUs). However, most studies addressing the diversity of bacteria associated with decaying leaf litter were limited to cultivable bacteria which were identified by traditional methods (Suberkropp and Klug 1976, Baldy *et al.* 1995, Hieber and Gessner 2002).

The present study tested the hypothesis that the bacterial biofilm is influenced by changes in environmental variables such as temperature and nutrient and also by presence of detritivores. DGGE analysis clearly showed a maximum of 25 bacterial ribotypes attached to birch leaves in our multifactorial study. This result is in agreement with previous findings by Das *et al.* (2007) that found 30 bacterial OTUs on decomposing sugar maple and white oak leaves in small-forested stream after 181 days and also by Duarte *et al.* (2010) that found 33-35 bacterial OTUs on decaying alder leaves in low-order stream after 56 days. In this study, analysis of DGGE profiles revealed no similarity between the bacterial community fingerprints in treatments with and without detritivores. Among physical variables, the role of temperature on bacterial community was more pronounced than nutrients. Specially, in treatments with detritivores, temperature separated the bacterial communities from each other. Surprisingly, bacterial community structure was not affected by different nutrient concentrations. In the first experiment the nutrient concentrations were 31.5 mg N L^{-1} and 1.5 mg P L^{-1} (Chapter I) and in the second one, 1.6 mg N L^{-1} and

0.1mg P L^{-1} were used (Chapter II) while bacterial ribotype richness was similar in both experiments.

Although this study was limited to one leaf type, similar bacterial community was detected on both sugar maple and white oak leaves in Das *et al*. (2007) study. The objective of this study was to examine the whole microbial community structure on decaying birch leaves. The DGGE analysis of fungal community structure showed 22 fungal ribotypes in comparison to 10 ribotypes on decaying leaves after four weeks (Nikolcheva *et al.* 2003), 33 ribotypes on decaying sugar maple and white oak leaves in small-forested stream after 181 days (Das *et al.* 2007) and 19-22 ribotypes on decaying alder leaves in low-order stream after 56 days (Duarte *et al.* 2010).While the composition of fungal communities on decomposing birch leaves varies between with and without detritivores treatments, the DGGE analysis showed identical band patterns between different treatment of temperature and nutrients level. Overall, the data from this DNA-based study yield the discriminating effect of detritivores on microbial community structure attached to decaying leaf litter in freshwater ecosystems (c.f. Ihnen & Zimmer 2008, for isopods).

Conclusion:

Leaf litter decomposition is a well-studied field in aquatic environments. This fundamental ecosystem process is controlled by the joint action of microbial decomposers and detritivores. In this thesis (chapter I) it was shown that simultaneous warming and nutrient-enrichment in temperate streams will interfere with grazing-pressure on bacterial biofilms by invertebrate detritivores, which provide a better picture of microbial and invertebrate decomposers under future global change scenario.

We believe that our data may have important implications for assessing the effect of these changes in the aquatic environment due to changed bacterial biofilm density and composition on leaf litter, particularly in processes in which bacterial biofilm are involved (e.g. leaf litter decomposition). We predict that

decomposition processes which depend on grazer-induced changes in bacterial biofilm composition could be affected by warming and, to a lesser degree, nutrient-enrichment. On the other hand, the feeding activity of invertebrates was more affected by increase in water temperature and nutrient-enrichment will mostly interfere with those processes that depend on bacterial density.

In chapter II, some questions about the wide field of microbial and invertebrate leaf litter decomposition were answered. The results indicate the strong effects of detritivores and nutrient-addition on leaf litter decomposition. This finding predicts the faster litter decomposition that may lead to food shortage in aquatic systems. . Both warming and nutrient-addition promoted bacterial density but detritivores grazing reduced the abundance of bacteria on litter surfaces. Moreover, fungal biomass appears to be stimulated not only by warming and nutrient-addition but also by detritivores which in turn, were not stimulated by warming and nutrient-enrichment. Therefore, we predict only little effect of environmental change on biological traits of detritivorous freshwater invertebrates. However, along with warming and nutrient-enrichment, detritivores affected the composition of litter-biofilm communities. Thus, the result of this study provides strong support for the pivotal role of microbial decomposers and also provides selective role of detritivores on bacterial and fungal communities on litter surfaces in freshwaters.

In future, more studies are necessary to assess the role of interactions between microbial decomposers under future climate change scenario in natural habitats. Also, it would be interesting to conduct studies to define bacterial and fungal species in microbial community structure.

References:

- Abelho, M. 2001. From litterfall to breakdown in streams: a review. *The Scientific World* 1, 656-680.
- Abelho, M., M. Moretti, J. França and M. Callisto. 2010. Nutrient addition does not enhance leaf decomposition in a Southeastern Brazilian stream (Espinhaço mountain range). *Brazilian Journal of Biology* 70, 747-754.
- Baldy, V. and M.O. Gessner. 1997. Towards a budget of leaf litter decomposition in a first-order woodland stream. *Comptes Rendues de l'Académie des Sciences Paris, Série III, Sciences de la vie* 320, 747-758
- Bärlocher, F. 1981. Fungi on the food and in the faeces of *Gammarus pulex*. *Transactions of the British Mycological Society* 76, 160-165.
- Bärlocher, F. 1992. Research on aquatic Hyphomycetes: historical background and overview. *In* The ecology of aquatic Hyphomycetes (F. Bärlocher, Ed.). Springer Verlag, Berlin, p. 1-15.
- Bärlocher, F. 2005. Freshwater fungal communities, In: The fungal community: its organization and role in the ecosystem (3rd ed.), (Deighton, J.; Oudemans, P. and White, J. Eds.). Taylor and Francis, CRC Press, Boca Raton, Florida, p. 39-59.
- Berg, B. and C. McClaugherty. 2003. Plant Litter, Decomposition, Humus Formation, Carbon Sequestration. Springer Verlag, Berlin.
- Bergfur, J. and N. Friberg. 2012. Trade-offs between fungal and bacterial respiration along gradients in temperature, nutrients and substrata: experiments with stream derived microbial communities. *Fungal Ecology* 5, 46-52.
- Blenckner, T., J.M. Malmaeus and K. Pettersson. 2006. Climatic change and the risk of lake eutrophication. *International Association of Theoretical and Applied Limnology* 29, 1837–1840.
- Brookshire, E.N.J., S. Gerber, J.R. Webster, J.M. Nose and W.T. Swank. 2011. Direct effects of temperature on forest nitrogen cycling revealed through analysis of long-term watershed records. *Global Change Biology* 17, 297-308.
- Burns, R.G. 1982. Enzyme activity in soil: location and possible role in microbial ecology. *Soil Biology and Biochemistry* 14,423-427
- Canhoto, C. and M. A. S. Graça. 2008. Interactions between fungi (aquatic Hyphomycetes) and invertebrates. In: Novel techniques and ideas in mycology (K. R. Sridhar, F.

Bärlocher, and Hyde K. D. Eds.). Fungal Diversity Research Series, 20. Fungal Diversity Press, Chiang Rai, p. 205–325.

- Chauvet, E. and K. Suberkropp. 1998. Temperature and sporulation of aquatic hyphomycetes. *Applied and Environmental Microbiology* 64, 1522-1525.

- Chung, N. and K. Suberkropp. 2008. Influence of shredder feeding and nuturients on fungal activity and community structure in headwater streams. *Fundamental and Applied Limnology Archiv für Hydrobiologie* 173, 35-46.

- Dang, C.K., M. Schindler, E. Chauvet, and M.O. Gessner. 2009. Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. *Ecology* 90, 122-131.

- Das, M., T.V. Royer and L.G. Leff. 2007. Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. *Applied and Environmental Microbiology* 73, 756-767.

- Duarte, S., C. Pascoal, A. Alves, A. Correia and F. Cássio. 2010. Assessing the dynamic of microbial communities during leaf decomposition in a low-order stream by microscopic and molecular techniques. *Microbiological Research* 165, 351-362.

- Duarte, S., I. Fernandes, F. Cássio and C. Pascoal. 2013. Temperature alters interspecific relationships among aquatic fungi. *Fungal Ecology* 6,187-191

- Fernandes I., B.Uzun,C. Pascoal and F. Cássio. 2009. Responses of aquatic fungal communities on leaf litter to temperature-change events. *International Review of Hydrobiology* 94, 410–418.

- Ferreira, V., V. Gulis and M. A. S. Graça. 2006. Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. *Oecologia* 149, 718–729.

- Ferreira, V. and E. Chauvet. 2011. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology* 17, 551–564.

- Ferreira, V., A.C. Encalada and M.A.S. Graça. 2012. Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. *Freshwater Science* 31, 945–962.

- Findlay SE and T.L. Arsuffi. 1989. Microbial growth and detritus transformations during decomposition of leaf litter in a stream. Freshwater Biology 21, 261–269

- Findlay, S., J. Tank, S. Dye, H.M. Valett, P.J. Mulholland, W.H. McDowell, S.L. Johnson, S.K. Hamilton, J. Edmonds, W.K. Dodds and W.B. Bowden. 2002. A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. *Microbial Ecology* 43, 55-66.

- Friberg, N., J.B. Dybkjær, J.S. Olafsson, G.M. Gislason, S.E. Larsen and T.L. Lauridsen. 2009. Relationships between structure and function in streams contrasting in temperature. *Freshwater Biology* 54, 2051-2068.

- Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger and M. A. Sutton. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320, 889-92.

- Gessner, M.O., E. Chauvet and M. Dobson. 1999. A perspective on leaf litter breakdown in streams. *Oikos* 85, 377-384.

- Gessner, M.O., V. Gulis, K.A. Kuehn, E. Chauvet and K. Suberkropp. 2007. Fungal decomposers of plant litter in aquatic ecosystems. In: The Mycota IV. Environmental and microbial relationships (2nd ed). (Kubicek C.P., Druzhinina I.S., Eds). Springer-Verlag, Berlin, p. 301-324.

- Gönczöl, J., P. Csontos, and Á. Révay. 2003. Catchment scale patterns of aquatic hyphomycetes. The role of physicochemical variables and substrate composition in structuring conidial communities. *Archiv für Hydrobiologie* 157, 249-266.

- Graça, M. A. S. 2001. The role of invertebrates on leaf litter decomposition in streams – A review. *International Review of Hydrobiol*ogy 86, 383-393.

- Grattan, R. M. and K. Suberkropp. 2001. Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. *Journal of North American Benthological Society* 20, 33–43.

- Gulis, V. and K. Suberkropp. 2003 a. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology* 48, 123–134.

- Gulis, V. and K. Suberkropp. 2003 b. Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. *Microbial Ecology* 45, 11-19.

- Gulis V. and K. Suberkropp. 2003 c. Interactions between stream fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. *Aquatic Microbial Ecology* 30, 149-157.
- Gulis, V. and K. Suberkropp. 2004. Effects of whole-stream nutrient enrichment on the concentration and abundance of aquatic hyphomycete conidia in transport. *Mycologia* 96, 57–65.

- Gulis, V., K. Suberkropp and A.D. Rosemond. 2008. Comparison of fungal activities on wood and leaf litter in unaltered and nutrient-enriched headwater streams. *Applied and Environmental Microbiology* 74, 1094-1101.

- Halcrow, K. and C.M. Boyd. 1967. The oxygen consumption and swimming activity of the amphipod *Gammarus oceanicus* at different temperatures. *Comparative Biochemistry and Physiology* 23, 233-242.

- Hieber, M. and M.O. Gessner. 2002. Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology* 83, 1026-1038.

- Huryn, A.D., V.M. Butz Huryn, C.J. Arbuckle and L. Tsomides. 2002. Catchment land use, macroinvertebrates and detritus processing in headwater streams: taxonomic richness versus function. *Freshwater Biology* 47, 401–415.
- Ihnen, K. and M. Zimmer. 2008. Selective consumption and digestion of litter microbes by *Porcellio scaber* (Isopoda: Oniscidea). *Pedobiologia* 51, 335-342.
- IPCC [International Panel on Climate Change]. 2007. Climate change 2007: the physical science basis. Cambridge University Press, Cambridge.

- Jeppesen, E., B. Kronvang, M. Meerhoff, M. Søndergaard, K.M. Hansen, H. E. Andersen, T. L. Lauridsen, M. Beklioglu, A. Özen and J. E. Olesen. 2009. Climate change effects on runoff, catchment phosphorus loading and lake ecological state, and potential adaptations. *Journal of Environmental Quality* 38, 1030–1041.

- Komínková, D., K.A. Kuehn, N. Bürsing, D. Steiner and M.O. Gessner. 2000. Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of large lake. *Aquatic Microbial Ecol*ogy 22, 271-282.

- Kosten, S., A. Kamarainen, E. Jeppesen, E.H. van Nes, E.T. H.M. Peeters, N. Mazzeo, L. Sass, J. Hauxwell, N. Hansel-Welch,T.L. Lauridsen, M. Søndergaard, R.W.

Bachmann, G. Lacerot, and M. Scheffer. 2009. Climate-related differences in the dominance of submerged macrophytes in shallow lakes. *Global Change Biology* 15, 2503-2517.

- Kuehn, K.A., M.J. Lemke, R.G. Wetzel and K. Suberkropp. 2000. Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effusus*. *Limnology and Oceanography* 45, 862–870.
- Langan, S.J., L. Johnston, M.J. Donaghy, A.F. Youngson, D.W. Hay, and C. Soulsby . 2001. Variation in river water temperatures in an upland stream over a 30-year period. *Science of the Total Environment* 265, 195–207.
- Lecerf, A., M. Dobson, C.K. Dang and E. Chauvet. 2005. Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. *Oecologia* 146, 432–442.
- Malmqvist, B. and S. Rundle. 2002. Threats to the running water ecosystems of the world. *Environmental Conservation* 29, 134-153.
- MEA (Millennium Ecosystem Assessment). 2005. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington D.C.
- Newell, R.C. and H.R. Northcroft. 1967. A re-interpretation of the effect of temperature on the metabolism of certain marine invertebrates. *Journal of Zoology (London)* 151, 277-298.
- Newell, R. C. 1966. The effect of temperature on the metabolism of poikilotherms. *Nature (London)* 212, 426-428.
- Nikolcheva, L.G., A.M. Cockshutt, and F. Bärlocher. 2003. Determining diversity of freshwater fungi on decaying leaves: comparison of traditional and molecular approaches. *Applied and Environmental Microbiology* 69, 2548-2554.
- Meyer J.L. and C. Johnson. 1983. The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. *Freshwater Biology*, 13, 177-183.
- Murdoch, P.S., J.S. Baron, and T.L. Miller. 2000. Potential effects of climate change on surface water quality in North America. *Journal of the American Water Resources Association* 36, 347–366.
- Nikolcheva, L.G., T. Bourque and F. Bärlocher. 2005. Fungal diversity during initial stage of leaf decomposition in a stream. *Mycological Research* 109, 246-253.

- Pascoal, C., F. Cássio, and P. Gomes. 2001. Leaf breakdown rates: a measure of water quality? *International Review of Hydrobiology* 86, 407-416.
- Pascoal, C., M. Pinho, F. Cássio, and P. Gomes. 2003. Assessing structural and functional ecosystem condition using leaf breakdown: studies on a polluted river. *Freshwater Biology* 48, 2033-2044.
- Pascoal, C. and F. Cássio. 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. *Applied and Environmental Microbiology* 70, 5266-5273.
- Rajashekar, M. and K.M. Kaveriappa. 2000. Effects of temperature and light on growth and sporulation of aquatic hyphomycetes. *Hydrobiologia* 441, 149-153.
- Rosemond, A. D., C. M. Pringle, A. Ramírez, M. J. Paul, and J. L. Meyer. 2002. Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. *Limnology and Oceanography* 47, 278–289.
- Royer, T. V. and G. W. Minshall. 2001. Effects of nutrient enrichment and leaf quality on the breakdown of leaves in a hardwater stream. *Freshwater Biology* 46, 603–610.
- Rustad, L.E., J.L.Campbell, G.M. Marion, R.J. Norby, M. Mitchell, A.E. Hartley, J.H.C. Cornellissen, and J. Gurevitch. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126, 543-562.
- Schmidt-Nielsen, K .1997. Animal physiology: adaptation and environment, 5th Ed. Cambridge University Press, Cambridge.
- Sridhar, K. R. and F. Bärlocher. 2000. Initial colonization, nutrient supply and fungal activity on leaves decaying in streams. – *Applied and Environmental Microbiology* 66, 1114-1119.
- Sridhar, K. R., S. Duarte, F. Cássio and C. Pascoal. 2009. The role of early fungal colonizers in leaf-litter decomposition in Portuguese streams impacted by agricultural runoff. *International Review of Hydrobiology* 94, 399-409.
- Sridhar, K.R., M. Beaton and F. Bärlocher. 2011. Fungal propagules and DNA in feces of two detritus feeding amphipods. *Microbial Ecology* 61, 31-40.
- Suberkropp, K. 1984. Effect of temperature on seasonal occurrence of aquatic hyphomycetes. *Transactions of the British Mycological Society* 82, 53-62.
- Suberkropp, K. 1992. Interactions with invertebrates. In: The ecology of aquatic hyphomycetes (F. Bärlocher Ed.), Springer Verlag, Berlin. p. 118–134.

- Suberkropp, K. 1998. Microorganisms and organic matter decomposition, In: River ecology and management: lessons from the Pacific coastal ecoregion (Naiman, R.J. and Bilby, R.E. Eds.), Springer, New York, p. 120-143.
- Suberkropp, K. and M.J. Klug.1976. Fungi and bacteria associated with leaves during processing in a woodland stream. *Ecology* 57, 707–719.
- Suberkropp, K. and M.J. Klug. 1980. The maceration of deciduous leaf litter by aquatic hyphomycetes. *Canadian Journal of Botany* 58, 1025-1031.
- Suberkropp, K. and E. Chauvet. 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. *Ecology* 76, 1433–1445.
- Tonin, A. M. and L. U. Hepp. 2011. Effects of nitrate enrichment on leaf litter decomposition. *Acta Limnologica Brasiliensia* 23, 86-94.
- Treplin, M. and M. Zimmer. 2012. Decomposition of autochthonous *versus* allochthonous detritus: a cross-habitat comparison. *Ecosystems* 15, 477-491.
- Wallace, J.B. and J.R. Webster .1996. The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology* 41, 115-139.
- Ward JV, Bretschko G, Brunke M, Danielopol D, Gibert J, Gonser T, Hildrew AG. 1998. The boundaries of river systems: the metazoan perspective. *Freshwater Biology* 40, 531- 69.
- Zimmer M, Kautz G, Topp W. 2003. Leaf litter-colonized microbiota: supplementary food source or indicator of food quality for *Porcellio scaber* (Isopoda: Oniscidea)? *European Journal of Soil Biology* 39, 209-216.

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Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig verfasst und keine anderen als die angegebenen Hilfsmittel und Quellen verwendet habe. Ich habe bisher keinen anderen Promotionversuch unternommen, und diese Arbeit hat weder ganz noch teilweise im Rahmen eines anderen Prüfungsverfahrens vorgelegen. Bei der Erstellung dieser Abhandlung habe ich mich an die Regeln guter wissenschaftlicher Praxis gehalten.

Kiel, den _____ _____