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Effects of Warming and Nutrient Enrichment on How Grazing Pressure Affects Leaf Litter–Colonizing Bacteria

Fatemeh Sanaei Moghadam* and Martin Zimmer

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 at 10°C and to a lesser degree at 15°C. However, the detritivore-induced decrease in bacterial numbers was compensated for by nutrient addition. After 31 d of incubation, amphipods reduced bacterial numbers only at 15°C, 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, whereas nutrient effects were stronger at high temperature after 31 d of incubation. Thus, warming without detritivores did not affect bacterial numbers under low-nutrient conditions (10 d). When detritivores were present, warming increased bacterial density significantly just under high-nutrient conditions ($P < 0.05$). After 31 d of incubation, warming did not affect bacterial density in detritivore-free controls in both nutrient conditions and did not affect bacterial density in both nutrient conditions in the presence of detritivores. Warming exhibited a significant effect on the composition of litter-associated bacterial communities irrespective of nutrient load ($P < 0.05$), whereas nutrients had less consistent effects. We conclude that warming, and to a lesser degree nutrient enrichment, may have influence on grazer-induced changes in bacterial biofilm composition, whereas nutrient enrichment may mostly interfere with those processes that depend on bacterial density.

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) and exoenzymes 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 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 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 have 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). Bacteria are capable of competing with fungi for leaf litter resources, and their contribution to leaf litter decay increases relative to fungal contributions upon nutrient enrichment (Gulis and Suberkropp, 2003b; Pascoal and Cássio, 2004).

Two of the main parameters controlling microbial communities and production are temperature and the availability of nutrients (Kirchman, 1994; Felip et al., 1996; Chauvet and Suberkropp, 1998; Rubin and Leff, 2007). A recent report from the Intergovernmental Panel on Climate Change (2007) predicts that global ambient temperature will increase 1.1 to 6.4°C by the end of this century, and freshwater temperatures are 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; however, its 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

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Abbreviations: DGGE, denaturing gradient gel electrophoresis; nMDS, nonmetric multidimensional scaling; OTU, operational taxonomic unit; PERMANOVA, permutational multivariate analysis of variance.

on litter surfaces but caused a shift in bacterial community structure (Flury and Gessner, 2011). In some studies, warming effects on microbial responses were stronger under high nutrient conditions (Ferreira and Chauvet, 2011; Villanueva et al., 2011). However, the effects of warming on decomposition processes in freshwaters, particularly in combination with other environmental changes (e.g., nutrient enrichment), are 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, soil to groundwater fluxes, and atmospheric deposition (Cross et al., 2005; Greenwood et al., 2007). Kunkel et al. (2008) showed nitrate concentrations above 100 mg NO_3 L^{-1} in soil leachates for the majority of agricultural land in Germany. Groundwaters 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 (EC, 1998) sets a maximum allowable concentration of 50 mg NO_3 L^{-1}, although in some groundwater sampling sites of Germany nitrate concentrations were above 50 mg NO_3 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), and fungi on leaf surface were twice as productive in a nutrient-enriched than in a control stream (Gulis et al., 2008). Moreover, nutrient enrichment increased the bacterial density on leaf litter (Gulis and Suberkropp, 2003; Pascoal and Cássio, 2004).

Whereas invertebrate shredders help leaf litter breakdown through feeding and digestion (Wallace and Webster, 1996; Gessner et al., 1999; Hieber and Gessner, 2002; Treplin and Zimmer, 2012), 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). The most direct effect that detritivorous invertebrates exert on microbial biofilms is their consumption, be it by shredders that consume entire leaf particles or by grazers that abrade biofilms from leaf surfaces. Although cell density is reduced during grazing, activity may increase through improved resource availability to the remaining cells, and community composition may change as a result of selective feeding. How invertebrate–microbe interactions during leaf litter decomposition are affected by environmental change (e.g., warming and eutrophication) is 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 simultaneous effects of multiple environmental changes (e.g., warming and nutrient enrichment) on biotic interactions been studied. Understanding the effect of multiple environmental changes on the interactions of detritivores and bacterial biofilm is a prerequisite to predicting effects of environmental changes on decomposition processes in temperate freshwaters.

The goal of the present study is to provide 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 warming and nutrient enrichment can change the bacterial density and their community structure by influencing detritivores' grazing pressure. To test this hypothesis, we designed an orthogonal experimental setup that independently combined the three factors of "grazers," "temperature," and "nutrients."

Materials and Methods

Experimental Set-up

Experiments were performed in microcosms (16 × 10 × 10 cm) that were supplied with 1 L of aerated diluted water from Russee creek (10°08′ E, 54°29′ N) near Kiel (northern Germany) containing 1.5 × 10^7 bacteria mL^{-1} through quantitative bacterial measurement. Creek water samples were kept at 10°C in 10-L bottles.

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 wk before introduction to the microcosms. Leaves were tested randomly for bacterial inoculation, and leaf disks (10 mm in diameter) were cut from similarly inoculated leaves using a cork borer. Nine disks were added to each microcosm.

Specimens of amphipods (*Gammarus pulex*) were collected from Russee creek. Nine specimens 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 1000 with a mean density of 506 individuals m^{-2} based on our observations. 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 a detritivore-free control.

Ammonium nitrate and potassium phosphate (Carl Roth) were added to half of the microcosms to achieve NO_3 and PO_4 concentrations of 1 mmol L^{-1} (NH_4NO_3; 90 mg L^{-1} is equivalent to 31.5 mg N L^{-1}) and 0.06 mmol L^{-1} (equimolar quantities of KH_2PO_4 and K_2HPO_4; 8.18 mg L^{-1} is equivalent to 1.5 mg P L^{-1}), respectively, according to their molar Redfield ratio of 16:1, to mimic a high nutrient load of headwater streams in agricultural land. Control microcosms were filled with 1 L creek water (ambient N and P concentrations <0.5 and <0.02 mg L^{-1}, respectively) diluted with tap water. Once a week, half of the water was replaced by a fresh-made mixture of tap and creek water with added nutrients. The other half of the microcosms served as controls.

Warming was mimicked by increasing the experimental temperature of 10°C (n = 20 × 2 for two sampling dates) to 15°C (n = 20 × 2) orthogonally to low (ambient) (n = 20 × 2) versus high (n = 20 × 2) nutrient concentration. Therefore, the treatments for the two dates of sampling were (i) control (ambient temperature, ambient nutrient load) (n = 10 × 2 for two sampling dates); (ii) increased temperature, ambient nutrient load (n = 10 × 2); (iii) ambient temperature, increased nutrient load (n = 10 × 2); and (iv) increased temperature, increased nutrient load (n = 10 × 2), which were run with (n = 5 for each of the above treatments and each sampling date) or without (n =

5) amphipods. Thus, 80 microcosms were set up to be sampled after 10 d (40 microcosms) and 31 d (40 microcosms).

After 10 and 31 d, five microcosms from each treatment were sacrificed. Subsets of four randomly chosen leaf disks from each microcosm were stored in formaldehyde at 4°C for the enumeration of bacteria; another subset of four leaf disks was kept frozen (−20°C) for the extraction of bacterial DNA and subsequent genetic fingerprinting.

Bacterial Density Measurements

To detach the bacteria from the leaves, two glass beads (5 mm) were added to a 2-mL tube containing four leaf disks in formaldehyde solution (Carl Roth), 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) (Sartorius Stedim, Millipore) 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 (Invitrogen) at a final concentration of 1 μg mL^{-1}. The filters were rinsed with sterile water and mounted onto glass slides. The slides were observed under 1000× magnification with an epifluorescence microscope (Axio scope A1, Zeiss). For statistical evaluation, 12 microscopic fields (100 × 100 μm^2) were counted per slide.

Extraction of Genomic DNA and 16S rRNA Gene Amplification

Total DNA was extracted from a subset of four frozen leaf disks with replication (n = 2) using the QiaAmp DNA Mini Kit (Qiagen) following the manufacturer's instructions. Amplification of bacterial 16S rRNA genes was performed using PureTaq Ready-To-Go PCR Beads (GE Healthcare) in a total PCR volume of 25 μL; 10 pmol of each bacterial primer 341F-GC (5′-[CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGC]CTACGGGAGGCAGCAG-3′) and 534R (5′-ATTACCGCGGCTGCTGG-3′) was used for amplification of suitable fragments for denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993). A GC clamp (sequence in square brackets) was attached to the 5′ end of the forward primer. Polymerase chain reaction 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 step was performed at 72°C for 5 min. The correct size of the amplified DNA fragments was verified by electrophoresis of the PCR product in 1.8% agarose and staining with ethidium bromide.

Denaturing Gradient Gel Electrophoresis

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

Statistical Analysis

The R statistical software environment (version 2.13.1; R Project for Statistical Computing [2014]) was used to perform quantitative data analyses. A three-factorial 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. The Shapiro-Wilk test was used to test for normality, and the Fligner-Killeen test was used for testing the homogeneity of variances.

Denaturing gradient gel electrophoresis 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). Bray-Curtis values without transformation were calculated. Sample similarities are shown by cluster analysis and nonmetric 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), which 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

Within 10 d of incubation (Fig. 1A), all experimental factors proved significant in ANOVA ($P < 0.05$) (Table 1) but mutually affected each other (statistical pairwise interactions; Table 1). In comparison to detritivore-free controls, litter-colonizing bacteria were reduced almost 2-fold when amphipods were present under low-temperature and low-nutrient conditions ($7.8 \pm 1.1 \times 10^7$ vs. $3.7 \pm 0.4 \times 10^7$). Warming by 5°C (15°C treatment) did not promote bacterial growth but reduced feeding pressure by amphipods; the reduction in bacterial density by amphipod presence was less pronounced, albeit still significant, whereas increasing temperature in detritivore-free controls did not affect bacterial counts ($7.8 \pm 1.1 \times 10^7$ vs. $7.8 \pm 1.3 \times 10^7$). 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 ($7.8 \pm 1.1 \times 10^7$ vs. $17.8 \pm 1.6 \times 10^7$), whereas no effect of nutrients was observed at high temperature ($7.8 \pm 1.3 \times 10^7$ vs. $7.5 \pm 1.3 \times 10^7$). Hence, nutrient addition entirely compensated for the loss of bacterial density through grazing by amphipods at low temperature

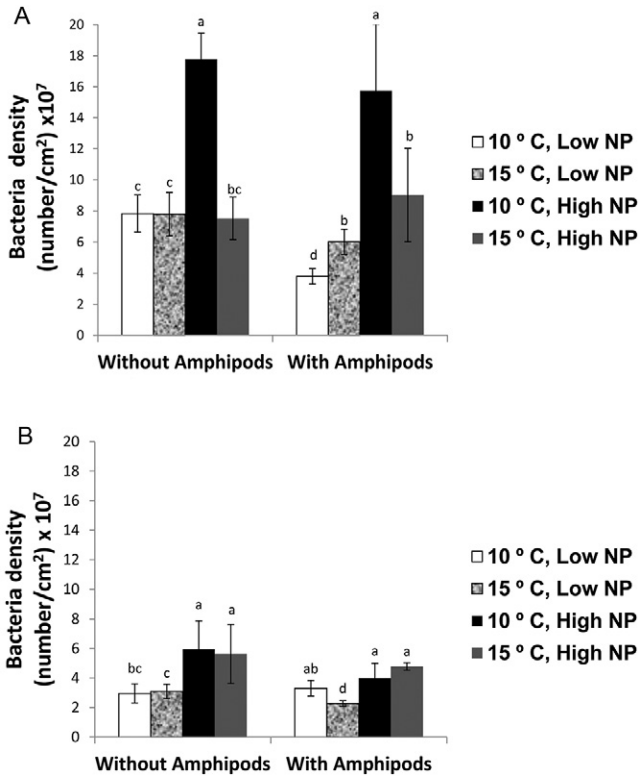

Fig. 1. Bacterial density on leaf litter (A) after 10 d of incubation and (B) after 31 d of incubation. Means ± standard deviation are plotted; each bar represents *n* = 5 for each treatment. NP, nitrate and phosphate. Means that share the same lowercase letter do not differ significantly ($\alpha = 0.05$).

($1.57 \pm 0.4 \times 10^8$ vs. $3.7 \pm 0.4 \times 10^7$) but did so only slightly at high temperature ($6.0 \pm 0.8 \times 10^7$ vs. $9.0 \pm 0.3 \times 10^7$). Low-nutrient/low-temperature versus high-nutrient/high-temperature treatments differed significantly in the presence of detritivores ($3.7 \pm 0.4 \times 10^7$ vs. $9.0 \pm 0.3 \times 10^7$), although bacterial densities were similar in detritivore-free treatments ($7.8 \pm 1.1 \times 10^7$ vs. $7.5 \pm 1.3 \times 10^7$) (Fig. 1A).

Beyond the responses of bacterial density to all experimental factors within 10 d of incubation, only detritivores and nutrients affected bacterial density after 31 d of incubation ($P < 0.001$ and $P = 0.02$) (Fig. 1B; Table 1). Feeding by amphipods did not affect bacterial density under low-temperature and low-nutrient conditions ($2.9 \pm 0.6 \times 10^7$ vs. $3.3 \pm 0.5 \times 10^7$). However, increasing temperature resulted in a decrease in bacterial density by about one-third through amphipods ($3.3 \pm 0.5 \times 10^7$ vs. $2.2 \pm 0.1 \times 10^7$). Without amphipods, warming did not influence bacterial density ($2.9 \pm 0.6 \times 10^7$ vs. $3.0 \pm 0.4 \times 10^7$ and $5.9 \pm 1.9 \times 10^7$ vs. $5.6 \pm 2.0 \times 10^7$), whereas nutrient addition doubled bacterial counts ($2.9 \pm 0.6 \times 10^7$ vs. $5.9 \pm 1.9 \times 10^7$ and $3.0 \pm 0.4 \times 10^7$ vs. $5.6 \pm 2.0 \times 10^7$). At high temperature, nutrient addition almost compensated for grazing by amphipods ($2.2 \pm 0.1 \times 10^7$ vs. $4.7 \pm 0.2 \times 10^7$), although such an effect of nutrients was not observed at low temperature ($3.3 \pm 0.5 \times 10^7$ vs. $3.9 \pm 1.0 \times 10^7$). Low-nutrient/low-temperature versus high-nutrient/high-temperature treatments differed significantly with and without detritivores ($3.3 \pm 0.5 \times 10^7$ vs. $4.7 \pm 0.2 \times 10^7$ and $2.9 \pm 0.6 \times 10^7$ vs. $5.6 \pm 2.0 \times 10^7$) (Fig. 1B).

Bacterial Community Composition

Denaturing gradient gel electrophoresis analysis of 16s rRNA genes from bacteria on birch leaves after 31 d of incubation allowed for discrimination of bacterial OTUs, which ranged from 12.5 ± 0.7 (n = 2) in low-temperature, low-nutrient treatment without detritivores to 24 ± 1.4 (n = 2) in high-temperature, high-nutrient treatment with detritivores. According to PERMANOVA, bacterial community composition was affected by all experimental factors and their interactions (Table 2). However, visually inspecting the nMDS plot and cluster analysis (Fig. 2 and 3) provides insight into the direction of how communities were affected: biofilm communities can clearly be distinguished on the basis of the presence or absence of detritivores and low or high temperature but less so by low versus high nutrient load. 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. 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).

Table 1. Analysis of variance comparison of bacterial density after 10 and 31 d of incubation.

Source of variation	Df	10-d incubation		31-d incubation	
		F value	*P* value	F value	*P* value
Nutrients† (N)	1	83.4929	<0.001*	38.9303	0.001*
Temp. (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				

* Significant at the 0.05 probability level.

† Nitrate and phosphate.

Table 2. Permutational multivariate analysis of variance comparison of bacterial communities derived from denaturing gradient gel electrophoresis band pattern analysis after 31 d. 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
Temp. (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			

† Pseudo F ratio of between-cluster variance to within cluster variance.

‡ Permutation *p* value.

§ Monte-Carlo *p* value.

Discussion

We assessed the simultaneous effects of a 5°C temperature increase and nutrient addition, simulating eutrophication in aquatic ecosystems (e.g., headwater streams) as caused by agricultural activities, atmospheric loading, and human sewage input, on the effects of detritivore grazing on bacterial communities on leaf litter surfaces. Within 10 d of incubation, the effects of nutrient addition on bacterial biofilm density were stronger than warming effects but were less pronounced at increased temperatures. The effects of nutrient addition within 31 d of incubation were stronger than those of warming irrespective of temperature. The increase in bacterial abundance in response to nutrient addition supports the importance of nutrients in aquatic systems (Meyer and Johnson, 1983; Suberkropp and Chauvet, 1995), especially at the low temperatures of the fall season when the nitrate concentration is high in the agricultural catchment (Poor and McDonnell, 2007). Additionally, nutrients tend to interact with the effects of detritivores and to enhance warming effects in the presence of detritivores. In terms of biofilm composition, nutrient addition exhibited significantly weaker effects than warming, whereas amphipods mediated warming effects on community composition.

Microbial production is mainly controlled by temperature and nutrient availability (Kirchman, 1994; Felip et al., 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 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). Because leaf litter decomposition is controlled by the joint action of microbial decomposers and animal detritivores and depends on environmental conditions and leaf litter quality (Gessner et al., 1999; Gessner et al., 2007), we present evidence for interaction effects of warming and nutrient enrichment on the detritivore–microbial interaction in freshwaters.

As expected, grazing by amphipods reduced bacterial densities but only under low-nutrient conditions. Nutrient addition effectively compensated for biofilm reduction on grazing. Warming, by contrast, only slightly counteracted the reduction in bacterial counts when amphipods were present (i.e., increased amphipod feeding activity). Within 31 d of incubation, bacterial density was slightly affected by grazing amphipods, and warming promoted density-reducing grazing effects.

Denaturing gradient gel electrophoresis analysis clearly showed a maximum of 24 bacterial ribotypes attached to birch leaves in our multifactorial study after 31 d. This result is in agreement with findings by Das et al. (2007), who found 30 bacterial OTUs on decomposing sugar maple and white oak leaves in a small-forested stream after 181 d and by Duarte et al. (2010), who found 33 to 35 bacterial OTUs on decaying alder leaves in a low-order stream after 56 d. In this study, analysis of DGGE profiles revealed little similarity between the bacterial community fingerprints in treatments with and without detritivores. Grazing, hence, clearly affected the bacterial biofilm composition (lower vs. upper part of nMDS plot). Except for the control (low nutrients/low temperature), all treatments with detritivores were highly similar in terms of biofilm composition (>80% OTU 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 through amphipod grazing. By contrast, bacterial community composition strongly depended on temperature (upper left vs. lower right part of nMDS plot), and temperature clearly separated communities within amphipod treatments from each other. These findings contrast findings presented by Ferreira and Chauvet (2011) on synergistic effects of nutrients and temperature on fungal communities associated with leaf litter.

Increases in bacterial biomass due to warming may provide additional food sources to grazing detritivores such as amphipods (see Ihnen and Zimmer [2008] for a terrestrial isopod). However, effects of detritivores depend on the leaf litter (e.g., birch) (Treplin and Zimmer, 2012), 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). Because 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 10 d of incubation effects, the presence of *Gammarus pulex* does not affect bacterial density on leaf litter after 31 d of incubation. 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,

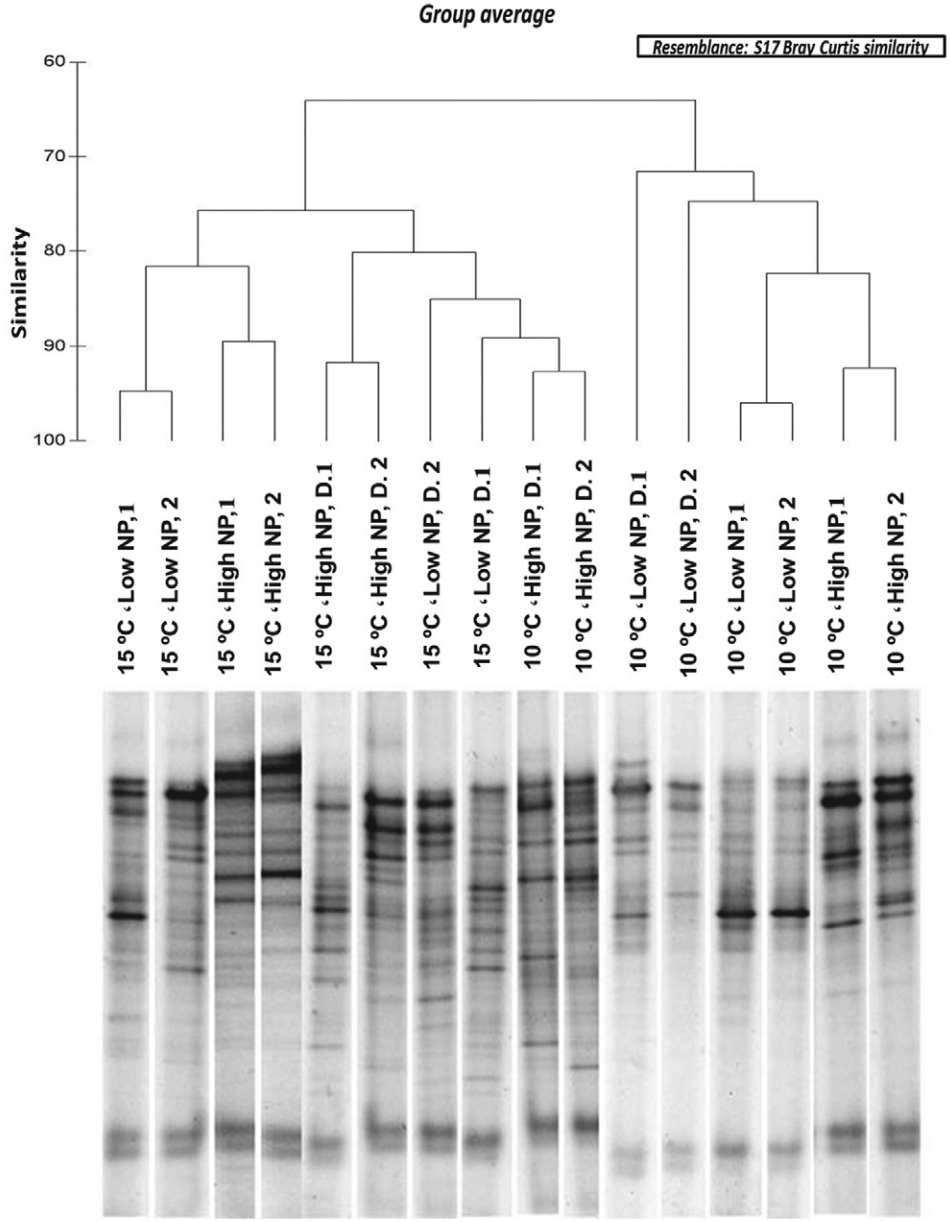

Fig. 2. Denaturing gradient gel electrophoresis (DGGE) and cluster analysis of bacterial biofilm community composition based on amplified 16S rRNA genes of birch leaves after 31 d. D, detritivores; NP, nitrate and phosphate. Cluster analysis of DGGE band patterns was performed using the Bray-Curtis similarity index; similarity values are given in % (*n* = 2 for each treatment).

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; Lyon and Ziegler, 2009; Hill et al., 2011). 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 (see Ihnen and Zimmer [2008] for a terrestrial isopod) and strain-specific responses to warming.

Water temperature and nutrient concentrations increase simultaneously in many streams (Murdoch et al., 2000; MEA, 2005). In our study, the simultaneous effects of warming and nutrient enrichment on bacterial abundance demonstrate that ecosystem response to nutrient addition increases with temperature, which is in line with observations of correlative studies by Blenckner et al. (2006) and Friberg et al. (2009). 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. On the basis of our present findings, we conclude 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

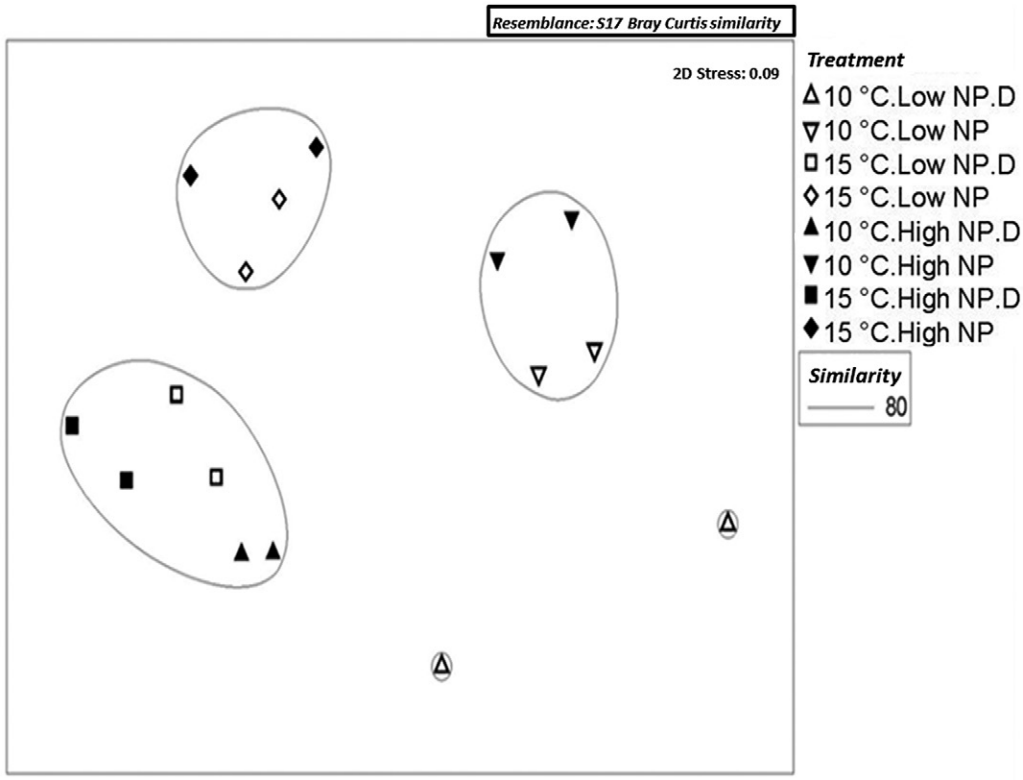

Fig. 3. Nonmetric multidimensional scaling of litter-colonizing bacterial communities after 31 d. D, detritivores; NP, nitrate and phosphate. Encircled symbols indicate 80% similarity level (*n* = 2 for each treatment).

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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