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Effects of invasive and indigenous amphipods on physico-chemical and microbial properties in freshwater substrates

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Abstract Native benthic amphipods play a key role in freshwater ecosystem services such as leaf litter decomposition. The replacement of native by invasive species with different functional traits has the potential to alter hyporheic processes, communities, and food webs. Despite the increasing number of publications on invasive species, there is a lack of studies that compare the effects of native versus invasive amphipods on physico-chemical habitat properties and microbial communities in the substrate. We compared the effects of an indigenous (*Gammarus roeseli*, Gervais 1835) and an invasive (*Dikerogammarus villosus*, Sowinsky 1894) amphipod on leaf litter decomposition, as well as on bacterial communities and physico-chemical conditions in freshwater substrata. We hypothesized that the different amphipod species distinctly alter habitat conditions and microbial community composition, depending on functional differences in leaf litter decomposition. We detected strong differences between *G. roeseli* and *D. villosus* in the feeding rates on alder leaves, with 11-fold greater decomposition of alder leaves by the native species. Additionally, our study revealed differences in microbial community composition between treatments at the substrate surface, but almost no differences in physico-chemical parameters in the interstitial and open water. These results support the hypothesis that the replacement of indigenous amphipod species by functionally different invasive amphipods can lead to a decrease in leaf litter decomposition and an altered microbial community composition with possible effects on benthic food webs. These effects on ecosystem services should be taken into account when assessing the impacts of invasive species on freshwater habitats.

Keywords Bacterial communities · *D. villosus* · *G. roeseli* · Ecosystem services · Limiting similarity · Leaf litter decomposition

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Introduction

Aquatic ecosystems are important to mankind due to the many ecosystem functions and services they provide (e.g. Geist 2011). In particular, biological communities in stream ecosystems are important for the decomposition of leaf litter and the recycling of nutrients via the microbial loop (Azam et al. 1983). Multiple anthropogenic impacts are hypothesized to alter the biological communities in streams, and consequently their ecosystem services. Globally, invasions by non-native species are among the five major threats to aquatic ecosystems (Dudgeon et al.

2006). Several non-native species have been identified that can directly impact indigenous species and communities, in turn affecting the structure (e.g. by invasive ecosystem engineers) and functioning (e.g. by disrupting food webs) of ecosystems (Allen et al. 2013 and references therein). Whilst many of the direct effects of invasive species on indigenous species such as competition and predation are well understood (reviewed in Simberloff et al. 2013), there is much less information on how functional differences between invasive and indigenous species alter ecosystem services (Levine et al. 2003; Dukes and Mooney 2004; Vellend et al. 2007). An assessment of threats to ecosystem services by alien species requires the identification of their impacts on keystone elements for the functioning of these systems, such as food web structure and the network of important services such as leaf litter decomposition, nutrient cycling and microbial communities as they affect the global metabolism of rivers (Marmonier et al. 2012).

In forested headwater streams with low primary production, the input and decomposition of allochthonous organic matter is crucial for the energy supply and the in-stream nutrient cycling (Vannote et al. 1980). Shredder organisms are particularly important in the recycling and bioavailability of nutrients because they break the organic matter up into finer particles. This increases the surface area for biofilms (Graca et al. 2001) which are essential in the further degradation and nutrient recycling in the microbial loop (Azam et al. 1983). In freshwater ecosystems, amphipods are among the most important shredder organisms and thus play a key role in leaf litter decomposition (Navel et al. 2010). These processes might get increasingly disturbed through the replacement of native species by their invasive relatives. For instance, the invasive Ponto–Caspian amphipod *D. villosus* has mostly replaced the indigenous *G. roeseli* in the Upper Danube River within a few years (Kley and Maier 2006; Brandner et al. 2013), with unknown consequences on ecosystem services. Many studies analysed the invasion success of *D. villosus* in relation to morphology, physiological tolerance, life-history strategies (all reviewed in Rewicz et al. 2014), anatomy (Mayer et al. 2009) and nutrition (Truhlar et al. 2014). All of those studies highlighted important aspects that explain the success of the species without taking the entire ecosystem functioning into account. In contrast, Piscart et al. (2011) reported on the impact of invasive amphipods on leaf litter recycling in aquatic ecosystems, but their study did not include the effects on physico-chemical habitat properties, or those on microbiota.

A holistic assessment of the replacement of native amphipods by non-native ones should also take the versatile effects on the supporting function of ecosystems and their services into account. Many theories in invasion biology try to explain the success of alien species (Catford et al. 2009), but some of them are restricted to certain aspects of ecology like the novel weapons or enemy release hypothesis (reviewed in Catford et al. 2009). Nevertheless, some are appropriate to evaluate the effects of invasive species on newly invaded areas in a holistic way (on different levels of taxonomic organization including abiotic factors) such as the limiting similarity hypothesis (MacArthur and Levins 1967). Similarity to native species can include morphologic, trophic, genetic as well as functional similarity. In plant invasions, like described for the Canada thistle (*Cirsium arvense*), potential invaders that are functionally similar to resident plant species are less likely to establish than those being functionally distinct (Larson et al. 2013). The aim of the present study was to evaluate the functional differences of an invasive (*D. villosus*) and a native (*G. roeseli*) amphipod species to draw conclusions on the effects on ecosystem services due to a replacement of native by invasive amphipods. To our knowledge, no study has yet analysed the effects of the invasive *D. villosus* on ecosystem services in direct comparison with the indigenous *G. roeseli* in a standardized and multivariate approach. The present study provides insight into the complex relationship of the presence and absence of invasive and indigenous amphipods on leaf litter decomposition, abiotic habitat properties and microbiota. A standardized microcosm experiment was set up with indigenous (*G. roeseli*) and invasive (*D. villosus*) amphipods and a control without organisms. Feeding rates, physico-chemical habitat properties (including standard ion concentrations) and microbial community composition were analysed with a multivariate statistical approach to determine the effects of an alteration in species composition on supporting ecosystem services such as leaf litter decomposition and nutrient turnover.

According to the limiting similarity hypothesis, suggesting that successful invaders are functionally distinct from species in the recipient community

(reviewed in Catford et al. 2009), we assumed that the invasive *D. villosus* is functionally different compared to the indigenous *G. roeseli*. These differences could be in burrowing behaviour and in leaf litter processing due to a different nutrition which in turn has the potential to affect physico-chemical habitat parameters, and indirectly microbial communities. Therefore, we compared the two species for (1) differences in the feeding rate on alder leaves, (2) the alteration of physico-chemical habitat properties in open and interstitial water, and consequently, (3) differences in microbial community composition on the substrate surface and at 5 cm substrate depth.

Methods

Study design

The effects of the indigenous (*G. roeseli*) and invasive (*D. villosus*) amphipods on abiotic habitat properties and microbial community composition were investigated in a 35-day laboratory experiment under standardized conditions. The experiment included nine biological replicates for each of the treatments and the control without animals.

Plastic boxes, solid on five sides (24.2 × 15.5 × 18.2 cm, Rotho Kunststoff AG, Würenlingen, Switzerland) and equipped with a lid of gauze (mesh width 500 µm, Aquacultur Fischtechnik GmbH, Nienburg, Germany), were filled with a 10.0-cm substrate layer. Experimental boxes were randomly placed (balanced latin square scheme) in three fibreglass-reinforced plastic channels (3.55 m × 0.45 m × 0.17 m, AGK Kronawitter GmbH, Wallersdorf, Germany) with constant water flow to ensure identical exposure temperatures. The substrate layer consisted of defined grain fractions (0.85, 0.63 and 0.063 mm in a proportion of 1.5:0.5:4.0) of washed substrate from the Moosach River (a calcareous river, 48°23′39.22″N; 11°43′26.65″E). Every microcosm was constantly supplied with water from the same river by a dripping system with a flow rate of 3.5–4.0 l/h per box. One redox electrode (ELANA, Boden und Wasser Monitoring, Arendsee, Germany) per box was installed 5 cm below the substrate surface to ensure non-invasive measurements. In each box, a perforated pipe (Volume 5 ml, Gardena GmbH, Ulm, Germany) with a control valve on one end was placed in each box at the same substrate depth as the redox electrode to collect water samples from the substrate.

To assess the leaf litter decomposition abilities by both amphipod species, alder leaves (*Alnus glutinosa*) were collected in autumn to feed the animals. For standardization, one triangular piece (4.62 cm^2; 0.035 ± 0.007 g dry weight) was cut out of every leaf at the same position and dried at room temperature. Before the test animals were fed with the triangular leaf pieces, the leaf pieces were pre-incubated for 7 days (Dedourge-Gafford et al. 2009) in water from the Moosach River. Every second day, 80 % of the water for leaf pre-incubation was replaced by freshwater from the Moosach River.

The experiment was performed during February and March 2014 with a constant 12-h light–dark cycle and a mean temperature in open water of 10.5 ± 1.2 °C. Due to the importance of similar initial conditions (i.e. physico-chemical parameters, microbial diversity), the microcosms were supplied with water for the first 15 days (day −15 to day 0) but not stocked with the amphipods. Each treatment consisted of nine biological replicates. On day 0, 30 *D. villosus* or 30 *G. roeseli* were introduced into each of nine boxes for the respective treatments. Nine control boxes remained unpopulated with amphipods. The invasive *D. villosus* were kick-sampled at the Upper Danube River (48°54′35.5″N, 11°51′07.4″E) 1 week before the introduction into the microcosms. The native *G. roeseli* were kick-sampled at the Moosach River (48°23′39.22″N; 11°43′26.65″E) which also belongs to the Danube drainage. For standardization, we used 30 test animals of the same size per box. Only specimens which were small enough to pass through a sieve with a mesh width of 3.0 mm, and large enough to be retained on a sieve with a mesh width of 1.5 mm were used. Before the experiments, all test animals were held under identical conditions for 1 week in basins at the Chair of Aquatic Systems Biology of the Technische Universität München, Germany, for acclimatization. The test animals remained in the experimental boxes for 20 days and were fed every day with one piece of a pre-incubated alder leaf per box. The remainder of the triangular piece of leaf from the previous day was taken out of the box and dried, to prevent from further decomposition by microorganisms, until analysis. All control boxes were treated in the same way as the boxes with test animals. To determine the feeding rate

of the amphipods, the remaining leaves were photographed and the areas measured with Image J (version 1.48v). For statistical analyses, the area of the leaf pieces from the control boxes was taken as 0 % feeding rate. Dead specimens were removed and replaced daily.

Analysis of physico-chemical parameters

Oxygen concentration and saturation, as well as redox potential as long-term oxygen indicator, were measured representing important habitat properties for bacterial metabolism. Values in redox potential >300 mV indicate oxic conditions, crucial for obligate aerobic bacteria. The redox potential in 5 cm substratum depth was measured using a platinum electrode fixed in a plastic tube as described in Geist and Auerswald (2007). Physico-chemical water parameters (pH, oxygen concentration and saturation, electric conductance, and redox potential) were measured with a hand-held WTW Multi 3430 SET G and a WTW pH 3110 SET-2 (Wissenschaftlich Technische Werkstätten, Weilheim, Germany) on days −15, −10, −5, 0, 5, 10, 15 and 20 of the experiment at nearly the same time between 9.00 am and 1.30 pm in random order. Parameters in open water were measured 5 cm above the substrate surface. Water samples (20 ml) of the interstitial zone were collected from each box using the valve of the installed and perforated pipe. To minimize sampling bias, 5 ml (the volume of the pipe) was purged before the interstitial water was sampled. Samples of interstitial water collected on experimental days 0, 10 and 20 were immediately frozen and stored at −20 °C. To evaluate treatment-related differences (including different forms of N indicative of N-cycling), these samples were used for ion detection (i.e. Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-}, SO_4^{2-} and F^-) using two ICS 1100 ion chromatographs, one with a AG-22 as guard column and AS-22 column for anion separation, and the other one with a CG-12 as guard column in line with a CS-12 column for cation separation (ion chromatographs and columns from Thermo Scientific, Dreieich, Germany). As eluent for anions, a mixture of 1.8 mM dinatriumcarbonate and 1.7 mM natriumhydrogencarbonate was used. 20 mM methanesulfonic acid was used for eluting cations.

Analysis of bacterial diversity

The bacterial community composition was analysed based on DNA-based terminal-restriction fragment length polymorphism (T-RFLP) fingerprinting. For investigating bacterial communities at 5 cm substrate depth, samples of interstitial water (taken on experiment days 0, 10 and 20) were filtered through 0.22-µm CME membrane filters (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), which were subsequently stored in sterile Petri dishes at −20 °C. For each treatment, nine biological replicates (one from each box) were analysed. Membrane filters were cut into pieces (0.5 cm^2) using a sterile scalpel for DNA isolation as described in Mueller et al. (2013). For investigating bacterial communities in the upper 1–3 mm of the substrate, a representative substrate sample was taken with a spoon by "writing" a *Z* on the substrate surface. The samples were put in sterile screw cap plastic vessels and stored at −20 °C. For DNA extraction, 0.8 g of each substrate sample with the adherent bacterial biofilm was put into a sterile 2.0-ml screw cap reaction tube filled with 0.l-mm zirconia/silica beads (1:1) and DNA was extracted as described in Lueders et al. (2004). DNA concentration was quantified using a ND-1000 Nanodrop Spectrophotometer (Peqlab, Erlangen, Germany), and samples were stored at −20 °C until further processing. As described in Lueders et al. (2006) and Pilloni et al. (2011), 16S rRNA gene-targeted T-RFLP fingerprinting was carried out but with an additional purification step using PCRExtract columns (PCRExtract Mini Kit, 5 Prime GmbH, Hamburg, Germany) according to the manufacturer's protocol. In total, a sum of 162 T-RFLP fingerprints were generated: nine biological replicates per treatment ($n = 3$), sampling time-point ($n = 3$) and substrate depth ($n = 2$). To evaluate electropherograms after capillary electrophoresis, the Gene Mapper 5.1 software (Applied Biosystems, Carlsbad, California, USA) was used and in the subsequential step T-RFLP data were analysed with the free online tool T-REX (Culman et al. 2009) using the peak heights to create the T-RF abundance data matrix. According to Abdo et al. (2006), a default factor of 1.2 was set for background noise filtering. The clustering threshold was set to 1.5 using the default alignment method of Smith et al. (2005) for peak alignments. To reduce data complexity, T-RFs that occurred in <10 % of total samples were

eliminated from the data set (Blackwood et al. 2007). To check which bacteria differ between treatments, and consequently, which functional differences result from our findings of distinct OTU compositions between the treatments, the terminated restriction fragments were further analysed with the MiCA database search. Bacterial lineages were assigned to the detected terminated restriction fragments using the Microbial Community Analysis III (MiCA) with a sensitivity of maximum two mismatches within two bases from the 5′ end of the primer in the RDP (R10, U27) 16S bacterial rRNA database (Shyu et al. 2007).

Data analysis

Multivariate data analyses were performed using the software PRIMER v.6 with PERMANOVA+ add on (Plymouth Marine Laboratory, Plymouth, United Kingdom). Based on the microbial community analysis, the DIVERSE function was used to compute different diversity indices [i.e. operational taxonomic unit (OTU) richness, Shannon diversity, Simpson and Evenness index including standard deviations (SD)]. SIMPER (similarity percentages) analyses using Euclidian distances were performed to reveal which TRFs contribute most to the average similarity per treatment. To test whether physico-chemical variables and T-RF compositions differ between treatments and sampling time-points (day 0, 10 and 20), PERMANOVAs (permutational multivariate analysis of variance) were performed using 999 permutations under the assumption of a reduced model using the sum of squares type III (partial). In the PERMANOVA design, the factor "treatment" was nested-fixed in the factor day (sampling time-point). To analyse the relationship between T-RF data and the physico-chemical parameters of open and interstitial water, as well as the ion composition in the interstitial water, separate distance-based linear models (DISTLMs) were computed. For calculations of the DISTLMs, a number of 999 permutations using Bray–Curtis similarities as distance measures were defined. A non-metric multidimensional scaling (NMDS) was plotted to display the differences between bacterial communities at the substrate surface and at 5 cm substrate depth. Differences between treatments and sampling time-points in abiotic variables and OTU diversity indices, as well as differences in feedings rates between treatments, were analysed using standard univariate statistics in R (version 3.0.2, www.r-project.com, 2013). Data were tested for normality (Shapiro–Wilk test) and homoscedasticity (Levene's test). Pairwise comparisons between treatments and sampling time-points as well as those of feeding rates between treatments were performed using the non-parametric pairwise Wilcoxon rank-sum test with Bonferroni correction since data were not normally distributed. Significance was accepted at $p \leq 0.05$ after Bonferroni correction.

Results

Differences in the feeding rate of *G. roeseli* and *D. villosus*

In line with our hypothesis, pronounced and significant differences in the feeding rates of invasive and indigenous amphipods on alder leaves were observed. The feeding rate of *G. roeseli* on the pre-incubated pieces of alder leaves was more than eleven times higher compared to that of *D. villosus*. The average area of the leaf pieces consumed by *G. roeseli* was 8.0 ± 2.5 mm^2 day^{-1} per individual, whereas that of *D. villosus* was only 0.7 ± 0.6 mm^2 day^{-1} per individual. Figure 1 displays the feeding rates on alder leaves observed in all treatments in mm^2 day^{-1} per individual.

Effects of invasive and non-invasive amphipods on microbial communities in the interstitial zone

In line with the hypotheses, the microbial community composition differed significantly between treatments at the substrate surface. These effects were less pronounced and not statistically significant at 5 cm substrate depth.

Before the introduction of amphipods, OTU composition between treatments was highly similar, both at the substrate surface (PERMANOVA, 2-factorial, nested fixed, $p > 0.05$), as well as at 5 cm substrate depth (PERMANOVA, 2-factorial, nested fixed, $p > 0.05$).

The OTU composition at the substrate surface differed most strongly between all treatments at day 20 [PERMANOVA, 2-factorial, nested fixed, p (control—*D. villosus*) <0.05; p (control—*G. roeseli*) <0.01; p (*D. villosus*–*G. roeseli*) ≤ 0.001]. This

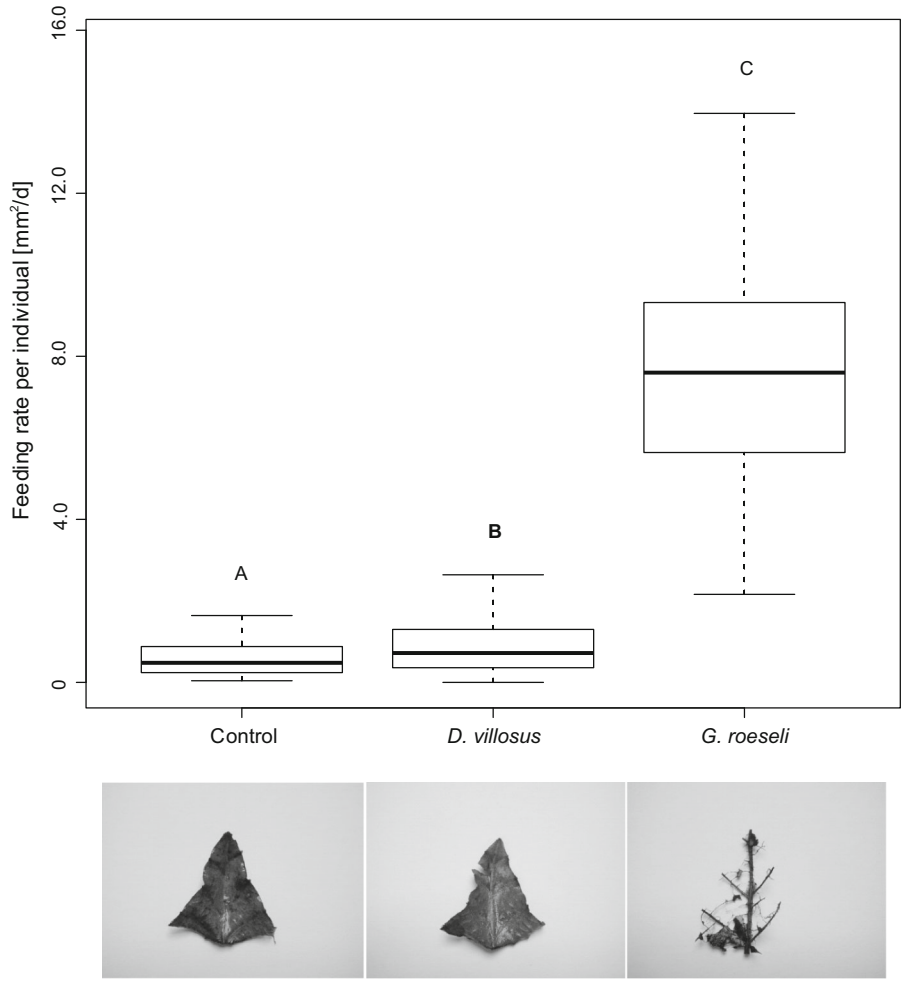


Fig. 1 Feeding rates of indigenous *G. roeseli* and invasive *D. villosus* on alder leaves after 24-h exposure compared to a control without animals. *Different letters* represent statistically significant differences between treatments (pairwise Wilcoxon rank-sum tests, Bonferroni-corrected, both $p < 0.001$). *Boxes* represent 0.25, median and 0.75, and whiskers represent 0.05 and 0.95. *Bottom*: representative photographs illustrate an alder leaf out of a control box (*left*, no feeding rate), the rest of leaf consumed by *D. villosus* (*middle*, low feeding rate) and *G. roeseli* (*right*, high feeding rate) after 24-h exposure

significant difference between treatments was not evident from comparisons of the common diversity indices (OTU richness, Evenness, Shannon, Simpson) (pairwise Wilcoxon rank-sum tests, Bonferroni-corrected; all p values >0.05). Additional differences in microbial community composition were detectable between the substrate surface and 5 cm substrate depth as evident from the number of different TRFs: 87 were found in samples collected from the substrate surface and 110 in those from 5 cm substrate depth. A PERMANOVA further substantiated the differences in OTU diversity between different substrate depths within all treatments (2-factorial, nested fixed, all $p \leq 0.001$) (Fig. 2). At 5 cm substrate depth, neither the OTU composition between treatments, nor that between sampling timepoints differed (PERMANOVA, 2-factorial, nested fixed, all $p > 0.05$). These findings were also confirmed by the comparison of calculated diversity indices which revealed no differences between treatments (pairwise Wilcoxon rank-sum tests, Bonferroni-corrected; all p values >0.05).

Distinct T-RFs contributed significantly to the observed microbial differences. At the substrate

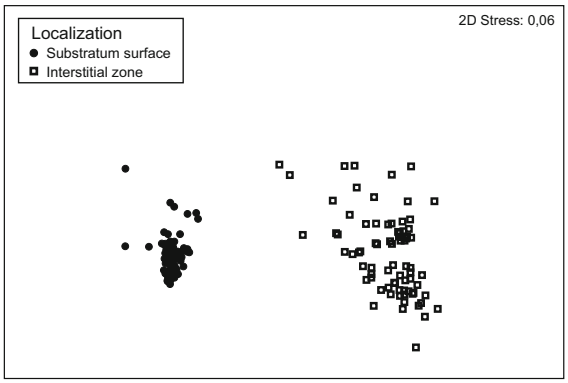


Fig. 2 Non-metric multidimensional scaling plot of operational taxonomic unit (OTU) composition of samples from the substrate surface (*filled circles*) and from 5 cm substrate depth (*empty quadrats*) overall sampling time-points and treatments. The OTU composition in different substrate depths differs strongly

surface, the 145- and 490-bp fragments contributed to the average similarity (SIMPER analysis) in the control and the *D. villosus* treatment, but they were not abundant in the *G. roeseli* treatment (Fig. 3a). In contrast, the 488-bp fragment contributed solely to the average similarity in the *G. roeseli* treatment. The 61- and 81-bp T-RFs were only abundant in treatments containing amphipods. Generally, the contribution of T-RFs to the average similarity was dominated by two fragments, which were detectable in all treatments: the 139-bp (SIMPER; 10.71 ± 0.16 %) and the 136-bp fragment (SIMPER; 7.40 ± 0.27), almost without differences in abundance between treatments. At 5 cm substrate depth, the 205-bp and 287-bp T-RFs were exclusively detected in the presence of *D. villosus* (Fig. 3b). Additionally, the 118- and the 160-bp fragments were solely present in samples of boxes populated with amphipods. The T-RF with 216 bp was most abundant in all treatments with slight differences between *D. villosus*, *G. roeseli* and the control (SIMPER; 9.96 ± 1.69 %), followed by the 490-bp fragment which exhibited no differences in abundance between treatments (SIMPER; 7.66 ± 0.25 %).

The variance in OTU composition can be partially explained by the electric conductivity at the substrate surface (5.49 %) and at 5 cm substrate depth (3.44 %) (DISTLMs, adjusted R^2, all $p < 0.05$). In the substrate, fluoride (5.1 %) followed by ammonium (3.6 %) and sulphate (2.9 %) influenced the composition of the microbial community (DISTLM, adjusted R^2, all $p < 0.05$).

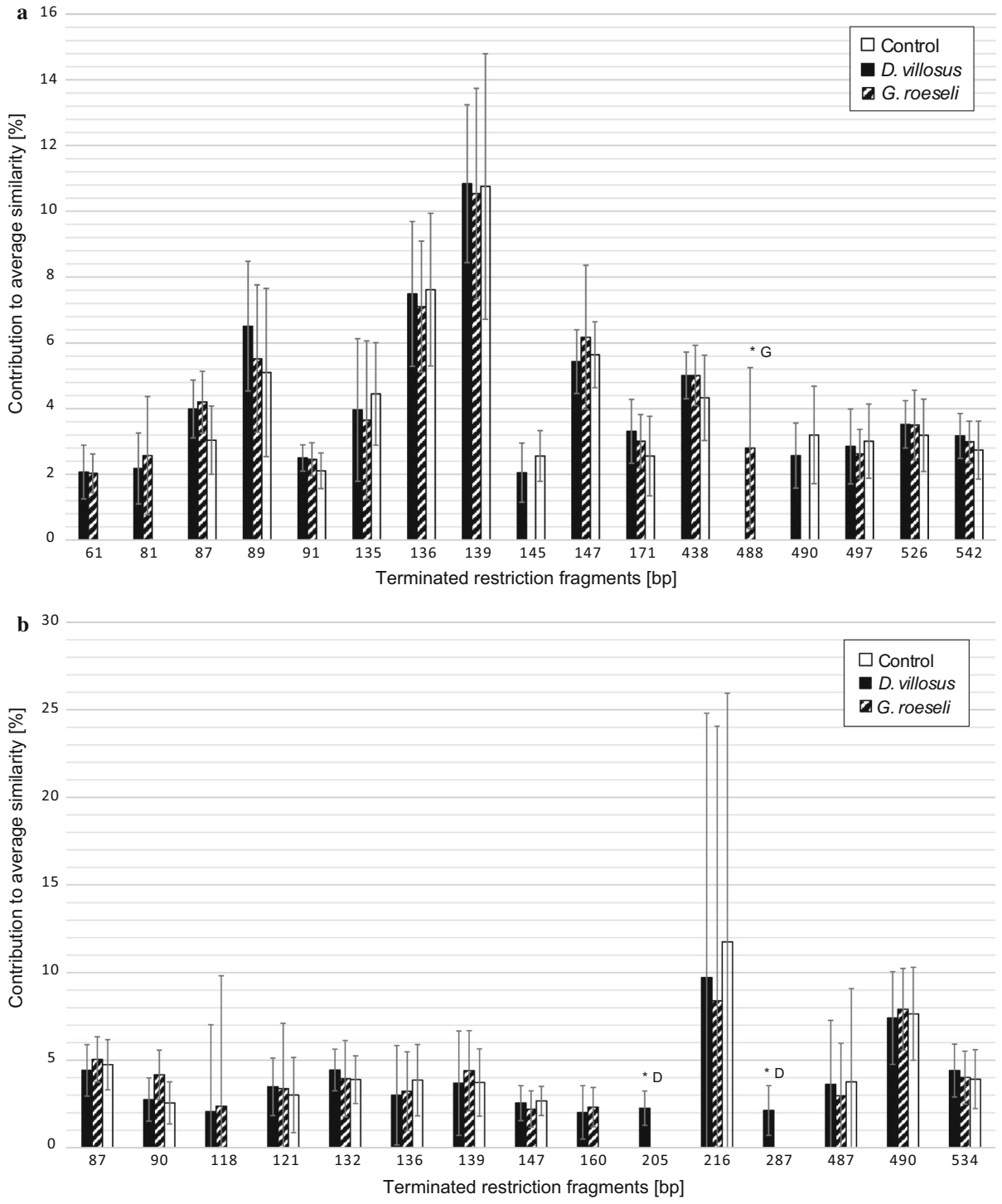
Based on the MiCA database assignments, aerobic as well as anaerobic bacteria (facultative and fastidious) were present in all treatments. The number of bacteria associated with plant decomposition and decaying matter processes (*Cytophaga* sp., *Caldicellulosiruptor* sp., *Brevibacillus* sp., *Gemella* sp.) was higher in the amphipod treatments compared to the control (*Spirochaeta smaragdine*.).

Alteration of physico-chemical habitat properties by invasive and non-invasive amphipods

Before the amphipods were introduced into the experimental boxes, neither the physico-chemical conditions in open water, nor those in interstitial water differed between treatments (PERMANOVA, treatment nested in day, for day 0 all $p > 0.05$), indicating highly similar starting conditions for the experiment.

Also after the introduction of amphipods, the physico-chemical habitat properties in open and interstitial water were mostly similar between the treatments (pairwise Bonferroni-corrected Wilcoxon rank-sum tests for pH, O_2 concentration, O_2 saturation, electric conductivity and redox potential, all $p > 0.05$) (Tables 1, 2). Over time, a trend towards a differentiation of abiotic habitat properties in the substrate between sampling time-points could be detected, but this development was largely independent of the treatment. Despite a lack of significance, there was a trend towards lower redox potentials and higher electric conductance in the substrate of those treatments with the highest leaf litter decomposition (i.e. boxes containing *G. roeseli*) compared to boxes containing *D. villosus* and the control. Open water conditions were generally similar throughout all treatments and exposure time.

In line with the physico-chemical measurements in the substrate, the ion composition in the substrate was similar between treatments (pairwise Wilcoxon rank-sum tests, Bonferroni-corrected, all $p > 0.05$), except for sulphate: in the control, the sulphate concentrations were lower (34.3 ± 7.6 mg/l) compared to the *D. villosus* treatment (41.5 ± 8.2 mg/l) and the *G. roeseli* treatment (40.0 ± 5.0 mg/l) (pairwise Wilcoxon rank-sum test, Bonferroni-corrected, all $p < 0.01$). Over time, ammonium (control: tenfold decrease), magnesium (control: 1.2-fold decrease) and fluoride



concentrations (control: decrease from 0.03 to nearly 0.0 mg/l) decreased (pairwise Wilcoxon rank-sum tests, Bonferroni-corrected, all $p < 0.05$). The chloride concentrations increased from day 0 to day 20 in all treatments (control: by 7 %) (pairwise Wilcoxon rank-sum test, Bonferroni-corrected, all $p < 0.01$).

◀**Fig. 3** Contribution of individual bacterial 16S rRNA gene T-RFs to the average similarity within treatments in a similarity percentage (SIMPER) analysis based on the abundance of terminated restriction fragments of samples from the substrate surface (**a**) and from 5 cm substrate depth (**b**). Presented are T-RFs contributing more than 2 % to the average similarity. The *error bars* represent the standard deviation of contribution to the average similarity between replicates. Different fillings of *bars* represent different treatments. T-RFs labelled with *G were exclusively present in the *G. roeseli* treatment and T-RFs labelled with *D were exclusively present in the *D. villosus* treatment

Discussion

The results of this study show that the rates of leaf litter decomposition by invasive and indigenous amphipods differ significantly under standardized laboratory conditions, in contrast to highly similar initial conditions among treatments. The present study provides first evidence that the microbial community composition can also be altered if indigenous amphipods are replaced by invasive species. Consequently, the currently occurring replacement of native *G. roeseli* populations by non-native *D. villosus* in many aquatic ecosystems likely results in ecologically relevant functional changes, affecting leaf litter decomposition and related ecosystem services such as water purification and nutrient cycling.

The observed strong differences in the feeding rate of the two amphipod species used in this study can be explained by their different functional roles in the food web, which in turn has an influence on ecosystem services such as leaf litter shredding and nutrient turnover. According to the literature, the dietary preferences and the trophic niche of *D. villosus* are controversial. On the one hand, *D. villosus* is often referred to as the carnivorous "killer shrimp" (see citations in MacNeil et al. 2013) which is considered a much more effective predator compared to *G. roeseli* (Dick and Platvoet 2000; Dick and Kelly 2002; Kinzler and Maier 2003; Krisp and Maier 2005). On the other hand, studies examining stable isotopes or fatty acids concluded that the dietary niche of *D. villosus* is not different from that of native amphipods but that *D. villosus* is opportunistic and omnivorous (Maazouzi et al. 2007, 2009; Koester and Gergs 2014). This latter theory is also supported by anatomical studies which suggest that the mouthparts of *D. villosus* do not indicate any specialization for a

Table 1 Abiotic habitat parameters in the open water and common diversity indices based on bacterial OTUs for all sampling time-points

Variable	Day 0			Day 10			Day 20		
	Control	*D. villosus*	*G. roeseli*	Control	*D. villosus*	*G. roeseli*	Control	*D. villosus*	*G. roeseli*
O_2 concentration (mg/l) ± SD	9.7 ± 0.2	9.8 ± 0.3	9.8 ± 0.1	9.6 ± 0.3	9.4 ± 0.1	9.2 ± 0.5	9.2 ± 0.2	8.8 ± 0.8	8.9 ± 0.4
O_2 saturation (%) ± SD	93.2 ± 1.6	93.0 ± 1.6	93.2 ± 1.4	91.3 ± 1.6	89.7 ± 1.6	89.0 ± 3.9	91.1 ± 1.2	86.7 ± 6.9	88.3 ± 3.5
pH ± SD	7.8 ± 0.1	7.8 ± 0.0	7.8 ± 0.1	7.9 ± 0.0	7.9 ± 0.0	7.9 ± 0.0	7.9 ± 0.0	7.9 ± 0.0	7.9 ± 0.0
Redox potential (mV) ± SD	494.9 ± 3.6	490.7 ± 14.8	493.7 ± 6.1	491.6 ± 7.0	493.6 ± 6.3	491.5 ± 8.1	504.3 ± 5.8	503.65 ± 5.0	504.6 ± 5.3
Electric conductance (µS/cm) ± SD	804.6 ± 3.5	809.1 ± 3.0	805.8 ± 4.1	791.8 ± 5.1	794.3 ± 1.2	794.0 ± 5.0	788.2 ± 6.5	790.9 ± 4.2	791.9 ± 3.8
OTU richness	12.4 ± 2.0	10.5 ± 2.1	12.6 ± 2.3	12.4 ± 2.9	11.2 ± 3.1	11.6 ± 3.7	12.7 ± 2.0	11.7 ± 1.9	11.1 ± 1.2
Evenness	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
Shannon	3.7 ± 0.2	3.6 ± 0.2	3.7 ± 0.1	3.2 ± 0.2	3.1 ± 0.3	3.1 ± 0.5	3.7 ± 0.2	3.6 ± 0.1	3.5 ± 0.1
Simpson	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0

Ions which were not different between treatments are not presented

Table 2 Abiotic habitat parameters and common diversity indices based on bacterial OTUs in the substrate for all sampling time-points

Variable	Day 0			Day 10			Day 20		
	Control	*D. villosus*	*G. roeseli*	Control	*D. villosus*	*G. roeseli*	Control	*D. villosus*	*G. roeseli*
O_2 concentration (mg/l) ± SD	2.6 ± 1.5	2.2 ± 0.3	2.0 ± 0.3	2.5 ± 1.8	2.0 ± 0.3	2.0 ± 0.3	2.1 ± 1.4	1.8 ± 0.2	1.7 ± 0.3
O_2 saturation (%) ± SD	26.1 ± 15.4	21.3 ± 3.1	20.2 ± 2.66	24.9 ± 18.7	19.7 ± 3.2	20.0 ± 3.4	21.2 ± 1.4	17.7 ± 1.8	17.5 ± 3.4
pH ± SD	7.6 ± 0.1	7.6 ± 0.1	7.6 ± 0.0	7.6 ± 0.1	7.6 ± 0.0	7.6 ± 0.0	7.6 ± 0.1	7.6 ± 0.1	7.6 ± 0.1
Redox potential (mV) ± SD	−108.3 ± 257.1	−228.8 ± 14.4	−228.1 ± 17.1	−94.0 ± 272.8	−125.0 ± 78.8	−139.6 ± 177.8	−68.6 ± 314.2	−123.9 ± 109.5	−203.3 ± 116.4
Electric conductance (µS/cm) ± SD	949.6 ± 113.2	929.9 ± 63.3	951.7 ± 79.2	900.9 ± 98.5	907.1 ± 59.7	925.9 ± 82.6	891.3 ± 83.2	874.4 ± 67.07	921.6 ± 69.1
Sulphate (mg/l)	36.2 ± 4.8	44.6 ± 7.9	42.9 ± 3.9	32.7 ± 11.9	41.4 ± 8.4	40.1 ± 5.0	34.1 ± 3.8	37.9 ± 7.7	36.6 ± 4.3
OTU richness	14.4 ± 2.7	14.8 ± 2.7	13.6 ± 4.1	13.1 ± 4.1	14.2 ± 2.5	14.6 ± 1.5	13.6 ± 2.8	12.5 ± 3.0	12.9 ± 4.6
Evenness	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.0	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Shannon	3.7 ± 0.4	3.6 ± 0.4	3.8 ± 0.3	3.0 ± 0.2	3.1 ± 0.3	3.0 ± 0.4	3.4 ± 0.4	3.4 ± 0.4	3.2 ± 0.5
Simpson	0.9 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.1

Ions which were not different between treatments are not presented

carnivorous diet (Mayer et al. 2008). For *G. roeseli*, Mayer et al. (2009) concluded that no specialized adaptations of mandibles for feeding on animal prey are present and that the antennulae and antennae are highly suitable for detritus feeding and collecting organic food. This is in line with Kelly et al. (2002) assigning the genus *Gammarus* to the shredder organisms because test animals exhibited leaf shredding in the presence of animal prey.

The results from our study suggest that *G. roeseli* is able to solely cover its energy demand by leaf shredding over a time period of at least a few weeks, whereas *D. villosus* exhibited a much lower feeding rate and a higher mortality rate, indicating that the animals starved due to the absence of animal prey or that the invasive amphipods exhibited cannibalism. Cannibalism might be possible since single dead individuals of *D. villosus* were not fully intact when collected after the 24-h intervals. No such observation was made in any of the *G. roeseli* treatments. Differences in size between species cannot explain the observed differences in feeding preferences since only individuals of the same size were used. Instead, the standardized animal size in the experiments might even have underestimated the dietary differences between the two species since *D. villosus* can reach greater sizes than *G. roeseli* and since larger specimens are generally more likely to be carnivorous than smaller ones. Additionally, under natural conditions, *D. villosus* would have been able to feed on animal prey in addition to leaf litter, which was not the case under the experimental conditions in the present study. Taken together, it is likely that *G. roeseli* and *D. villosus* occupy different niches in the food web, which would be expected according to the limiting similarity hypothesis (reviewed in Catford et al. 2009). This is also relevant for the ecosystem services both species provide: if *D. villosus* is a scavenger among other nutrition habits, then it probably provides ecosystem services such as water purification and the containment of diseases transmitted by carrion, whereas *G. roeseli* is more likely to affect the nutrient cycling and microbial biofilm formation.

As indicated by the results for the physico-chemical parameters, our hypothesis that the amphipods would affect the microbial community by altering physico-chemical habitat properties has to be rejected. Instead, a direct effect of the different leaf-shredding activities in the two species

on microbial communities is likely, as evident from the observed OTU differences at the substrate surface. It is well established that the gut microflora depends on the dietary composition (Pedrotti et al. 2015 and references therein), and the excretion of different microbiota by the two species as a consequence of the differences in their diets could explain the observed microbial differences between the treatments. Considering the higher feeding rate on alder leaves of *G. roeseli*, their gut microbiota must be highly influenced by the herbivorous diet. On the contrary, *D. villosus* of the same size exhibited an 11-fold lower feeding rate on alder leaves compared to *G. roeseli* and occasional cannibalism was observed in *D. villosus*. These differences in dietary composition are likely to result in differences in gut microflora between the invasive and indigenous species. Since the gut microflora is also present in faeces due to the contact to the colon epithelium (Choy et al. 2014), the observed differences in OTU composition likely represent differences in the gut microflora excreted to the substrate surface. In addition to the bacteria surviving the digestive tract and consequently being present in faecal pellets, the latter are colonized rapidly by external bacteria since the microbes are possibly attracted by leaching DOM (reviewed in Wotton and Malmqvist 2001). After the described colonization with microbes, the pellets are broken down over time, and in this colonized and protein-enriched state (Shepard and Minshall 1981) they serve as high-quality food resource for detritivorous invertebrates (discussed in Wotton and Malmqvist 2001; Joyce et al. 2007; Joyce and Wotton 2008). Thus, native amphipods provide essential ecosystem services in freshwater ecosystems: in addition to the leaf litter degradation, the egestion of faecal pellets is indispensable for primary food web structure.

The explanation that the differences in OTU composition are likely to be induced by differences in nutrition, and therefore gut microbiota, would be also in line with the observation that differences in OTU composition between treatments were only detected at the substrate surface, but not deeper in the substrate. Due to the observed low sediment reworking behaviour of both amphipods, no or very low amounts of faeces were transferred deeper into the substrate which may be different if other species are present. The consequences of the observed alteration of microbial community composition at the substrate surface may have an impact on grazers feeding on these biofilms and thus on the food web, as well as on the nutrient cycling and the ability for biological degradation of organic contaminants.

Besides the classic effects on ecosystems (e.g. on the food web), there are many more effects which should be considered when assessing the impact of invasive species on supporting ecosystem services. Our standardized experimental conditions did not fully imitate naturally occurring conditions concerning the amounts of organic matter input into freshwater ecosystems, and other species which also contribute to oxygen consumption within the substrate were excluded. This may have underestimated the effects on physico-chemical conditions. For instance, streams in temperate regions are characterized by a singular input of large amounts of leaves within a short time period during autumn. Based on the observed differences in leaf litter decomposition rates, the replacement of *G. roeseli* by *D. villosus* can have strong effects on the shredded amounts of leaf litter input, and consequently on the timeline of oxygen consumption during this process. The time it takes to degrade all fallen leaves in a freshwater ecosystem would be more than eleven times longer in the presence of *D. villosus* compared to *G. roeseli* with possible adverse effects on habitat quality. The decomposition of the naturally occurring large amounts of leaf litter affects the N-cycling, the ion composition and oxygen availability. The longer it takes to shred the large amounts of leaf litter, the longer the time period in autumn and winter when a potential oxygen depletion (because of an increased oxygen demand due to the remineralization of organic matter) can occur, with potential negative consequences for all species depending on certain concentrations of oxygen. This might not be of great importance in the open water of oxygen-rich streams with high current, but the situation can be different upstream of weirs and dams with lake-like hydrological properties (Nykänen et al. 2012). Furthermore, the large amounts of leaves which are not shredded sink down to the substrate surface and can clog the pores of the stream bed, decreasing the exchange between open and interstitial water. If this happens, the reproductive success of lithophilic fish species depending on an oxygen-rich stream bed is adverse affected, as in the case of salmonid eggs which mostly develop in the interstitial zone over winter (Hancock 2002 and references therein; Sternecker et al. 2013a, b). Similarly, juvenile stages of highly

endangered freshwater mussels (Unionoida) also strongly depend on oxygen-rich substrates with high exchange rates to the open water (Geist and Auerswald 2007; Denic et al. 2014 and references therein). As obvious from the physico-chemical data from different sampling time-points, the conditions within the substrate change over time. The results for physico-chemical variables, which were not significantly different between treatments, likely underestimate the complexity of the naturally occurring situation. However, mean values are probably less important than extremes, e.g. in the case of oxygen minima.

In summary, our results indicate that the observed effects of invasive amphipods are greater and more diverse than currently assumed, especially if the invasive species replace keystone fauna which are indispensable for the overall organic matter processing and nutrient turnover in freshwater ecosystems, such as amphipods. In many aquatic ecosystems in Europe, native amphipods have already been largely replaced by non-native ones which is also true in the natural habitat where the test organisms were sampled (Kley et al. 2006). The presented alterations in leaf litter decomposition and microbial community composition can have negative impacts on ecosystem functioning and thus on supporting ecosystem services, which in turn can also affect the human well-being.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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