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Detritivore physiology and growth benefit from algal presence during microbial leaf colonization

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Abstract

In headwater streams, riparian leaf litter is the primary food source for detritivores. While it is well known that aquatic fungi improve the nutritious quality of leaves, our understanding on whether and how benthic algae influence this process remains limited. Here, we hypothesized that the interplay between algae and fungi, termed "algal priming", further enhances food quality. In a 40-d microcosm experiment, we fed *Gammarus fossarum* of two size classes with *Fagus sylvatica* leaves of varying qualities: pure leaves (low quality), leaves colonized by fungi (intermediate quality), and leaves colonized by fungi in the presence of a diatom (high quality). Our results revealed that *Gammarus*' ingestion rates increased (55–164%) with food quality, spurring accelerated growth (4–14%), regardless of the size class. Furthermore, we observed a tendency for *Gammarus*' overall fatty acid (FA) quantity to rise with higher-quality food (12–318%), with the FA profile exhibiting increased proportions of specific polyunsaturated FAs that are essential for detritivores. These observations can likely be attributed to leaf-associated fungi, which are more readily assimilated than the leaves and are known as a source of FA. This enhancing effect by fungi was further amplified in the presence of diatoms, presumably through the positive effect of algal-derived labile organic carbon, which supports fungal growth. Despite reduced autochthonous primary production in shaded headwater streams, the experimental findings from this study indicate a potential of enhanced secondary production and energy transfer to higher trophic levels within the aquatic ecosystem.

The provision of a high-quality diet is vital for various aspects of consumers' life within food webs. It significantly impacts their survival, somatic growth, maintenance, and reproduction, ultimately influencing their ecological interactions with other organisms in any (meta-)ecosystem (Simpson and Raubenheimer 2012). A high-quality diet ensures that consumers receive essential nutrients, energy, and resources necessary for their overall well-being and successful functioning within the ecosystem (Simpson and Raubenheimer 2012). For instance, invertebrate detritivores that inhabit headwater streams heavily rely on allochthonous detritus, primarily in the form of leaf litter shed from riparian trees, as their main source of energy (Wallace et al. 1997). However, plain leaf litter represents a low-quality diet for detritivores due to its toughness and high content of indigestible substances (Bärlocher 1985). The presence of leaf-associated aquatic hyphomycetes (a polyphyletic group of fungi), acts as the "peanut butter on the cracker" for detritivores (sensu Cummins 1974). These fungi enhance the nutrient content, are more readily assimilated than other microbes (Price et al. 2021) and improve the fatty acid (FA) content (Arce Funck et al. 2015) of the leaves, making it a food source of higher quality for detritivores.

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Additional Supporting Information may be found in the online version of this article.

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Although considered as the "peanut butter on the cracker", aquatic hyphomycetes lack the ability to synthesize essential long-chain polyunsaturated fatty acids (LC-PUFAs) such as arachidonic acid (ARA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5n-3) that play a critical role in the physiological functioning of organisms (including detritivores) and are strongly linked to their growth (e.g., Guo et al. 2016a, 2016b; Crenier et al. 2017; Trochine et al. 2021). Therefore, detritivores rely on sources other than leaf-associated fungi to fulfill their nutritional needs for ARA and EPA. In this context, diatoms may serve as a source of ARA and EPA for detritivores, as they likewise colonize leaf litter in streams and produce LC-PUFAs (Guo et al. 2016*a*; Crenier et al. 2017; Grieve and Lau 2018). Previous studies have shown that consuming higher shares of algal food supports higher growth rates and reproduction of animals (Müller-Navarra 1995; Müller-Navarra et al. 2000), ultimately facilitating the trophic transfer of energy through the food web (Müller-Navarra et al. 2000; Kainz et al. 2004; Gladyshev et al. 2012). Furthermore, the consumption of higher-quality food can increase their own nutritious quality for higher trophic levels (Müller-Navarra et al. 2000), for example, detritivores serving as prey for fish (Macneil et al. 1999).

However, research on the role of algal PUFAs has primarily been conducted in lakes, with only recently growing interest in their role in streams (e.g., Crenier et al. 2017; Guo et al. 2021; Trochine et al. 2021). Trochine et al. (2021), for instance, emphasized the significance of diatoms associated with macrophytes for the detritivore *Hyalella patagonica*. They found that consuming diatom-colonized macrophytes led to increased growth and higher levels of PUFAs in detritivores. Similarly, Crenier et al. (2017) observed that the detritivore *Gammarus fossarum* enhanced its survival and somatic growth, along with increased levels of PUFAs, when consuming a mixed diet comprising leaves, aquatic hyphomycetes, and algae. However, the use of agarose pellets as food source, instead of using entire leaf material, questions the environmental realism of this study.

Considering this background, we assessed how the presence of algae affects the quality of microbially-conditioned leaf litter as a food source for *G. fossarum*, a European key species in the ecosystem process leaf litter decomposition (Dangles et al. 2004). To ensure ecological bearing and better mimic interactions between detritivores and their food sources (in comparison to Crenier et al. 2017), *G. fossarum* specimens from two size classes were fed over a period of 40 d with three food types of distinct quality based on the evidence described above: plain leaf material (low-quality food), leaf material colonized by aquatic hyphomycetes (intermediate-quality food), and leaf material colonized by aquatic hyphomycetes in the presence of an alga (high-quality food). We quantified the effects of food quality on detritivores by measuring survival rates, assimilation efficiency, somatic growth (quantified as biomass gain), and acquisition of FA (FA quantity and FA profiles). Our hypotheses were as follows: (a) fungal colonization of leaves will improve the quality of food for detritivores compared to plain leaves, due to the presence of readily assimilable microbial biomass and higher FA quantities (Bärlocher 1985; Arce Funck et al. 2015); (b) algal presence during fungal leaf colonization will further enhance the quality of food for detritivores by increasing leaf-associated fungal biomass (sensu Kuzyakov 2010) and FA quantity, particularly the levels of ARA and EPA (Trochine et al. 2021); (c) feeding on higher-quality food will lead to reduced mortality, higher assimilation efficiency, higher somatic growth, and improved physiological fitness of the detritivore (Crenier et al. 2017; Guo et al. 2021; Trochine et al. 2021).

Materials and methods

Overview of the study design

Our study design closely followed Zubrod et al. (2011) and consisted of two main steps: a leaf litter colonization phase to provide *Gammarus* with food of distinct nutritional qualities, followed by a 40-d *Gammarus* bioassay. To offer *G. fossarum* food of distinct nutritional qualities, we used the following leaf types: (1) plain *Fagus sylvatica* leaves (low-quality food) after 48 h of leaching in ultrapure water to avoid affecting leaf mass loss calculations due to the loss of soluble leaf components (Petersen and Cummins 1974); (2) *F. sylvatica* leaves colonized by aquatic hyphomycetes (intermediate-quality food); or (3) *F. sylvatica* leaves colonized by aquatic hyphomycetes in the presence of the diatom *Nitzschia palea* (high-quality food). These food items were fed to two size classes of *Gammarus* (small and large) in a fully crossed design, resulting in a total of six tested treatments. As response variables of *Gammarus*, we measured survival rates, assimilation efficiency, somatic growth (quantified as biomass gain), and acquisition of FA (FA quantity and FA profiles).

Sources of aquatic hyphomycetes, diatoms, and *G. fossarum*

We used in-house cultures of three aquatic hyphomycete species: *Alatospora acuminata* Ingold (isolate DSM 104360), *Clavariopsis aquatica* de Wild. (isolate DSM 104362), and *Neonectria lugdunensis* (Sacc. and Therry) L. Lombard and Crous (isolate DSM 104361). We chose these aquatic hyphomycete species as they are known for synthesizing PUFAs (Arce Funck et al. 2015). The cultures were maintained in axenic conditions on 2% malt extract agar (MEA). Two weeks before starting each microbial leaf colonization run (*see* "Leaf conditioning and bioassay"), we inoculated sterile 1% MEA using approximately 0.5 × 0.5 cm agar plugs from the in-house cultures for their use in the leaf conditioning runs.

We obtained a *N. palea* strain (isolate TCC139-1) from the Institut National de la Recherche Agronomique (Thonon-les-Bains, France) and acclimatized it to the conditioning medium (adapted after Guillard and Lorenzen 1972; Dang et al. 2005;

Supporting Information Table S1) and laboratory conditions for 4 weeks before the first microbial leaf colonization run. The conditioning medium included 3-(*N*-morpholino)propanesulfonic acid, which could have made a slight contribution to the pool of dissolved organic carbon in the water (*see* Supporting Information), however, at a level fourfold lower than measured in forested headwater streams (Graeber et al. 2012). Laboratory conditions included a temperature of $14.0 \pm 0.3°C$ (mean ± standard error; $n = 5$; measured every 30 min over 30 d using data loggers; HOBO) and a 16 : 8 h light : dark rhythm. The intensity of the photosynthetically active radiation (PAR) corresponded to the irradiance on streambeds during summer months ($\sim 40\ \mu mol\ m^{-2}\ s^{-1}$ PAR; Hill and Dimick 2002). We exchanged the medium weekly to ensure a constant nutrient supply. Cell densities in the algal cultures were quantified fluorometrically following Feckler et al. (2023).

Fourteen days before starting the bioassay, we kick-sampled *G. fossarum* from the near-natural stream Hainbach (Germany, 49°14′N, 8°03′E), which harbors a population that is entirely composed of the cryptic lineage B (Feckler et al. 2014). We used a passive underwater separation technique (Franke 1977) to divide animals into two size classes based on diameter: 1.4–1.6 mm (small) and 1.7–2.0 mm (large). We only selected specimens that were free of acanthocephalan parasites visually, to minimize variability in the ingestion behavior during the bioassay (Pascoe et al. 1995). The selected animals were gradually acclimated to the test medium (Borgmann et al. 1998) and the test conditions of $14 \pm 0.3°C$ and darkness. During the acclimation period, the medium was constantly aerated, and animals were provided with leaves (mostly *F. sylvatica*; minor shares of *Castanea sativa* and *Pinus* spp.) ad libitum, collected in-stream at the sampling site of *G. fossarum*. At the beginning of the bioassay, we shock-froze 50 animals per size class in liquid nitrogen, followed by lyophilization and weighing to the nearest 0.01 mg, to establish a baseline for biomass gain measurements and FA compositions (storage at −80°C).

Leaf conditioning and bioassay

During autumn 2019, we picked senescent *F. sylvatica* leaves from trees near Landau, Germany (e.g., 49°12′N, 8°13′E). To achieve intermediate- and high-quality food, we submerged leached leaves in 14 liters of aerated conditioning medium (14 d; medium renewal after 7 d) within BPA-free polypropylene tanks (volume = 28 liters; $n = 3$). The tanks were equipped with a Perspex® middle lamella and flow pumps (PfG GmbH) to generate water movement at approximately $0.6\ m\ s^{-1}$ (Fig. 1a). The intermediate-quality food was attained by adding six agar plugs (diameter = 10 mm; ~ 3.7–4.6 ng fungal biomass) from each of the three aquatic hyphomycete species into stainless-steel cages (mesh size = 1 mm) positioned in front of the flow pump (Fig. 1a). For the high-quality food, we also introduced approximately 500,000 *N. palea* cells per tank directly into the medium at both the start of the conditioning (0 d) and the medium renewal (7 d). To minimize location effects, we shuffled the leaves within each tank daily, and to reduce intra-treatment variation, we exchanged leaves among tanks of the same

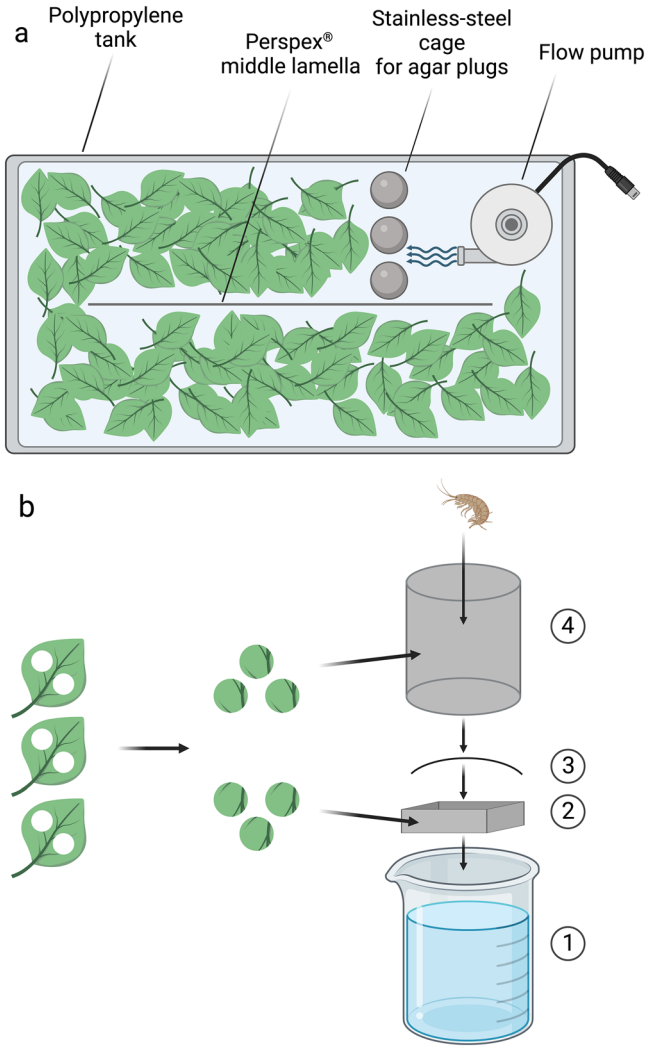

Fig. 1. (**a**) Scheme illustrating the setup of the conditioning tanks: We divided polypropylene tanks, filled with conditioning medium, with Perspex® middle lamellae and equipped them with flow pumps to generate water movement. In front of the flow pumps, we positioned stainless-steel cages containing agar plugs from three aquatic hyphomycete species. Depending on the microbial treatment, 500,000 *Nitzschia palea* cells were added to the conditioning medium. We submerged leached *Fagus sylvatica* leaves in the conditioning medium for 14 d to promote the growth of microbial biomass. (**b**) Scheme illustrating the setup of the microcosms: At the bottom of 250-mL glass beakers, filled with 200 mL of test medium (item 1), we placed cuboid stainless-steel cages (item 2) containing three leaf discs from different leaves. These cages were covered with watch glasses (item 3). On top of the watch glasses, we positioned cylindrical stainless-steel cages (item 4) containing the corresponding three leaf discs from the same leaves, along with one *Gammarus fossarum* (see text for more details). Created with BioRender.com.

treatment every other day. The microbial leaf colonization process was carried out in a temperature-controlled room maintained at 14 ± 0.3°C, either in darkness (intermediate quality) or under illumination (high quality) at a 16 : 8 h light : dark rhythm. After the colonization phase, we cut one leaf disc (diameter = 20 mm) from each side of the leaves' midribs and immediately introduced them into the bioassay or stored them for analyses of leaf-associated microbial assemblages (stored at −20°C; Fig. 1b). To ensure a constant food quality during the bioassay, we conducted four independent leaf colonization runs, each starting 14 d before the respective leaf discs were introduced into the bioassay (bioassay start or food renewal).

For the bioassay, we set up 30 microcosms for each combination of *Gammarus* size class and food quality ($N = 180$). Each microcosm consisted of a 250-mL glass beaker filled with 200 mL of test medium (item 1 in Fig. 1b; Borgmann et al. 1998). Inside the beaker, we placed a stainless-steel cylindrical cage (mesh size = 1.0 mm) containing one *Gammarus* and three leaf discs obtained from three different leaves (item 4 in Fig. 1b). These cylindrical cages facilitated the careful transfer of the animals to new microcosms during food renewal and prevented the animals from coprophagy. In addition, within each microcosm, we placed a cuboid cage made from the same stainless-steel mesh (item 2 in Fig. 1b). The cuboid cage contained the corresponding three leaf discs, originating from the same three leaves as the leaf discs offered as food to the *G. fossarum* specimen. As these discs were protected from feeding, they allowed us controlling for abiotic and microbial leaf mass loss in each replicate (cf. Zubrod et al. 2011). To prevent interaction between fecal pellets and the leaf discs within the cuboid cage, we separated the two cages with a watch glass (item 3 in Fig. 1b). The beakers were arranged randomly in a temperature-controlled room maintained at 14 ± 0.3°C in darkness. We implemented a flow-through system, which allowed a gentle renewal of the test medium (without disturbing the fecal pellets) twice a day, ensuring constant water quality throughout the experiment.

Every 10 d, we assessed *Gammarus'* mortality by visually inspecting the activity of the animals and renewed the leaf material. If any mortality occurred, the respective microcosms were excluded from further analyses. Otherwise, we retrieved the remaining leaf material and any shredded off leaf tissue from both cages. The retrieved material was dried separately at 60°C for 24 h and weighed to the nearest 0.01 mg to quantify *Gammarus'* ingestion rates (Zubrod et al. 2011). Furthermore, we filtered the old test medium through combusted and preweighed glass fiber filters (GF/6; Whatman, Dassel, Germany) to quantify *Gammarus'* egestion rate after drying and weighing of the filters, following the procedure described above. Using the data obtained for ingestion and egestion, we calculated *Gammarus*' assimilation efficiency as the percentage ratio between assimilated (ingestion–egestion) and ingested food (Waldbauer 1968). At the termination of the bioassay, all surviving *Gammarus* were shock-frozen in liquid nitrogen, followed by lyophilization and weighing to the nearest 0.01 mg.

Analyses of leaf-associated microbial assemblages

We used DNA extracts for quantitative real-time polymerase chain reaction (qPCR) analyses to achieve two objectives: (1) to estimate the biomasses of each of the aquatic hyphomycete species used and (2) to estimate the number of bacterial operon copies as a proxy for bacterial abundance. For this purpose, we extracted DNA from 10 to 20 mg of leaf material (dry weight [DW] to the nearest 0.01 mg) per conditioning tank and sampling date (i.e., at the end of each individual colonization run; $n = 3$) using the FastDNA® Spin Kit for Soil and the FastPrep™-24 5G (both MP Biomedicals).

To estimate the biomasses of individual aquatic hyphomycete species, we followed the assays as described in Baudy et al. (2019). Tenfold diluted extracts were used for quantifying DNA of the model fungi in species-specific TaqMan® qPCR reactions (Applied Biosystems). Further details regarding the species-specific TaqMan® assays, reaction compositions, cycling conditions, and data analysis can be found in Baudy et al. (2019).

Bacterial operon copy numbers were quantified following the protocol described in Manerkar et al. (2008) using the primer pair E8F/E533R (Baker et al. 2003). DNA extracts were 50-fold diluted and utilized in 10-μL qPCR reactions comprising 2 μL extract, 0.1 μL of each primer (100 μM; Biomers.net GmbH), 5 μL PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific GmbH), and 2.8 μL ddH_2O. Calibration curves covering a gradient of 10^5 to 10^9 copies of a model amplicon of the bacteria *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919 (Supporting Information Table S2) were run in parallel using the same assay. The qPCR program consisted of an initial uracil-*N*-glucosylase incubation step of 2 min at 50°C, a DNA polymerase activation step of 10 min at 95°C, and 40 cycles of denaturation for 15 s at 95°C and stepwise hybridization and extension for 20, 30, and 15 s at 55°C, 72°C, and 83°C, respectively. The fluorescence intensity was measured at the end of each cycle. Melting curve analysis was carried out at the end of each qPCR run to test the specificity of the assays, with an initial denaturation step for 15 s at 95°C, followed by a steady temperature increase for 20 min from 60°C to 95°C. The cycle of quantification was determined for each sample as a measure of DNA quantity. All qPCR reactions were performed on a Mastercycler® ep gradient S (Eppendorf) using 0.2-mL eight-tube strips covered with clear optical eight-cap strips (Sarstedt AG & Co. KG). The results were dry-normalized to the respective leaf discs.

For the quantification of diatoms associated with leaf material and diatoms present in the medium as well as those associated with the walls of the leaf conditioning tanks, we employed high-performance liquid chromatography (HPLC) using fucoxanthin as a biomarker (Johansen et al. 1974).

Fucoxanthin was chosen as a biomarker for diatoms instead of the commonly used chlorophyll *a*, as the latter may have also originated from the leaf material, leading to confounded results. The sampling procedures, sample preparation steps, and analytical procedures followed those described in Feckler et al. (2023).

FA analyses

We conducted FA profile analyses for both the leaf litter used as food sources and the detritivore *G. fossarum* at the beginning and termination of the experiment. For the leaf material, we extracted lipids from lyophilized and weighed samples (26–35 mg; $n = 3$) using a chloroform/methanol mixture (2 : 1; $v : v$) in the presence of the internal standard triacylglycerol with three deuterated 18:0 FAs, as described by Pietz et al. (2023). The samples were stored at −20°C overnight, followed by lipid hydrolysis and FA methylation to obtain fatty acid methyl esters (FAMEs) using 3 N methanolic HCl (Sigma-Aldrich) at 70°C for 20 min. The FAMEs were stored in 100 μL of dichloromethane until measurement.

Regarding *Gammarus*, we focused on neutral lipid FAs (NLFAs), as they represent a primary energy storage in invertebrates (Azeez et al. 2014) and their composition and content change more rapidly than phospholipid FAs in response to shifting food quality (Koussoroplis et al. 2014). We manually crushed lyophilized and weighed *Gammarus* samples (1.5–5.5 mg; $n = 5$) using glass Pasteur pipettes and extracted lipids using a chloroform/methanol/Milli-Q water mixture (final ratio of 2 : 2 : 1.8, $v : v : v$). Acting as an internal standard, Tristearin-D105 was added, and the lipids were extracted overnight at 4°C. Neutral lipids were separated from glycolipids and phospholipids by solid-phase extraction using polar modified polystyrene/divinylbenzene copolymer cartridges (Chromabond, easy polypropylene columns; Macherey-Nagel). The neutral lipids were then hydrolyzed, and FAs were methylated to FAMEs using trimethylsulfonium hydroxide (Sigma-Aldrich). Gas chromatography (GC) with flame-ionization detection (Trace GC Ultra; Thermo Fisher Scientific), employing a Restek FAMEWAX capillary GC column (30 m × 0.25 mm, 0.25 μm film thickness) and helium (1.4 mL min^{-1}) as carrier gas, was utilized for the identification and quantification of FAMEs (Pietz et al. 2023).

Data analysis

Considering the ongoing debate on null hypothesis significance testing (Nuzzo 2014), we primarily employed Bayesian methods for data analyses. The R package "brms" (Bürkner 2018) was used to assess the individual and combined effects of the parameters "*Gammarus* size" (two levels) and "Food quality" (three levels) on all variables, except *Gammarus* mortality and FA profiles, using the "brm" function (*see* model notation in the Supporting Information). We used weakly informative priors for all model coefficients (details in the Supporting Information; Table S3) and a normal distribution of errors after visual inspection of the data. The model ran with four Markov chains, 20,000 iterations, a warm-up period of 10,000 iterations, and a thinning factor of 5. We checked model convergence, fit, and posterior distributions (PDs) through trace plots for visual assessment of convergence among the four Markov chains, Pareto smoothed importance-sampling leave-one-out cross-validation (Pareto *k* diagnostic; all k values below 0.7; Vehtari et al. 2017), and posterior predictive plots, respectively (*see* Supporting Information; Figs. S1–S10; Tables S4–S12).

From the PDs, we calculated the absolute effect sizes (ES) as the difference between the posterior of treatments (*see* Feckler et al. 2018 for details). These ES were then used to derive the Bayesian 95% credible intervals (CRIs) and the proportion of the PD range above 0 (no-effect level). The latter serves as a measure of certainty in the direction of an estimated effect, with values around 0.5 considered biologically irrelevant (Hobbs and Hooten 2015). Values deviating from 0.5 (approaching 0 or 1) indicate increasing biological relevance. In addition, we calculated Bayes factors (BF) to assess the likelihood of the alternative hypothesis over the null hypothesis, providing a clearer estimate of the evidence in the data (Keysers et al. 2020). The BF can be interpreted as the ratio contrasting the likelihood of the data under the alternative hypothesis with that under the null hypothesis, with higher BF values indicating stronger evidence in favor of the alternative hypothesis (Jarosz and Wiley 2014). We followed the terminology of Jarosz and Wiley (2014) on the interpretation of BF as evidence for the alternative hypothesis compared to the null hypothesis (*see* Supporting Information Table S13 for further clarification of the terminology).

To evaluate the effect of food quality on *Gammarus* mortality, we used a Bayesian test of association (a Bayesian alternative to the chi-square test) with the "contingencyTableBF" function in the R package "BayesFactor" (Morey and Rouder 2021), using default priors and an independent multinomial sampling plan. We assessed the effect of food quality on mortality within each *Gammarus* size class and then compared mortality between individual food qualities for the two *Gammarus* size classes.

For multivariate data (absolute amounts of individual FAs or groups), we created nonmetric multidimensional scaling (NMDS) ordination plots for both food sources and *Gammarus* specimens to represent dissimilarities among groups (Clarke 1993). Multivariate homogeneity of group dispersions (variances) was quantified using the "betadisper" function in the R package "vegan" (Oksanen et al. 2022). Given that Bayesian methods are not yet readily available for multivariate community analyses, we assessed the statistical significance of the evaluated parameters ("*Gammarus* size" and "Food quality") and their interactions using a permutational multivariate analysis of variances (PERMANOVA), performed on square-root-transformed data to mitigate the effect of dominant FAs (Clarke and Warwick 2001) and employing

Euclidean distances between the groups. We analyzed data and created figures using the open-source statistical software R (R Core Team 2022) with add-on packages.

Results

Microbial treatment effects on leaf litter

Diatoms were not detected on leaves in any of the conditioning runs using HPLC: all measurements were below the level of detection (0.12 μg fucoxanthin L^{-1}). However, diatoms were found in the medium after scraping off the conditioning tanks' walls, with mean concentrations of up to 0.19 ($\pm$ 0.04) μg fucoxanthin L^{-1}.

Leaf-associated bacterial abundances varied based on the microbial treatment, with an increase from low-quality food (mean: 0.7×10^7 bacterial operon copies, 95% CRI: −1.6 to 3.0) to intermediate-quality food (mean: 7.9×10^7 bacterial operon copies, 95% CRI: 5.7–10.1) and high-quality food (mean: 8.9×10^7 bacterial operon copies, 95% CRI: 6.4–11.3). Bacterial abundance increased decisively from low- to intermediate-quality food (ES: 7.2×10^7 bacterial operon copies, 95% CRI: 4.5 to 9.9, BF: ∞, PD > 0: 1), and from low- to high-quality food (ES: 7.9×10^7 bacterial operon copies, 95% CRI: 5.3 to 10.6, BF: ∞, PD > 0: 1). However, the increase in the bacterial abundance from intermediate- to high-quality food (ES: 0.7×10^7 bacterial operon copies, 95% CRI: −2.0 to 3.4, BF: 2.08, PD > 0: 0.68) was only anecdotal.

Measured DNA concentrations indicated microbial treatment effects on the biomasses of the individual aquatic hyphomycete species. On leaf material of low-quality food, no biomass of the aquatic hyphomycetes was detected. However, we observed decisive and very strong increases in the biomasses of *A. acuminata* (ES: 0.9 μg g leaf DW^{-1}, 95% CRI: 0.44–1.35, BF: 570.4, PD > 0: 1) and *C. aquatica* (ES: 0.05 μg g leaf DW^{-1}, 95% CRI: 0.01–0.1, BF: 39.8, PD > 0: 0.98; Table 1) from intermediate- to high-quality food, respectively. Conversely, for *N. lugdunensis*, we observed the opposite pattern, with a decisively higher biomass associated with leaf material from intermediate-quality food compared to high-quality food (ES: 0.06 μg g leaf DW^{-1}, 95% CRI: 0.02–0.1, BF: 120, PD > 0: 0.99; Table 1).

Table 1. DNA concentrations (μg g leaf DW^{-1}, $\pm$ 95% credible intervals, $n = 12$) of *Alatospora acuminata*, *Clavariopsis aquatica*, and *Neonectria lugdunensis* associated with leaf material of three qualities.

Food quality	*A. acuminata*	*C. aquatica*	*N. lugdunensis*
Low	n.d.	n.d.	n.d.
Intermediate	0.57 ± 0.40	0.03 ± 0.03	0.11 ± 0.03
High	1.47 ± 0.37	0.09 ± 0.04	0.05 ± 0.04

n.d., not detected.
Limits of detection: *A. acuminata*—3.57 fg, *C. aquatica*—0.65 fg, *N. lugdunensis*—3.52 fg; Baudy et al. 2019.

Table 2. FA profiles (μg mg DW^{-1}; $\pm$ 95% CRI), n-3/n-6 ratios, and total FA quantities (μg mg DW^{-1}; $\pm$ 95% CRI) of food sources (*Fagus sylvatica* at three qualities; $n = 3$) and *Gammarus fossarum* at two size classes fed with the food sources of distinct qualities ($n = 5$).

Species	Size	Food quality	16:0	16:1n-7	18:1n-9	18:2n-6	18:3n-3	20:4n-6	20:5n-3	SAFA	MUFA	PUFA	n-3	n-6	n-3/n-6	FA quantity
Fagus	—	Low	3.53 ± 1.78	0.29 ± 0.05	0.46 ± 0.22	0.80 ± 0.57	5.42 ± 4.97	n.d.	n.d.	4.16 ± 2.04	0.78 ± 0.26	6.07 ± 3.79	5.24 ± 3.38	0.79 ± 0.56	6.6	11.0 ± 5.4
sylvatica	—	Intermediate	4.64 ± 1.79	0.31 ± 0.05	0.49 ± 0.22	0.81 ± 0.56	7.62 ± 5.47	n.d.	n.d.	5.33 ± 1.94	0.85 ± 0.27	8.75 ± 3.85	7.94 ± 3.53	0.81 ± 0.58	9.8	14.9 ± 5.5
	—	High	6.20 ± 1.87	0.23 ± 0.05	0.48 ± 0.22	0.83 ± 0.55	8.01 ± 5.43	n.d.	n.d.	7.22 ± 1.99	0.77 ± 0.26	13.33 ± 4.03	12.49 ± 3.48	0.83 ± 0.55	21.5	21.2 ± 5.5
Gammarus	Small	-(Start)	20.84 ± 16.78	5.30 ± 3.97	22.24 ± 14.43	3.73 ± 2.79	21.06 ± 5.23	0.89 ± 0.38	0.98 ± 0.45	33.03 ± 21.37	20.04 ± 12.56	25.76 ± 10.84	6.18 ± 6.10	3.07 ± 3.01	2.1	56.7 ± 12.0
fossarum		Low	43.05 ± 16.21	11.73 ± 3.97	33.29 ± 14.81	7.25 ± 3.57	30.22 ± 20.22	0.94 ± 0.39	1.03 ± 0.44	64.85 ± 20.94	30.49 ± 19.45	42.65 ± 25.88	33.37 ± 21.53	9.18 ± 4.46	3.6	138.7 ± 20.2
		Intermediate	59.59 ± 16.89	7.21 ± 4.02	22.56 ± 14.56	9.29 ± 3.67	41.16 ± 20.62	1.03 ± 0.39	1.16 ± 0.44	80.52 ± 21.06	49.54 ± 20.25	55.77 ± 26.06	44.39 ± 22.16	11.52 ± 4.34	3.8	186.7 ± 43.9
		High	41.75 ± 16.70	2.28 ± 3.99	14.06 ± 14.07	6.58 ± 3.59	47.53 ± 20.78	1.07 ± 0.39	1.27 ± 0.45	64.23 ± 21.56	30.25 ± 19.52	60.06 ± 25.66	50.97 ± 22.02	8.56 ± 4.38	6.0	154.7 ± 31.2
	Large	-(Start)	20.55 ± 12.25	3.93 ± 1.14	11.90 ± 6.39	3.37 ± 1.75	13.95 ± 2.78	1.27 ± 0.37	1.10 ± 0.48	33.59 ± 14.40	17.54 ± 9.07	18.43 ± 7.93	3.98 ± 3.43	3.41 ± 1.63	1.2	51.8 ± 6.8
		Low	16.99 ± 12.36	3.82 ± 0.74	12.18 ± 2.99	3.42 ± 1.10	13.77 ± 2.92	0.74 ± 0.37	0.80 ± 0.49	32.96 ± 13.97	17.22 ± 4.19	18.53 ± 6.13	3.97 ± 2.95	1.99 ± 0.69	2.0	44.9 ± 2.5
		Intermediate	41.19 ± 12.05	4.77 ± 4.02	19.58 ± 12.28	5.45 ± 3.45	33.08 ± 13.63	1.27 ± 0.38	0.99 ± 0.48	64.99 ± 14.29	27.89 ± 17.98	44.18 ± 17.63	36.28 ± 14.98	7.63 ± 3.63	4.8	138.4 ± 17.9
		High	52.08 ± 12.05	9.65 ± 3.80	29.44 ± 12.03	7.73 ± 3.35	48.23 ± 13.88	1.68 ± 0.38	1.79 ± 0.48	78.46 ± 14.52	43.51 ± 17.60	63.85 ± 18.38	52.58 ± 14.67	10.72 ± 3.60	4.9	187.2 ± 41.2

n-3, omega-3 FA; n-6, omega-6 FA; n.d., not detected.

The highest FA quantity occurred at the high-quality food (mean: 21.2 μg mg leaf DW^{-1}, 95% CRI: 15.7–26.7), followed by the intermediate-quality food (mean: 14.9 μg mg leaf DW^{-1}, 95% CRI: 9.4–20.4) and low-quality food (mean: 11.0 μg mg leaf DW^{-1}, 95% CRI: 5.6–16.4; Table 2). Consequently, the total leaf-associated FA quantities differed substantially to decisively among the microbial treatments (ES: ≥ 3.92 μg mg leaf DW^{-1}, BF: 6–112, PD > 0: 0.87–0.99). FA profiles also differed significantly among the food qualities ($p = 0.019$: Fig. 2): high-quality food showed highest proportions of PUFAs and n-3 FAs, while monounsaturated FAs (MUFAs), n-6 FAs, and individual FAs (16:1n-7 and18:1n-9) were highest on intermediate- and low-quality food (Fig. 2; Table 2). Linoleic acid (18:2n-6; LIN) and α-linolenic acid (18:3n-3; ALA) were highest on intermediate-quality and high-quality food. The n-3/n-6 ratio increased along the food quality gradient from 6.6 at low quality up to 21.5 at high quality. However, we did not detect ARA or EPA in any of the tested food qualities (Table 2), which supports the absence of diatoms on leaf material, given that *Nitzschia* sp. typically contain high levels of C20-PUFAs (e.g., Renaud et al. 1995).

Food quality effects on *G. fossarum*

Food quality affected *Gammarus'* ingestion rates and assimilation efficiencies. Ingestion rates showed decisive increases from low- to intermediate- (≥ 58%) and high-quality (≥ 73%) food for both size classes (BF: ∞, PD > 0: 1) as well as substantial (73%; BF: 6.7, PD > 0: 0.87) and decisive (157%; BF: ∞, PD > 0: 1) increases from intermediate- to high-quality food for small and large *Gammarus*, respectively (Fig. 3a; Supporting Information Table S14). When comparing ingestion rates between size classes at the same food quality, a slightly lower ingestion rate occurred for the large size class than the small size class at low-quality food (15%; BF: 10, PD > 0: 0.91), while the opposite pattern was observed at high-quality food (26%; BF: 999, PD > 0: 1; Fig. 3a; Supporting Information Table S15).

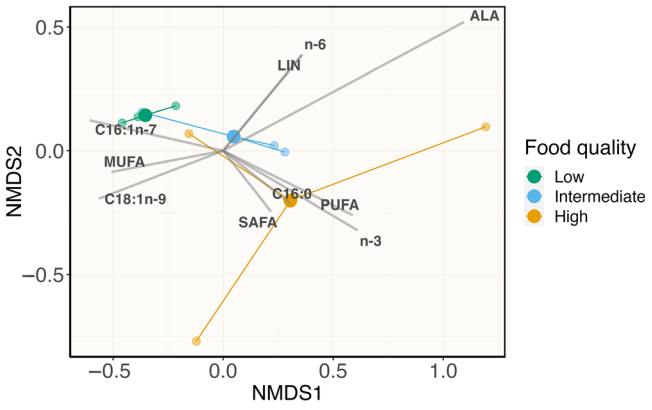

Fig. 2. NMDS ordination plot for FA profiles associated with leaf material of low-, intermediate-, and high-quality (small symbols; food qualities depicted by colors; $n = 3$). The group centroids (large symbols) of the individual food qualities are also displayed, connecting the respective replicates via spider webs. The NMDS stress value was below 0.2, indicating a reasonable fit of the data (Clarke 1993).

Assimilation efficiencies for the small size class exhibited a very strong decrease from low- to high-quality food (≥ 6%; BF: 22–60, PD > 0: 0.96–0.98), while for the large size class, it substantially decreased (≥ 8%; BF: 4–7, PD > 0: 0.80–0.88; Fig. 3b; Supporting Information Table S14). Comparing the assimilation efficiencies between the two size classes at the same food qualities showed only anecdotal differences (≥ 5%; BF: 1–2, PD > 0: 0.50–0.66; Fig. 3b; Supporting Information Table S15). Our rough approximation of assimilation, however, does not account for energy spend on processes such as movement and heating (being quantified using more complex measurements), which would result in assimilation efficiencies and biomass gain lower than those reported here.

Biomass gain of *Gammarus*, measured as the difference in weight between start and termination of the bioassay, was influenced by food quality (Fig. 3c). Both size classes showed substantial to strong increases in biomass gain from low- to high-quality food (≥ 35%; BF: 7–16, PD > 0: 0.87–0.94; Fig. 3c). The absolute biomass gain was slightly to strongly higher for the small size class than for the large size class, regardless of food quality (≥ 25%; BF: 1 to 18, PD > 0: 0.58 to 0.95; Supporting Information Table S15).

The total NLFA quantities in *Gammarus* varied based on the quality of food they were fed, but the FA patterns differed between the two size classes (Table 2). In the small size class, NLFA quantities were similar at all food qualities, which were consistently higher (≥ 145%) than those observed at the start of the bioassay. On the other hand, in the large size class, *Gammarus* had similar NLFA quantities at the bioassay's start and when feeding on low-quality food, but these quantities differed (≥ 208%) when feeding on food of intermediate and high qualities (Table 2). These distinctive patterns became also visible in the NMDS ordination plots (Fig. 4). Specimens from all food treatments in the small size class were clustered together separately from the initial specimen. Conversely, specimens from the test start and those feeding on low-quality food were separated from those feeding on intermediate- and high-quality food in the large size class (Fig. 4). In small *Gammarus*, we found highest proportions of ARA, and EPA at the test start. Specimens feeding intermediate-quality food were characterized by highest proportions of MUFAs, 16:1n-7, and 18:1n-9. On the other hand, specimens feeding on high-quality food contained highest proportions of PUFAs, n-3 and n-6 NLFAs, LIN, and ALA (Fig. 4; Table 2). In large *Gammarus*, highest proportions of ARA and EPA were found at the test start. Specimens feeding on low-quality food were characterized by highest proportions of saturated FAs (SAFAs), while those feeding on intermediate- and high-quality food contained highest proportions of MUFAs, PUFAs, n-3 NLFAs, 18:1n-9, and ALA (Fig. 4; Table 2). The n-3/n-6 ratio increased in both size classes with increasing food quality, mirroring the trend observed in the food sources. Overall, food quality

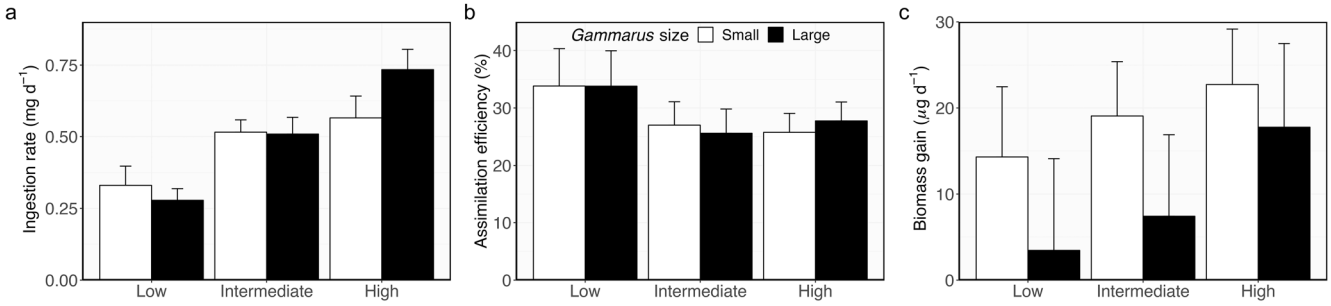

Fig. 3. Means (± 95% CRI; $n = 20–29$) of (**a**) ingestion rate (mg d^{-1}), (**b**) assimilation efficiency (%), and (**c**) biomass gain (μg d^{-1}) by small (white) and large (black) *Gammarus fossarum* when feeding on low-, intermediate-, and high-quality leaf material for 40 d.

influenced the NLFA profiles of *Gammarus* significantly, irrespective of the size class ($p \leq 0.045$).

Food quality did not affect *Gammarus* mortality within the two assessed size classes (BF ≤ 1; Supporting Information Table S16). However, when comparing *Gammarus* mortality between the two size classes at low-quality food, we observed strong evidence indicating higher mortality in the small size class compared to the large size class (BF = 24). At the intermediate- and high-quality food, on the other hand, we only found an anecdotal effect on *Gammarus* mortality (BF ≤ 1).

Discussion

Our findings supported our hypotheses, revealing that algal presence during microbial leaf colonization indirectly enhanced the quality of leaves as food for detritivores by enhanced fungal growth. Consuming such higher quality food resulted in substantially improved survival particularly of small specimens, while the survival of large specimens remained consistently high (≥ 90%) across all food qualities. Moreover, the consumption of higher-quality food sources positively affected the ingestion rate, somatic growth, physiological fitness (indicated by FAs), and the presence of essential MUFAs and PUFAs in the detritivore, aligning with the nutritional gradient proposed by Anderson and Cummins (1979).

The decisively increased ingestion rates for intermediate- and high-quality food by both *Gammarus* size classes, compared to the ingestion of sole leaf material, can be attributed to microbial activity. Fungi colonizing leaf litter convert indigestible leaf substances into digestible subunits and soften the leaf tissue through fungal catalysis (Bärlocher 1985). These microbial processes transform leaf litter into a more attractive food source for detritivores (Graça et al. 1993). In addition, fungal production subsidizes the nutritionally poor leaves with readily assimilable biomass (here observed as an increase

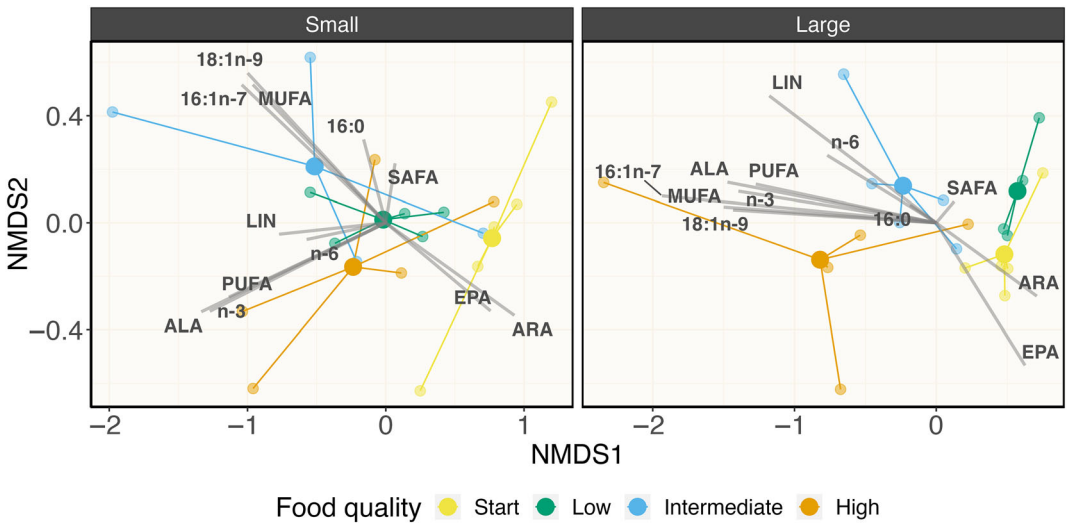

Fig. 4. NMDS ordination plots for NLFA profiles of small and large *Gammarus fossarum* at the start of the experiment and after feeding on leaf material of low-, intermediate-, and high-quality food (small symbols; food qualities depicted by colors; $n = 5$) for 40 d. The group centroids (large symbols) of each individual leaf quality are also displayed, connecting the respective replicates via spider webs. The NMDS stress values were below 0.2, indicating reasonable fits of the data (Clarke 1993). Note that PUFA encompasses not only the cumulative quantities of LIN, ALA, ARA, and EPA, but also encompasses the quantities of other PUFAs (*see* Supporting Information Table S17).

in the leaf-associated DNA content of the used aquatic hyphomycetes), complementing the positive effect of leaf softening (Bärlocher 1985; Graça et al. 1993). Furthermore, the overall increase in FA quantities and the shifts in FA profiles likely played a role in the enhanced ingestion of intermediate and high-quality food. Arce Funk et al. (2015) demonstrated that aquatic hyphomycetes serve as sources of n-3 and n-6 PUFAs for detritivores. Since these FAs cannot be synthesized de novo by *Gammarus* and other invertebrates (Nelson 2011), aquatic hyphomycetes are indeed crucial for the dietary provisioning of PUFAs to enhance organisms' somatic growth. In this context, these fungi serve as sources of ALA and LIN, which are precursors for the bioconversion into EPA (Brett and Müller-Navarra 1997; Monroig and Kabeya 2018). In addition, certain lipid classes, such as triglycerides and PUFAs, are known as feeding stimulants for some aquatic invertebrates (Cargill et al. 1985). Therefore, the higher overall FA quantities and higher proportions of ALA and PUFAs associated with intermediate- and high-quality leaf litter could act as triggers for the observed ingestion pattern. Ultimately, the increased ingestion of these lipids led to a higher total NLFA quantity in *Gammarus*.

The absence of diatoms, a highly nutritious microbial group and source of essential FAs for detritivores (e.g., Crenier et al. 2017; Trochine et al. 2021), on leaves despite its presence in the medium, was reported before (Feckler et al. 2023). Several hypothetical mechanisms could explain this observation that, however, need further empirical support: first, heterotrophs are more efficient competitors for inorganic nutrients compared to autotrophs (Currie and Kalff 1984), possibly reducing autotrophs' colonization success on leaf litter under nutrient limited conditions, as tested in our study. Second, besides nutrient competition, autotrophs and heterotrophs compete for substrate, leading to spatial competition and potentially causing allelopathic interactions among them (Li et al. 2014; Gerphagnon et al. 2015). Third, the high velocity in the colonization tanks may have hindered an adhesion of the diatom species *N. palea* to the leaves (Gordon et al. 2009). Nevertheless, the presence of diatoms during microbial colonization of leaf litter supported fungal growth, likely by providing labile organic carbon that is used by heterotrophs as an easily accessible source of energy, a mechanism known as "priming" (Fontaine et al. 2003). Autotrophic priming is believed to be a two-step process: initially, the availability of labile organic carbon increases the turnover of fast-growing organisms such as bacteria (Kuzyakov 2010), as observed in our study and elsewhere (Feckler et al. 2023). These bacteria either die or become dormant after substrate depletion (Morris and Blackwood 2005). Subsequently, slower-growing fungi not only derive energy and nutrients from the leaves but also from the less recalcitrant bacterial necromass promoting fungal growth (sensu Kuzyakov 2010). The latter is also supported by our data pointing to a higher fungal biomass associated with high-quality food compared to the intermediate quality, resulting in increased availabilities of easily assimilable microbial biomass and PUFAs for *Gammarus*.

Despite the increased ingestion with higher food quality, the assimilation efficiency decreased at the intermediate- and high-quality food compared to the low-quality food. The low ingestion rate of plain leaf material should have extended its gut residence time, likely allowing gut enzymes to degrade leaf structural components (Nilsson 1974)—a process typically facilitated by leaf-associated fungi (Bärlocher 1985). This strategy should enable detritivores to meet short-term energy requirements with low-nutritional food sources. However, in order to gain a more comprehensive understanding of whether and to what extent gut enzymes can break down structural components of leaves, we need to delve further into detritivores' gut microbiome and its ability to adjust to differing food quality. Conversely, when conditioned leaf litter was available, the high ingestion rates likely caused large quantities of material to pass through the gut rapidly, leading to a reduction in assimilation efficiency due to higher egestion rates. This feeding strategy is advantageous for organisms not limited by organic energy sources, as it maximizes the ingestion of proteins, FAs and other essential micronutrients for growth (Golladay et al. 1983). The conditioned leaf litter, already partially degraded by fungal activity and containing easily assimilable microbial biomass, can be more readily digested by detritivores (Bärlocher 1985). Thus, while the gross assimilation efficiency decreased with increasing food quality, the assimilation efficiency for proteins, FAs and other essential micronutrients can be assumed to be high (Bärlocher and Kendrick 1975; Prus 1976). However, it remains unclear whether and to which extent the assimilation efficiency in response to the food quality is mirrored in the quality of the fecal pellets for its consumers (reviewed in Bundschuh and McKie 2016).

As a result of ingesting higher-quality food as well as assimilating FAs and other essential micronutrients, *Gammarus* experienced increased survival and substantial somatic growth, particularly in the small size class. EPA and ARA are important PUFA for the functioning of detritivores and are strongly linked to their growth (as represented by biomass gain; e.g., Guo et al. 2016a, 2016b; Crenier et al. 2017; Trochine et al. 2021). We observed the highest EPA and ARA proportions in *Gammarus* at the start of the bioassay, while these proportions decreased in specimens fed with leaf litter independent of its quality. Three mechanisms potentially explain these findings: first, *Gammarus* did not take up EPA and ARA from the offered food, as indicated by the absence of leaf-associated diatoms capable of synthesizing these FAs de novo (Taipale et al. 2013). Second, although *Gammarus* can bioconvert precursors like ALA and LIN into EPA and ARA, they might not have invested substantial energy into this highly energy-demanding and physically not required process (Parrish 2009). This assumption is supported by the decreasing EPA and ARA proportions despite an increase in ALA and LIN

proportions with higher food quality. Third, the available EPA and ARA were most likely invested in growth, regardless of the size class.

However, in this study, we focused solely on quantifying neutral FAs, which are presumed to change their composition and content more rapidly than phospholipid FAs in response to shifting food quality (Koussoroplis et al. 2014). In addition, quantifying polar lipid FAs should be considered during future studies, as this would allow tracking whether *Gammarus* allocated EPA and ARA from storage to polar lipids over the course of the study, and aid in interpreting shifts in the consumers' physiology. The finding of higher somatic growth in small compared to large specimens, irrespective of the food quality, suggests differing energy allocations for physiological processes such as growth, maturation, and reproduction, in accordance with the dynamic energy budget theory (Kooijman 2000). Smaller *Gammarus* likely had a higher scope for growth, representing the energy potentially available for somatic production (Naylor et al. 1989), as they were striving for biomass gain and were not sexually mature during the experiment. On the other hand, larger *Gammarus* likely allocated more energy to reproduction (formation of eggs and spermatozoa), leaving less energy available for somatic growth.

Moreover, food quality explained the variation in the NLFA profiles of the consumer. The absolute FA quantities present in the food sources were partly reflected in the absolute NLFA quantities found in *Gammarus* consuming the respective food sources. This indicates that specimens consuming higher-quality food could build up higher lipid storages, resulting in improved physiological fitness (Koop et al. 2011). The enhancement of the consumers' fitness was further supported by an increase in n-3/n-6 ratio (sensu Twining et al. 2016), which mirrored the values observed in the food sources. Higher n-3/n-6 ratios are considered indicators of improved physiological fitness, as n-3 PUFAs are essential for animals, being unable to synthesize them de novo in many taxa, yet they are crucial for maintaining vital physiological processes (Cook and McMaster 2002; Twining et al. 2016). Therefore, the higher n-3/n-6 ratios in *Gammarus* feeding on higher quality food indicate their improved physiological fitness, as they were able to store certain n-3 FAs despite using such PUFAs for higher somatic growth.

Conclusions

Our findings confirm the significance of leaf-associated aquatic hyphomycetes in enhancing the quality of this food item concerning nutrients and biomolecules for detritivores in stream ecosystems (e.g., Bärlocher 1985; Arce Funck et al. 2015). In addition, we demonstrated that not only the consumption of algae (cf. Crenier et al. 2017; Trochine et al. 2021) but also the mere algal presence during microbial leaf conditioning can indirectly enhance secondary production. These findings are likely driven by the stimulation of heterotrophic activity by algae, a process known as algal priming. Therefore, algal priming could hold pivotal importance by fueling secondary production and energy transfer to higher trophic levels, both within the aquatic ecosystem and across its boundaries. Presumably, observable effects on detritivore growth and physiology might have been even more pronounced if algae would have been growing directly on the leaves, however, further evidence is needed to support this assumption.

Therefore, future studies should not only focus on the impacts of autotrophic-heterotrophic interactions on energy pathways within aquatic ecosystems, but also delve into shifts in the physiology of merolimnic, detritivorous insects such as caddisflies, potentially influencing their quality as prey for riparian predators (cf. Pietz et al. 2023). In this context, it seems crucial to consider biomolecules beyond FAs, such as amino acids and sterols, to obtain a more holistic understanding of the effects of food quality on consumers and the potential subsequent repercussions throughout the food web.

Data availability statement

The data that support the findings of this study are openly available on GitHub (https://github.com/aflandau/10.1002-lno.12530). Data, associated metadata, and calculation tools are also available from the corresponding author upon reasonable request (alexander.feckler@rptu.de).

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Conflict of Interest

None declared.

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