Multiple stressor effects act primarily on microbial leaf decomposers in stream mesocosms

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ABSTRACT

At the global level, stream ecosystems are impacted by multiple anthropogenic stressors such as eutrophication, habitat deterioration, and water scarcity. Multiple stressor effects on stream biodiversity are well documented, but multiple stressor effects on stream ecosystem processes have received only limited attention. We conducted one mesocosm (stream channel) and one microcosm (feeding trial) experiment to study how combinations of reduced flow, increased nutrient concentrations, and increased fine sediment cover would influence fungal and macroinvertebrate decomposer assemblages and their active contribution to leaf decomposition. In the stream channels, increased fine sediment cover significantly reduced fungal biomass, occurrence frequencies of most hyphomycete species, and microbial leaf decomposition rates compared to untreated controls. Macroinvertebrate mediated leaf decomposition rates were mainly correlated to total fungal biomass and resemblance in fungal community composition to untreated controls. Neither increased nutrient concentrations, nor reduced flow conditions significantly influenced leaf decomposer communities or decomposition rates. The feeding trials revealed significantly reduced leaf consumption in the freshwater amphipod *Gammarus pulex* when feeding on leaf material from treatments with increased fine sediment cover in the mesocosm experiment. When offered a food choice between sterile, unconditioned leaf material and leaf material from treatments with increased fine sediment cover, *G. pulex* foraged mainly on sterile material. This study showed that increased fine sediment cover can alter the flux of energy and material in the detrital food chain through bottom-up regulation of leaf conditioning by fungal decomposers. Our results suggest that increasing attention should be given to mitigate fine sediment transport and deposition in stream systems to preserve ecosystem functioning within the detrital food chain.

1. INTRODUCTION

The United Nations Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) has emphasised that natural resources are severely overexploited and that land use change and intensity are primary causes of the accelerating biodiversity decline and loss of ecosystem services (IPBES, 2019). Relative to their global area, freshwater ecosystems support a disproportionately high amount of the global biodiversity (Strayer & Dudgeon, 2010) but are severely under pressure by drivers of global change (Vörösmarty et al., 2010).

In streams, anthropogenic stressors are particularly intense and complex in terms of their composition and spatiotemporal variation (Birk et al., 2020; Lemm et al., 2021; Ormerod et al., 2010). Dominant stressors include, increased nutrient (Smith, 2003), contaminant (e.g. Malaj et al., 2014; Niyogi et al., 2007; Schäfer et al., 2016), or suspended sediment loads (Walling, 2006), habitat deterioration (e.g. Vörösmarty et al., 2010; Wagenhoff et al., 2011), and intensity, duration, and frequency of low flows (e.g. Hannaford & Buys, 2012; Xu et al., 2022). All of these stressors, especially frequency and duration of critical low flows, are expected to become more widespread and intense within coming decades threatening the structure and functioning of stream ecosystems through direct or indirect mechanisms (IPCC, 2022).

While multiple stressor effects have been intensely studied in context of ecosystem structure (including ecological quality assessments), ecosystem functioning has received substantially less attention (Birk et al., 2020; Feckler & Bundschuh, 2020). Moreover, relationships between ecosystem structure and function are not always clear-cut (Feckler & Bundschuh, 2020; Mulder et al., 2015; Verdonschot et al., 2020). The decomposition of organic matter is one of the most important processes in stream ecosystems and is integral to trophic ecosystem

dynamics (Handa et al., 2014; Marks, 2019). Leaf litter constitutes the most important part of allochthonous particulate organic matter input to streams, and its decomposition has been frequently used to quantify single stressor effects on stream ecosystem functioning (e.g. Chauvet et al., 2016; Gessner & Chauvet, 2002).

Leaf litter decomposes through abiotic and biotic processes that occur sequentially or simultaneously including leaching, microbial decomposition, and consumption by macroinvertebrate shredders (Abelho, 2001). Aquatic fungi (especially aquatic hyphomycetes) dominate microbial decomposition, typically comprising > 90% of the total litter-associated microbial biomass (Marks, 2019). The colonisation by aquatic fungi enhances the nutritional value and palatability of the leaf litter for macroinvertebrate shredders (Abelho, 2001; Gessner & Chauvet, 1994). Macroinvertebrate consumption of leaf litter produces fine particulate organic matter (FPOM) which is utilised by macroinvertebrate filter and deposit feeders, forming the primary link to higher trophic levels, such as fish (Bundschuh & McKie, 2016; Graca, 2001).

Anthropogenic stressors can impact leaf litter decomposition through effects on different trophic decomposer levels (Bärlocher et al., 2010; Ferreira et al., 2015; Marks, 2019): Elevated nutrient concentrations typically stimulate fungal activity and decomposition rates (Ferreira et al., 2015; Woodward et al., 2012). Fine sediment deposition can impair fungal colonisation and growth, especially if interstitial flow and oxygen concentrations are reduced (Bollinger et al., 2022; Bruder et al., 2016; Cornut et al., 2010; Medeiros et al., 2009). Combinations of restricted access for macroinvertebrate shredders and reduced nutritional value of buried leaf litter can reduce overall leaf decomposition rates (Piggott, Niyogi, et al., 2015; Wagenhoff et al., 2011). Conversely, leaf litter burial in coarse substrates under oxygen

saturated conditions in interstitial water may accelerate microbial decomposition probably due to additional physical abrasion mediated by the substrates (Danger et al., 2012; Jyvasjarvi et al., 2021; Piggott, Townsend, et al., 2015). Reduced flow velocity can increase the boundary layer thickness of substrates reducing fluxes of oxygen and nutrients to fungal decomposers. Consequently, fungal growth and activity may be inhibited governing an overall reduction in leaf litter decomposition (Bruder et al., 2016; Medeiros et al., 2009). Moreover, reduced flow velocity can increase sedimentation rates of fine particles, leading to burial of deposited leaf litter.

Combinations of elevated nutrient concentrations, fine sediment deposition, and reduced flow velocity may generate non-linear and interactive effects on leaf litter decomposition rates. Stimulating effects of elevated nutrient concentrations on microbial decomposers may overrule negative effects of reduced flow velocities and fine sediment deposition. However, stressor interactions appear to be context dependent as a function of, for example, individual stressor intensities and oxygen reducing potential of the deposited sediments (Bruder et al., 2016; Danger et al., 2012; Jyvasjarvi et al., 2021; Matthaei et al., 2010; Piggott, Niyogi, et al., 2015; Piggott, Townsend, et al., 2015). Most studies addressing multiple stressor effects on leaf litter decomposition focused on macroinvertebrate-mediated leaf decomposition, whereas microbial decomposers have been studied less (Bollinger et al., 2022; Bruder et al., 2016; Mustonen et al., 2016). Moreover, multiple stressor effects on mechanistic interactions between microbial and macroinvertebrate decomposers have, to our knowledge, not been studied.

To address these knowledge gaps, we performed two experiments. We used a stream mesocosm experiment to examine effects of low flow, elevated nutrient concentrations, and

fine sediment deposition on fungal decomposer communities and on microbial and macroinvertebrate-mediated leaf litter decomposition. Leaf litter from mesocosm treatments were, subsequently, used in a microcosm experiment to study specific feeding preferences of macroinvertebrate shredders providing mechanistic insights of interactions between microbial and macroinvertebrate leaf decomposers. The shredding freshwater amphipod *Gammarus pulex* (L.), common in Northern European streams (Marchant, 1981) and abundant in the stream mesocosms, was selected as model organism for the microcosm experiment.

Overall, we hypothesised that treatments would alter leaf litter decomposition rates, primarily directly, and indirectly, through changes in fungal communities. Our specific hypotheses were: i) aquatic hyphomycete community structure would change in response to low flow and elevated fine sediment deposition towards a community dominated by few tolerant taxa, ii) fungal biomass would decrease in response to low flow and elevated fine sediment deposition, iii) elevated nutrient concentration would increase fungal biomass and mitigate negative effects from low flow and elevated fine sediment deposition, iv) microbial leaf litter decomposition would increase with increasing fungal biomass, v) macroinvertebrate-mediated leaf litter decomposition would track treatment-based differences in microbial leaf litter decomposition, vi) in the absence of a food choice (one leaf disc from stream mesocosm treatments), *G. pulex* would compensate for low nutritious value by increasing feeding activity, and vii) in the presence of an alternative food source with low nutritional value (i.e., sterile beech leaf discs) *G. pulex* would exert active preference for conditioned leaf discs from stream mesocosm treatments.

2. MATERIAL AND METHODS

2.1 Rationale behind the dual-experiment approach

Mesocosm experiments provide opportunities for testing stressor effects on complex biological systems that resemble natural conditions while maintaining reasonable control of stressor levels and deployed community assemblages. However, the increased realism gained from using e.g., complex introduced macroinvertebrate assemblages comes at the cost of losing mechanistic insights behind observed effects. While some species of stream macroinvertebrates utilise mainly one food resource, most species are somewhat plastic in terms of their ability to utilise different food resources (Cummins, 2018). Similarly, the dominant shredder in our mesocosm experiment (*G. pulex*) has a high feeding plasticity and can act as shredder, predator, and even grazer (Kelly et al., 2002). Consequently, reduced leaf litter decomposition in the mesocosm experiment could indicate a partial or full change in preferred food resources or a remaining preference for leaf litter although coupled with reduced feeding activity.

Microcosm experiments aimed to provide insights into the mechanisms behind altered leaf decomposition rates observed in the mesocosm experiment. Leaf material subjected to treatments in the mesocosm experiment was offered to *G. pulex* as single food resource or in combination with other food resources with high (*Alnus glutinosa*) or low (autoclaved and unconditioned *Fagus sylvatica*) nutritional value. Hereby, potentially changed feeding preferences in *G. pulex* could provide mechanistic insights into observed changes in altered leaf decomposition rates in the mesocosm experiment.

2.2 Stream mesocosm experiment

2.2.1 Stream mesocosms

The experiment was conducted during summer in 2015 at the Lemming stream mesocosm facility, Denmark (56°4’ N, 9°31’ E). The stream channels (n = 12) were constructed of

stainless steel and dimensioned with length, width, and height of 12 m, 60 cm, and 30 cm, respectively. Inorganic sediments were deployed to create pool-riffle sequences (three riffles per stream channel), and the distribution of particle size classes resembled headwater streams in Denmark (Pedersen et al., 2004) (see Appendix S1 for details on stream channel set-up).

Unfiltered stream water from a neighbouring forest stream was continuously supplied to the stream channels using a main feeder pump via 1,000 L plastic feeder tanks (n = 12). Each stream channel discharged into a 1,000 L plastic receiver tank. An additional recycling pump was deployed in each receiver tank to increase discharge when needed. This pump recirculated defined volumes of water from the receiver tank to the feeder tank, allowing to fine-tune the discharge in each stream channel. The maximum discharge capacity was approximately 6 L s^{-1} (see Appendix S1 for details).

2.2.2 Stream mesocosm experimental design

The experiment consisted of three succeeding phases: pre-treatment, normal flow, and low flow (see Appendix S1 for details). The pre-treatment phase was initiated on June 15th 2015 and lasted eight weeks intending to condition inorganic substrates with benthic microorganisms and to deploy macroinvertebrate assemblages. All stream channels received discharge corresponding to the maximum capacity of the main feeder pump and the recycling pumps (target discharge = 6 L s^{-1}). Macroinvertebrates were collected from Lemming stream using a kick sampling net (mesh size 500 µm) and transferred to the stream channels after two weeks of the pre-treatment phase. Five kick samples from riffles and five from depositional areas were transferred to each stream channel.

198 The normal-flow phase lasted four weeks and was initiated as a continuation of the pre-
199 treatment phase (target discharge = 6 L s^{-1}). Six randomly chosen stream channels were
200 enriched with additional nutrients using nitrogen and phosphorous fertilisers (SweDane NPK
201 21-3-10 and GrowHow NS 24-6, purchased from DLG, Copenhagen, Denmark).
202 Consequently, the normal-flow phase comprised two treatment groups: Control and nutrient
203 enriched (termed NP) (n = 6) (Fig. S2). In brief, fertilizers were mixed with water from
204 Lemming stream in a 600 L plastic tank. From this tank, peristaltic pumps transported the
205 nutrient-enriched water to the feeder tanks, allowing for mixing with stream water, thus
206 reaching the target nutrient concentration before the water entered the stream channels. This
207 procedure ensured a continuous supply of water with elevated nutrient concentrations to the
208 designated stream channels (see Appendix S1 for details). We intended to elevate background
209 concentrations of nitrate-N, ammonium-N, and phosphate-P by a factor 2, 20, and 4,
210 respectively. These nutrient concentrations correspond to typical Danish lowland agricultural
211 streams (Wiberg-Larsen et al., 2012).
212
213 The low-flow phase lasted four weeks and was initiated as an immediate continuation of the
214 normal-flow phase. In this phase, discharge was equally reduced in all channels to
215 approximately 1 L/s while maintaining the water depth from the normal-flow phase (see
216 Appendix S1 for details). The necessary flow reduction was achieved by turning off the
217 recycling pumps and reducing the pumping capacity of the main feeder pump. The NP
218 treatment was continued with adjustments in the supply of nutrient enriched water
219 corresponding to the reduced discharge in order to maintain target values of elevated nutrient
220 concentrations. In addition, we added organic rich fine sediment to six randomly chosen
221 channels creating four treatments (n = 3): control, NP, fine sediment addition (FS), and
222 combined nutrient enrichment and fine sediment addition (NP+FS) (Fig. S2). The fine

sediment was collected from depositional areas in Lemming stream and deployed in the respective stream channels until a FS cover > 90 % was reached (see Appendix S1 for details).

2.2.3 Flow, water chemistry, and fine sediment cover in the stream mesocosms

In each experimental phase, discharge was measured at weekly intervals, and current velocity and water depth were measured once during each experimental phase (see Appendix S1 for details). Temperature was measured at 10 min intervals throughout the experiment using loggers (HOBO Pendant UA-001, Onset, USA). Fine sediment cover in all stream channels was visually quantified the last week of the normal-flow phase and with weekly intervals in the subsequent low-flow phase (see Appendix S1 for details). Water samples for water chemistry measurements were collected in 1 L glass bottles in each stream channel with three-day intervals during all experimental phases (see Appendix S1 for details). Analytical methods and applied standards for the water chemistry analyses are described in Appendix S1.

On average, discharge was reduced from 4.65 L s^{-1} in the normal-flow phase to 1.05 L s^{-1} in the low-flow phase. Concentrations of nitrate-N, ammonium-N and phosphate-P were elevated by a factor of 2.5, 20, and 4, respectively, in the NP treatments compared to treatments without added nutrients. Fine sediment cover was elevated by a factor of approximately 4 in the FS treatments compared to treatments without fine sediment additions (Appendix S1, Table S2).

2.2.4 Macroinvertebrates

247 Macroinvertebrates were sampled from the stream channels using surber sampler (sampling
248 area = 195 cm^2, mesh size 200 µm). One surber sample was collected on riffle and pool
249 habitats, respectively, in each stream channel one week before the start of the normal-flow
250 phase and with weekly intervals during the normal- and low-flow phases (9 sampling
251 occasions in total). The number of surber samples was restricted to avoid removing an excess
252 number of individuals which could influence the results.
253
254 All macroinvertebrate samples were preserved in 96% ethanol. The macroinvertebrates were
255 identified to species level except Chironomidae (sub-family), Oligochaeta (class), and
256 Empididae, Tipulidae, and Simuliidae (all to family). Feeding preferences were ascribed to
257 each taxon according to Tachet et al. (2002) allowing quantification of shredder densities.
258
259 Importantly, the established macroinvertebrate assemblages resembled the taxon-specific
260 densities and composition of macroinvertebrate communities in Lemming stream (Graeber et
261 al., 2017).
262
263 *2.2.5 Leaf bags*
264 Leaf material of beech (*Fagus sylvatica*) was used to study leaf decomposition rates as
265 function of the stream mesocosm treatments. The leaf material was collected directly from
266 trees before abscission in November 2014, airdried for one week and stored at -20 °C until
267 the start of the mesocosm experiment. A total of 1 ± 0.01 g DW leaf material was transferred
268 to each leaf bag, and 12 +12 leaf bags with coarse (15 mm) and fine mesh (500 µm),
269 respectively, were deployed in each stream channel at the start of the normal flow phase (total
270 n = 288) (see Appendix S1 for details). Two leaf bags with coarse mesh and two with fine
271 mesh were collected from each stream channel after experimental weeks 1, 2, and 4 of the

normal-flow phase. All remaining leaf bags (n = 144) were removed after experimental week 4 in the transition towards the low-flow phase. At the start of the low-flow phase, another 12 + 12 leaf bags with coarse and fine mesh, respectively, were deployed in each stream channel, and leaf bag collection followed the sampling protocol for the normal-flow phase. Note that remaining leaf bags with fine mesh size (n = 72) were subsequently used in the microcosm experiment (see Methods section 2.2 below).

All leaf bags were conditioned in Lemming stream for 7 d before deployment (see Appendix S1 for details). The conditioning of leaf material in Lemming stream was conducted using nets (500 µm mesh size) to exclude macroinvertebrate shredders. Collected leaf bags were stored in separate plastic bags and stored in a cooling box while transported to the laboratory and immediately subjected to further treatments as described below.

2.2.6 Processing of leaf material

The leaf material in each leaf bag collected on experimental weeks 1, 2, and 4 during normal- and low-flow phases was carefully rinsed in tap water to remove fine sediment and macroinvertebrates. For leaf bags with coarse mesh size, remaining leaf material from each leaf bag was transferred to a paper bag, dried at 60 ºC for 72 h and weighed on a Mettler Toledo scale (accuracy 0.1 mg). For each leaf bag with fine mesh size, 25 leaf discs (diameter = 10 mm) were punched with a cork borer. The cork borer, cutting board, and tweezers were rinsed in 96% ethanol and, subsequently, in tap water between cutting procedures for each leaf bag to avoid cross contamination of fungi. The leaf discs were used for hyphomycete sporulation (n = 5), weight measurements (n = 5), and quantification of ergosterol (n = 15). Leaf discs for hyphomycete sporulation were processed immediately following the descriptions below. Leaf discs for weight measurements were transferred to paper bags, dried

297 at 60 ºC for 72 h and weighed. Leaf discs for ergosterol measurements were freeze-dried and
298 stored frozen (- 20 ºC) until analysis. The remaining leaf material from each leaf bag was
299 transferred to paper bags, dried at 60 ºC for 72 h and weighed.
300
301 *2.2.7 Hyphomycetes sporulation and spore identification*
302 The five leaf discs from each leaf pack were transferred to a 50 mL Erlenmeyer flask
303 containing 20 mL of treatment water (collected in 1 L glass bottles from the stream channels).
304 All Erlenmeyer flasks were sealed with cotton to allow air flow but prevent cross
305 contamination and placed on an orbital shaker (120 rpm) in darkness at 10 ºC. After 48 h,
306 produced conidia were prevented from agglomerating by adding 25 µL of 0.5 % Tween80
307 and fixed by adding 1.14 mL 37 % formaldehyde to obtain a final concentration of 2 %. The
308 samples were stored at 4 ºC. An aliquot of the conidia suspension was homogeneously
309 filtered over a gridded membrane filter (0.45 µm), and retained conidia were stained with
310 lactophenol cotton blue. At least 300 conidia were identified for each replicate (100 – 400x
311 magnification) primarily using the key provided by Gulis et al. (2005). The number of
312 counted conidia was normalized to the total filter surface, sample volume, and dry weight of
313 the respective set of leaf discs.
314
315 *2.2.8 Quantification of ergosterol*
316 Ergosterol, a component of eumycotic cell walls, was used as proxy for fungal biomass
317 following Gessner (2005). Briefly, using alkaline methanol, ergosterol was extracted from
318 freeze-dried leaf material and subsequently purified by solid-phase extraction (Sep-Pak® Vac
319 RC tC18 500 mg sorbent; Waters, Milford, USA). The ergosterol concentration was
320 quantified by high-performance liquid chromatography at a wavelength of 282 nm.
321

322 *2.3 Microcosm experiment*

323 *2.3.1 Microcosm setup*

324 The microcosm experiment was conducted as an immediate continuation of the mesocosm
325 experiment in summer 2015. The microcosm setup comprised 100 small polystyrene
326 containers (LxWxD = 10x10x1.2 cm), and the experiment was conducted in a cooling
327 chamber (Aarhus University, Campus Silkeborg) at 10 °C with a 12h:12h light/darkness
328 cycle. Each container was supplied with 100 mL unfiltered water from Lemming stream.

329

330 *2.3.2 Microcosm experimental design*

331 A 72h feeding trial was conducted using the freshwater amphipod *G. pulex* as model
332 organism. One individual of *G. pulex* was introduced to each container (n = 100). Individuals
333 of *G. pulex* were sampled in Lemming stream with a kick-net, and individuals with a body
334 length of approximately 1 cm (visual inspection) were collected for the experiment using
335 plastic pipettes. All selected individuals were acclimatised and starved for 48 h at 10 °C in
336 darkness in a 100 L aquarium containing water from Lemming stream.

337

338 Each container was, additionally, supplied with one (n = 50) or two (n = 50) leaf discs (20
339 mm diameter) providing five treatments with single and paired leaf discs, respectively (n =
340 10) (overview of treatments available in Appendix S1, Table S3). The leaf discs were
341 produced from 1) leaf material from remaining fine meshed leaf bags (*F. sylvatica*) of the
342 mesocosm experiment, collected immediately after terminating the low-flow phase. A total of
343 20 leaf discs were produced for each treatment of the mesocosm experiment (C, NP, FS, and
344 NP+FS), 2) unconditioned beech leaf material collected directly from trees before abscission
345 in November 2014, airdried for one week and stored at -20 °C until the start of the
346 microcosm experiment. To ensure absence of living microorganisms, these leaf discs (n =

100) were autoclaved for 60 minutes at 121 °C (15 psi), and 3) alder (*Alnus glutinosa*) leaf discs were produced from material collected directly from trees just before abscission in October 2014 and stored frozen (-20 ºC) until the microcosm experiment. A total of 10 alder leaf discs were produced. All leaf discs were produced using a cork borer and following the method described in section 2.2.6.

Before the feeding trial, all leaf discs were dried to constant weight at 15 °C and weighed (accuracy 0.1 mg) and, subsequently, soaked in tap water and deployed in the containers. After the 72h feeding trial, all leaf discs and individuals of *G. pulex* were dried at 60 °C for 72 h and weighed on a Mettler Toledo scale (accuracy 0.1 mg).

2.4 Data treatment

All data analyses were performed using R (version 4.3) (R Core Team 2023). See Appendix S1 for details on packages, functions, and tests of assumptions.

Differences in leaf decomposition rates (k) among treatments in the mesocosm experiment (last week of each flow phase) and feeding trials using Analysis of variance (ANOVA). A one-way ANOVA was used for the normal and low flow phase and the feeding trials. Moreover, differences among treatments in ergosterol concentrations and decomposition rates in leaf bags with coarse mesh size, corrected for microbial leaf decomposition ($k_{\text{invertebrate}}$), were tested separately for each flow phase using one-way ANOVA as described above. Tukey's honest significant differences was used to assess pairwise differences in cases of a significant ANOVA ($p < 0.05$).

Linear models were used to assess correlations between $k_{microbial}$ and ergosterol concentration and between $k_{invertebrate}$ and shredder abundance, respectively. Ergosterol concentrations from experimental week 4, integrating the entire experimental period, was selected as time-integrative representative for the experimental period of each treatment. Ergosterol concentration had to be log-transformed to reach normal distribution of the residuals.

We used principal response curves (PRC) to analyse differences among treatments in temporal developments of freshwater hyphomycete community composition in the stream channels (Van den Brink & Ter Braak, 1999). The PRC model is based on the first axis of a principal coordinate analysis using a measure of community similarity to generate species scores (Oksanen et al., 2018). The PRC analysis provides treatment scores and species weights, where treatment scores represent community responses to a treatment and species weights represent species specific responses to treatment patterns in the PRC (Van den Brink & Ter Braak, 1999). Species weights approximating zero represent no or weak treatment-related responses, and high negative or positive species weights represent strong negative or positive treatment-related responses, respectively (Van den Brink & Ter Braak, 1999). We only show species with species weight scores > 0.2 or < - 0.2 (a more conservative score cut off of 0.5 was proposed by Van den Brink (1999)).

Since hyphomycete community composition data was comprised by spore production data, and since spore production potential varies by several orders of magnitude among species (Bärlocher, 2009), we used Jaccard's dissimilarity based on presence-absence as input data for the PRC models (please see Appendix S1 for details on the procedure).

For all PRC models, we used an ANOVA-like permutation test with 999 iterations to test if the PRC model (first axis of the principal-coordinates model described above) explained a significant proportion of the variation in community composition among treatments. Separate PRC models were produced for the normal and low flow phases. In cases of significant PRC models, we tested the correlation between hyphomycete community composition (PRC scores) and $k_{\text{microbial}}$ using a linear model.

3. RESULTS

3.1 Mesocosm experiment

3.1.1 Hyphomycete community structure

During the normal-flow phase, aquatic hyphomycete community structure was not significantly different between the NP treatment and untreated controls (PRC, $F = 1.66$, $p = 0.16$, Fig. 1A). In contrast, aquatic hyphomycete community structure was significantly different among treatments during the low-flow phase (PRC, $F = 3.11$, $p = 0.036$, Fig. 1B). Increased fine sediment (FS) cover negatively influenced the presence of all hyphomycete species with the exception of *Anguillospora crassa* that was more frequently occurring in treatments with increased fine sediment cover (FS and NP + FS) (Fig. 1B; PRC score < 0). Moreover, the hyphomycetes community composition in the FS treatment strongly deviated from untreated controls, while a treatment effect was less pronounced at elevated nutrient concentrations (NP + FS) (Fig. 1B). In fact, the community-based PRC scores indicated that hyphomycete community structure deviated from untreated controls by a factor of two more in the FS treatment compared to the NP + FS treatment (Fig. 1B) indicative of an almost complete absence of hyphomycete species (Appendix S2).

3.1.2 Fungal biomass

Total fungal biomass (ergosterol concentration) generally increased through the normal and low-flow phases of the mesocosm experiment except in the FS treatment (low flow) (Fig. S4) in which average ergosterol concentrations remained constantly low (40-50 μg g^{-1} leaf material (DW)). During the normal and low-flow phases, ergosterol concentrations in NP treatments and untreated controls increased by 70-100%, whereas ergosterol concentrations in the NP + FS treatment during low flow only increased by approximately 50% (Fig. S4). After four weeks, we found a significant treatment effect on ergosterol concentrations under low flow (one-way ANOVA, $p = 0.039$, Fig. S4) but not under normal flow (one-way ANOVA, $p = 0.73$, Fig. S4). Thus, the average ergosterol concentrations were significantly lower in the FS treatment compared to the NP treatment and untreated controls (Tukey's test, $p = 0.003$ and $p < 0.001$, respectively, Fig. S4) after four weeks of treatment under low flow conditions.

3.1.3 Leaf litter decomposition

During normal flow, we found no significant treatment effect on leaf decomposition rates in bags with coarse or fine mesh size (one-way ANOVA, $p = 0.36$ and $p = 0.34$, respectively, Fig. 2A and 2B). During low flow, however, we found significant treatment effects on leaf decomposition rates in bags with coarse and fine mesh size (one-way ANOVA, $p = 0.001$ and $p = 0.0007$, respectively) (Fig. 2C and 2D). For leaf bags with coarse mesh size, leaf decomposition rates were significantly reduced in FS and NP+FS treatments compared to untreated controls (Tukey's test, $p < 0.05$, Fig. 2C). For leaf bags with fine mesh size, leaf decomposition rates were significantly lower in the FS treatment compared to the NP treatment and untreated controls (Tukey's test, $p < 0.05$, Fig. 2D). Notably, when decomposition rates in leaf bags with coarse mesh size were corrected for microbial induced decomposition (bags with fine mesh size) to obtain macroinvertebrate induced decomposition

($k_{invertebrate}$), the treatment effect became insignificant (one-way ANOVA, p = 0.10, Fig. S5). Moreover, $k_{invertebrate}$ was not significantly correlated to total shredder densities in the stream channels (Pearson r = 0.03, p = 0.65, data not shown) or abundance of the most dominant macroinvertebrate shredder, *G. pulex* (Pearson r < 0.01, p = 0.97, Fig. S6)

Including all leaf bags with fine mesh size across all treatments during low flow, we found a significant linear relationship between normalised decomposition rates ($\Delta k_{treatment\text{-}control}$) and hyphomycetes community composition (PRC scores) (Fig. 3, $r^2 = 0.49$, $p < 0.001$). In this context, higher dissimilarities in hyphomycetes community composition between treatments and untreated controls was correlated with reduced decomposition rates. Moreover, normalised ergosterol concentrations (treatment:control) were significantly and positively correlated with normalised decomposition rates (Fig. 3, $r^2 = 0.57$, $p < 0.001$).

3.2 Microcosm experiment

3.2.1 Feeding trials

In feeding trials with one leaf disc offered to *G. pulex*, we found significantly different leaf consumption among treatments (Fig. 4A, one-way ANOVA, p = 0.004). Consumption of leaves from control treatments was significantly higher compared to consumption of autoclaved beech leaves (sterile) and of leaves from the FS and NP + FS treatments (Fig. 4A, Tukey's test, $p < 0.05$). Consumption of leaves from the NP treatment was higher compared to sterile beech leaves and to leaves from the FS and NP + FS treatments, but the differences were not statistically significant (Tukey's test, $p > 0.05$).

In feeding trials with paired leaf discs, we found significant treatment effects on the leaf consumption by *G. pulex* (Fig. 4B, one-way ANOVA, p = 0.001). In general, *G. pulex*

consumed more leaf material from unconditioned alder and leaf discs from NP, NP + FS, and control treatments when compared to leaf consumption of sterile beech leaf discs (Fig. 4B). In contrast, *G. pulex* consumed more leaf material from sterile beech leaf discs compared to leaf discs from FS treatments (Fig. 4B). Consumption of alder and leaf discs from the NP treatment were significantly higher compared to leaf discs from the FS treatment (Fig. 4B, Tukey's test, $p < 0.01$). Moreover, consumption of alder leaf discs was significantly higher compared to leaf discs from control and NP + FS treatments (Fig. 4B, Tukey's test, $p < 0.05$).

4. DISCUSSION

4.1 Mesocosm experiment

4.1.1 Hyphomycete community structure

Generally, hyphomycete species occurring in control treatments during normal flow also occurred in control treatments during low flow although supplemented by several additional species belonging to the genus *Anguillospora* (Fig. 1). Partly contradicting our first hypothesis, these results suggest that the reduced flow conditions did not influence community composition of aquatic hyphomycetes compared to normal-flow conditions. Moreover, all occurring hyphomycete species in control treatments of the normal and low-flow phases have been characterised as generalists with no particular sensitivity or tolerance towards critical low-flow episodes (Arias-Real et al., 2023). However, the relatively short experimental period may have acted as temporal filter, generally favouring generalist colonisers.

During low-flow, increased fine sediment cover governed significant changes in hyphomycete community composition generally reducing occurrence frequencies of all but one species (*Anguillospora crassa*). Partly confirming our first hypothesis, these results

suggest that fine sediment deposition might have inhibited colonisation and/or growth of hyphomycetes. However, since the community composition data was produced based on forced sporulation, these results should be interpreted with care. The observed differences in community composition may originate from reduced oxygen availability beneath the deposited fine sediment directly impeding colonisation and growth, but other factors such as reduced surface area available for colonisation or modified leaf surface characteristics may have influenced colonisation or sporulation activity of the hyphomycetes. However, we observed several black patches on leaf material in treatments with increased fine sediment cover which is indicative of hydrogen sulphide and thus local anoxic conditions beneath the deposited fine sediment. Supporting our results, the single hyphomycete species with increased occurrence frequency in treatments with increased fine sediment cover (*A. crassa*) has been previously characterised as tolerant to burial within the hyporheic zone (Arias-Real et al., 2023; Cornut et al., 2012).

In general, colonisation and growth of microbial decomposers on buried leaf litter strongly depends on the flow of oxygen-rich water in remaining interstitial spaces. When interstitial flow can provide sufficient oxygen and nutrients, the structure and growth of microbial decomposer communities can be relatively unaltered compared to unexposed communities (Cornut et al., 2012; Danger et al., 2012). However, even small reductions in oxygen concentrations in interstitial water can impede colonisation and growth of freshwater fungi (Bruder et al., 2016; Cornut et al., 2010; Medeiros et al., 2009) or change microbial decomposer communities towards dominance of terrestrial endophytes (Mustonen et al., 2016). Consequently, fine organic sediments commonly deposited in agricultural and urban streams (Paul & Meyer, 2001; Wagenhoff et al., 2011) and similar to the fine sediments

519 applied in our study, may have substantial effects on microbial decomposer communities
520 mediated through interstitial water with poor oxygen conditions (Bollinger et al., 2022).
521
522 *4.1.2 Fungal biomass*
523 In contrast to our second hypothesis, ergosterol concentrations (serving as proxy for fungal
524 biomass) increased through the experiment in control treatments of both normal and low-flow
525 conditions reaching approximately 150 µg g^{-1} showing no clear effect of flow reduction.
526 Partly confirming our second hypothesis, however, ergosterol concentrations remained at
527 approximately 50 µg g^{-1} throughout the low flow phase when exposed to fine sediment
528 deposition, which was significantly lower than in the controls. This result further suggests
529 that hyphomycete colonisation and/or growth was reduced by fine sediment deposition
530 probably due to insufficient interstitial flow and oxygen supply beneath the deposited fine
531 sediment. Several studies support that biomass accrual or respiration for microbial
532 decomposers is reduced when buried in stream sediments (e.g., Bruder et al., 2016; Cornut et
533 al., 2010; Medeiros et al., 2009; Piggott, Niyogi, et al., 2015). However, burial in stream
534 sediments does not necessarily impede fungal growth when interstitial flow and oxygen
535 supply is sufficient to support metabolic requirements (Bollinger et al., 2022; Cornut et al.,
536 2012; Danger et al., 2012).
537
538 Elevated nutrient concentrations did not significantly increase ergosterol concentrations
539 during normal or low-flow conditions. Partly confirming our third hypothesis, however,
540 elevated nutrient concentrations reduced (although not significantly) negative effects of
541 increased fine sediment cover on fungal biomass. Similar trends were found for the
542 hyphomycete community composition with reduced occurrence frequency of most
543 hyphomycete species in treatments with increased fine sediment cover. These results

544 congruently suggest that moderately elevated nutrient concentrations to limited extent can
545 reduce negative effects of increased fine sediment cover on hyphomycete communities.
546 Stimulating effects of elevated nutrient concentrations on hyphomycete growth has, however,
547 been consistently confirmed in numerous controlled experimental studies (Bruder et al.,
548 2016; Ferreira et al., 2015; Ferreira & Graca, 2016; Piggott, Niyogi, et al., 2015) and in the
549 field (Gulis et al., 2006; Noel et al., 2016; Robinson & Gessner, 2000). Based on a meta-
550 analysis from Woodward et al. (2012), we propose that nutrient concentrations in our control
551 treatments probably provided optimal conditions for hyphomycete growth. Consequently, if
552 untreated controls provided optimal growth conditions, this would plausibly explain why
553 further elevated nutrient concentrations did not govern significantly increased fungal biomass
554 production.
555
556 *4.1.3 Leaf litter decomposition*
557 Microbial leaf litter decomposition was significantly different among treatments during low-
558 flow but not during normal-flow conditions. During low-flow, increased fine sediment cover
559 significantly reduced microbial leaf litter decomposition compared to controls, and elevated
560 nutrient concentrations only partially mitigated this effect. These results mirror the treatment
561 effects on ergosterol concentrations suggesting that higher fungal biomass governed higher
562 microbial leaf litter decomposition rates. This is fully in line with our fourth hypothesis and
563 numerous existing studies (e.g., Feckler & Bundschuh, 2020; Ferreira et al., 2015; Gessner &
564 Chauvet, 1994; Mustonen et al., 2016; Noel et al., 2016).
565
566 Leaf litter decomposition in leaf bags with coarse mesh size was significantly different
567 among treatments during low-flow but not during normal-flow conditions. During low-flow,
568 increased fine sediment cover significantly reduced decomposition rates, even in the presence

of elevated nutrient concentrations. However, macroinvertebrate induced leaf litter decomposition was not significantly different among treatments during low-flow conditions. Instead, and confirming our fifth hypothesis, macroinvertebrate mediated decomposition in individual stream channels significantly increased with increasing average ergosterol concentrations and with resemblance of the hyphomycete community composition to untreated controls (Fig. 3). These results probably reflect a combination of increased nutritional value and palatability of the leaf material due to increased fungal biomass, and macroinvertebrate shredder preference for specific hyphomycete species.

4.2 Microcosm experiment

4.2.1 Feeding trials

Confirming our sixth hypothesis, when offered single leaf discs from stream channel treatments, or a sterile beech leaf disc, leaf litter consumption by *G. pulex* was consistent with observed differences in leaf litter decomposition in the stream channels. Leaf litter consumption by *G. pulex* was significantly reduced, and to similar extent, for leaf discs from fine sediment treatments and for sterile leaf discs when compared to controls (Fig. 4). In other words, *G. pulex* foraged less on leaf discs with lower nutritional value. Since no alternative food sources were available, this suggests that *G. pulex* probably minimised activity levels to reduce metabolic requirements (sensu the scope for growth concept (Naylor et al., 1989)). This is further supported by Graca et al. (1993) who found that *G. pulex* could maintain their growth rates when feeding on unconditioned leaf material, and the authors suggested that *G. pulex* can reduce metabolic costs allocated to body maintenance when fed on low quality food sources.

In contrast to the seventh hypothesis, *G. pulex* did not consistently prefer feeding on leaf discs from the low-flow stream channel treatments over sterile beech leaf discs. Instead, *G. pulex* consumed more sterile leaf material when the alternative food source was leaf material from the treatment with increased fine sediment cover (FS). However, leaf discs from stream channels with increased fine sediment cover and elevated nutrient concentrations (NP + FS) were preferred over sterile leaf discs. Since fungal biomass more or less remained at constant low levels on leaf material from the treatment with deposited fine sediment, the nutritional value (C:N:P ratio) was probably comparable to sterile leaf discs. This indicates a repelling effect of leaf material from the treatment with increased fine sediment cover on *G. pulex*. Such repelling effects could originate from specific hyphomycete species (e.g., *A. crassa*) occurring in the treatment with increased fine sediment cover or from bacterial communities established under partly anaerobic conditions (e.g., hydrogen sulphide producing species), but this remains unexplored in existing literature.

5. CONCLUSIONS AND PERSPECTIVES

In this study, we showed that increased fine sediment cover can alter the flux of energy and material in the detrital food chain through bottom-up regulation of leaf conditioning by fungal decomposers. The observed reduction in biomass and occurrence frequencies of most species of fungal decomposers in treatments with fine sediment deposition prompted a substantial change in food preference by macroinvertebrate shredders where *G. pulex* even preferred sterile leaf material over leaf material from the fine sediment treatments.

Freshwater fungi, typically accounting for > 90% of the microbial biomass of submerged leaf material (Baldy et al., 1995; Marks, 2019), produce extracellular enzymes to break down complex compounds and contain essential nutrients for macroinvertebrate shredders that are

not found in the leaf material (Marks, 2019). In addition, mycelia of freshwater fungi that penetrate the leaf material can serve as vector for colonising bacteria into otherwise inaccessible leaf tissue (Gessner & Chauvet, 1994). As such, the absence of fungi on leaf material likely has detrimental consequences for detrital stream food webs causing increased C:N:P ratios of the leaf material and absence of essential nutrients available for macroinvertebrate shredders. This may further propagate into reduced growth and reproduction of the shredders (Abelho, 2001).

Faster decomposition of leaf material does not necessarily equal increased ecosystem health, however (Marks, 2019; Woodward et al., 2012). A broader suite of different leaf species with different elemental composition and associated decomposition rates can ensure that palatable and nutritious leaf material is available for decomposers for longer windows of time. In this context, well oxygenated hyporheic zones plays an important role in the prolongation of these temporal windows (Marks, 2019). Conversely, fine sediment deposition can lead to anoxic hyporheic zones converting deposited leaf material from nutritional sources to sinks (Marks, 2019) and partly obstructing microbial conditioning and general energy flux within the detrital food chain.

CRediT Author Contribution Statement

Jes J Rasmussen: Writing – original draft, Conceptualisation, Formal analysis, Investigation, Methodology, Supervision. **Mirco Bundschuh**: Writing – review & editing, Investigation. **Tinna Mia Jensen**: Writing – review & editing, Investigation. **Peter Wiberg-Larsen**: Writing - review & editing, Methodology, Investig (Placeholder1)ation. **Annette Baattrup-Pedersen**: Writing - review & editing, Conceptualisation, Project administration, Supervision. **Nikolai Friberg**: Writing – review & editing, Funding acquisition, Project

administration. **Daniel Gräber**: Writing – original draft, Conceptualisation, Project administration, Supervision, Investigation, Formal analysis, Methodology, Visualisation.

Data availability statement

All R codes and data are available in the following open repository under the BSD-3 clause license (code) or the license of the journal (data): https://git.ufz.de/graeber/leaf-litter-degradation-mesocosm-microcosm

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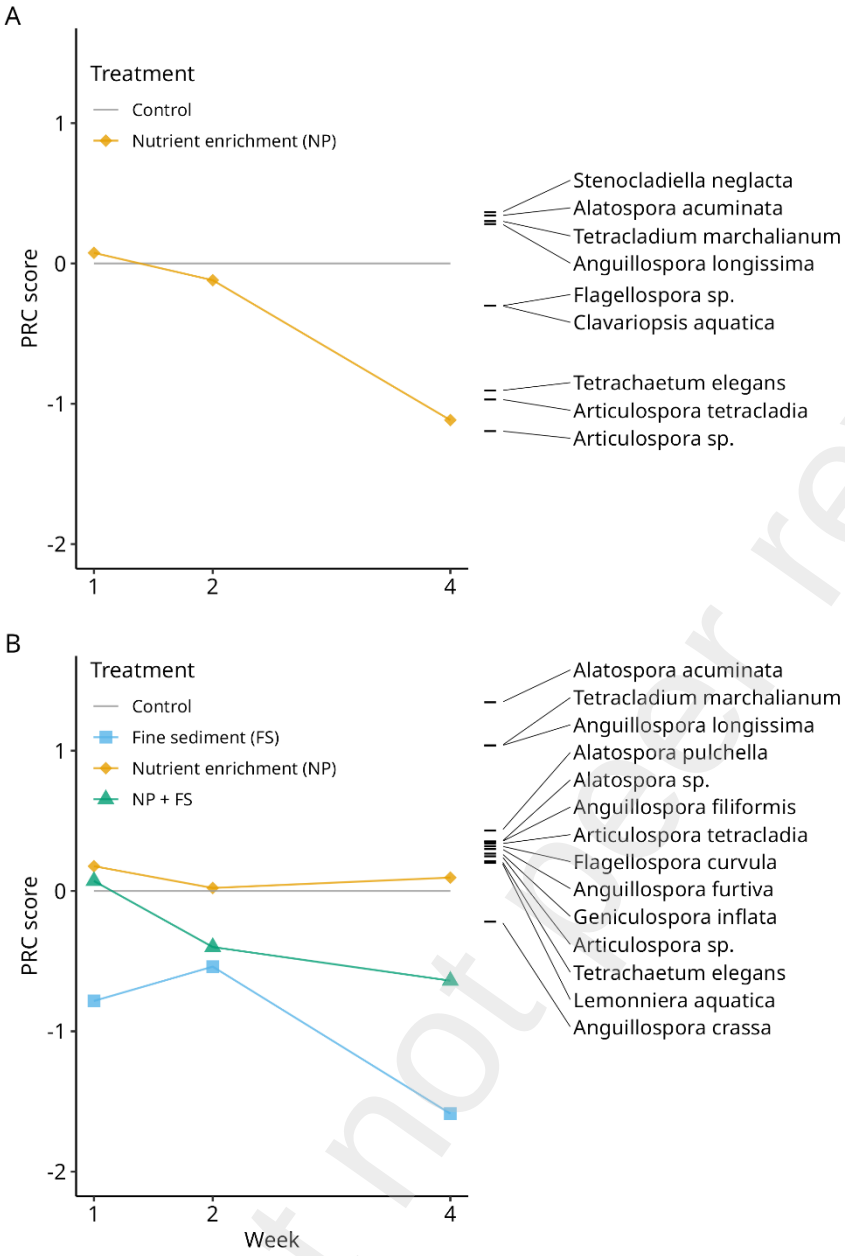
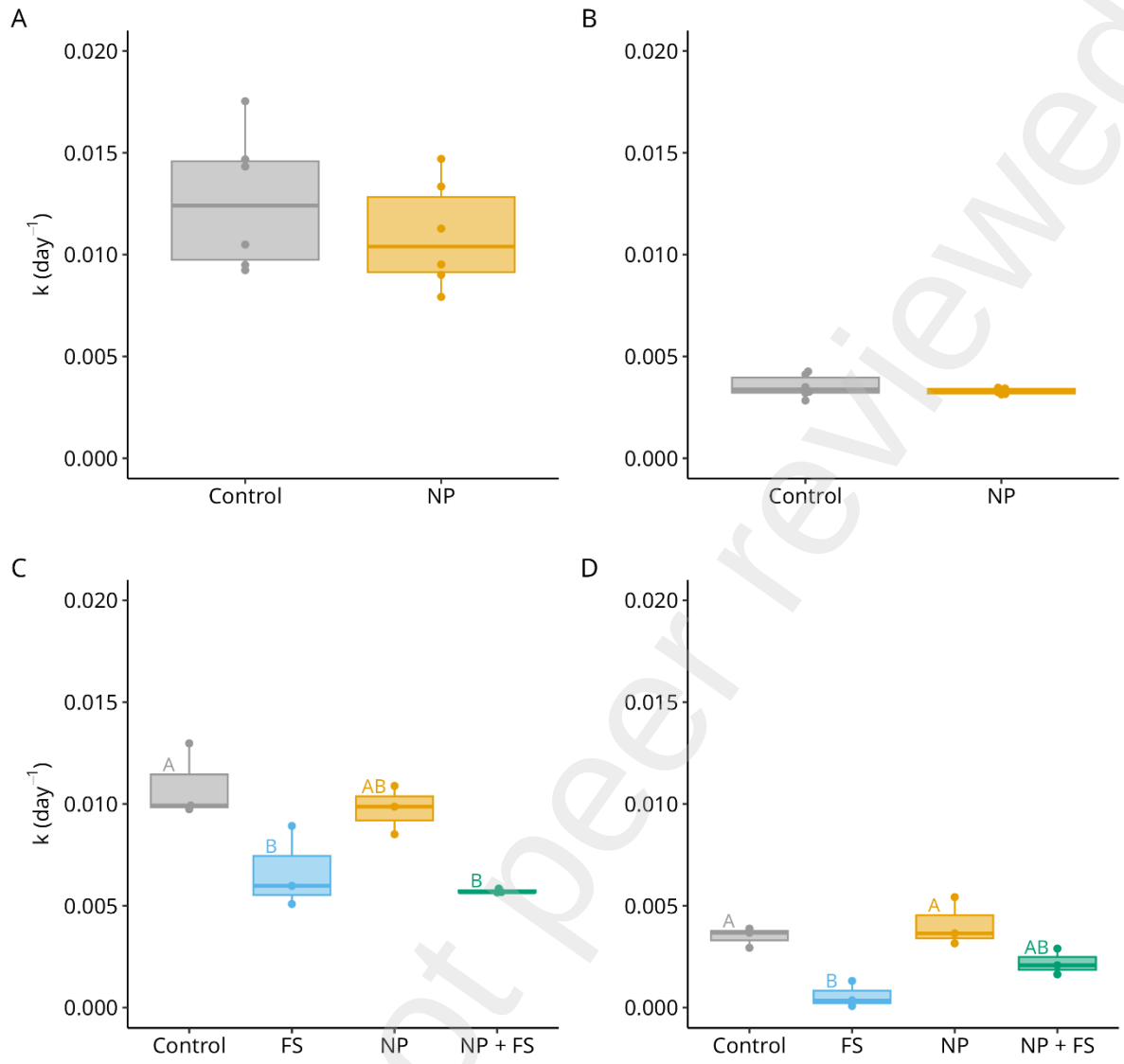


Fig. 1. Principal response curves (PRC) of hyphomycete community composition affiliated with leaf material in leaf bags with fine mesh size. Hyphomycete communities were characterised on experimental weeks 1, 2, and 4 during normal (A) and low (B) flow. The PRC curves represent treatments with increased nutrient concentrations (NP), increased fine sediment cover (FS), increased nutrient concentrations and fine sediment cover (NP + FS), and untreated controls.



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891 Fig 2. Decomposition rates (*k*) of beech leaves in bags with coarse (A and C) and fine (B and
892 D) mesh size, respectively. The box plot shows decomposition rates during normal (A and B)
893 and low flow conditions (C and D) with treatments of increased nutrient concentrations (NP),
894 increased fine sediment cover (FS), increased nutrient concentrations and fine sediment cover
895 (NP + FS), and untreated controls. Capital letters indicate significantly different leaf
896 decomposition rates ($p<0.05$). Bold lines indicate median values, upper and lower box edges
897 represent 25 and 75 percentiles, respectively, and error bars indicate 95% confidence limits.

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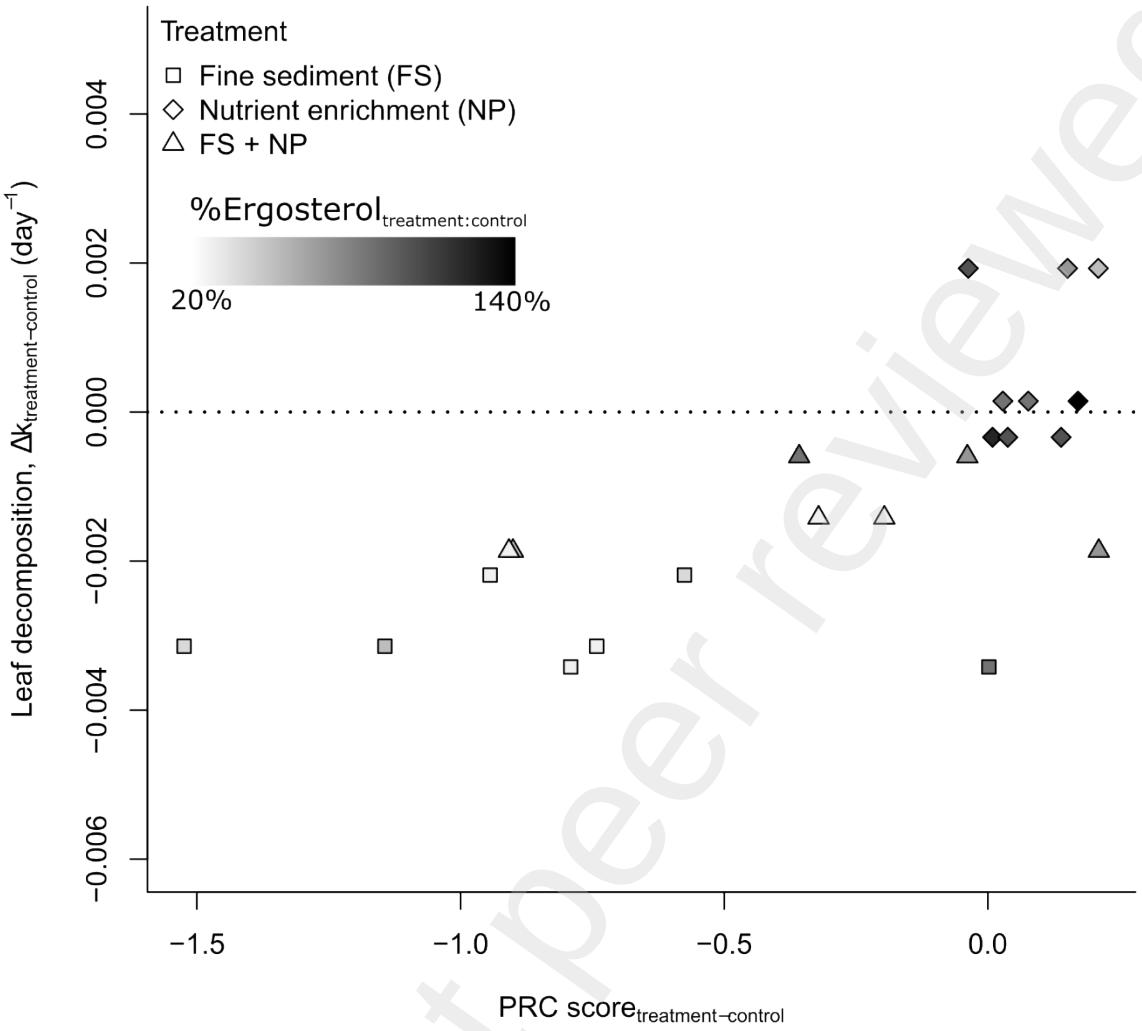

900

901 Fig. 3. Normalised leaf decomposition rates (*k*) in leaf bags with fine mesh size as function of
902 normalised PRC scores for the hyphomycete communities. Normalised leaf decomposition
903 rates and PRC scores represent differences between untreated controls and NP, FS, and
904 NP+FS treatments, respectively. Relative ergosterol concentrations (normalised to untreated
905 controls) for the respective leaf bags are depicted using a colour gradient. All data points
906 represent leaf bags collected after experimental week 4 in the low flow phase.

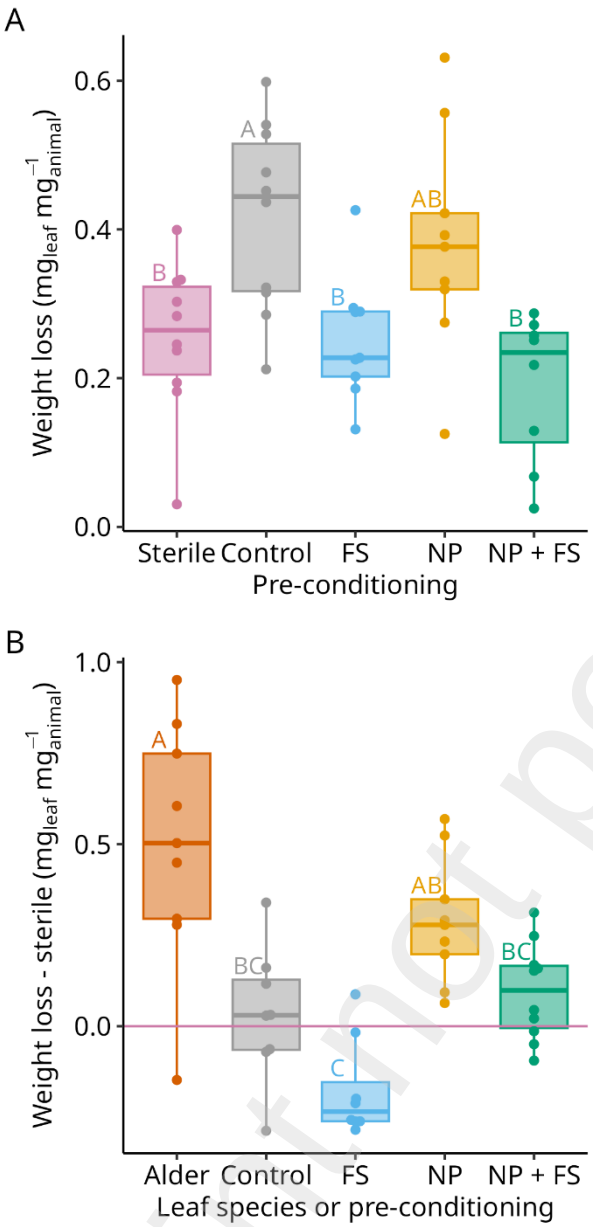

Fig. 4. Weight loss of leaf discs due to *G. pulex* leaf consumption. Leaf discs originating from experimental treatments were offered to *G. pulex* as the only food source (A) or in paired combination with a sterile beech leaf disc (B). Weight loss from leaf disc pairs is presented as the difference between treatment and sterile leaf discs. Hence, negative values indicate higher consumption of sterile leaf discs compared to treatment leaf discs. Experimental treatments include increased nutrient concentrations (NP), increased fine sediment cover (FS), increased

916 nutrient concentrations and fine sediment cover (NP + FS), and untreated controls. The leaf
917 material was retrieved after 4 experimental weeks in the low flow phase. Capital letters
918 indicate significantly different leaf decomposition rates ($p<0.05$).