Running Head

Flow intermittence alters carbon processing

Title

Flow intermittence alters carbon processing in rivers through chemical diversification of leaf litter

List of authors

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Abstract

The dry phase of intermittent rivers promotes the emergence of diverse terrestrial and aquatic habitats where large amounts of leaf litter can accumulate. This environmental heterogeneity can cause diverse chemical alterations in leaf litter by the co-occurrence of multiple physical and biological degradation processes across these different habitats. After flow resumption, these

chemically diversified leaves are mixed and continue decomposition downstream in fully aquatic conditions. Here, we (i) test experimentally the hypothesis that environmental heterogeneity during the dry phase can translate into a chemical diversification of leaf litter, and (ii) investigate how chemical diversity may affect leaf litter decomposition in re-established lotic conditions. To do so, we simulated with laboratory treatments the exposure of a single leaf litter species (*Alnus glutinosa*) to various terrestrial and aquatic habitats typically found during the dry phase. Then, we assembled leaves to create mixtures with increasing treatment richness and measured their decomposition in a perennial river reach. Our laboratory treatments mimicking dry-phase habitats caused a strong chemical diversification of leaf litter, which accelerated its decomposition in the river. The mixing of chemically diversified leaves stimulated both microbial decomposition and detritivore activity, likely by facilitating their acquisition of essential nutritional components. These results suggest that intermittent river reaches may act as hotspots of organic matter diversification with potential implications on C processing at river-network scale.

Keywords:

Intermittent rivers, dry phase, chemical diversity, C cycling, river networks, ecosystem functioning, biodiversity, organic matter

Introduction

The decomposition of riparian leaf litter is an essential ecosystem process in rivers, supporting heterotrophic food webs and driving the cycling of carbon (C) and nutrients (Webster & Benfield 1986). At river-network scale, decomposition interacts with downstream transport (Battin et al.

2008). The relative importance of decomposition over transport depends on leaf litter retention mechanisms, which are intimately related to river size and hydrological dynamics (Larrañaga et al. 2003). An extreme example of this interplay is epitomized by intermittent rivers. From a biogeochemical perspective, they act as pulsed bioreactors where the alternation of dry and wet phases results in the processing of organic matter in repeated cycles of accumulation, transport, and decomposition (Larned et al. 2010). This model considers the wet phase as the only period of actual organic matter decomposition (Corti et al. 2011, Datry et al. 2018). In contrast, during the dry phase, riparian leaf litter is accumulated on dry riverbeds, with little or no decomposition (Sanpera-Calbet et al. 2016; Datry et al. 2018). Afterwards, when water flow resumes, leaf litter is quickly and massively transported downstream (Corti & Datry 2012), where decomposition continues. During the last years, this simple model has been challenged at reach and network scales, mainly because the exposure of leaf litter to various environmental conditions during the dry phase (in this context also referred to as "preconditioning") can trigger chemical changes, which ultimately affect leaf decomposition after flow resumption (Dieter et al. 2013; Mora-Gómez et al. 2019).

Indeed, environmental conditions at reach scale affect leaf litter chemistry (del Campo et al. 2019). The exposure to solar radiation on dry riverbeds can cause an increase in leaf litter biodegradability due to the loss of lignin by photodegradation (Austin et al. 2016; del Campo & Gómez 2016). In contrast, leaf litter accumulated in stagnant isolated pools can decrease in biodegradability due to leaching of labile compounds and accumulation of phenols (Dieter et al. 2013; Abril et al. 2016). These are two examples from a great variety of terrestrial and aquatic habitats that emerge during the fragmentation of water flow (e.g. wet and shaded remnant sediments, pools connected to hyporheos, etc.) (Larned et al. 2010; Stanley et al. 1997). The

accumulation and preconditioning of leaf litter across this mosaic of aquatic-terrestrial habitats might result in a chemical diversification of leaf litter. At network scale, the re-establishment of water flow and the reconnection of dry tributaries trigger the mixing of the variously preconditioned leaf litter during downstream transport (see here for an example during a rewetting event in the Albarine river, France). Finally, upon retention, decomposition proceeds in reassembled, chemically diversified litter packs in environmentally homogeneous, fully lotic conditions (Corti & Datry 2012).

Under aquatic conditions, decomposition is mainly controlled by leaf litter traits (Zhang et al. 2018). Leaf species rich in labile C compounds or nutrients decompose quickly, while high concentrations of lignin or polyphenols curb decomposition (Talbot & Treseder 2012). The mixing of various species can have non-additive effects on decomposition (Gessner et al. 2010), meaning that the decomposition rate of mixtures is either below or above those expected from individual species` rates (Gartner & Cardon 2004). Negative effects of litter diversity are associated to inhibition of decomposer activity by secondary metabolites like polyphenols (Chomel et al. 2016). Positive effects, in contrast, are often attributed to fungal-driven nutrient transfer or nutritional complementarity among leaves with contrasting chemical qualities (Tonin et al. 2017; López-Rojo et al. 2020). Owing to the increased chance of obtaining essential nutrients and C compounds for decomposer metabolism, the chemical diversity (a form of functional diversity) of leaf mixtures *per se* may accelerate decomposition (Lecerf et al. 2011; Stoler et al. 2016).

Despite the abundance of studies showing the relevance of biodiversity for ecosystem functioning (Hooper et al. 2005), our understanding of chemical (bio)diversity effects on important ecological processes such as decomposition is still incomplete. In particular, little

93 evidence exists from natural mechanisms that could promote chemical diversity in particulate
94 organic matter beyond the diversity of leaf species (but see Wickings et al. 2012). The
95 hydrological dynamics of intermittent tributaries could create a powerful mechanism of chemical
96 diversification of leaf litter with unknown consequences for decomposition dynamics at river-
97 network scale. Indeed, accounting for dry-phase-associated diversity effects could be critical to
98 achieve mechanistic understanding and realistic modelling capacity for C fluxes in river
99 networks (Marcé el al. 2019). The latter is a pending challenge, especially considering that
100 intermittent rivers represent over half of the length of global river networks, and that this fraction
101 will likely increase due to climate and global change (IPCC 2013).
102 Here we test the hypothesis that environmental heterogeneity occurring during the dry
103 phase of intermittent rivers can promote the chemical diversification of accumulated leaf litter
104 and thus, affect its decomposition in downstream rivers, once lotic conditions are re-established.
105 To do so, we first simulated the preconditioning of a single leaf litter species (*Alnus glutinosa*)
106 under various environmental conditions typically found during the dry phase of intermittent
107 rivers. Then, we measured the decomposition of leaf litter mixtures assembled using an
108 increasing number of preconditioning situations. We predict that the increase of chemical
109 diversity in mixtures of preconditioned leaves will accelerate decomposition in aquatic
110 conditions.
111
112 **Materials and methods**
113 *Leaf litter preconditioning and preparation of mixtures*
114 We collected fresh leaves of *Alnus glutinosa* (alder) directly from several trees in the floodplain
115 of the Löcknitz river (Brandenburg, Germany) and let them air-dry for two weeks in a dark

room. Approximately one kg of air-dried leaves was distributed among the seven preconditioning treatments (see Table 1). Following preconditioning, we prepared fine- and coarse-mesh bags (0.5 and 8 mm, respectively. 15 x 15 cm size) containing leaves of single treatments (7 treatments x 4 replicates) and mixtures of leaves of increasing treatment richness in all possible combinations of 2, 4, and 6 treatments. This design resulted in 4 richness levels comprising a total of 91 bags (28 single-treatments + 21 2-treatment combinations + 35 4-treatment combinations + 7 6-treatment combinations) for each mesh size. We filled each litterbag with 12 leaves. In mixtures, the 12 leaves were evenly partitioned across the component treatments. All leaves were scanned prior to the bag assembly to later measure treatment-specific leaf areas by digital image analysis (ImageJ, https://imagej.nih.gov/ij/) and compute the exact contributions of component treatments on a dry mass (DM) basis. For this, we established conversion factors of leaf area to DM (48h, 105 °C) for every treatment from 20 leaves.

Leaf litter chemical composition and calculation of chemical diversity

Following preconditioning, sub-samples of all treatments were freeze-dried, ground using a ball mill and analyzed for C- and N-content (Elementar vario EL C/N elemental analyzer, Germany), other nutrients such as P, Ca, Mg and K by ICP-OES (Thermo Scientific, iCAP 6500, USA), and macromolecular organic C moieties by FTIR (FTIR-8300, Shimadzu, Japan). For FTIR, 1.75 mg (± 0.05 mg) of ground, freeze-dried leaf litter were mixed with 400 mg of KBr, homogenized in a ball mill for 30 s and pressed to a pellet. FTIR spectra (as absorbance in units of cm^{-1}) were measured for wavenumbers of 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1} with 200 scans per sample. We subtracted a blank spectrum (pure KBr pellet) measured as a background every 5 samples. We normalized the raw spectra by dividing each peak by the square root of the sum of

all peaks, and corrected the baseline drift by the iterative restricted least squares method. Then, we extracted heights of seventeen peaks from 800 to 1800 cm^{-1} corresponding to main functional groups of carbohydrates, cellulose, lignin and phenolic compounds (Duboc et al. 2012; Liu et al. 2016).

We combined information from FTIR peaks and elemental analysis to perform a single principal component analyses (PCA). All chemical variables were z-standardized prior to PCA. Average scores of each single treatment on the first two PCA axes served as a 2-dimensional proxy of chemical composition. To capture composition of leaf mixtures we computed community-weighted means from the average of each involved treatment´s PCA scores weighted by its relative abundance in the mixture (see Stoler et al. 2016). As a proxy of the chemical diversity of leaf litter, we computed Rao's quadratic entropy (RaoQ; Laliberte & Legendre 2010) according to Stoler et al. (2016). RaoQ is a functional diversity measure that indicates the mean functional distance among a group of species weighted by their relative abundance. In our case, RaoQ is based on the mean Euclidean distance of the chemical traits (i.e. the PCA scores) of the treatments present in a litterbag:

$$RaoQ = \sum_{i=1}^{R}\sum_{j=1}^{R} \rho_i \rho_j d_{ij}$$

where d_{ij} is defined as the Euclidean distance between treatments *i* and *j* included in a set of *R* treatments, and ρ is the relative abundance of each treatment. RaoQ was considered 0 for litterbags containing single treatments. RaoQ was calculated using the package FD (Laliberte & Legendre 2010) in R 3.2.1 (R Core Team 2015).

Aquatic decomposition experiment

To measure aquatic decomposition of single treatments and mixtures, we incubated all litterbags in the Löcknitz River (52°24'43.7"N, 13°49'33.6"E) for 23 days at the end of August in 2014. Löcknitz is a forested, 3rd-order lowland river in the Elbe catchment (Germany). Litterbags were tied to iron rods and fixed on the riverbed in four randomly selected reaches of 50 m with running water and homogeneous substrate, depth and flow conditions. During incubation the water temperature oscillated between 13 and 17 °C, average dissolved oxygen concentration was always higher than 6.5 mg L^{-1}, conductivity and pH averaged 560 µS cm^{-1} and 7.5, respectively.

After retrieving the litterbags at approximately 50% average mass loss, leaves were washed individually in the laboratory with tap water above a 250 µm sieve to collect invertebrates, which were preserved in 70% ethanol. Individuals were counted, identified to family level and classified by guilds. The density of shredders was expressed as number of individuals per DM of leaf litter. The leaves from each litterbag were dried (105 °C, 48h) to obtain the final DM and then, compute leaf litter mass loss as the difference between initial and final litterbag DM divided by initial DM.

From leaves in fine-mesh bags we cut a set of 12 discs with a cork borer (10 mm) to measure fungal biomass as ergosterol according to Gessner et al. (2005). These leaf discs were frozen at -80 °C pending lyophilization and weighing. Lipids were extracted using a KOH-methanol solution at 80 °C for 30 min and purified using solid-phase extraction cartridges (Waters Sep-Pak®, Vac RC, 500 mg, tC18 cartridges, Waters Corp., USA). Ergosterol was eluted using isopropanol and quantified by HPLC with absorbance detection at 282 nm (Dionex UltiMate 3000 LC, USA). Values of ergosterol were expressed as µg g^{-1} DM.

Microbial respiration assay

184 Parallel to the decomposition experiment, we measured oxygen consumption rates of the
185 preconditioned leaves as a proxy for microbial respiration. To that end, we incubated 12 leaf
186 discs by mixture or single treatment in 250 mL sealed bottles filled with mineral water (Volvic)
187 at room temperature in a water bath. As microbial inoculum we used 10 mL of river water
188 filtered by 0.7 µm pre-combusted glass fiber filters (Whatman GF/F, Maidstone, UK). Dissolved
189 oxygen concentrations were measured 13 times over 24 days with a needle-based micro-optode
190 (Oxygen Microsensor PM-PSt7 mounted on a Microx 4 trace meter; PreSens, Germany). 10
191 bottles were filled with plain water as a control. Oxygen consumption rates (day^{-1}) were
192 computed as first order oxygen decay rates from log-linear regression models.
193
194 *Data analysis*
195 To analyze the response of leaf litter decomposition to the increase of treatment richness in
196 mixtures we used generalized additive models for location, scale and shape (GAMLSS) (Rigby
197 & Stasinopoulos 2005). Models were built using the treatment richness (1, 2, 4, 6 treatments) as
198 explanatory variable and for the response variables mass loss, fungal biomass, shredder density
199 and microbial respiration. GAMLSS allow to model effects on the average values (µ) of the
200 response variable as well as its variance (σ). We also applied GAMLSS to test for the
201 relationship between treatment richness and chemical diversity in mixtures.
202 We estimated expected values of all response variables for each mixture using observed
203 values in single treatments, and compared those to observed values in mixtures. Expected values
204 were computed as the weighted average of observed values of component treatments on a DM
205 basis. Non-additive effects of mixing were considered synergistic when observed values were

206 significantly higher than expected ones based on paired Wilcoxon signed rank tests (Gartner &
207 Cardon 2004).
208 Finally, to analyze the influence of chemical diversity and chemical composition on the
209 decomposition of both single treatments and mixtures we used general linear models that
210 included RaoQ (chemical diversity), PC1 and PC2 (summary of the chemical composition traits)
211 as predictors. For each response variable, we built an initial model that included all main effects
212 and first-order interactions between RaoQ and each PCA axis and then selected a top list of the
213 most parsimonious models using a multi-model inference approach (Grueber et al. 2011) using
214 the R package MuMIm (Bartón 2016). The top model set kept all models with delta AICc < 2 to
215 the best model. Finally, we used the natural method of model averaging to generate an average
216 model from the top model set. This way we obtained a robust, weighted mean for each predictor
217 coefficient and its errors based on AIC weights (Grueber et al. 2011). All explanatory variables
218 were z-standardized to obtain scaled, comparable average predictor coefficients. We finally
219 evaluated the effect of chemical diversity and chemical composition on leaf litter decomposition
220 by comparing the absolute magnitude and direction of the averaged predictor coefficients and
221 checking whether their 95% confidence intervals spanned zero.
222
223 **Results**
224 *Chemical diversification of leaf litter during preconditioning situations*
225 The PCA based on the chemical traits of leaf litter clearly separated the various preconditioning
226 treatments (Fig. 1A and 1B). PC1 (56 % of the total variance) principally separated treatments
227 T1 and T2 with the highest abundance of carbohydrates from T6 and T7, which had the highest
228 N- and P-content, and FTIR-peaks indicating the presence of lignin-like and phenolic-like

compounds. PC2 (28.8% of total variance) was mainly formed by the abundance of structural polysaccharides such as cellulose, and separated T5 from the other treatments. T3 and T4 were characterized by intermediate values of nutrients and structural C compounds.

As expected, GAMLSS identified a significant increase of the average and a reduction of the variance of chemical diversity with increasing treatment richness in mixtures (Fig. 1C), meaning that leaf litter mixtures of 2-treatment combinations had a lower average diversity but a higher variability than 6-treatment combinations.

Effect of treatment richness on aquatic decomposition of leaf litter mixtures

Increasing treatment richness in mixtures caused significant increases of the average values of leaf litter mass loss in coarse- and fine-mesh bags, of microbial respiration and of fungal biomass (Fig. 2A and 3). Shredder density was the only response variable not affected by treatment richness. Alongside effects on the average response, increasing treatment richness significantly reduced the variance of mass loss in fine-mesh bags and of fungal biomass (Fig. 3A & 3C). In contrast to observed values, expected values of mass loss did not respond to treatment richness in their mean but showed a decrease of the variance (Fig. 2B). This pattern was identical for all other response variables (data not shown). Positive, non-additive effects of mixing leaf litter were further identified for all response variables through significant differences between observed and expected values by Wilcoxon signed-ranked tests (Fig. S1).

Effects of chemical composition and chemical diversity on aquatic decomposition of mixtures

Model averaging identified chemical diversity (RaoQ) as the most important predictor explaining mass loss in coarse-mesh bags, fungal biomass and microbial respiration in single treatments and

mixtures (Table S1). For these three response variables, chemical diversity had the highest model-averaged coefficients, and its confidence intervals excluded 0, indicating a significantly positive effect (Fig. 4). In concert with other predictors, chemical diversity also had a significantly positive effect on mass loss in fine-mesh bags, and a positive (but not significant) influence on shredder density.

The chemical composition of leaf litter (average scores of PC1 and PC2) was the main predictor explaining mass loss in fine-mesh bags (significantly positive effect of PC2) and microbial respiration (significantly negative effect of PC1) (Fig. 4, Table S1). For these two response variables, model averaging also identified significant interaction of chemical diversity with chemical composition.

Discussion

Our laboratory treatments – intended to mimic the environmental heterogeneity typically emerging in intermittent rivers during drying – resulted in the strong chemical diversification of leaf litter as expected from previous works (Dieter et al. 2013; del Campo et al. 2019; Mora-Gómez et al. 2019). The greatest chemical differentiation in the PCA based on elemental and macromolecular composition of leaf litter (changes observed along PC1; Fig. 1B) was achieved between distinct terrestrial and aquatic habitat conditions, which are known to support contrasting rates of litter decomposition (Abril et al. 2016). Untreated leaf litter (T1: leaves entering water directly from riparian vegetation) or preconditioned under terrestrial conditions (T2: exposure to UVB radiation) retained a high content of labile C compounds such as carbohydrates, likely because of the limitation of microbial activity by water scarcity (Abril et al. 2016). Conversely, leaves in aquatic habitats with high temperature and nutrient concentration

(T6 and T7), lost carbohydrates due to leaching and microbial degradation (Dieter et al. 2013, Abril et al. 2016), and gained in nutrients and phenols by microbial immobilization (Webster & Benfield 1986; Mora-Gómez et al. 2019). In contrast, acidic and anoxic conditions in stagnant pools (T5) might curb microbial degradation and just induce the leaching loss of more soluble C compounds, resulting in leaf litter with high content of structural polysaccharides such as cellulose (Dieter et al. 2013), as reflected by the second axis of chemical differentiation in the PCA. Although we recognize the limitations of reproducing leaf litter preconditioning under laboratory conditions, our results challenge the general assumption that intermittent rivers are biogeochemically static during the dry phase; instead, this can be a critical period of chemical diversification of organic matter at the river reach and network scale.

Flow resumption reconnects dry tributaries to the river network and prompts the mixing of variously preconditioned leaf litter; further downstream, the mixed leaf litter may be retained again and subjected to further decomposition (Corti & Datry 2012). Our findings show that such diversified leaf litter packs experience accelerated decomposition under fully aquatic conditions through positive non-additive effects in a similar way as reported for mixtures of riparian leaf litter species (Gessner et al. 2010; Lecerf et al. 2011). Increasing preconditioning treatment richness in leaf litter mixtures accelerated mass loss (Fig. 2A and 3A) and stimulated fungal biomass and microbial respiration (Fig. 3); this was not true for any response variable´s expected values computed from single treatments (Fig 2B). Shredder density did not increase significantly along the gradient of treatment richness; but higher than expected shredder densities in leaf litter mixtures indicate a positive effect of leaf litter diversity on the detritivore community as well (Fig. S1I). These results demonstrate that the heterogeneity of preconditioning situations during

297 the dry phase facilitates increased activity of both microbial decomposers and detritivores
298 involved in later aquatic litter decomposition.

299 Increasing treatment richness of leaf litter mixtures also caused a decrease of the
300 variability in fungal biomass and mass loss in fine-mesh bags (Fig. 3A and 3C). This often-
301 observed outcome of manipulating resource (or species) richness emerges by dampening of
302 extreme contributions in more complex mixtures (see Dang et al. 2005; Lecerf et al. 2007). A
303 reduced variability in fungal-mediated decomposition with higher heterogeneity of
304 preconditioning situations translates to decreased spatial variability and increased stability of this
305 ecosystem process in downstream aquatic systems. We could not confirm this decrease in
306 variance with treatment richness for shredder density or mass loss in coarse-mesh bags as
307 response variables. This result may point to strong influence of individual leaf litter types on the
308 consumption of detritivores, which usually tend to preferentially consume leaf litter species
309 richer in labile C compounds or nutrients when present in mixtures (Swan & Palmer 2006;
310 López-Rojo et al. 2020).

311 Synergistic effects of leaf litter mixing on decomposition can arise mainly from
312 facilitative interactions among litter components with contrasting chemical composition (for
313 instance, by the transfer of nutrients by fungi from nutrient-rich to nutrient-poor leaves; Tonin et
314 al. 2017), or from complementary acquisition of resources (for example, by different leaf litter
315 species providing complementary, essential C compounds; Stoler et al. 2016). With our
316 experimental design, we cannot identify which mechanism drives the acceleration of leaf litter
317 decomposition by mixing; however, our results suggest chemical diversity as the main factor
318 stimulating decomposition. The increase in treatment richness in mixtures implied an increase in
319 chemical diversity (Fig. 1C). More importantly, chemical diversity was the main predictor in

averaged-models explaining mass loss in coarse-mesh bags, microbial respiration and fungal biomass (Fig. 4). These results are in line with previous studies where chemical diversity had a predominant influence on the decomposition of leaf litter mixtures (Lecerf et al. 2011; Stoler et al. 2016). We suggest that chemical diversity in mixtures of preconditioned leaves enhanced the activity of microbial communities by facilitating the acquisition of essential nutritional components for their growth and metabolism from multiple sources, such as nutrients, labile C compounds like carbohydrates, or long-lasting resources like cellulose (Gessner et al. 2010).

Our results also show a great importance of chemical composition controlling the microbial decomposition of leaf litter mixtures besides chemical diversity, as also found elsewhere (Frainer et al. 2015; López-Rojo et al. 2020). The averaged models identified a stronger influence of the chemical composition (PCA scores) than chemical diversity on the mass loss of leaf litter in fine-mesh bags and microbial respiration (Fig. 4). Specifically, PC2 scores had a positive effect on the mass loss in fine-mesh bags indicating a higher microbial activity associated with treatments and mixtures rich in cellulose (see Fig. 1B) (Talbot & Treseder 2012). On the other hand, the negative effect of PC1 on microbial respiration could be due to either inhibition by lignin and phenolic compounds (Talbot & Treseder 2012; Chomel et al. 2016), and/or positive influence of carbohydrates (Stoler et al. 2016).

Although intermittent streams and rivers are much less studied than perennial running waters, there is a growing body of evidence highlighting the role of these systems in the processing of terrestrial organic matter in drainage networks (Datry et al. 2018; Marcé et al. 2019). This is mostly because they accumulate large amounts of organic matter during the dry phase, which in turn, can trigger hot moments of microbial activity during flow resumption due to release of nutrients, particulate, and dissolved organic matter from riverbeds (Datry et al.

343 2018; Shumilova et al. 2019). Indeed, Datry et al. (2018) estimate that the inclusion of
344 intermittent and ephemeral rivers in organic matter decomposition models would increase annual
345 estimates of global CO_2 emissions from streams and rivers by 7-152%. All of these studies
346 associate hot moments during flow resumption to the quantity of organic matter accumulated on
347 dry riverbeds, but they do not consider the potential role of organic matter chemical quality as a
348 modulator of microbial activity. Our results demonstrate chemical alteration and diversification
349 of accumulated organic matter in the dry phase may have potentially far-reaching implications.
350 In fact, our results suggest that flow re-establishment in intermittent rivers triggers not only a
351 pulse of organic matter, but a pulse of chemical diversity, which is transported downstream
352 across the river network, and consequently may alter organic matter fluxes at regional scale
353 (Datry et al. 2017). As mixing of variously preconditioned leaf litter accelerates its
354 decomposition, the length of organic matter transport along the river network decreases. This
355 means a spatial compression of organic matter processing along the river continuum, which, in
356 fact, counteracts the classical view of the pulsed bioreactor model, where the organic-matter
357 processing length is considered to increase due to the little decomposition activity during the dry
358 phase and the far-reaching transport by flashy flow during rewetting events. A key element of
359 this hypothesis is the existence of a mosaic of terrestrial and aquatic habitats creating
360 environmental heterogeneity during the dry phase. Whether such diversified habitat conditions
361 exist and for how long is difficult to predict, as drying patterns in intermittent rivers vary among
362 years, bioclimatic regions and depending on catchment hydrology (Stanley et al. 1997). For
363 instance, in arid climates, the total drying of riverbeds is usually completed after a few days from
364 the beginning of flow fragmentation due to high summer temperatures (Gómez et al. 2005).
365 Consequently, in these rivers the short duration of the terrestrial-aquatic habitat mosaic may limit

366 chemical diversification of accumulating leaf litter. In any case, our results, together with the
367 expectations of future prevalence of intermittent rivers in river networks, reinforce the potential
368 relevance of intermittent rivers in global C cycling and the necessity (and difficulties) of
369 integrating them in larger scale modelling efforts.

370 The experimental character of our study precludes a strong assessment of implications.
371 Surely, future studies will have to achieve this under natural conditions, also considering the
372 influence of other factors acting at catchment scale such as land use or vegetation types (as these
373 may have a diversifying impact on leaf litter as well). However, to the best of our knowledge, we
374 prove for the first time that environmental diversity can promote chemical diversity, which in
375 turn has similar effects on important ecosystem processes such as decomposition as biodiversity
376 (Tanentzap et al. 2019).

377

378 **Acknowledgements**

379 We thank Jörg Gelbrecht and the staff of the Chemical Lab of the IGB-Berlin for their assistance
380 with laboratory analyses and experimental set up, Mark Gessner and his team for support with
381 ergosterol analysis and fruitful discussions, and Matthew Talluto for revising English. RdC was
382 funded by a Ph.D. contract (FPU R-269/2014) from the University of Murcia and supported by
383 the COST Action CA15113 (SMIRES, Science and Management of Intermittent Rivers and
384 Ephemeral Streams, www.smires.eu). RC was funded by the IGB Fellowship Program in
385 Freshwater Science. GS and RC conceived the study idea and designed the experiment; RC
386 conducted the field and laboratory experiments and collected the data; RC and RdC performed
387 laboratory analyses. RdC led data analysis with inputs of RC and GS; RdC led the writing of the

manuscript; GS and RC revised the manuscript critically and gave their final approval for publication. The authors declare no conflict of interest.

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508 Table 1. Summary of the preconditioning treatments used in the study to mimic the terrestrial-aquatic habitat mosaic appearing in

509 intermittent rivers during the dry phase.

Treatments	Riverbed habitat during the dry phase	Laboratory simulation	Physicochemical conditions in aquatic habitats
T1 Untreated	Vertical input of leaf litter shortly before flow resumption	Initially collected leaves, air-dried and kept at room temperature and in darkness.	
T2* UV	Dry riverbed exposed to intense solar irradiation	Irradiation for 12 h/day with a UV lamp (Cosmedico Arimed B6, Osram Biolux 965, Germany; with 31% UVB of total UV) at room temperature.	
T3 Moist	Shaded and humid riverbed habitats	Container with soil from the Löcknitz river floodplain moistened with 500 mL of tap water every 4 days and kept at room temperature.	
T4 Cold pool	Pool connected to hyporheic flow paths with cold and nutrient-poor water supporting limited algal growth	Aquarium filled with mineral water and stones with biofilm from the Löcknitz river. The aquarium was continuously illuminated, oxygenated by air-bubbling and kept at constant low temperature.	T = 15.3 °C DO = 9.45 mg L^{-1} pH = 7.94 Cond = 925 µS cm^{-1}
T5 Anoxic pool	Anoxic, stagnant pool	Container filled with mineral water and 8 mg of Na_2SO_3 per mg dissolved oxygen to create anoxic conditions, kept at room temperature and in darkness.	T = 24.6 °C DO = 0.15 mg L^{-1} pH = 5.5 Cond = 1650 µS cm^{-1}
T6* Wet/dry	Habitats subjected to wet/dry cycles associated to rain events	Alternating T2 and T3 every 7 days.	
T7 Hot pool	Disconnected pool with warm and nutrient-rich water supporting algal growth	Same conditions as T4, except that the aquarium was kept at room temperature and a nutrient solution (0.6 g L^{-1} of $NaNO_3$ and 0.3 g L^{-1} of KH_2PO_4) was added.	T = 25.1°C DO = 6.72 mg L^{-1} pH = 7.66 Cond = 800 µS cm^{-1}

510 T: water temperature, DO: dissolved oxygen, Cond: water conductivity. *The duration of preconditioning treatments was 21 days except for T2

511 and T6, which extended for 60 days, since terrestrial decomposition processes occur at a longer time scales than aquatic ones.

Figure legends

Figure 1. PCA describing changes in the chemical composition of leaf litter due to preconditioning under different treatments (A and B). (A) Variable loadings defining the PCA space. (B) Distribution of the preconditioning treatments across the PCA space. In (B), the colors of treatments represent their positions in PCA-space; similar chemical compositions of two treatments (e.g. T6 - T7) translates to similar colors and vice versa (e.g. T1 - T7) – this allows chemical interpretation of color in subsequent figures. (C) GAMLSS identified a significant increase of the average (μ, black line) and a significant reduction of the variance (σ, grey percentile lines) of chemical diversity (RaoQ) with increasing richness or preconditioning treatments. The colors in the pie charts used as symbols for mixtures indicate chemical composition as identified in (B).

Figure 2. Observed (A) and expected (B) values of mass loss in coarse-mesh bags in single treatments and mixtures along the treatment richness gradient. GAMLSS identified a significant increase of the mean (μ, black line) but no change in the variance (σ, grey percentile lines) of observed values of mass loss with increasing richness, while there was no change in the mean but a decrease in variance of the expected values. Colors indicate the identity of single treatments (simple dots) or the treatment composition of mixtures (pie charts); color codes in Fig. 1B.

Figure 3. Observed values of mass loss in fine-mesh bags (A), microbial respiration (B), fungal biomass in leaf litter (C) and shredder density (D) in single treatments and mixtures along the treatment richness gradient. GAMLSS identified a significant increase of the mean (μ, black line) for all four variables with increasing richness, but a decrease in the variance (σ, grey lines) only for mass loss in fine mesh bags and fungal biomass. Colors indicate the identity of single

534 treatments (simple dots) or the treatment composition of mixtures (pie charts); color codes in
535 Fig. 1B.
536 Figure 4. Model-averaged coefficients (mean ± 95% CI) of predictors explaining the mass loss,
537 microbial respiration, fungal biomass and shredder density of single treatments and mixtures.
538 Chemical diversity (estimated through RaoQ) was the most important predictor for mass loss in
539 coarse-mesh bags, fungal biomass and shredder density, while chemical composition features
540 (estimated through the average score of PC1 and PC2) were more important explaining mass loss
541 in fine-mesh bags (PC2) and microbial respiration (PC1).
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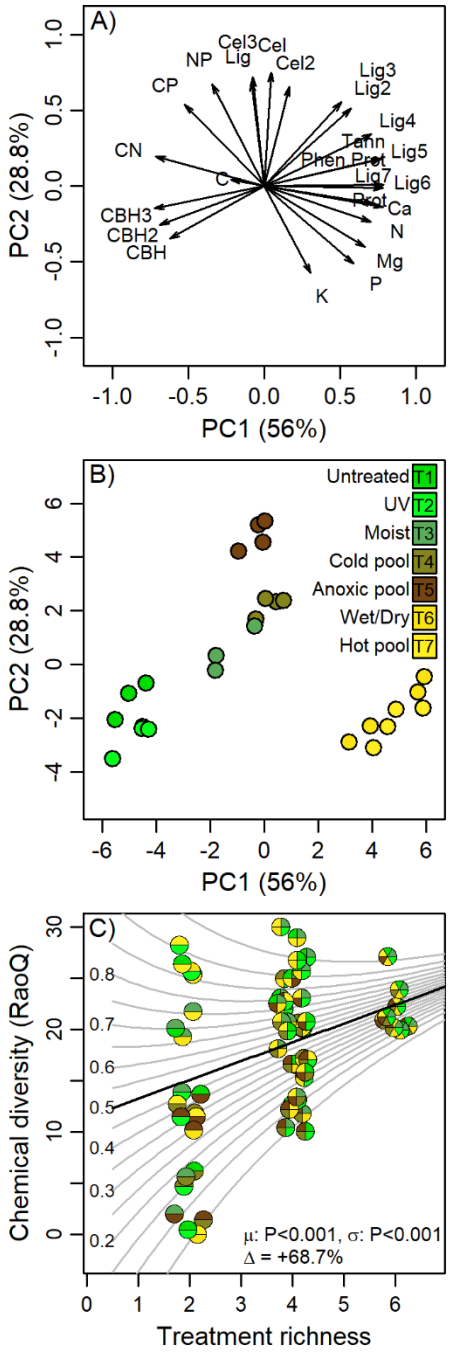
557 Figure 1.



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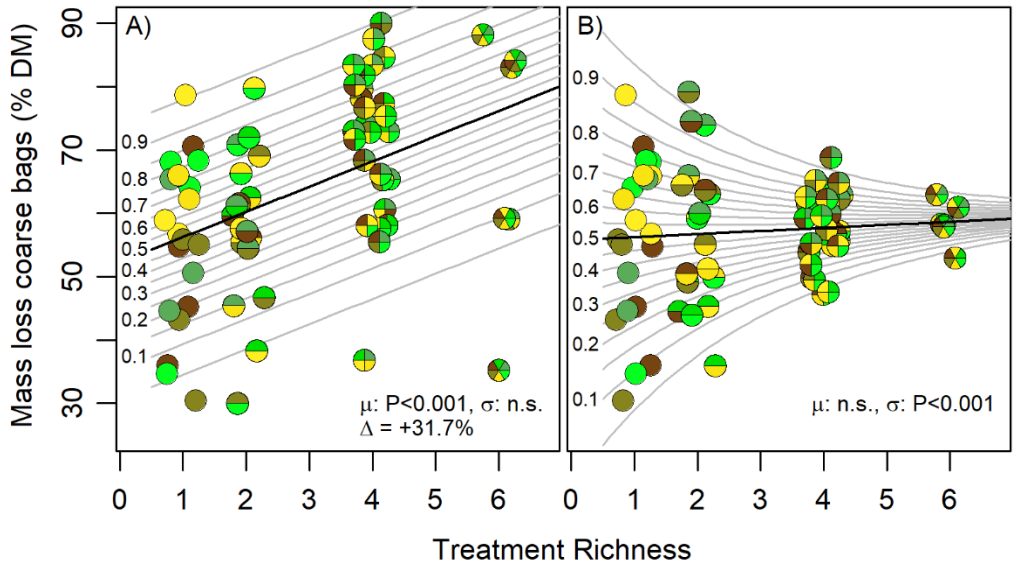
562 Figure 2



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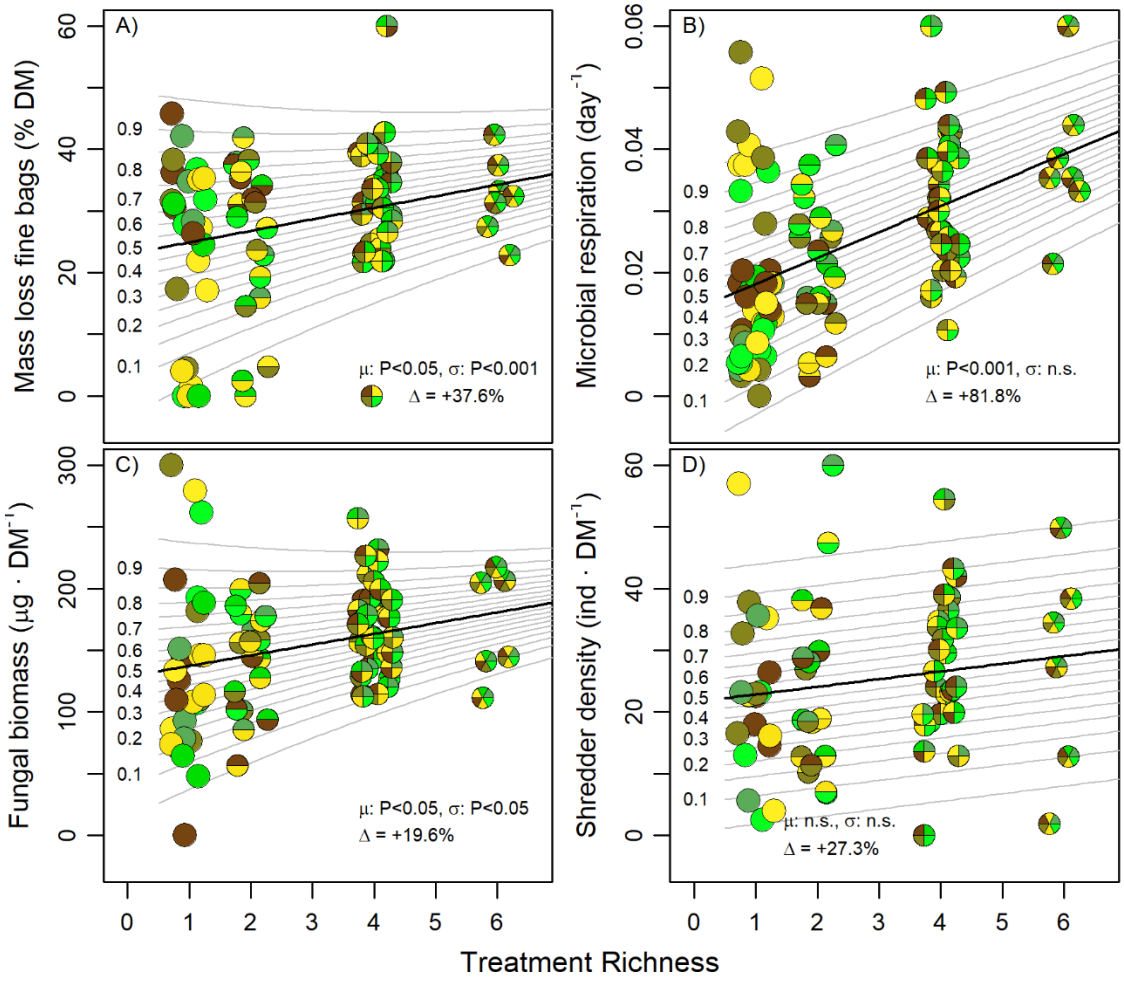
574 Figure 3



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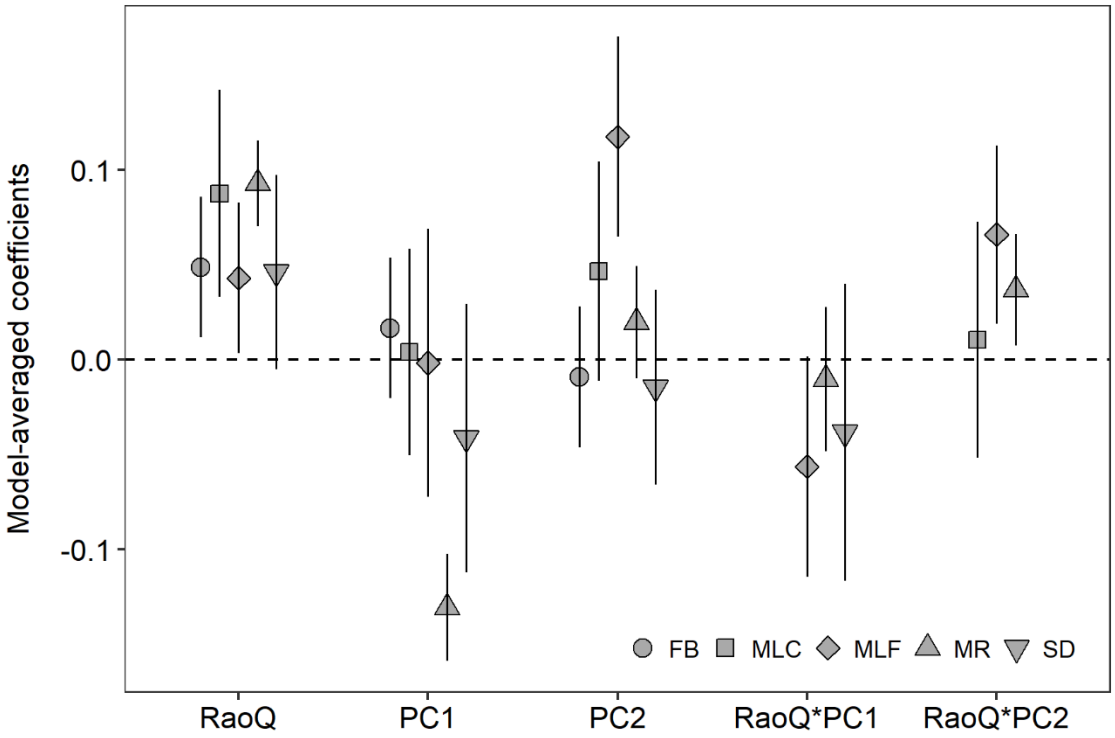
582 Figure 4


Model-averaged coefficients
0.1
0.0
-0.1
FB
MLC
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MR
SD
RaoQ
PC1
PC2
RaoQ*PC1
RaoQ*PC2

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