Title: Spatio-temporal patterns of multi-trophic biodiversity and food-web characteristics across a river catchment

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Abstract

Accurate characterisation of ecological communities with respect to their biodiversity and food-web structure is essential for conservation. However, combined empirical study of biodiversity and multi-trophic food webs at a large spatial and temporal resolution has been prohibited by the lack of appropriate access to such data from natural systems. Here, we assessed biodiversity and food-web characteristics across a 700 km^2 riverine network through time using environmental DNA. We find contrasting biodiversity patterns, with richness (α-diversity) of fish increasing towards downstream positions within the catchment, while freshwater bacteria and invertebrates having an invariant and minimal decrease in richness, respectively, with downstream position. Food-web characteristics, such as link density and nestedness, however, were relatively conserved across space, but varied over season. Patterns of biodiversity across major taxonomic groups are thus not directly scalable to food-web structures at the same spatial and temporal scales, indicating that effective conservation measures must consider them jointly.

Introduction

The study of biodiversity patterns[1–3] and the characterisation of food-web structures[4,5] are essential, yet often disconnected goals in ecology. Understanding these patterns is not only of fundamental interest, but also needed to predict stability, functioning and resilience of natural ecosystems and to bend the curve of biodiversity loss in the context of anthropogenic pressures including contemporary global change[6].

Studies on biodiversity predominantly focus on analyses of α-, β- and γ -diversity and possible underlying fundamental drivers of their spatial or temporal patterns[7]. Freshwater rivers are highly spatially structured systems[8–10] in which theoretical and empirical studies have identified characteristic patterns of biodiversity for specific groups. For example, fish α-diversity has been found to increase with distance downstream[11], whereas headwaters often show high endemic bacterial species richness[12]. Aquatic invertebrate biodiversity exhibits more complicated overall patterns with disproportionately high biodiversity being found in headwaters[13] and a significant increase in biodiversity linked to catchment size[14]. However, these group-specific biodiversity patterns have been mostly studied in isolation from one another, although species are present within the same system and trophically interact with each other. Indeed, recent theoretical work shows that contrasting patterns driven by species' resource competition are possible[15]. Therefore, to ensure optimal strategies for conservation and understanding of biodiversity patterns across different organismal groups, an ensemble approach integrating major taxonomic and trophic groups is crucial to reveal how species are linked through trophic interactions[16].

Trophic interactions and food webs by definition encompass multiple groups of organisms. Individual freshwater food webs are well-resolved[17], and often exhibiting distinct features, such as highly nested structures[18] and prevalent omnivory[17,19]. Nevertheless, food-web studies often have a localised perspective due to methodological limitations of sampling food-web interactions and organismal occurrence in a standardized and comparable manner across different places and organismal groups[20–22]. Due to the same reason, these studies also tend to focus on simple spatial and environmental gradients[23] or temporal change[24] when spatio-temporal influences should be considered in conjunction[25,26]. This is particularly problematic in freshwater riverine ecosystems, that are characterised by a high spatio-temporal structure: they exhibit characteristic spatial structures, and have strong seasonal variations driven by changing abiotic conditions[27] and pronounced life-cycle changes of key taxa inhabiting these systems. The variation in dynamics over the course of a year remains a significant gap in our understanding of freshwater food webs[17,28].

To effectively conserve riverine biodiversity, we must encompass spatio-temporal variation of multiple trophic levels to understand the underlying dynamics of both biodiversity patterns and food-web characteristics[4,25,26]. In particular, molecular monitoring techniques may now provide a suitable solution to break through the above-mentioned methodological constraints caused by sampling based primarily on species sight or capture. Environmental DNA, or eDNA, is the collection of DNA extracted from an environmental sample such as water, air or sediment[29]. By collecting eDNA we can screen samples for multiple taxonomic groups via metabarcoding[30], thereby creating a biodiversity assessment suitable for food-web reconstruction.

Here, we use eDNA metabarcoding to assess patterns of biodiversity and reconstruct local food webs via a metaweb-based approach[31]. The metaweb approach is suitable to capture food-web patterns based on taxa co-occurrence data[24,32] in both terrestrial and aquatic systems. We used high-throughput sequencing and a multi-marker approach to examine three taxonomically relevant groups, namely fish (via the 12S barcode region), invertebrates (cytochrome *c* oxidase I - COI) and bacteria (16S) in a large-scale river network over the course of three seasons (spring, summer and autumn). This approach allowed us to test for association of biodiversity patterns and food-web structures with network location and seasonal change. We found contrasting effects on biodiversity patterns and food-web structure from spatial and temporal influences, respectively, providing insight into the underlying changing ecosystem dynamics and indicating that effective and targeted conservation measures must consider them jointly.

Results

Data collection and community construction

We collected water samples from the upper Thur catchment, Switzerland, which covers approximately 700 km^2 and is made up of three sub-catchments: River Thur, Necker and Glatt. Seventy-three field sites were selected to allow for maximum coverage across the catchment area, comprising of a broad size range of upstream drainage sizes (i.e., the drainage area size indicates the location of the site within the catchment: upstream sites having a small drainage area and lowland sites having a larger drainage, see Fig. 1). Water samples were filtered on site and DNA extracted in a specialist clean lab environment at Eawag, Switzerland. A total of 12.29 million, 14.32 million and 14.02 million raw reads were produced from the 12S, COI and 16S

libraries, respectively. After bioinformatic processing the average sequencing read depth per sample was: 43,176, 50,704 and 49,857 for 12S, COI and 16S, respectively. This included 159 Zero-radius Operational Taxonomic Units (ZOTUs), 3179 ZOTUs and 11,320 ZOTUs for 12S, COI and 16S respectively (see Methods and Supplementary Information Table S1 for details on laboratory and bioinformatic analysis). For further analysis, ZOTUs were merged to genus level and only fish genera, invertebrate genera with an aquatic life stage and bacteria associated with freshwater were kept for further analysis (See Methods for further details). Analysis was performed on presence/absence data to merge the three libraries for the complete freshwater community and exclude a possible influence of uneven sample read depth generated from multiple markers (see also [33]).

To quantify biodiversity, we calculated α-diversity (local richness) at genus level at each site and compared β-diversity (variation in community composition between sites) by using Jaccard dissimilarity. Jaccard dissimilarity was also partitioned into taxon replacement (turnover) and taxon loss (nestedness) components to assess the mechanisms contributing to the variation in community assemblage across the catchment.

To measure food webs and functional characteristics of the community, we constructed a metaweb based on known interactions of genera classified into different functional feeding groups (Fig. 2; see also Methods and Supplementary Information Table S2). We defined local food webs at each field site based on genera co-occurrence and the corresponding subset of interactions from the metaweb, to determine broad changes in the community over time and

space. With this approach, variation in food-web structures and function feeding groups emerge from the spatio-temporal differences in genus composition (See Methods for further details).

To assess the relationship between α-diversity of each group (fish, invertebrates and bacteria) and food-web structures with site location within the catchment, we ran linear mixed model analysis. Drainage area (km^2) was log transformed to fit model assumptions. For each dependent variable, drainage area and season were the fixed effects, while site was the random effect. We determined the overall effect of both factors using analysis of variance and contrast testing of estimated marginal means to determine the influence of seasonal changes on all α-diversity and food-web elements (see Methods for details). To examine the effect of river distance on β-diversity we constructed a matrix of pairwise distances for sites that were connected along the fluvial network and to examine the effect of river distance on β-diversity we performed a Mantel test (see Methods and Supplementary information).

Spatial and temporal biodiversity patterns

In total we detected 374 genera across all organismal groups associated with freshwater, including 12 fish genera, 80 invertebrate genera and 282 bacteria genera. When combining all seasons, α-diversity (genus richness) ranged between 8–96 genera with all taxonomic groups combined (Fig. 1). Over the different seasons, mean local α-diversity was 70 (range 10–92) in spring, 48 (range 8–85) in summer, and 63 (range 19–96) in autumn (See Supporting Information Fig. S1 and Table S3). We used mixed models to assess the influence of drainage area and season on the local α-diversity of each group (Fig. 3, for model output see Supporting Information Table S4–S6). Of the three taxonomic groups, only fish α-diversity significantly

154 increased with the size of the drainage area ($p < 0.001$, Fig. 3a and Supporting Information for
155 model output Table S4– S6), while for bacteria and invertebrates there was no significant
156 relationship ($p = 0.670$ and $p = 0.239$, respectively, Fig. 3c and 3e). There was, however, a
157 significant seasonal effect in α-diversity on invertebrate and bacteria genera ($p < 0.001$ for both
158 groups, Fig. 3d and 3f, see Supporting Information Table S4–S6). The influence of seasons can
159 be seen further in the contrast testing (Fig. 3b, d and f and Supporting Information Table S7 for
160 full contrast testing results), which shows invertebrate α-diversity in spring was significantly
161 higher compared to summer and autumn ($p < 0.001$, Fig. 3d), and bacteria α-diversity in summer
162 was significantly lower compared to spring and autumn ($p < 0.001$, Fig. 3f). Fish α-diversity
163 however did not vary significantly between seasons, with the mean number of 2 (range 0–6) fish
164 genera remaining constant (Fig. 3b).
165
166 Regarding the β-diversity across the catchment, the Jaccard's dissimilarity significantly
167 increased with the river distance among sites in all organismal groups across all seasons, apart
168 from bacteria in spring and autumn (fish Mantel statistics: Spring 0.143, $p < 0.01$, Summer
169 0.171, $p = 0.001$, Autumn 0.321, $p = 0.001$; invertebrate Mantel statistics: Spring 0.095, $p < 0.05$,
170 Summer 0.169, $p = 0.001$ and Autumn 0.114, $p < 0.05$; bacteria Mantel statistics: Spring -0.058,
171 $p = 0.834$, Summer 0.179, $p < 0.005$ and Autumn 0.069, $p = 0.12$, See Supplementary
172 Information Table S8). Further partitioned analyses on taxon replacement and loss revealed
173 contrasting patterns. Taxon replacement between sites increased over river distance for all
174 groups in most seasons (significant or marginally significant, see Table S8), apart from fish in
175 spring (Mantel statistics 0.07, $p = 0.891$) invertebrates in spring (Mantel statistics 0.066, $p =$
176 0.051) and bacteria in spring and summer (Mantel statistics 0.016, $p = 0.34$ and Mantel statistics

177 0.07, p = 0.068, respectively). In contrast, taxon loss was only found to significantly increase
178 over river distance for fish in spring and autumn (Mantel statistics 0.219, p = 0.001 and 0.059, p
179 = 0.05, respectively) and bacteria in summer (Mantel statistics 0.089, $p < 0.05$).
180
181 *Spatial and temporal changes in food-web structure and functional characteristics*
182 We examined commonly used food-web structural characteristics (link density, connectance,
183 nestedness, omnivory, coherence, number of links, modularity, and robustness), as well as
184 functional characteristics (functional diversity and redundancy). Functional characteristics were
185 examined by using the designated functional feeding groups (e.g., shredders and omnivorous
186 fish), based on specialised feeding behaviours (Fig. 2 & Supporting Information Fig. S2). Here
187 we describe the results of link density, connectance, nestedness and omnivory as the most
188 ecologically important food-web descriptors in our study, while the results from the remaining
189 descriptors (coherence, number of links, modularity, and robustness) are presented in the
190 Supplementary Information (See Fig. S3). Drainage area did not significantly influence any of
191 the food-web structures looked at (Fig. S3 and S4). However, season had a significant influence
192 on the change in food-web structures (Fig. 4 and S4), namely, link density ($p < 0.001$, Fig. 4a),
193 connectance ($p < 0.001$, Fig. 4b), nestedness ($p < 0.001$, Fig. 4c), and omnivory ($p < 0.001$, Fig.
194 4c and see Supporting Information Table S4 - S6 for full results), indicating that seasonal
195 variation is of more importance in food-web dynamics than site location within the riverine
196 network. To examine seasonal variation further, we carried out contrast testing, which showed a
197 range of seasonal change in food-web dynamics. In particular, spring had significantly higher
198 link density than summer and autumn ($p < 0.001$ and $p < 0.001$, respectively, Fig. 4a); autumn
199 had significantly lower connectance than spring and summer ($p < 0.001$ and $p < 0.001$,

respectively, Fig. 4b), and the same is true for nestedness ($p < 0.01$ and $p < 0.01$, respectively, Fig. 4c); finally, summer had significantly higher omnivory than autumn ($p = 0.001$, Fig 4d, see Supporting Information Table S7 for full contrast testing results).

Functional diversity (defined as the change in function feeding groups used in this study) significantly decreased with drainage area and was also significantly different across seasons ($p < 0.05$ Fig. 5a and $p < 0.001$ Fig. 5b, respectively, see Supporting Information Table S4 - S6 for full results), whereas only season has a significant influence on the functional redundancy ($p < 0.001$, Fig. 5d). To further examine the changes in seasonal influence we compared seasonal values for all functional characteristics. Functional diversity in spring was significantly higher than in summer and autumn ($p = 0.001$ and $p = 0.001$, respectively, Fig. 5b), while functional redundancy in summer was significantly lower than in spring and autumn ($p < 0.001$ and $p < 0.001$, respectively, Fig. 5d and Supporting Information Table S7).

Discussion

Studies of biodiversity and food-web assemblages in riverine networks are often constrained to local scales or aggregated to a single time point, which in essence fails to capture the spatial processes and the temporal fluctuations that together play a key role in community dynamics present within a river network[8]. Our study is a first assessment utilising data derived from eDNA metabarcoding to detect patterns of biodiversity and food-web characteristics across three major taxonomic groups, namely fish, invertebrates and bacteria, in a whole river network at a spatial and temporally large scale. In our study we find contrasting patterns of biodiversity across these

223 groups, which indicates different mechanisms may shape these organismal communities. By
224 using a metaweb approach, we showed a strong signature of seasonality in food-web structures
225 across the river network. We also showed that functional characteristics are influenced by both
226 spatial and temporal changes. Overall, our study supports the need to include both spatial and
227 temporal scales in order to understand changes in ecosystems, particularly as we see increased
228 effects of contemporary global change.
229
230 Aquatic biodiversity is subject to fluvial influences within a dendritic network, whereby spatial
231 patterns of α-diversity and β-diversity are known for key groups, such as fish, invertebrates and
232 microbes[11–14,34]. Our data is congruent with previous studies on fish diversity[11,33], in that both α-
233 diversity significantly increased downstream, and β-diversity (community dissimilarity)
234 significantly increased with river distance in all three seasons. However, patterns for
235 invertebrates and bacteria differed from the predictions, and exhibited no significant influence of
236 drainage size on α-diversity but were influenced by seasonal change. For invertebrates, seasonal
237 variation can be linked directly to the emergence of the non-aquatic adult life stage of several
238 macroinvertebrate genera we detected (Diptera, Ephemeroptera, Plecoptera and Trichoptera),
239 which takes place in the late spring and summer months[27], and literally removes these organisms
240 from the aquatic food-web as they become air-bound and often end up in terrestrial food-webs.
241 For the spatial patterns, past studies often looked at spatial scales much larger or much smaller,
242 and thus disparity may be due to a mismatch in scale looked at here. For example, Finn and
243 colleagues[13] suggested headwaters harbour disproportionately high invertebrates compared to
244 lower points in the catchment, but their studied focused on small (1–2) and mid (3–4) stream
245 orders only, whereas the sites in our study ranged from small to much larger rivers (stream

246 orders 1–7, [35]). Contrastingly, when looking at scales about 50-fold larger, Altermatt and
247 colleagues[14] showed the number of key aquatic invertebrate taxa (Ephemeroptera, Plecoptera and
248 Trichoptera) increased with catchment size, however they also found a combination of local
249 factors (catchment areas, drainage area, elevation and network centrality) had the greatest
250 influence on local invertebrate α-diversity. Possibly, the scale at which we study invertebrate α-
251 diversity and contributing local factors falls between such small-scale vs large-scale perspective,
252 but instead we detected regional patterns where α-diversity remains relatively constant
253 throughout the catchment. Similarly, drainage area did not have a significant effect on bacterial
254 α-diversity, and β-diversity increased with river distance in summer only. We can therefore
255 postulate that aquatic bacteria are able to persist as they disperse through the catchment;
256 however, the number of bacteria genera (the α-diversity) that do persist are subject to seasonal
257 influences. Our findings expand on past studies, highlighting the influences of scale and
258 temporal variation on different groups, which is of utmost importance when trying to conserve
259 biodiversity and understand the differing drivers behind these patterns in a river network.
260
261 By resolving the fundamental trophic relationships among broad feeding groups, we established
262 a trophic interaction metaweb for the three taxonomic groups examined in this study. The meta-
263 web with genus co-occurrence data thus allowed our investigation of both spatial and temporal
264 influences on the characteristics of local food webs. We found that the most significant factor
265 driving freshwater riverine food-web characteristics was season. All food-web structural
266 characteristics (apart from the number of links and robustness) were lowest in autumn, despite
267 overall α-diversity being lowest in summer. This indicates that the food-web structural variation
268 we detected is not merely reflecting the genera richness dynamics over seasons, but the genera

composition dynamics instead. In other words, the structures of the food webs are more influenced by those genera absent in autumn. Indeed, previous studies on freshwater food webs that have examined temporal changes have often found reduced productivity as the main driver behind a declined food-web structure in winter vs summer[20,36]. Thus, the decrease in structure we captured in this study could be the start of the productivity restriction seen in food webs over the winter months. The less-connected and less-nested food-web structure also implies weaker resource competition among consumers, which may be necessary for their coexistence in the food web[37], especially when competition becomes more costly with lower resource productivity in autumn.

Moving from broad food-web patterns to examining functional feeding groups enables us to study fundamental changes within the community and possible effects at the ecosystem level[17]. Interestingly, and contrary to the biodiversity patterns and food-web dynamics, we see significant effects of both drainage area and season on the functional diversity, which we found to be higher in smaller drainage areas and in spring. The spatial part of such a pattern along the hierarchical river network is demonstrated in the River Continuum Concept, which shows increased diversity of invertebrate functional feeding groups at low stream orders[38,39]. Whereas both drainage area and season influences functional diversity, season is the only significant influence on functional redundancy, with fewer genera per functional feeding group observed in summer rather than in autumn. These results indicate that in summer the genera absent are across functional groups, whereas in autumn the genera absent include whole functional feeding groups leading to the constriction of local food webs. The inconsistent temporal patterns of food-web structure versus functional characteristics further imply that multiple feeding groups share

similar trophic roles in the food web (Fig. S2), though they each adopt specialised feeding behaviour within the catchment and thus perform distinctive ecological functions (e.g., shredders, collectors, filterers). In other words, the structure and function of a food web does not necessarily match and synchronise (*sensu stricto*[22,40]). These results are particularly encouraging, because the patterns of both food-web structure and functional diversity are known to be important for ecosystem health assessment and identifying potential vulnerability to perturbation[17,26,41]. Addressing the consistency of their patterns across spatial and temporal scales will likely lead to novel and comprehensive understanding of biodiversity and ecosystem function loss due to environmental change.

In our study, the functional feeding groups were defined at the level of genus, while we expect investigations at finer resolutions will be promising for future work that can reveal not only more accurate patterns, but also the influences of sampling taxonomic resolution. Similarly, our selection of focal taxa may have influenced the food-web patterns we detected. For example, algae become an increasingly important resource when moving from allochthonous inputs in headwaters to larger streams with increased light levels further down the catchment[38,39]. With the selected three key taxonomic groups in riverine ecosystems, we present a broad and relevant view capturing trophic roles from basal resources (cyanobacteria) to top consumers (piscivorous fish), captured by three relatively broad metabarcoding markers. However, we also by default excluded some further groups, such as algae or terrestrial taxa, which could have been relevant as primary producers and as terrestrial-aquatic linkages, respectively. The choice of taxonomic groups looked at was both methodologically defined, as well as driven by the goal to have an

overseeable and clearly aquatic-focused view on food webs. Thereby, it captures by default a subset from the real-world food web in which more species are involved.

By using the eDNA technique, we gain three notable advantages: its scalability for monitoring complex and large systems, its reusable nature, and its being a non-invasive method of collecting biodiversity information[42,43]. Our understanding of how the information we ascertain from eDNA sample collection has greatly improved in recent years due to studies on the hydrological influences[44] and a general understanding of the rate of eDNA persistence in lotic ecosystems[45]. However, the successful detection of taxa with eDNA is also linked to the ecology of individual species[45], and some seasonal variation in the detection of several taxonomic groups is known[46–49]. Therefore, it is possible that some taxa that were not detected in the colder season (autumn) in this study were false-negative records. However, these non-detections are likely linked to low abundance or low metabolic rates, and thus these species are, while not physically absent, at least "relatively absent" in ecological terms.

In summary, our work showed we can construct comprehensible food webs at a large scale and over time by using eDNA sampling and combining multiple markers. Based on the biodiversity patterns we observed that spatial and temporal influences are different across groups. Furthermore, temporal influences were the significant driver of change for commonly used food web descriptors. Our approach is a first demonstration of the application of eDNA to a complex river network for the reconstruction of food web patterns and can be easily replicated in other systems worldwide. As biodiversity in freshwater systems face huge threats from anthropogenic pressures, including global climate change, establishing vital information on the changes in

biodiversity and food web composition over spatio-temporal scales is essential for the detection of these stressors in order to protect and conserve river systems.

Methods

Site selection and eDNA sample collection

Environmental DNA samples were collected from the edge of the waterbody using single use disposable 50 ml syringes. At each site on each sampling event, a total of 1 L of water was filtered through two 0.22 µm sterivex filters (Merck Millipore, Merck KgaA, Darmstadt, Germany), sealed with luer caps and placed in individually labelled bags. Sampling was carried out over five consecutive days in May (spring), August (summer) and October (autumn) in 2018. During the summer sampling campaign, four sites were dry and could not be sampled (total samples across all seasons is n = 215). Negative field control samples were collected on each sampling day by filtering 500 ml of ddH_2O through a sterivex filter in the same way as field samples were collected (n = 15). Negative field samples were processed alongside field samples. All samples were placed in labelled bags and cooled in a cool box until frozen at -20 °C on return to the laboratory.

Extraction and library preparation

DNA extraction and first round PCR set up was carried out in a specialist eDNA clean lab, with separate lab facilities for post PCR workflows. Samples were extracted using DNeasy PowerWater Sterivex Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Prior to extraction, each sterivex was defrosted at room temperature and then wiped with 10%

bleach, then 70% ethanol solution prior to extraction to remove any DNA from the outside of the filters. Extraction controls were carried out alongside sample extractions and consisted of blank sterivex filters (n = 5). Samples selected at random were analysed using a QuBit 3.0 fluorometer for double stranded DNA concentration, values measured between 0.317 - 27.5 ng/µl. All negative controls (field and extraction) were tested and recorded below detection limits.

Samples were sequenced with the following markers: a 106 bp fragment of the mitochondrial 12S marker ([50], hereafter referred to as 12S) used to amplify vertebrate DNA, a 313 bp fragment of the mitochondrial cytochrome oxidase I marker ([51] and [52], hereafter referred to as COI) used to amplify metazoan DNA and a 450 bp fragment of the V3-V4 region of the 16S marker (hereafter, 16S) used to amplify bacteria and archaea DNA (See Supporting Information Table S8 for primer sequences). Positive controls (n = 6 per library prep, see Supporting Information Table S9) and PCR negative controls (2 µl of ddH_2O, n = 11 per library prep) were included in each library. Each library consisted of 252 samples in total (including positive and negative controls).

Library preparation followed a two-step PCR process for both the 12S and COI markers, the 16S library was carried out using a three-step PCR[53] (See Supporting Information Methods for full details), all samples were amplified in triplicate. After the initial amplification, where Nextera® transposase sequences (Microsynth, AG, Balgach, Switzerland) are added to the PCR product, all samples were tested for amplification success with the AM320 method on the QiAxcel Screening Cartridge (Qiagen, Germany). PCR products were cleaned using ZR-96 Plate clean-up Kit (Zymo Research) following the manufacturers protocol with the minor modification by which

the elution step was prolonged to 2 minutes at 4000 g. The clean amplicons were indexed using the Illumina Nextera XT Index Kit A, C and D following the manufacturer's protocol (Illumina, Inc., San Diego, CA, USA). A reaction contained 25 µL 2x KAPA HIFI HotStart ReadyMix (Kapa Biosystems, Inc., USA), 5 µL of each of the Nextera XT Index adaptors and 15 µL of the DNA templates. The final PCR products were then cleaned using the Thermo MG Magjet bead clean up kit (Thermo Fisher Scientific Inc., MA, USA) and a customized programme for the KingFisher Flex Purification System (Thermo Fisher Scientific Inc., MA, USA) in order to remove excessive Nextera XT adaptors. The cleaned product of 50 µl was then eluted into a new plate and stored at 4 °C prior to quantification. For each library, the clean indexed amplicons were quantified in duplicate on a Spark Multimode Microplate Reader (Tecan, US Inc. USA) prior to equimolar pooling using the BRAND Liquid Handling Station (BRAND GMBH + CO KG, Wertheim, GE). Negative extraction and PCR controls were added according to their concentration. Library concentration was quantified on the Agilent 4200 TapeStation (Agilent Technologies, Inc., USA) and verified by the Qubit the HS Assay Kit. Paired-end sequencing was performed on an Illumina MiSeq (Illumina, Inc. San Diego, CA, USA) at the Genetic Diversity Centre at ETH (See supporting information Table S1 for library loading information).

Bioinformatics

After each of the libraries were sequenced, the data was demultiplexed and reads were quality checked using usearch v11.0.667 [54] and FastQC[55]. Raw reads were first end-trimmed, merged and full-length primer sites were removed using usearch v11.0.667 [54] (16S reads were merged using Flash v1.2.11, [56]). The merged and primer trimmed reads were quality filtered using prinseq-lite (0.20.4). The UNOISE3 (usearch v11.0.667) workflow with an additional clustering

of 99% (usearch v11.0.667) identity was applied to obtain error corrected and chimera-filtered sequence variants ZOTUs. The ZOTUs were then clustered using a 97 %, similarity approach and taxonomic assignment with a 0.85 confidence threshold was performed using SINTAX in the usearch software v11.0.667 [54] with following databases: 12S: NCBI BLAST (v200416), COI: Custom reference database (Including MIDORI un-trimmed (V20180221) and 16S: GreenGenes (V13.5), RDP (160411) and SILVA (V128). See Supporting Information Methods for all bioinformatic parameters and reference database information used for each library. Prior to downstream analysis, 0.1% of reads were removed from each sample to reduce errors caused by tag-jumping or minor contamination in library preparation[57].

Biodiversity patterns

We calculated α-diversity (genus richness) at each site and compared β-diversity between sites by using Jaccard dissimilarity using the betapart R-package[58]. We constructed a matrix of pairwise distances between sites along the fluvial network with the igraph R-package[59] and compared the similarity between β-diversity and river distance using the Mantel test with the vegan R-package[60]. To further partition β-diversity into species loss and replacement, sites which did not record genus from a target group were removed from the analysis of that group only.

Feeding group assignment

Feeding groups were determined based on literature, species inventories and targeted expert knowledge[61,62] for fish and invertebrates. Genera of bacteria were included if the phyla they belong to has a strong association with freshwater habitat[63], bacteria were then broadly divided into heterotrophic and cyanobacteria, the latter constituting a basal resource. A constant basal

resource of detritus was also included in all food webs (See Supporting Information Table S2 for assignment of genera to feeding groups).

Food-web structure

We constructed a metaweb based on known trophic relationships among feeding groups (Supplementary Table S2), then defined local food webs using this metaweb at each field site, based on co-occurrence of genera (nodes) and their interactions (links). The metaweb approach has been applied to identify food-web characteristic change across spatial gradients in terrestrial ecosystems[32], and temporal changes in aquatic ecosystems[24], but has yet to be used at a large spatial scale in an aquatic ecosystem over time.

We then quantified common food-web structural characteristics for each local food web generated. These included fundamental node-link composition features, i.e., number of links (L), link density (L/S, the number of feedings links per taxa divided by the genus α-diversity at each site) and connectance (L/S^2, the proportion of realised interactions). To further explore the holistic topology of these food webs and their change over space and time, we adopted the following indices to determine some more key features. Nestedness is an indication that the diets of specialist taxa are subsets of generalist taxa's diets, and was calculated using the nestedness metric based on Overlap and Decreasing Fill (NODF) function in vegan[60]. Modularity is indication of a less nesteded network[64] in that the nodes are characterised into modules which, unlike nested structures, do not overlap, and was calculated using the multilevel.community() function from the igraph package[59]. Omnivory is defined as the proportion of taxa with a mixed trophic level diet. Thus, the more omnivores a community has, the less coherent the food web[65].

Omnivory is often debated as a measure of food-web stability, and weak omnivorous interactions are likely to lead to a more stable food web[66]. Trophic level of genera and omnivory were calculated using the methods stated in William and Martinez[21]. Coherence is defined as the overall degree of homogeneity of the difference between trophic levels of every consumer-resource pair within the local food web. As described by [65], the coherence of a network can reliably be used to establish the stability of a network by looking at the distribution of trophic distances over all links in each network. For example, a perfectly coherent network (q = 0) implies that each taxon within the food-web only occupies a single trophic level. Coherence was calculated using the methods stated in Johnson et al[65]. Robustness was defined by Dunne and Williams[5] as the "*proportion of species subjected to primary removals that resulted in a total loss … of some specified proportion of the species*". In our study we used 50% as the proportion of species lost and excluded basal resources for primary removal. Robustness was calculated using the methods stated in Dunne and Williams[5].

To explore the change in functional characteristics within the food webs, we used the broad feeding groups as described when constructing the interaction network (See Fig. 1 and Fig. S2). Functional diversity was calculated as the number of feeding groups that had at least one genus present in the sample. Functional redundancy was calculated as α-diversity divided by functional diversity, reflecting the average number of genera within a feeding group.

Data analysis

We ran linear mixed model analysis to assess the relationship between group genus α-diversity (bacteria, invertebrates and fish), food-web structural characteristics (coherence, connectance,

475 link density, number of links, omnivory, modularity, nestedness and robustness) and functional
476 characteristics (functional diversity and redundancy) with site location within the catchment.
477 Drainage area (km^2) was log transformed to fit model assumptions. For each dependent variable,
478 drainage area and season were the fixed effects. We included site as a random effect to account
479 for repeated sampling of sites (e.g., genus α-diversity ~ drainage area + season + (1 | Site)).
480 Significance was calculated using the lmerTest R-package[67], which applies Satterthwaite's
481 method to estimate degrees of freedom and generate p-values for mixed models (Table S4 and
482 S5). We then used anova() to see the overall effect of both factors: Drainage area and Season
483 (Table S6) and follow up contrast testing were carried out using the emmeans R-package [68] to
484 test for significant differences between seasons (Table S7).
485
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494
495 **Author contributions:**
496 R.C.B and F.A conceived the study. R.C.B performed the genomic lab analyses and J.C.W.
497 performed the bioinformatics. R.C.B assigned trophic levels to all taxa and constructed the

metaweb, and H.H performed food-web analysis. R.C.B performed all statistical analyses. R.C.B produced all figures and illustrations. All authors contributed to the interpretation of the networks and discussed and commented on the paper.

Competing interests:

The authors confirm there are no competing interests.

Materials & Correspondence:

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Data availability

Sequencing data generated during this study will be made available in a public repository upon publication.

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601 and applications of global eDNA. *Global Ecology and Conservation* **17**, e00547 (2019).
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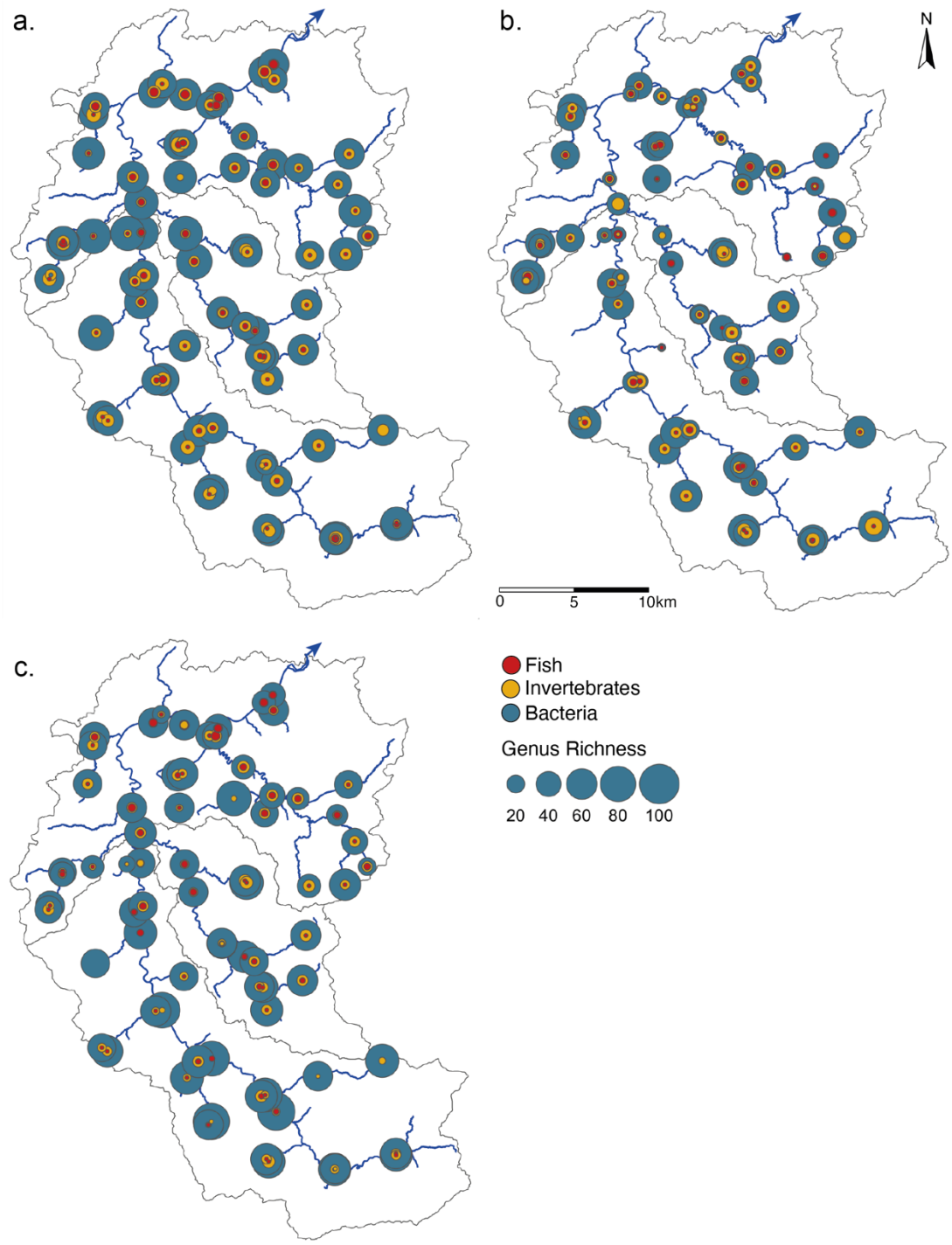
661 **Figures:**

662 **Figure 1: Genus α-diversity of bacteria, invertebrates and fish collected from eDNA**
663 **samples in the Thur catchment in (a) spring, (b) summer and (c) autumn.** The circles are
664 scaled proportionate to the α-diversity of each group. Grey lines give the sub-catchments Glatt,
665 Necker, and Thur, respectively and the blue arrow shows the direction of flow.



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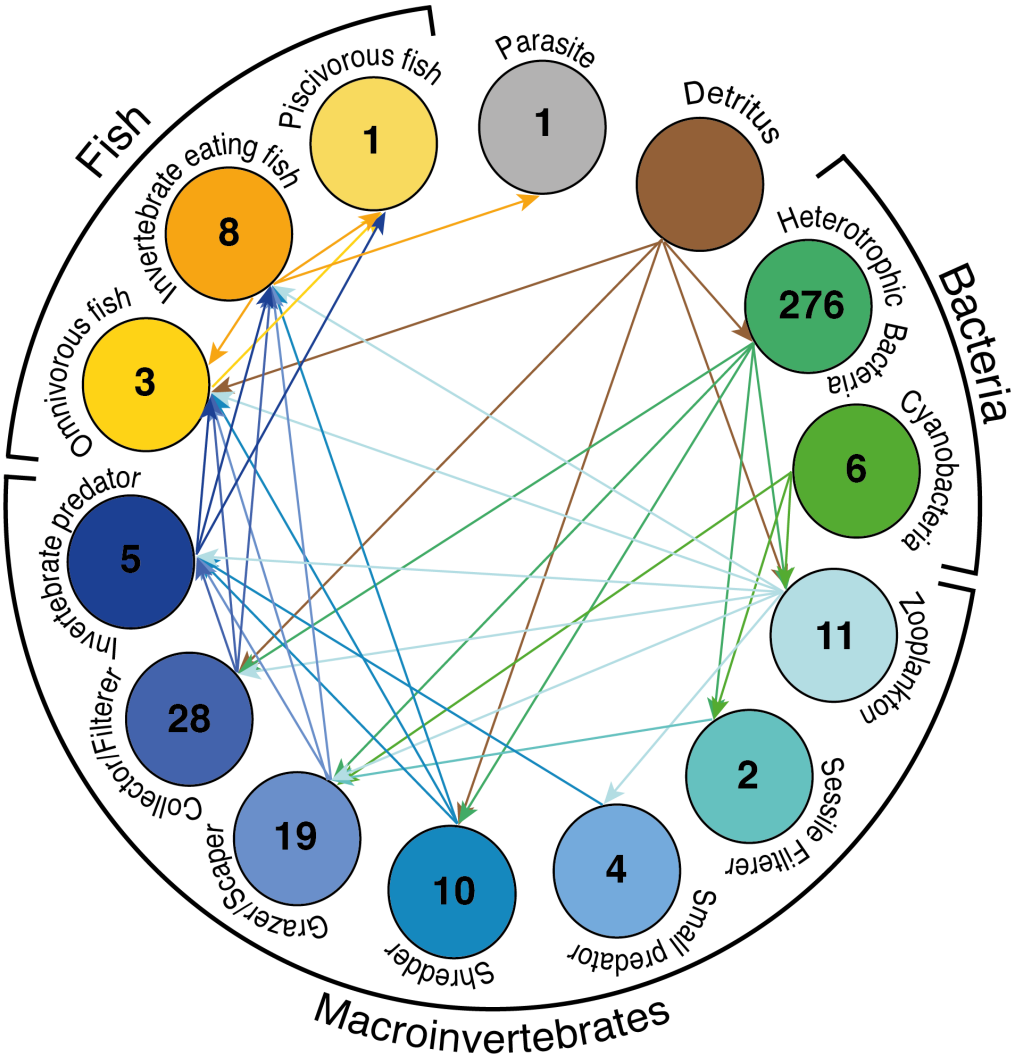
668 **Figure 2: Food-web network generated from eDNA data.** All genera were categorised into
669 feeding groups dependant on their dominant feeding behaviour, we then used this information to
670 construct a trophic interaction metaweb using these links. Number of genera in each group is
671 shown in circles, arrows indicate energy flow.



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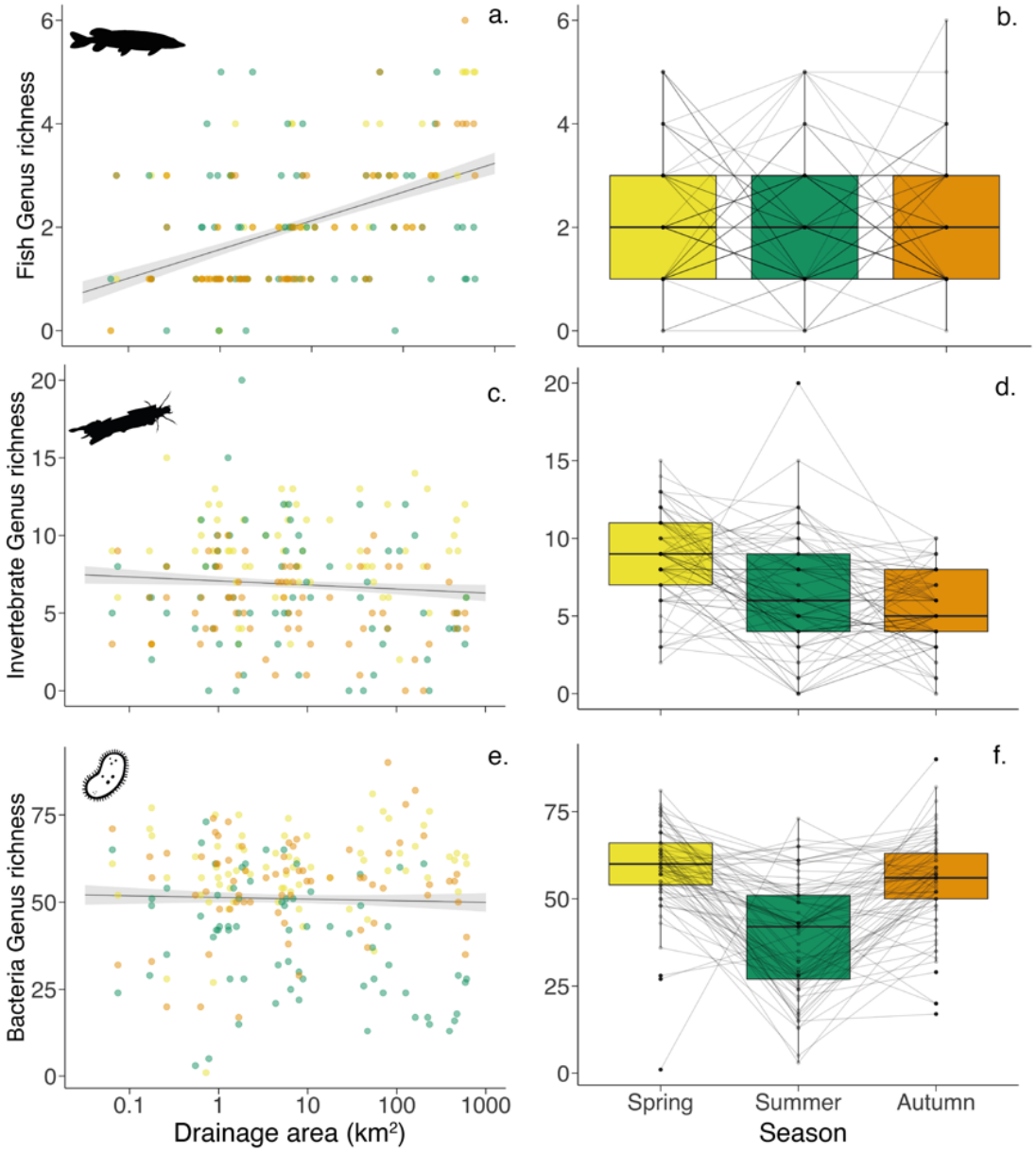
674 **Figure 3: Genus α-diversity in space and time.** Plots a, c and e show α-diversity in each group
675 as a function of drainage area. Line indicates linear mixed model with shaded area showing 95%
676 confidence intervals as calculated using the model predictions and standard error. Plots b, d and f
677 show change in α-diversity in each group over season. Grey lines indicate link sample points
678 over the three sampling seasons. In all plots colour represents season: yellow – Spring, green –
679 Summer and orange – Autumn.



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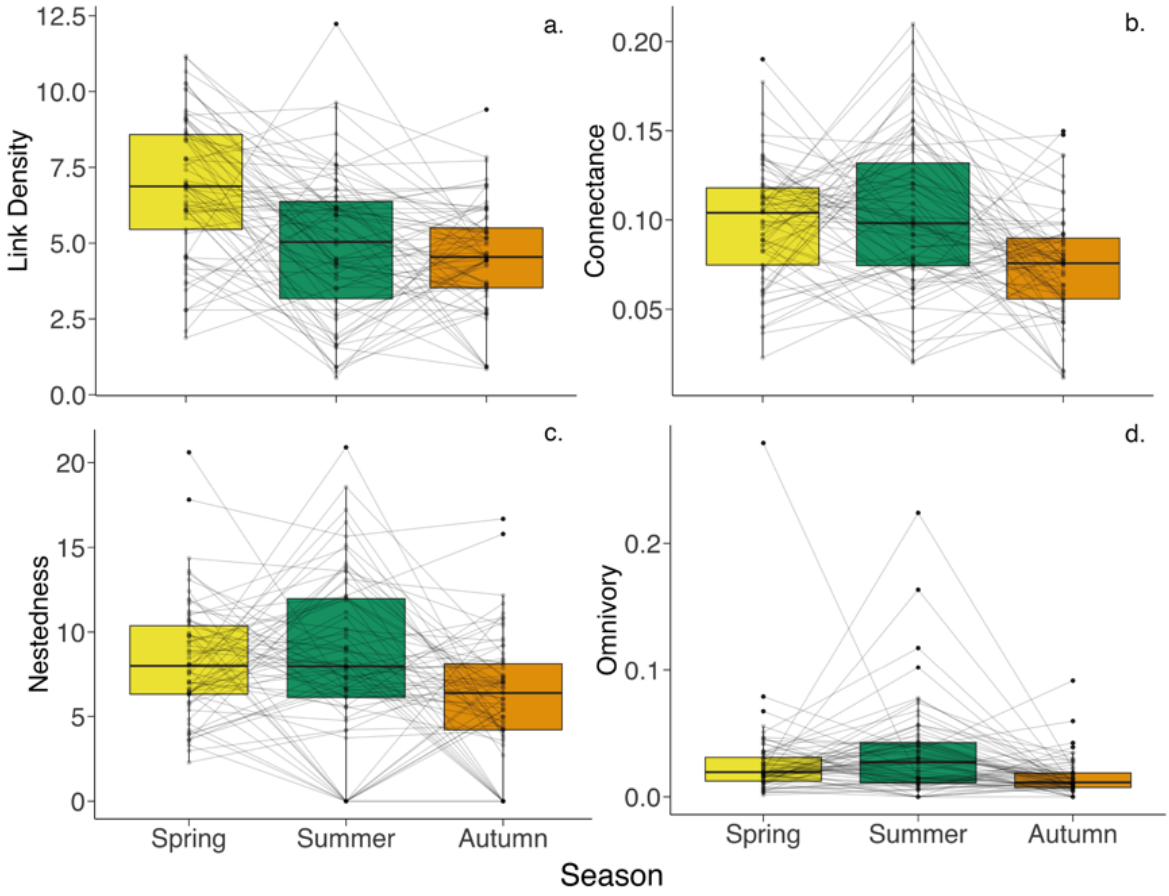
681 **Figure 4: Food-web structural characteristics:** Box plots showing the change in each food-
682 web structural characteristic over the three seasons: a: Link Density, b: Connectance, c:
683 Nestedness and d: Omnivory. Grey lines indicate link sample points over the three sampling
684 seasons and colour represents season: yellow – Spring, green – Summer and orange – Autumn.



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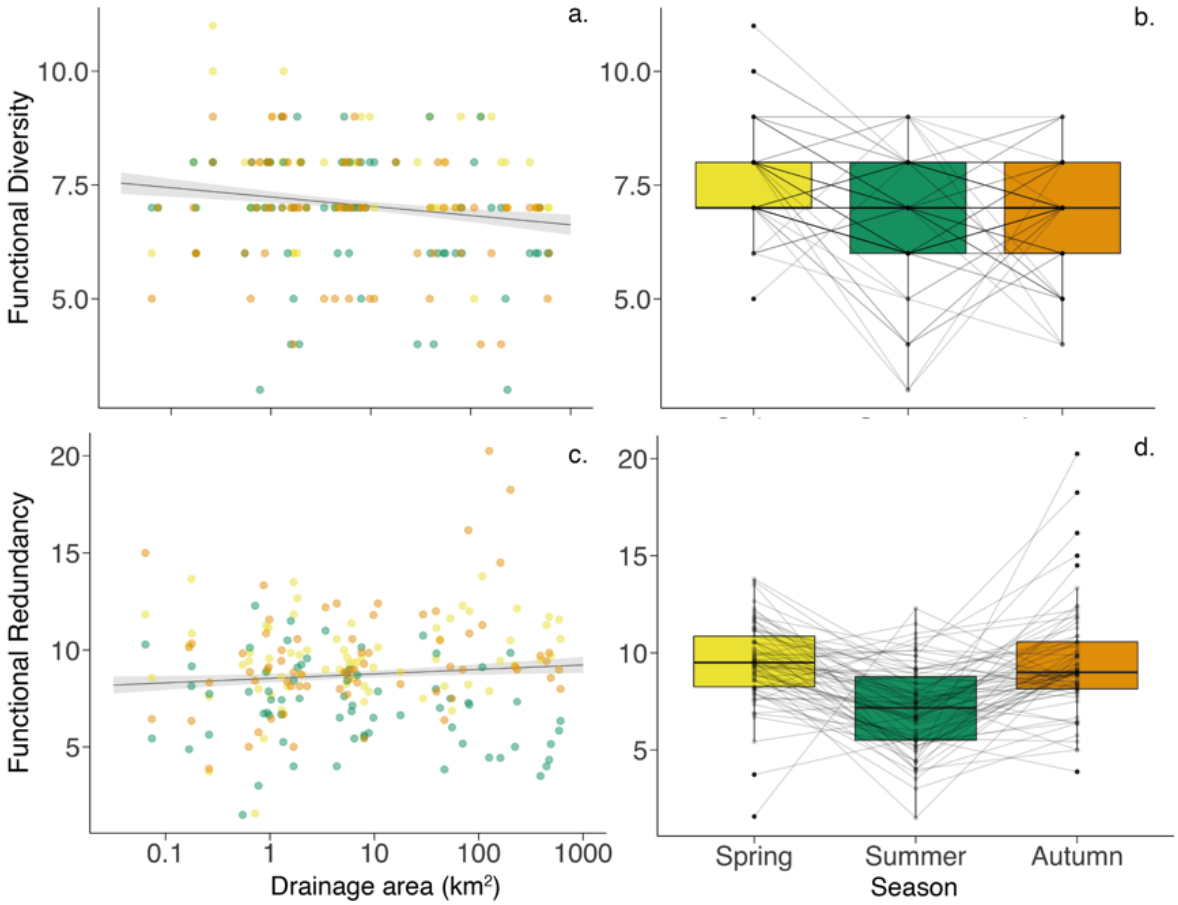
687 **Figure 5: Functional diversity and functional redundancy.** a and b: Functional Diversity, c
688 and d: Functional Redundancy. Plots a and c show the change over drainage area. Line indicates
689 linear mixed model with shaded area showing 95% confidence intervals as calculated using the
690 model predictions and standard error. Plots b and d show change over season. Grey lines indicate
691 link sample points over the three sampling seasons and colour represents season: yellow –
692 Spring, green – Summer and orange – Autumn.



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