Title:

Spatio-temporal patterns of multi-trophic biodiversity and food-web characteristics uncovered across a river catchment using environmental DNA

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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 over seasons using environmental DNA. We found contrasting biodiversity patterns between major taxonomic groups; local richness showed seasonally dependent and statistically significant increases and decreases towards downstream positions within the catchment for fish and bacteria respectively, while invertebrate richness remained unchanged with increased downstream position. The local food-webs, formed by these taxonomic groups, also showed a variation in their structure, such as link density and nestedness, to both space and time, yet these patterns did not necessarily mirror those of biodiversity and functional feeding characteristics. In order to conserve species diversity as well as their functional trophic integrity of communities, patterns of biodiversity and food-web characteristics must thus be jointly studied, as our results suggest that they are not directly scalable to each other even at the same spatial and temporal scales.

Introduction

The study of biodiversity patterns[1–3] and the characterisation of food-web structures[4,5] are essential, yet often disconnected elements of the study of 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 and 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 increasing catchment area (i.e., distance downstream)[11], whereas headwaters often show high endemic bacterial species richness[12]. Aquatic invertebrate biodiversity has been widely studied and exhibits complex pattern with studies demonstrating both disproportionately high biodiversity found in headwaters compared to downstream[13] and also the reverse: a significant increase in biodiversity linked to a greater catchment size[14], which is generally a combination of opposing natural and anthropogenic drivers. However, group-specific biodiversity patterns have been mostly studied in isolation from one another, although all 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 levels is crucial to reveal how species are linked through trophic interactions[16] and how food-web structures unfold.

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

91 sampling based primarily on species sight or capture. Environmental DNA, or eDNA, is the
92 collection of DNA extracted from an environmental sample such as water, air or sediment[29]. By
93 collecting eDNA we can screen samples for multiple taxonomic groups via metabarcoding[30],
94 thereby creating a biodiversity assessment suitable for food-web reconstruction.
95
96 Here, we use eDNA metabarcoding to assess patterns of biodiversity and reconstruct local food-
97 webs via a metaweb-based approach[31]. We used high-throughput sequencing and a multi-marker
98 approach to examine the occurrence of three taxonomically relevant groups, namely fish (via the
99 106 bp fragment of the 12S barcode region), invertebrates (a 313 bp fragment of the cytochrome
100 *c* oxidase I region, COI) and bacteria (a 450 bp fragment of the 16S barcode region) in a large-
101 scale river network over the course of three seasons (spring, summer and autumn). A metaweb
102 details all possible interactions of those species found in a dataset, and then each local food-web
103 is inferred from the subset of those species and interactions found at each site. The metaweb
104 approach is suitable to capture food-web patterns based on taxa co-occurrence data in both
105 terrestrial and aquatic systems[24,32-35]. This allowed us to test for association of biodiversity and
106 food-web structures with network location, season, and the interaction between these two factors.
107 We found contrasting effects on biodiversity patterns and food-web structure from spatial and
108 temporal influences and their interaction, providing insight into the underlying changing
109 ecosystem dynamics and indicating that effective and targeted conservation measures must
110 consider them jointly.
111

112 **Results**

113

114 *Data collection and community construction*

115 We collected water samples from the upper Thur catchment, Switzerland, which covers
116 approximately 700 km^2 and is made up of three sub-catchments: River Thur, Necker and Glatt.
117 Seventy-three field sites were selected to allow for maximum coverage across the catchment
118 area, comprising of a broad size range of upstream drainage sizes (i.e., the drainage area size
119 indicates the location of the site within the catchment: upstream sites having a small drainage
120 area and lowland sites having a larger drainage, see Supplementary Fig. S1). Water samples were
121 filtered on site, sealed with luer fittings, and placed in individual sealed bags in a cool box for
122 transport to the lab, where they were stored at −20 °C until further processing. DNA extraction
123 was performed in a specialist clean lab environment at Eawag, Switzerland and subsequent
124 library preparation followed a uni-directional lab processing protocol where PCR product was
125 not returned to the cleaned lab. A total of 12.29 million, 14.32 million and 14.02 million raw
126 reads were produced from the 12S, COI and 16S libraries, respectively. After bioinformatic
127 processing the average (±sd) sequencing read depth per sample was: 42,189 ±19,057, 46,573
128 ±407,659 and 24,646 ±27,612 for 12S, COI and 16S, respectively (see Methods and
129 Supplementary Table S1 and bioinformatics workflow). For further analysis, ZOTUs were
130 merged to genus level and only fish genera, invertebrate genera with an aquatic life stage and
131 bacteria associated with freshwater were kept for further analysis (see Methods for further
132 details). Analysis was performed on presence/absence data to merge the three libraries for the
133 complete freshwater community and exclude a possible influence of uneven sample read depth
134 generated from multiple markers (see also [36]).

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[37].

To measure structural food-web and functional characteristics of communities, we constructed a metaweb based on known interactions of genera classified into different functional feeding groups (Fig. 2; see also Methods and Supplementary Table S2 and S3). We defined a local food-web at each field site based on genera co-occurrence and the corresponding subset of interactions from the metaweb. With this approach, variation in food-web structural and functional characteristics emerge from the spatio-temporal differences in genus and thus feeding-group composition, which then determines broad changes in food-webs over time and space (See Methods for further details).

To assess the relationship between α-diversity of each group (fish, invertebrates and bacteria), food-web structural and functional characteristics with site location within the catchment, we conducted linear mixed-effects model analyses. Drainage area (km^2) was log transformed to aid model assumptions for normality. For each dependant variable, drainage area and season were the fixed effects, while site was the random effect. We ran models with and without an interaction of the two fixed effects to determine the overall effect of factors, and report below the results based on the interaction models (see supplementary Table S5). As post hoc contrast

158 testing, we ran estimated means of linear trends to compare the slopes from the mixed-effects
159 models and estimated marginal means of data over season only to determine the influence of
160 seasonal changes on all α-diversity and food-web measurements (see Methods for further
161 details). To examine the effect of river distance on β-diversity we constructed a matrix of
162 pairwise distances for sites that were connected along the fluvial network and to examine the
163 effect of river distance on β-diversity we performed a Mantel test (see Methods and
164 Supplementary Table S11).
165
166 *Spatial and temporal biodiversity patterns*
167 In total we detected 374 genera across three organismal groups associated with freshwater,
168 including 12 fish genera, 80 invertebrate genera and 282 bacteria genera. When combining all
169 seasons, α-diversity (genus richness) ranged between 8–96 genera with all taxonomic groups
170 combined (Fig. 1). Over the different seasons, mean local α-diversity was 70 (range 10–92) in
171 spring, 48 (range 8–85) in summer, and 63 (range 19–96) in autumn (Supplementary Fig. S2 and
172 Table S4). We used mixed-effects models to assess the influence of drainage area and season on
173 the local α-diversity of each group (Fig. 3, for detailed model output see Supplementary Table
174 S5–S8). Of the three taxonomic groups, interaction between drainage area and season was found
175 to be significant for both fish and bacteria α-diversity ($p < 0.001$ for fish and bacteria, Table S8),
176 in this study this indicates that the effect of drainage area is modulated by the season rather than
177 vice-versa. By examining the slopes generated from the mixed-effect models for each season, the
178 effect of drainage area differs across the seasons in both fish and bacteria α-diversity (Fig. 3a and
179 e, Supplementary Table S9). Fish α-diversity in summer has a flatter slope and is significantly
180 different compared to spring and autumn ($p < 0.001$ for both) both with steep increases in

richness with increase of drainage area (Fig. 3a). Bacteria α-diversity also showed a similar trend such that summer diversity spatial pattern is significantly different from both spring and autumn sampling seasons ($p < 0.001$ and $p < 0.01$, respectively; Supplementary Table S9); however, this is mostly because of a sharp decline in richness with increased drainage area in summer (Fig. 3e). Examining the mean α-diversity of genus across seasons (i.e., collapsing the spatial axis) shows no significant change in fish (mean number of 2 genera and range 0–6, Fig. 3b), but for bacteria we find a significantly lower α-diversity in summer compared to spring and autumn ($p < 0.001$ for both, Fig. 3f and Supplementary Table S10). Looking at invertebrate α-diversity we do not see a significant interaction of drainage area and season ($p = 0.295$, Fig. 3c and Supplementary Table S5), but season as a significant main effect on it ($p < 0.05$, Supplementary Table S8). Examining the means across the seasons further shows a significant drop in α-diversity of invertebrates from spring to summer ($p < 0.001$) and summer and autumn ($p< 0.0001$, Fig 3d and Supplementary Table S10).

Regarding the analysis of β-diversity across the catchment, the Jaccard's dissimilarity significantly increased with increasing pairwise river distance among sites in all organismal groups across all seasons, apart from bacteria in spring and autumn (fish Mantel statistics Spring: 0.143, $p < 0.01$, Summer: 0.171, $p = 0.001$, Autumn: 0.321, $p = 0.001$; invertebrate Mantel statistics Spring: 0.095, $p < 0.05$, Summer: 0.169, $p = 0.001$ and Autumn: 0.114, $p < 0.05$; bacteria Mantel statistics Spring: -0.058, $p = 0.834$, Summer: 0.179, $p < 0.01$ and Autumn: 0.069, $p = 0.12$, See Supplementary Table S11). Further partitioned analyses on taxon replacement and loss revealed contrasting patterns. Taxon replacement between sites increased over river distance for all groups in most seasons (significant or marginally significant, see

Supplementary Table S11), apart from fish (Mantel statistics: 0.07, $p = 0.891$) and bacteria (Mantel statistics: 0.016, $p = 0.34$) in spring. In contrast, taxon loss was only found to significantly increase over river distance for fish in spring and autumn (Mantel statistics: 0.219, $p = 0.001$ and 0.059, $p = 0.05$, respectively) and bacteria in summer (Mantel statistics: 0.089, $p < 0.05$).

Spatial and temporal changes in food-web structural and functional characteristics

We examined commonly used food-web structural characteristics (link density, connectance, nestedness, level of omnivory, coherence, number of links, modularity, and robustness), as well as functional characteristics (functional diversity and redundancy, Fig. 2 & Supplementary Fig. S2 and Table S3). For food-web structure, here we describe the results of link density, connectance, nestedness and level of omnivory (hereinafter, omnivory) as the most ecologically important food-web descriptors in our study, while the results from the remaining descriptors are presented in the Supplementary Information (Table S5–10 and Fig. S4). As with the biodiversity patterns, we find a significant interaction between drainage area and season for connectance, nestedness, and marginally for omnivory, but not for link density ($p < 0.05$, $p < 0.001$, $p = 0.066$ and $p = 0.138$, respectively; Fig 4 and Supplementary Table S8). Food-web connectance, nestedness and omnivory increased with increasing drainage area, particularly in summer, and in contrast showed a decreasing trend in spring and autumn (Fig. 4c, e, and g). There, summer patterns were significantly or marginally different from the ones of autumn ($p < 0.05$, $p < 0.001$, and $p = 0.074$, respectively; Supplementary Table S9). Whereas link density showed only a marginally significant decrease with drainage area ($p = 0.064$; Supplementary Table S8) and a significant effect of season ($p < 0.05$; Fig. 4a and Supplementary Table S8). When we examined

227 mean food-web measurements across the seasons, link density was significantly higher in spring
228 than in summer and autumn ($p < 0.001$ for both, Fig. 4b and Supplementary Table S10) whereas
229 connectance and nestedness were significantly lower in autumn than in spring and summer (all p
230 < 0.01 or 0.001, Fig. 4d and f and Supplementary Table S10), and omnivory was significantly
231 higher in summer than in autumn ($p < 0.001$, Fig 4h and Supplementary Table S10).
232
233 Functional diversity showed no significant interaction between drainage area and season ($p =$
234 0.354, Supplementary Table S8), but a marginally significant decrease with increasing drainage
235 area while exhibiting no change with season ($p < 0.05$ and $p = 0.147$, respectively; Fig. 5a and b
236 and Supplementary Table S8). Functional redundancy showed a significant interaction between
237 drainage area and season ($p < 0.01$, Supplementary Table S8). Across seasons, the mean
238 functional redundancy was significantly lower in summer than in autumn ($p < 0.05$, Fig. 5d and
239 Supplementary Table S10).
240
241 **Discussion**
242
243 Studies of biodiversity and food-web assemblages in riverine networks are often constrained to
244 local scales or aggregated to a single time point, which in essence fails to capture the spatial
245 processes and the temporal fluctuations that together play a key role in community dynamics
246 present within a river network[8]. Our study is one of the first assessments utilising data derived
247 from eDNA metabarcoding to describe biodiversity patterns and food-web characteristics[38-40] in
248 a whole river network at a spatially and temporally large scale. By taking this approach, we were
249 able to detect contrasting group-specific spatial and temporal biodiversity patterns, as well as

250 food-web structural and functional characteristics whose spatial responses also showed strong
251 dependence on the season, yet in different ways. Overall, our findings support the need to
252 include multifaceted biomonitoring, i.e., at taxa and community levels and regarding richness as
253 well as biological interactions, conducting across both spatial and temporal scales, to better
254 understand changes in ecosystems[41]. Such a comprehension is particularly crucial as we are
255 facing contemporary global change and biodiversity loss.
256
257 Aquatic biodiversity is subject to fluvial influences within a dendritic network, whereby spatial
258 patterns of α-diversity and β-diversity are known for key groups, such as fish, invertebrates and
259 microbes[11–14,42]. Our data is congruent with previous studies on fish diversity[11,36], in that both α-
260 diversity significantly increased downstream, and β-diversity (community dissimilarity)
261 significantly increased with river distance in all three seasons. Bacterial richness was highest at
262 the top of catchment, which was also in line with previous studies[37], albeit only in the summer
263 sampling campaign. Similarly, bacterial β-diversity also increased with river distance during this
264 season. We therefore postulate that bacteria are able to disperse through the focal catchment
265 during higher flows (spring and autumn), which makes bacterial spatial diversity patterns subject
266 to seasonal influences. Unlike the former two groups, invertebrate richness exhibited no
267 significant influence of drainage size but a seasonal change. The seasonal variation in
268 invertebrate diversity can be linked directly to the emergence of the non-aquatic adult life stage
269 of several macroinvertebrate genera detected (Diptera, Ephemeroptera, Plecoptera and
270 Trichoptera), which takes place in the late spring and summer months[27], and literally removes
271 these organisms from the aquatic communities as they become air bound. For the lack of spatial
272 effect, this result is contrary to previous studies which have shown clear, although variable,

273 spatial patterns for invertebrate communities in river systems[13,14,43]. As previous studies often
274 looked at spatial scales much larger or much smaller than our, this disparity may be due to a
275 mismatch in scale looked at here, or reflect different strengths of anthropogenic drivers that may
276 have opposing effects. For example, Finn and colleagues[13] suggested headwaters harbour
277 disproportionately high invertebrates compared to lower points in the catchment, but their
278 studied focused on small (1–2) and mid (3–4) stream orders only, whereas the sites in our study
279 ranged from small to much larger rivers (stream orders 1–7, [44]). Contrastingly, when looking at
280 scales about 50-fold larger, Altermatt and colleagues[14] showed the number of key aquatic
281 invertebrate taxa (Ephemeroptera, Plecoptera and Trichoptera) increased with catchment size,
282 however they also found a combination of local factors (catchment areas, drainage area,
283 elevation and network centrality) had the greatest influence on local invertebrate α-diversity.
284 Possibly, the scale at which we study invertebrate α-diversity and contributing local factors falls
285 between such small-scale vs. large-scale perspective, such that we detected regional patterns
286 where α-diversity remains relatively constant throughout the catchment. These findings thus
287 expand on past studies, highlighting the influences of spatio-temporal interactions on freshwater
288 biodiversity, and the importance of choosing a scale at which we monitor biodiversity patterns.
289
290 By resolving the fundamental trophic relationships among broad feeding groups, we established
291 a trophic interaction metaweb for the three taxonomic groups examined in this study. The
292 metaweb with genus co-occurrence data thus allowed our investigation of both spatial and
293 temporal influences on the characteristics of local food-webs. With regard to food-web structure,
294 we found a significant interaction between drainage area and season that associated with
295 connectance, nestedness and omnivory, whereas link density was influenced in isolation

296 significantly by season and marginally by drainage area. Overall, the impact of season turns out
297 to be prevalent in all four focal structural characteristics, highlighting the importance of
298 considering seasonality of food-web structure. By definition, a lower link density indicates a
299 higher proportion of trophic specialist (who interact with relatively fewer other taxa) in the food-
300 web. Therefore, combining observed link density and α-diversity patterns together, reveals that
301 communities are larger and have more specialists toward downstream in wetter seasons (spring
302 and autumn), while smaller and fewer generalists are found towards downstream in the dry
303 season (summer). Such changes are likely driven mainly by bacteria given their dominance in
304 the communities. Thus, the unique summer spatial patterns (in comparison to spring and autumn)
305 for food-web connectance, nestedness and omnivory may reflect the similarly unique bacterial α-
306 diversity summer spatial pattern. Temporally, all four focal food-web structural characteristics
307 were lowest in autumn, despite overall α-diversity being lowest in summer. This indicates that
308 the seasonal variation in food-web structure found in this study does not merely reflect the
309 overall richness (i.e., community size) change, but rather the change in genera and thus feeding-
310 group composition. Previous studies on freshwater food-webs that have examined temporal
311 changes have often found reduced productivity as the main driver behind a declined food-web
312 structure in winter vs. summer[20,45]. Thus, the restrained food-web structural characteristics we
313 see in autumn may capture the start of the productivity restriction seen in food-webs over the
314 winter months[46], allowing only some the feeding groups to persist (as further suggested by our
315 functional redundancy results, see below). Indeed, the detected less-connected and less-nested
316 food-web structure also implies weaker resource competition among consumers, which may be
317 necessary for their coexistence in the food-web[47], especially when competition becomes more
318 costly with lower resource productivity in autumn.

319

320 Moving from broad food-web patterns to examining feeding groups enables us to study
321 fundamental functional changes within the community and possible effects at the ecosystem
322 level[17]. We find a marginally significant effect of drainage area on the functional diversity,
323 which is highest upstream. Such a spatial pattern along the hierarchical river network is
324 congruent with to the River Continuum Concept (RCC), which states increased diversity of
325 invertebrate functional feeding groups at low stream orders[48,49] (i.e., upstream). In river systems
326 allochthonous material (i.e., the dominant organic material) in forested headwaters is processed
327 by specialist shredder, collector/filterers, and grazer/scrapers, while downstream autochthonous
328 organic material is utilized by fewer functional feeding groups[48]. Therefore, the pattern we
329 observed is to be expected and shows the suitability of our approach of using single overarching
330 functional feeding groups (i.e., a single dominant functional feeding group per genera), which
331 simplified the life cycle and changes in feeding behaviour observed in invertebrates (the
332 dominant group within the functional feeding groups). Interestingly, the functional redundancy
333 we observe gives us more information about the changes exhibited at a community level.
334 Functional redundancy, i.e., genera per functional feeding group, was interactively influenced by
335 drainage area and season, showing a unique decrease toward downstream in summer, contrasting
336 the opposite spatial trend in spring and autumn. Also, the functional redundancy was lower in
337 summer than in autumn. Since this measurement accounts for the number of genera, its spatio-
338 temporal patterns could again largely reflect the variation in bacteria richness. Nonetheless, the
339 particularly high functional redundancy in autumn suggests that, although fish and bacteria
340 richness increase toward downstream, some key invertebrate taxa of well-connected feeding
341 groups (i.e., interact with many other groups) can become absent particularly in this season, and

342 losing such feeding groups cause a constriction of local food-web structure as we observed.
343 Furthermore, although both the food-web structural and functional characteristics are determined
344 by genera occurrence, their inconsistent and variable temporal patterns imply that multiple
345 feeding groups share similar trophic roles in the food-web (Fig. S3), though they each adopt
346 specialised feeding behaviour within the catchment and thus perform distinctive ecological
347 functions (e.g., shredders, collectors, filterers). In other words, the structure and function of a
348 food-web does not necessarily match and synchronise (*sensu stricto*[22,50]). These results are
349 particularly encouraging, because the patterns of both food-web structure and functional
350 diversity are known to be important for ecosystem health assessment and identifying potential
351 vulnerability to perturbation[17,26,51]. Addressing the consistency of their patterns across spatial and
352 temporal scales will likely lead to novel and comprehensive understanding of biodiversity and
353 ecosystem function loss due to environmental change.
354
355 In our study, the functional feeding groups were defined at the level of genus, while we expect
356 investigations at finer resolutions will be promising for future work that can reveal not only more
357 accurate patterns, but also the influences of sampling taxonomic resolution. Similarly, our
358 selection of focal taxa may have influenced the food-web patterns we detected. With the selected
359 three key taxonomic groups in riverine ecosystems, we present a broad and relevant view
360 capturing trophic roles from basal resources (cyanobacteria) to top consumers (piscivorous fish),
361 captured by three relatively broad metabarcoding markers. However, we also by default
362 excluded some further groups, such as algae or terrestrial taxa, which could have been relevant
363 as primary producers and as terrestrial-aquatic linkages, respectively. For example, it is known
364 that algae become an increasingly important resource when moving from allochthonous inputs in

365 headwaters to larger streams with increased light levels further down the catchment[48,49]. The
366 choice of taxonomic groups looked at was both methodologically defined, as well as driven by
367 the goal to have an overseeable and clearly aquatic-focused view on food-webs. Thereby, we
368 here capture the spatio-temporal patterns of an (dominant) subset from the real-world
369 communities and food-webs in which even more species are involved.
370
371 By using the eDNA technique, we gain three notable advantages: its scalability for monitoring
372 complex and large systems, its reusable nature, and its being a non-invasive method of collecting
373 biodiversity information[41,52]. Our understanding of how the information we ascertain from eDNA
374 sample collection has greatly improved in recent years due to studies on the hydrological
375 influences[53] and a general understanding of the rate of eDNA persistence in lotic ecosystems[54].
376 However, the successful detection of taxa with eDNA is also linked to the ecology of individual
377 species[54], and some seasonal variation in the detection of several taxonomic groups is known[55–
378 58]. Therefore, it is possible that some taxa that were not detected in the colder season (autumn) in
379 this study were false-negative records. However, these non-detections are likely linked to low
380 abundance or low metabolic rates, and thus these species are, while not physically absent, at least
381 "relatively absent" in ecological terms.
382
383 In summary, we reconstruct comprehensible multi-trophic community and evaluate their
384 characteristics at a large spatial scale (i.e., the catchment) and over time (i.e., seasons), by
385 exploiting broad species occurrence information derived by eDNA sampling and combining
386 multiple markers. For both biodiversity and food-web characteristic, we detect spatio-temporal
387 patterns, and those of the former are different across taxonomic groups while the latter across

388 measurements. Specifically, we identify inconsistent patterns between food-web structural and
389 functional feeding characteristics, as well as a reduction in local food-web structure particularly
390 in autumn. Our approach provides a first demonstration of the application of eDNA to a complex
391 river network for the monitoring of not only biodiversity but also food-web patterns, paving a
392 way for establishing long-term, repeated monitoring of complex communities, and potentially
393 also in other systems worldwide. Biodiversity in freshwater systems face huge threats from
394 anthropogenic pressures, including global climate change. To protect and conserve the systems,
395 it is essential to establish vital information on the changes in biodiversity and food-web
396 composition over spatio-temporal scales for the detection of these stressors.

397 **Methods**

398

399 *Site selection and eDNA sample collection*

400 Environmental DNA water samples were collected from the edge of the waterbody and filtered
401 on site using single use disposable 50 ml syringes. At each site the syringe was filled and
402 refilled 10 times from the river and the water was filtered through a 0.22 µm sterivex filters
403 (Merck Millipore, Merck KgaA, Darmstadt, Germany). Two syringes and two sterivex filters
404 were used per site, equating to 1 L of samples water per site. The filters were then sealed with
405 luer caps, placed in individually labelled bags in a cool box for transport to the lab, where they
406 were stored at −20 °C until further processing. Sampling was carried out over five consecutive
407 days in May (spring), August (summer) and October (autumn) in 2018. During the summer
408 sampling campaign, four sites were dry and could not be sampled (total samples across all
409 seasons is n = 215). Negative field control samples were collected on each sampling day by
410 filtering 500 ml of ddH_2O through a sterivex filter in the same way as field samples were
411 collected (n = 15). Negative field samples were processed alongside field samples. All samples
412 were placed in labelled bags and cooled in a cool box until frozen at -20 °C on return to the
413 laboratory.

414

415 *Extraction and library preparation*

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

420 bleach, then 70% ethanol solution prior to extraction to remove any DNA from the outside of
421 the filters. Extraction controls were carried out alongside sample extractions and consisted of
422 blank sterivex filters (n = 5). Samples selected at random were analysed using a QuBit 3.0
423 fluorometer for double stranded DNA concentration, values measured between 0.317 - 27.5
424 ng/µl. All negative controls (field and extraction) were tested and recorded below detection
425 limits.
426
427 Sample replicated were pooled for subsequent sequencing with the following markers: a 106 bp
428 fragment of the mitochondrial 12S marker ([59], hereafter referred to as 12S) used to amplify
429 vertebrate DNA, a 313 bp fragment of the mitochondrial cytochrome oxidase I marker ([60] and
430 [61], hereafter referred to as COI) used to amplify metazoan DNA and a 450 bp fragment of the
431 V3-V4 region of the 16S marker ([62], hereafter, 16S) used to amplify bacteria and archaea DNA
432 (See Supplementary Information Table S8 for primer sequences). Positive controls (n = 6 per
433 library prep, see Supplementary Information Table S9) and PCR negative controls (2 µl of
434 ddH_2O, n = 11 per library prep) were included in each library. Each library consisted of 252
435 samples in total (including positive and negative controls).
436
437 Library preparation followed a two-step PCR process for both the 12S and COI markers, the
438 16S library was carried out using a three-step PCR[63] (See Supplementary Information Methods
439 for full details), all samples were amplified in triplicate. After the initial amplification, where
440 Nextera® transposase sequences (Microsynth, AG, Balgach, Switzerland) are added to the PCR
441 product, all samples were tested for amplification success with the AM320 method on the
442 QiAxcel Screening Cartridge (Qiagen, Germany). PCR products were cleaned using ZR-96

443 Plate clean-up Kit (Zymo Research) following the manufacturers protocol with the minor
444 modification by which the elution step was prolonged to 2 minutes at 4000 g. The clean
445 amplicons were indexed using unique combinations of the Illumina Nextera XT Index Kit A, C
446 and D following the manufacturer's protocol (Illumina, Inc., San Diego, CA, USA). A reaction
447 contained 25 µL 2x KAPA HIFI HotStart ReadyMix (Kapa Biosystems, Inc., USA), 5 µL of
448 each of the Nextera XT Index adaptors and 15 µL of the DNA templates. The final PCR
449 products were then cleaned using the Thermo MG Magjet bead clean up kit (Thermo Fisher
450 Scientific Inc., MA, USA) and a customized programme for the KingFisher Flex Purification
451 System (Thermo Fisher Scientific Inc., MA, USA) in order to remove excessive Nextera XT
452 adaptors. The cleaned product of 50 µl was then eluted into a new plate and stored at 4 °C prior
453 to quantification. For each library, the clean indexed amplicons were quantified in duplicate on
454 a Spark Multimode Microplate Reader (Tecan, US Inc. USA) prior to equimolar pooling using
455 the BRAND Liquid Handling Station (BRAND GMBH + CO KG, Wertheim, GE). Negative
456 controls were used here to determine if any contamination had occurred at either the PCR1 or
457 PCR2 stage. No such amplification was detected when the samples were run on the QIAxcel
458 and the maximum amount of each negative sample was pooled with the other samples for
459 library preparation. Library concentration was quantified on the Agilent 4200 TapeStation
460 (Agilent Technologies, Inc., USA) and verified by the Qubit the HS Assay Kit. Paired-end
461 sequencing was performed on an Illumina MiSeq (Illumina, Inc. San Diego, CA, USA) at the
462 Genetic Diversity Centre at ETH (See supplementary information Table S1 for library loading
463 information).
464

465 *Bioinformatics*

466 After each of the libraries were sequenced, the data was demultiplexed and reads were quality
467 checked using usearch v11.0.667[64] and FastQC[65]. Raw reads were first end-trimmed, merged
468 and full-length primer sites were removed using usearch v11.0.667[64] (16S reads were merged
469 using Flash v1.2.11,[66]). The merged and primer trimmed reads were quality filtered using
470 prinseq-lite (0.20.4). The UNOISE3 (usearch v11.0.667) workflow with an additional clustering
471 of 99% (usearch v11.0.667) identity was applied to obtain error corrected and chimera-filtered
472 sequence variants ZOTUs. The ZOTUs were then clustered using a 97 %, similarity approach
473 and taxonomic assignment with a 0.85 confidence threshold was performed using SINTAX in
474 the usearch software v11.0.667[64] with following databases: 12S: NCBI BLAST (v200416),
475 COI: Custom reference database (Including MIDORI un-trimmed (V20180221) and 16S:
476 SILVA (V128). See Supplementary Information Methods for all bioinformatic parameters and
477 reference database information used for each library. Prior to downstream analysis, positive
478 samples were examined from each library to see signs of potential contamination. Once we had
479 removed terrestrial genera (such as *Bos taurus*, which was identified due to the use of BSA in
480 the 12S library preparation), we applied a minor contamination of 0.1% to reduce errors caused
481 by tag-jumping or minor contamination in library preparation[67].

482

483 *Biodiversity patterns*

484 We calculated α-diversity (genus richness) at each site and compared β-diversity between sites
485 by using Jaccard dissimilarity using the betapart R-package[37]. We constructed a matrix of
486 pairwise distances between sites along the fluvial network with the igraph R-package[68] and
487 compared the similarity between β-diversity (including further partition β-diversity into species

488 loss and replacement) and river distance using the Mantel test with the vegan R-package[69]. Sites
489 which did not record genus from a target group were removed from this analysis: 12S dataset: 4
490 sites were removed from Spring, Summer and Autumn, the COI dataset 4 sites were removed
491 from the Summer and 3 from the Autumn analysis, no further sites were removed from the 16S
492 Bacteria dataset.
493
494 *Feeding group assignment*
495 Functional feeding groups were determined based on literature, species inventories and targeted
496 expert knowledge[70,71] for fish and invertebrates. Genera of bacteria were included if the phyla
497 they belong to has a strong association with freshwater habitat[72], bacteria were then broadly
498 divided into heterotrophic and cyanobacteria, the latter constituting a basal resource. An
499 assumptive constant basal resource of detritus was also included in all food-webs. Genera were
500 designated into one of eleven feeding groups: Parasite, Piscivorous fish, Invertebrate eating fish,
501 Omnivorous fish, Invertebrate predator, Collector/Filterer, Grazer/Scraper, Shredder, Small
502 predator, Sessile filterer, Zooplankton, Cyanobacteria and Heterotrophic bacteria (see
503 Supplementary Table S2 for descriptions of the feeding-group categories and Table S3 for
504 assignment of genera to each feeding group).
505
506 *Food-web structure*
507 We constructed a metaweb based on known trophic relationships among feeding groups
508 (Supplementary Table S2), then defined local food-webs using this metaweb at each field site,
509 based on co-occurrence of genera (nodes) and their interactions (links) (see Supplementary Fig.
510 S5 for site food-web examples and associated GitHub repository for information on for feeding-

511 group occurrence found in each sample). The metaweb approach has been applied to identify
512 food-web characteristic change across spatial gradients in terrestrial ecosystems[32], and temporal
513 changes in individual aquatic ecosystem[24], but has yet to be used at a large spatial scale in an
514 aquatic ecosystem over time.
515
516 We then quantified common food-web structural characteristics for each local food-web
517 generated. These included fundamental node-link composition features, i.e., number of links
518 (L), link density (L/S, the number of feedings links per taxa divided by the genus α-diversity at
519 each site) and connectance (L/S^2, the proportion of realised interactions). To further explore the
520 holistic topology of these food-webs and their change over space and time, we adopted the
521 following indices to determine some more features. Nestedness is an indication that the diets of
522 specialist taxa are subsets of generalist taxa's diets, and was calculated using the nestedness
523 metric based on Overlap and Decreasing Fill (NODF) function in vegan[69]. Modularity is
524 indication of a less nesteded network[73] in that the nodes are characterised into modules which,
525 unlike nested structures, do not overlap, and was calculated using the multilevel.community()
526 function from the igraph package[68]. Omnivory is defined as having a mixed trophic-level diet.
527 Thus, the more omnivores a community has, the less coherent (see below) the food-web[74]. The
528 level of omnivory is often debated as a measure of food-web stability, and weak omnivorous
529 interactions are likely to lead to a more stable food-web[75]. Trophic level and level of omnivory
530 of genera were calculated using the methods stated in William and Martinez[19], and omnivory
531 was averaged over all consumers in the food-web as a community-level index. Coherence is
532 defined as the overall degree of homogeneity of the difference between trophic levels of every
533 consumer resource pair within the local food-web. As described by [74], 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 [74]. 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 the commonly adopted 50% (i.e., R_{50}) as the threshold proportion of species lost at which we evaluated robustness, and excluded basal resources for primary removal. Robustness was calculated using the methods stated in Dunne and Williams[5].

Functional characteristics

We explored functional characteristics of local food-webs shaped by the broad functional feeding groups as described in the previous subsection (see Fig. 1 and Fig. S2). The feeding ecology (i.e., behaviour and diet) that defines each of these groups are further detailed in Supplementary Table S2. To calculate the local functional diversity, we used 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-effects model analysis to assess the relationship between group-specific genus α-diversity (bacteria, invertebrates and fish), food-web structural characteristics (coherence, connectance, link density, number of links, omnivory, modularity, nestedness and

robustness) and functional characteristics (functional diversity and redundancy) with site location within the catchment. Drainage area (km^2) was log transformed to fit model assumptions (normality). For each dependant variable, drainage area and season were the fixed effects. We included site as a random effect to account for repeated sampling of sites. We tested models with and without interactions to determine the significance of this potential interaction (see Supplementary Table S5):

Interactive model: genus α-diversity ~ drainage area * season + (1 | Site)

No Interaction model: genus α-diversity ~ drainage area + season + (1 | Site)

Significance was calculated using the lmerTest R-package[76], which applies Satterthwaite's method to estimate degrees of freedom and generate p-values for mixed models (Supplementary Table S6 and S7). We then used anova() to see the overall effect of both drainage area and season (Supplementary Table S8). Subsequent contrast testing were carried out using the emmeans R-package[77] with the function emtrends() for mixed-effects model slopes contrast testing (Supplementary Table S9), and the function emmeans() for testing significant differences between seasons only (i.e., collapsing the drainage area axis; Supplementary Table S10).

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Author contributions:

R.C.B and F.A conceived the study. R.C.B performed the genomic lab analyses and J.C.W. 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 and the data files used in the analysis will be made available upon publication on the following GitHub repository: https://github.com/RosettaBlackman/RiverDNA

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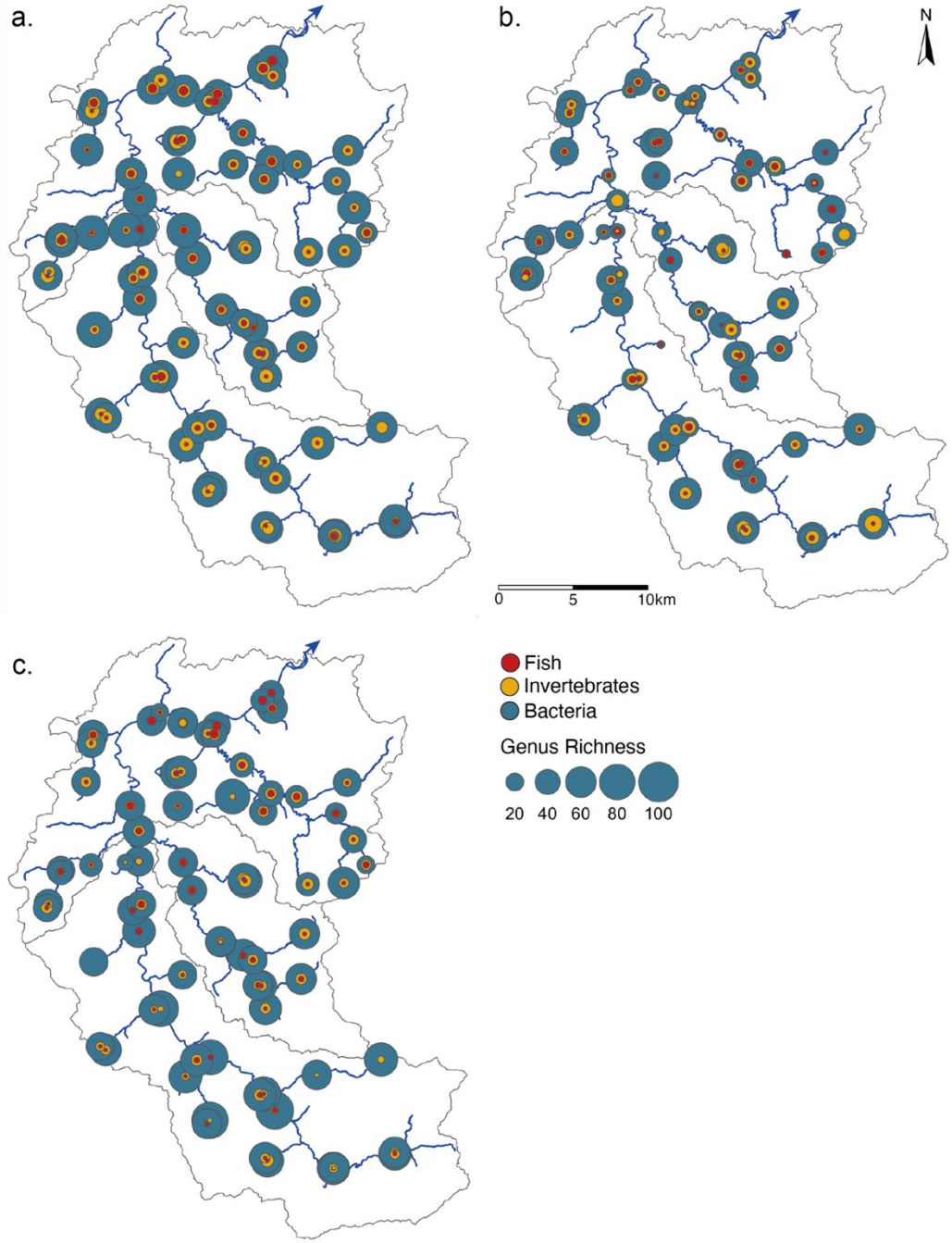
773 **Figures:**

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



778

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

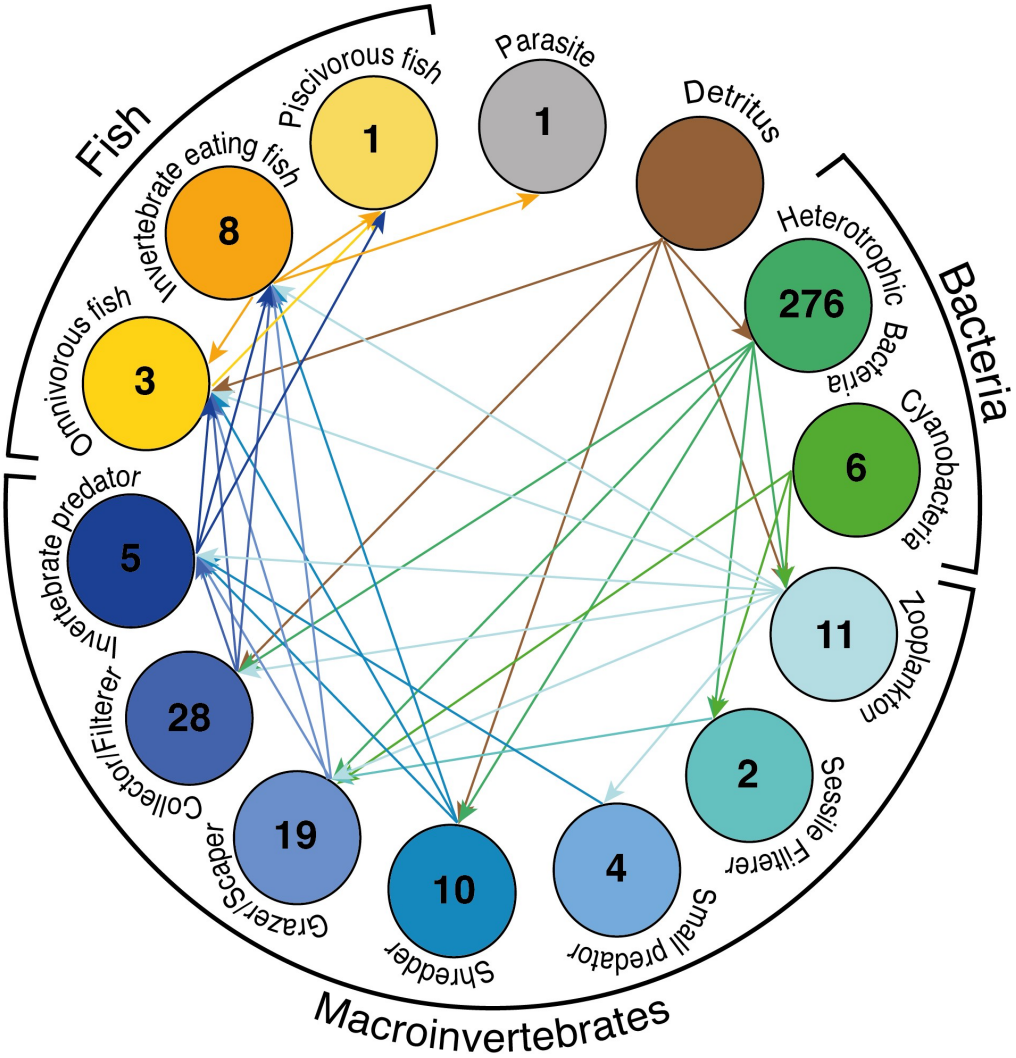


Figure 3: Genus α-diversity in space and time. Plots a, c and e show α-diversity in each group as a function of drainage area. Lines indicate linear mixed-effects models with shaded area showing 95% confidence intervals as calculated using the model predictions and standard error. Plots a and e show the models with a significant interaction between drainage area and season with the difference across each season (colour represents season). Plot c shows no significant effect of this interaction, the line indicates the model output of all data as a function of drainage area which is not significant (blue). Plots b, d and f show change in α-diversity in each group over season with samples sites linked by grey lines over the three sampling seasons. Colour represents season: yellow – Spring, green – Summer and orange – Autumn.

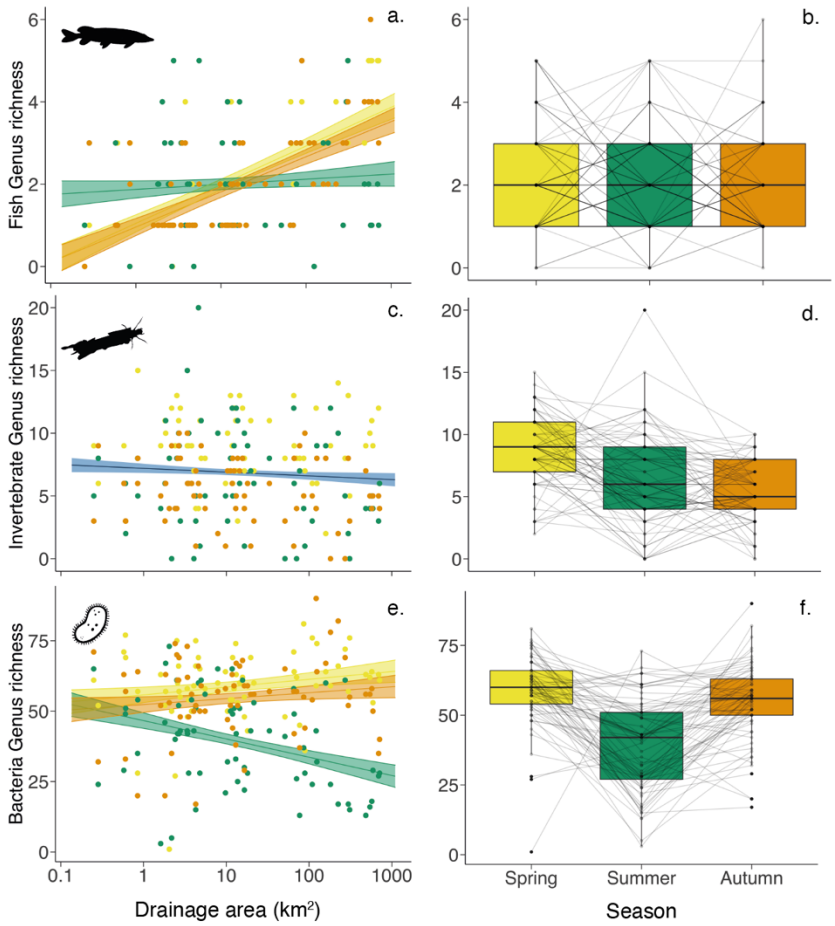


Figure 4: Food-web structural characteristics: Plots show food-web structural characteristic: a and b: Link Density, c and d: Connectance, e and f: Nestedness and g and h: Omnivory. Lines indicate linear mixed-effects models with shaded area showing 95% confidence intervals as calculated using the model predictions and standard error. Plots a shows no significant interaction between drainage area and season, therefore the line indicates the model output of all the data as a function of drainage area which is marginally significant (blue). Plots c, e and g show the models with a significant interaction with the difference across each season (colour represents season). Plots b, d, f and h show the change in characteristic over season with samples sites linked by grey lines over the three sampling seasons. Colour represents season: yellow – Spring, green – Summer and orange – Autumn.

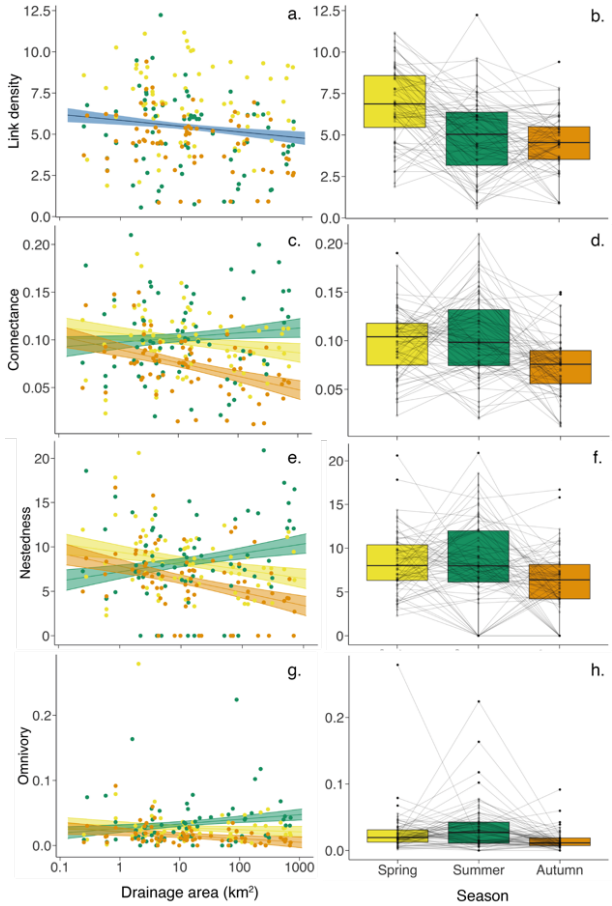
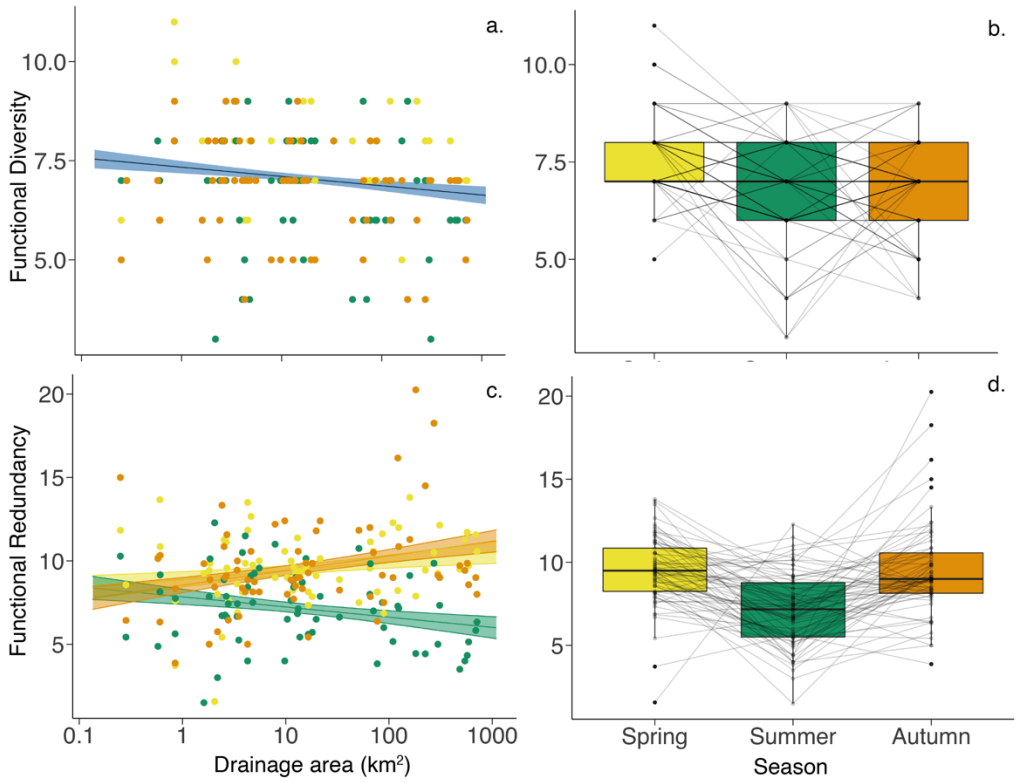



Figure 5: Functional diversity and functional redundancy. Plots show functional dynamics: a and b: functional diversity and c and d: functional redundancy. Lines indicate linear mixed effect models with shaded area showing 95% confidence intervals as calculated using the model predictions and standard error. Plot a shows no significant effect of the interaction between drainage area and season therefore the line indicates the model output of all the data as a function of drainage area which is marginally significant (blue). Plot c shows the model with a significant interaction with the difference across each season (colour represents season). Plots b and d show the change over season with samples sites linked by grey lines over the three sampling seasons. Colour represents season: yellow – Spring, green – Summer and orange – Autumn and blue – all seasons combined.