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

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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 km² riverine network over seasons using environmental DNA. We found contrasting biodiversity patterns between major taxonomic groups. Local richness showed statistically significant, season-dependent increases and decreases towards downstream location within the catchment for fish and bacteria, respectively. Meanwhile, invertebrate richness remained spatially unchanged but varied across seasons. The structure of local food webs, such as link density and nestedness, also varied across space and time. However, these patterns did not necessarily mirror those observed for biodiversity and functional feeding characteristics. Our results suggest that biodiversity patterns and food-web dynamics are not directly scalable to each other even at the same spatial and temporal scales. In order to conserve species diversity as well as the functional trophic integrity of communities, patterns of biodiversity and food-web characteristics must thus be jointly studied.

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The study of biodiversity patterns1–3 and the characterisation of food-web structures4–6 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 the stability, functioning and resilience of ecosystems. Furthermore, in the context of anthropogenic pressures and contemporary global change it is vital to bend the curve of biodiversity loss6.

Studies on biodiversity predominantly focus on analyses of α-, β- and γ-diversity, and possible underlying fundamental drivers of their spatial or temporal patterns2. Rivers are highly spatially structured systems8–10, 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 richness12. Aquatic invertebrate biodiversity has been widely studied and exhibits complex patterns with studies demonstrating both disproportionately high biodiversity found in headwaters compared to downstream17 and also the reverse: a significant increase in biodiversity linked to the greater catchment size14, 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 may trophically interact with each other. Indeed, recent theoretical work shows that contrasting biodiversity patterns are possible especially in disturbed systems through altered outcomes of species’ resource competition15. Therefore, to ensure optimal strategies for conservation and understanding of biodiversity patterns across different organisational groups, an ensemble approach integrating major taxonomic and trophic levels is crucial to reveal how species are linked through trophic interactions16 and how food-web structures unfold.

Trophic interactions and food webs by definition encompass multiple groups of organisms. Freshwater food webs are well-resolved17 and frequently exhibit distinct features, such as highly nested structures18 and prevalent omnivory17,19. Nevertheless, food-web studies often have a localised perspective due to methodological limitations of sampling food-web interactions and organisational occurrence in a standardised and comparable manner across different places and organisational groups20–22. Due to the same reason, these studies also tend to focus on simple spatial and environmental gradients23 or temporal change24 when spatio-temporal influences should be considered in conjunction15,26. This is particularly problematic in riverine ecosystems, which are characterised by a high spatio-temporal structure: they exhibit characteristic spatial structures, and have strong seasonal variations driven by changing abiotic conditions25 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 webs17,28.

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 characteristics4,25,26. In particular, molecular monitoring techniques may now provide a suitable solution to break through current methodological constraints. Whereby, conventional methods focus on individual taxonomic groups in isolation (kick-net, electrofishing, etc.), and can be costly both in time and financially, molecular tools can be used at highly resolved spatial and temporal scales and encompass multiple groups simultaneously. Environmental DNA, or eDNA, is the collection of DNA extracted from an environmental sample such as water, air or sediment29. By collecting eDNA we can screen samples for multiple taxonomic groups via metabarcoding30, thereby creating a biodiversity assessment suitable for food-web reconstruction.

Here, our specific objectives were to assess patterns of biodiversity and food-web characteristics at a spatially and temporally large scale. Based on eDNA derived taxa occurrence data, we used a metaweb-based approach to examine local food-web dynamics31. A metaweb details all possible interactions of those species found in a dataset, and then each local food web is inferred from the subset of species and their potential interactions found at each site. The metaweb approach is suitable to capture food-web patterns based on taxa co-occurrence data in both terrestrial and aquatic systems24,32–35. This allowed us to test for the association of biodiversity and food-web structures with network location (drainage area), season, and the interaction between these two factors. We used high-throughput sequencing and a multi-marker approach to examine the occurrence of three taxonomic groups, namely fish (via the 106 bp fragment of the 12S barcode region), invertebrates (a 313 bp fragment of the cyanobacterial c oxidase I region, COI) and bacteria (a 450 bp fragment of the 16S barcode region) in a large-scale river network over the course of three seasons (spring, summer and autumn). We found contrasting effects on biodiversity patterns and food-web structure from spatial and temporal influences and their interaction, 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 km² 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 Supplementary Fig. S1). Water samples were filtered on site, sealed with luer fittings, and placed in individual sealed bags in a cool box for transport to the lab, where they were stored at −20 °C until further processing. DNA extraction was performed in a specialist clean lab environment at Eawag, Switzerland and subsequent library preparation followed a uni-directional lab processing protocol, where PCR product was not returned to the cleaned lab. 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 (±sd) sequencing read depth per sample was: 42,189 ± 19,057, 46,573 ± 407,659 and 24,646 ± 27,612 for 12S, COI and 16S, respectively (see Methods and Supplementary Table S1 and bioinformatics workflow). 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 and Supplementary Table S2 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 ref. 36).

To quantify biodiversity, we calculated α-diversity (local richness) at the 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 catchment37.

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
feeding-group composition, which then determines broad changes emerge from the spatio-temporal differences in genus and thus variation in food-web structural and functional characteristics subset of interactions from the metaweb. With this approach, the results indicate energy flow. Number of genera in each group is shown in circles, arrows indicate energy flow.

groups (Fig. 1; see also “Methods” and Supplementary Table S3 and Supplementary Data file 1). 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 (km²) 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 (Supplementary Table S4). As post hoc contrast testing, we ran estimated means of linear trends to compare the slopes from the mixed-effects models and estimated marginal means of data over season only to determine the influence of seasonal changes on all α-diversity and food-web measurements (see Supplementary Tables S5–S9 for further 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 Table S10).

Spatial and temporal biodiversity patterns. In total, we detected 374 genera across three organisal 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. 2). 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 (Supplementary Fig. S2 and Table S2). We used mixed-effects models to assess the influence of drainage area and season on the local α-diversity of each group (Fig. 3, for detailed model output see Supplementary Tables S5–S7). Of the three taxonomic groups, interaction between drainage area and season was found to be significant for both fish and bacteria α-diversity (p < 0.001 for both, Table S7), in this study this indicates that the effect of drainage area is modulated by the season rather than vice-versa. By examining the slopes generated from the mixed-effect models for each season, the effect of drainage area differs across the seasons in both fish and bacteria α-diversity (Fig. 3a, e, Supplementary Table S8). Fish α-diversity in summer has a flatter slope and is significantly 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 S8); 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) showed no significant change in fish (mean number of 2 genera and range 0–6, Fig. 3b), but for bacteria we found a significantly lower α-diversity in summer compared to spring and autumn (p < 0.001 for both, Fig. 3f and Supplementary Table S9). Looking at invertebrate α-diversity we do not see a significant interaction between drainage area and season (p = 0.295, Fig. 3c and Supplementary Table S7), but rather season as a significant main effect (p < 0.05, Supplementary Table S7). Examining the means across the seasons further showed 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 S9).

Regarding the analysis of β-diversity across the catchment, the Jaccard’s dissimilarity significantly increased with increasing pairwise river distance among sites in all organisal 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, Supplementary Table S10). 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, for all β-diversity results see Supplementary Table S10), 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. 1 and Supplementary Fig. S3 and Supplementary Data file 1). For food-web structure, here we describe the results of link density, connectance, nestedness and level of omnivory (hereinafter, omnivory) as the most ecologically

Fig. 1 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.
important food-web descriptors in our study, while the results from the remaining descriptors are presented in the Supplementary Information (see Tables S4–9 and Fig. S4). As with the biodiversity patterns, we found 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 S7). 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 S8). Whereas link density showed only a marginally significant decrease with drainage area ($p = 0.064$; Supplementary Table S7) and a significant effect of season ($p < 0.05$; Fig. 4a and Supplementary Table S7). When we examined mean food-web measurements across the seasons, link density was significantly higher in spring than in summer and autumn ($p < 0.001$ for both, Fig. 4b and Supplementary Table S9) whereas connectance and nestedness were significantly lower in autumn than in spring and summer (all $p < 0.01$ or 0.001, Fig. 4d, f and Supplementary Table S9), and omnivory was significantly higher in summer than in autumn ($p < 0.001$, Fig. 4h and Supplementary Table S9).

Functional diversity showed no significant interaction between drainage area and season ($p = 0.354$, Supplementary Table S7), but a marginally significant decrease with increasing drainage area while exhibiting no change with season ($p < 0.05$ and $p = 0.147$, respectively; Fig. 5a, b and Supplementary Table S7). Functional redundancy showed a significant interaction between drainage area and season ($p < 0.01$, Supplementary Table S7). Across seasons, the mean functional redundancy was significantly lower in summer than in autumn ($p < 0.05$, Fig. 5d and Supplementary Table S9).

**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

**Fig. 2 Genus α-diversity of bacteria, invertebrates and fish collected from eDNA samples in the Thur catchment.** Maps show the α-diversity of each taxonomic group in (a) spring, (b) summer and (c) autumn. The circles are scaled proportionate to the α-diversity of each group. Grey lines give the subcatchments Glatt, Necker, and Thur, respectively and the blue arrow shows the direction of flow.
processes and the temporal fluctuations that together play a key role in community dynamics present within a river network. Our study is one of the first assessments utilising data derived from eDNA metabarcoding to describe biodiversity patterns and food-web characteristics in a whole river network at a spatially and temporally large scale. By taking this approach, we were able to detect contrasting group-specific spatial and temporal biodiversity patterns, as well as food-web structural and functional characteristics whose spatial responses also showed strong dependence on the season, yet in different ways. Overall, our findings support the need to include multifaceted biomonitoring, i.e., at taxa and community levels and regarding richness as well as biological interactions, conducting across both spatial and temporal scales, to better understand changes in ecosystems.

Aquatic biodiversity is subject to fluvial influences within a dendritic network, whereby spatial patterns of α-diversity and β-diversity are known for key groups, such as fish, invertebrates and microbes. Our data is congruent with previous studies on fish diversity, in that both α-diversity significantly increased downstream, and β-diversity (community dissimilarity) significantly increased with river distance in all three seasons. Bacterial richness was highest at the top of catchment, which was also in line with previous studies, albeit only in the summer sampling campaign. Similarly, bacterial β-diversity also increased with river distance during this season. We therefore postulate that bacteria can disperse through the focal catchment during higher flows (spring and autumn), which makes bacterial spatial diversity patterns more subjected to seasonal influences. Unlike the former two groups, invertebrate richness exhibited no significant influence of drainage size but a seasonal change. The seasonal variation in invertebrate diversity can be linked directly to the emergence of the non-aquatic adult life stage of several macro-invertebrate genera detected (Diptera, Ephemeroptera, Plecoptera and Trichoptera), which takes place in the late spring and summer months, and literally removes these organisms from the aquatic communities as they become air bound. The lack of a spatial effect is contrary to previous studies that have shown clear, although variable, spatial patterns for invertebrate communities in river systems. As previous studies often looked at spatial scales much larger or much smaller than ours, this disparity may be due to a mismatch in scale looked at here, or reflect different strengths of anthropogenic drivers that may have opposing effects. For example, Finn and colleagues suggested headwaters harbour disproportionately high invertebrates compared...
to lower points in the catchment, but their studied focused on small (1–2) and mid (3–4) stream orders only, whereas the sites in our study ranged from small to much larger rivers (stream orders 1–7). Contrastingly, when looking at scales about 50-fold larger, Altermatt and colleagues showed the number of key aquatic invertebrate taxa (Ephemeroptera, Plecoptera and Trichoptera) increased with catchment size; however, they also found a combination of local factors (catchment areas, drainage area, elevation and network centrality) had the greatest influence on local invertebrate $\alpha$-diversity. Possibly, the scale at which we study invertebrate $\alpha$-diversity and contributing local factors falls between such small-scale vs. large-scale perspective, such that we detected regional patterns where $\alpha$-diversity remains relatively constant throughout the catchment.

By resolving the fundamental trophic relationships among broad feeding groups, we established a trophic interaction metaweb for the three taxonomic groups examined in this study. The metaweb with genus co-occurrence data thus allowed our investigation of both spatial and temporal influences on the characteristics of local food webs. As our local food webs were mostly stemmed from simplified basal resource (i.e., assumptive detritus and cyanobacteria genera; Fig. S5), the detected food-web patterns were mostly contributed by the variation in the consumer trophic levels. With regards the food-web structure, we found a significant

Fig. 4 Food-web structural characteristics. Plots show food-web structural characteristic: (a, b) Link Density, (c, d) Connectance, (e, f) Nestedness and (g, h) Omnivory. Lines indicate linear mixed-effects models with shaded area showing 95% confidence intervals as calculated using the model predictions and standard error. Plot 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.
interaction between drainage area and season associated with connectance, nestedness and omnivory, whereas link density was influenced in isolation significantly by season and marginally by drainage area. Overall, the impact of season was prevalent in all four focal structural characteristics, highlighting the importance of considering seasonality of food-web structure. By definition, a lower link density indicates a higher proportion of trophic specialist (who interact with relatively fewer other taxa) in the food web. Therefore, combining observed link density and α-diversity patterns together, reveals that communities are larger and have more specialists toward downstream in wetter seasons (spring and autumn), while smaller communities with fewer generalists are found towards downstream in the dry season (summer). Such changes are likely driven mainly by bacteria given their dominance in the communities. Thus, the unique summer spatial patterns (in comparison to spring and autumn) for food-web connectance, nestedness and omnivory may reflect the similarly unique bacterial α-diversity summer spatial pattern. Temporally, all four focal food-web structural characteristics were lowest in autumn, despite overall α-diversity being lowest in summer. This indicates that the seasonal variation in food-web structure found in this study does not merely reflect the overall richness change (i.e., community size), but rather the change in genera and thus feeding-group composition. 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. Thus, the restrained food-web structural characteristics we see in autumn may capture the start of the productivity restriction seen in food webs over the winter months, allowing only some of the feeding groups to persist (as further suggested by our functional redundancy results, see below). Indeed, the detected 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 especially when competition becomes more costly with lower resource productivity in autumn.

Moving from broad food-web patterns to examining feeding groups enables us to study fundamental functional changes within the community and possible effects at the ecosystem level. We found a marginally significant effect of drainage area on the functional diversity, in which the functional diversity was highest upstream. Such a spatial pattern along the hierarchical river network is congruent with the River Continuum Concept (RCC), which states increased diversity of invertebrate functional feeding groups at low stream orders (i.e., upstream). In river systems allochthonous material (i.e., the dominant organic material) in forested headwaters is processed by specialist shredder, collector/filterer, and grazer/scaper, while downstream autochthonous organic material is utilised by fewer functional feeding groups. Therefore, the pattern we observed is to be expected and showed the suitability of our approach of using single overarching functional feeding groups (i.e., a single dominant functional feeding-group per genera), which simplified the life cycle and changes in feeding behaviour observed in invertebrates (the dominant group within the functional feeding groups). Interestingly, the functional redundancy we observe gives us more information about the changes exhibited at a community level. Functional redundancy, i.e., genera per functional feeding group, was interactively influenced by drainage area and season, showing a unique decrease toward downstream in summer, contrasting the opposite spatial trend in spring and autumn. Also, the functional redundancy was lower in summer than in autumn. Since this measurement accounts for the number of genera, its spatio-temporal patterns could again largely reflect the variation in bacteria richness. Nonetheless, the particularly high functional redundancy in autumn suggests that, although fish and bacteria richness increase toward downstream, some key invertebrate taxa of well-connected feeding groups (i.e., interact
with many other groups) can become absent particularly in this season, and losing such feeding groups causes a constriction of local food-web structure as we observed. Furthermore, although both the food-web structural and functional characteristics are determined by genera occurrence, their inconsistent and variable temporal patterns imply that multiple feeding groups share similar trophic roles in the food web (Fig. S3), 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 stricto22-25). 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 perturbation17,26,51. 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 taxa, 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. 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. For example, it is known that algae become an increasingly important resource when moving from allochthonous inputs in headwaters to larger streams with increased light levels further down the catchment48,49. The choice of taxonomic groups looked at was both methodologically defined, as well as driven by the goal to have an observable and clearly aquatic-focused view on food webs. Hereby, we here capture the spatio-temporal patterns of a (dominant) subset from the real-world communities and food webs in which even more species are involved.

By using the eDNA technique, we gain three notable advantages: scalability for monitoring complex and large systems, reusability and a non-invasive method of collecting biodiversity information41,52. Our understanding of how the information we ascertain from eDNA sample collection has greatly improved in recent years due to studies on the hydrological influences53 and a general understanding of the rate of eDNA persistence in lotic ecosystems54. However, the successful detection of taxa with eDNA is also linked to the ecology of individual species54, and some seasonal variation in the detection of several taxonomic groups is known55-58. 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, we reconstruct comprehensible multi-trophic communities and evaluate their characteristics at a large spatial scale (i.e., the catchment) and over time (i.e., seasons) by exploiting broad species occurrence information derived by eDNA sampling and combining multiple markers. For both biodiversity and food-web characteristic, we detect spatio-temporal patterns, and those of the former are different across taxonomic groups while the latter across measurements. Specifically, we identify inconsistent patterns between food-web structural and functional feeding characteristics, as well as a reduction in local food-web structure particularly in autumn. Our approach provides a first demonstration of the application of eDNA to a complex river network for the monitoring of not only biodiversity but also food-web patterns, paving a way for establishing long-term, repeated monitoring of complex communities, and potentially also in other systems worldwide.

**Methods**

**Site selection and eDNA sample collection.** Environmental DNA water samples were collected from the edge of the waterbody and filtered on site using single use disposable 50 ml syringes. At each site the syringe was filled and refilled 10 times through a 0.2 μm filter (Merck Millipore, Merck KGaA, Darmstadt, Germany). Two syringes and two steriver filters were used per site, equating to 1 L of samples water per site. The filters were then sealed with luer caps, placed in individually labelled bags in a cool box for transport to the lab, where they were stored at −20 °C until further processing. 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 dH2O through a steriver filter in the same way as field samples were collected (n = 15). Negative controls (n = 5). Samples selected at random were analysed using a Qubit 3.0 fluorometer for double stranded DNA concentration, values measured between 0.317 and 27.5 ng/μL. All negative controls (field and extraction) were tested and recorded below detection limits.

Sample replicated were pooled for subsequent sequencing with the following markers: a 106 bp fragment of the mitochondrial 12S marker (59, hereafter referred to as 12S) used to amplify cytochrome oxidase I marker (60 and 61, hereafter referred to as COI) used to amplify metazoan DNA and a 450 bp fragment of the V3-V4 region of the 16S marker (62, hereafter, 16S) used to amplify bacteria and archaea DNA (See Supplementary Information Table S8 for primer sequences). Positive controls (n = 6 per library prep, see Supplementary Information Table S9) and PCR negative controls (2 μL of dH2O, 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 PCR53 (See Supplementary Information Methodology Information for full details). All libraries 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 Zymo Rapid Clean-Up Kit (Zymo Research) following the manufacturer’s protocol with the minor modification by which the elution step was prolonged to 2 min at 4000 g. The clean amplicons were indexed using unique combinations of 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 5′ end-indexed (n = 1,000 Pairs) and 15 μL of the DNA templates. The final PCR products were then cleaned using the Thermo MG Magiet bead clean up kit (Thermo Fisher Scientific Inc., MA, USA) and a customised programme for the KingFisher Flex Purification System (Thermo Fisher Scientific Inc., MA, USA) in order to remove excessive Nextera XT adapters. 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 controls were included here to determine if any contamination had occurred at either the PCR1 or PCR2 stage. No such amplification was detected when the samples were run on the QIAxcel and the maximum amount of each negative sample was pooled with the other samples for library preparation. Library concentration was quantified on the Agilent 4200 TapeStation (Agilent Technologies, Inc., USA) and verified by the Qubit 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 supplementary information Table S1 for library loading information).
Bioinformatics. After each of the libraries were sequenced, the data was demul-
tiplixed and reads were quality checked using usearch v11.0.6674 and FastQC65. Raw reads were then primed trimmed, merged and full-length primer sites were
removed using usearch v11.0.6674 (16S reads were merged using Flash v2.1.2.166). 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
chimeras removed ZOTU. The ZOTUs were then taxonomically annotated using
a 97%, similarity approach and taxonomic assignment with a 0.85 confidence
threshold was performed using SINTAX in the usearch software v11.0.6674 with
following databases: 12S: NCBI BLAST (v200416), COI: Custom reference database
(including MIDORI un-trimmed (V20180223)) and SILVA (V128). See Supplementary
Information Methods for all bioinformatic parameters and refer-
dence database information used for each library. Prior to downstream analysis,
positive samples were examined from each library to see signs of potential con-
tamination. Once we had removed terrestrial genera (such as Bos taurus, which was
identified due to the use of BSAs in the 12S library preparation), we applied a minor
contamination of 0.1% to reduce errors caused by tag-jumping or minor con-
tamination in library preparation66.

Biodiversity patterns. We calculated α-diversity (genus richness) at each site and
compared β-diversity between sites by using Jaccard dissimilarity using the beta-
part R-package37. We constructed a matrix of pairwise distances between sites
along the fluvial network with the igraph R-package68 and compared the similarity
between β-diversity (including further partition β-diversity into species loss and
replacement) and river distance using the Mantel test with the vegan R-package69. Sites
which did not record genus from a target group were removed from this
analysis: 12S dataset: 4 sites were removed from Spring, Summer and Autumn, the
COI dataset 4 sites were removed from the Summer and 3 from the Autumn
analysis, no further sites were removed from the 16S Bacteria dataset.

Feeding-group assignment. Functional feeding groups were determined based on
literature, species inventories and targeted expert knowledge39,41 for fish and
invertebrates. Genera of bacteria were included if the phyla they belong to has a
strong association with freshwater habitat72, bacteria were then broadly divided
into heterotrophic and cyanobacteria, the latter constituting a basal resource. An
assumptive constant basal resource of detritus was also included in all food webs.
Genera were designated into one of eleven feeding groups: Parasite, Piscivorous
fish, Invertebrate predator, Collector/Filterer, Grazer/Scraper, Shredder, Small predator, Sessile
filter feeders, Omnivorous, Collector/Filterer, Omnivorous, Collector/Filterer, Collector/Filterer,
Collector/Filterer, Collector/Filterer, Collector/Filterer. Genera of bacteria were included if the phyla they belong to has a
strong association with freshwater habitat72, bacteria were then broadly divided
into heterotrophic and cyanobacteria, the latter constituting a basal resource. An
assumptive constant basal resource of detritus was also included in all food webs.

Food-web structure. We constructed a metaweb based on known trophic relation-
ships among feeding groups (Supplementary Table S2), then defined local food webs
using these metaweb at each field site, based on co-occurrence of general
(nodes) and their interactions (links) (see Supplementary Fig. S5 for site food web
examples and associated GitHub repository for information on for feeding-group occurrence found in each sample). The metaweb approach has been applied to
identify food-web characteristic change across spatial gradients in terrestrial
ecosystem35, and temporal changes in individual aquatic ecosystem35, 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 a diversity at each site) and connectance (L/S2, 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 features. Nesteredness is an indication that the diets of
specialist taxa are subsets of generalist taxa’s diets, and was calculated using the
nesteredness metric, Overlap and Decreasing FI (ODFI) in vegan85. Modularity is
indication of a less nesteded network73 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 package68.
Omnivory is defined as having a mixed trophic-level diet. Thus, the more
omnivorous a community has, the less coherent (see below) the food web4
3. The level of omnivory is often debated as a measure of food-web stability, and weak
omnivorous interactions are likely to lead to a more stable food web57. Trophic
level and level of omnivory of genera were calculated using the methods stated in
William and Martinez45, and omnivory was averaged over all consumers in the
two systems as a community-level index. Coherence is defined as the overall degree
of homogeneity of the difference between trophic levels of each consumer resource
pair within the local food web. As described by4, 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
4, Robustness was defined by Dunne and Williams59 as the “proportion of species
subjected to primary removals that resulted in a total loss ... of some spec-
tified proportion of the species”. In our study, we used the commonly adopted 50% (i.e.,
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 Williams59.

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 relation-
ship 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 char-
acteristics (functional diversity and redundancy) with site location within
the catchment. Drainage area (km2) 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 also included interaction term between season and drainage area to
determine the significance of this potential interaction (see Supplementary Table S5):

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

2. No Interaction model: genus α-diversity – drainage area + season + (1

| Site)

Significance was calculated using the lme4 Test R-package76, which applies
Satterthwaite’s method to estimate degrees of freedom and generate p-values
for mixed models (Supplementary Tables S6 and S7). We then used anova() to see
the overall effect of both drainage area and season (Supplementary Table S8).
Subsequent contrast testing was carried out using the emmeans R-package77 (with the
function emmeans()) 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). All analysis was completed in R studio version 4.0.478.

Reporting summary. Further information on research design is available in the Nature
Research Reporting Summary linked to this article.

Data availability
Sequencing data generated during this study is available on the European Nucleotide
Archive under accession project numbers PRJEB50676 (12S), PRJEB50363 (COI), and
PRJEB50654 (16S).

Code availability
The data and analysis files used in this study are available on the following GitHub
repository: https://github.com/RosettaBlackman/RiverDNA.

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References
1. Whittaker, R. H. Vegetation of the Siskiyou mountains, Oregon and California. Ecol. Monogr. 30, 279–338 (1960).
2. Wilson, R. J., Thomas, C. D., Fox, R., Roy, D. B. & Kunin, W. E. Spatial patterns in species distributions reveal biodiversity change. Nature 432, 393–396 (2004).
3. Newbold, T. et al. Global effects of land use on local terrestrial biodiversity. Nature 520, 45–50 (2015).
4. Ings, T. C. et al. Ecological networks—beyond food webs. J. Anim. Ecol. 78, 253–269 (2009).
5. Dunne, J. A. & Williams, R. J. Cascading extinctions and community collapse in model food webs. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 364, 1711–1723 (2009).
6. Leclère, D. et al. Bending the curve of terrestrial biodiversity needs an integrated strategy. Nature 585, 551–556 (2020).
7. Vellend, M. The Theory of Ecological Communities Vol. 57 229 (Princeton University Press, 2016).
8. Altermatt, F. Diversity in riverine metacommunities: a network perspective. Aquat. Ecol. 47, 365–377 (2013).

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COMMUNICATIONS BIOLOGY | https://doi.org/10.1038/s42003-022-03216-z | www.nature.com/commsbio
Harvey, E., Gounand, I., Fronhofer, E. A. & Altermatt, F. Disturbance reverses diversity patterns in Mississippi-Missouri basin. *Nature* **453**, 220–222 (2008).

Besemer, K. et al. Headwaters are critical reservoirs of microbial diversity for fluvial networks. *Proc. Natl Acad. Sci. USA* **111**, 12979–12984 (2014).

Seymour, M. et al. Environmental DNA provides higher resolution assessment of riverine biodiversity and ecosystem function via spatio-temporal nestedness analyses in a temperate wetland. *Environ. DNA* **1**, 290–301 (2019).

Mächler, E. et al. Assessing different components of diversity across a river network using eDNA. *Environ. DNA* **1**, 290–301 (2019).

Peralta-Maraver, I., López-Rodríguez, M. J. & de Figueroa, J. M. T. Structure, dynamics and stability of a Mediterranean river river web. *Mar. Freshw. Res.* **65**, 2206–2217 (2014).

Woodward, G. et al. Ecological networks in a changing climate. *Ecol. Netw.* **42**, 71–138 (2010).

Kondoh, M., Kato, S. & Sakato, Y. Food webs are built up with nested subwebs. *Ecology* **91**, 3123–3130 (2010).

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R. & Cushing, C. E. Fishes of the River Continuum Concept. *Can. J. Fish. Aquat. Sci.* **37**, 130–137 (1980).

Power, M. E. & Dietrich, W. E. Food webs in river networks. *Ecol. Res. https://doi.org/10.4319/er.2012.1.01950.x* (2002).

Montoya, D., Yallop, M. L. & Memmott, J. Functional group diversity increases with modularity in complex food webs. *Nat. Commun.* **6**, 7379 (2015).

Gravel, D., Aubouy, C. & Thuiller, W. The meaning of functional trait composition of food webs for ecosystem functioning. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **371**, 20150268 (2016).

Ruppert, K. M., Kline, R. J. & Rahman, M. S. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: a systematic review in methods, monitoring, and applications of global eDNA. *Glob. Ecol. Conserv.* **9**, 458–468 (2017).

Carraro, L., Mächler, E., Wüthrich, R. & Altermatt, F. Environmental DNA allows upscaling spatial patterns of biodiversity in freshwater ecosystems. *Nat. Commun.* **11**, 3585 (2020).

Barnes, M. A. & Turner, C. R. The ecology of environmental DNA and implications for conservation genetics. *Conserv. Genet.** 17**, 1–17 (2016).

Buita, I. et al. Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. *Nat. Commun.* **8**, 14087 (2017).

Ericksen, R. A., Merkes, C. M., Jackson, C. A., Goforth, R. R. & Amberg, J. B. Seasonal trends in eDNA detection and occupancy of bigheaded carps. *J. Great Lakes Res.* **43**, 762–770 (2017).

Teoth, C. R., Sweet, M. J., Nightingale, J. & Burian, A. Seasonality, DNA degradation and spatial heterogeneity as drivers of eDNA detection dynamics. *Sci. Total Environ.* **768**, 144466 (2021).

Thalinger, B. et al. The effect of activity, energy use, and species identity on seasonal trends in eDNA detection and occupancy of bigheaded carps. *Front. Ecol. Evol.* **9**, 73 (2021).

Kelly, R. P., Port, J. A., Yamahara, K. M. & Crowder, L. B. Using environmental DNA to census marine fishes in a large mesocosm. *PLoS ONE* **9**, e86175 (2014).

Barnes, M. A. & Turner, C. R. The ecology of environmental DNA and implications for conservation genetics. *Conserv. Genet.** 17**, 1–17 (2016).

Buita, I. et al. Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. *Nat. Commun.* **8**, 14087 (2017).

Ericksen, R. A., Merkes, C. M., Jackson, C. A., Goforth, R. R. & Amberg, J. B. Seasonal trends in eDNA detection and occupancy of bigheaded carps. *J. Great Lakes Res.* **43**, 762–770 (2017).

Thalinger, B. et al. The effect of activity, energy use, and species identity on seasonal trends in eDNA detection and occupancy of bigheaded carps. *Front. Ecol. Evol.* **9**, 73 (2021).

Kelly, R. P., Port, J. A., Yamahara, K. M. & Crowder, L. B. Using environmental DNA to census marine fishes in a large mesocosm. *PLoS ONE* **9**, e86175 (2014).

Barnes, M. A. & Turner, C. R. The ecology of environmental DNA and implications for conservation genetics. *Conserv. Genet.** 17**, 1–17 (2016).

Buita, I. et al. Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. *Nat. Commun.* **8**, 14087 (2017).

Ericksen, R. A., Merkes, C. M., Jackson, C. A., Goforth, R. R. & Amberg, J. B. Seasonal trends in eDNA detection and occupancy of bigheaded carps. *J. Great Lakes Res.* **43**, 762–770 (2017).

Thalinger, B. et al. The effect of activity, energy use, and species identity on seasonal trends in eDNA detection and occupancy of bigheaded carps. *Front. Ecol. Evol.* **9**, 73 (2021).

Kelly, R. P., Port, J. A., Yamahara, K. M. & Crowder, L. B. Using environmental DNA to census marine fishes in a large mesocosm. *PLoS ONE* **9**, e86175 (2014).

Barnes, M. A. & Turner, C. R. The ecology of environmental DNA and implications for conservation genetics. *Conserv. Genet.** 17**, 1–17 (2016).

Buita, I. et al. Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. *Nat. Commun.* **8**, 14087 (2017).

Ericksen, R. A., Merkes, C. M., Jackson, C. A., Goforth, R. R. & Amberg, J. B. Seasonal trends in eDNA detection and occupancy of bigheaded carps. *J. Great Lakes Res.* **43**, 762–770 (2017).

Thalinger, B. et al. The effect of activity, energy use, and species identity on seasonal trends in eDNA detection and occupancy of bigheaded carps. *Front. Ecol. Evol.* **9**, 73 (2021).

Kelly, R. P., Port, J. A., Yamahara, K. M. & Crowder, L. B. Using environmental DNA to census marine fishes in a large mesocosm. *PLoS ONE* **9**, e86175 (2014).

Barnes, M. A. & Turner, C. R. The ecology of environmental DNA and implications for conservation genetics. *Conserv. Genet.** 17**, 1–17 (2016).

Buita, I. et al. Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. *Nat. Commun.* **8**, 14087 (2017).

Ericksen, R. A., Merkes, C. M., Jackson, C. A., Goforth, R. R. & Amberg, J. B. Seasonal trends in eDNA detection and occupancy of bigheaded carps. *J. Great Lakes Res.* **43**, 762–770 (2017).

Thalinger, B. et al. The effect of activity, energy use, and species identity on seasonal trends in eDNA detection and occupancy of bigheaded carps. *Front. Ecol. Evol.* **9**, 73 (2021).
74. Johnson, S., Domínguez-García, V., Donetti, L. & Muñoz, M. A. Trophic coherence determines food-web stability. *Proc. Natl Acad. Sci. USA* **111**, 17923–17928 (2014).
75. Wootton, K. L. Omnivory and stability in freshwater habitats: Does theory match reality? *Freshw. Biol.* **62**, 821–832 (2017).
76. Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. lmeTest package: tests in linear mixed effects models. *J. Stat. Softw., Artic.* **82**, 1–26 (2017).
77. Lenth, R. V. Estimated Marginal Means, aka Least-Squares Means [R package emmeans version 1.6.1] (2021).
78. RStudio Team RStudio: Integrated development for R. RStudio, PBC, Boston, MA. R version 4.0.4 Retrieved from http://www.rstudio.com/ (2021).

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**Author contributions**
R.C.B. and F.A. conceived the study. R.C.B. carried out the fieldwork and 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 declare no competing interests.

**Additional information**

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