

Beyond aquatic and terrestrial: a multitaxon, large-scale perspective reveals the distinct biodiversity of non-perennial rivers

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Beyond aquatic and terrestrial: a multitaxon, large-scale perspective reveals the distinct biodiversity of non-perennial rivers

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79

80 **ABSTRACT**

81 Non-perennial rivers (NPRs), which alternate between flowing and dry conditions, constitute a
 82 major component of river networks yet their contribution to biodiversity across the aquatic–
 83 terrestrial continuum remains insufficiently understood. Their highly variable hydrology has
 84 hindered consistent global assessments of NPR biodiversity. Here we address this gap using a
 85 large-scale collaborative network and environmental DNA metabarcoding to characterize
 86 biodiversity across both flowing and dry NPRs, alongside perennial rivers (PRs) and adjacent
 87 riparian soils. Our results show that NPRs support high biodiversity across multiple taxonomic
 88 groups and are not depauperate versions of perennial systems. Instead, they host
 89 compositionally distinct communities with different taxonomic groups following distinct
 90 response pathways to flow intermittence. While microbial communities occupied intermediate

compositional space between aquatic and terrestrial systems across flowing and dry conditions, sediment-associated taxa reorganize into different communities that remain distinct from both perennial rivers and adjacent soils. These patterns are modulated by climate, with warmer and drier conditions amplifying divergence of NPR communities from the aquatic–terrestrial continuum. Our findings reposition NPRs not as transitional systems between aquatic and terrestrial states, but as complex ecological entities, calling for a re-evaluation of how biodiversity is conceptualized and monitored in river networks.

MAIN TEXT

1. Introduction

River networks cover <1% of the Earth’s surface, but contain ~13% of described species and deliver key ecosystem services including the provision of drinking water, energy production, climate regulation and recreational activities^{1,2}. However, rivers are among the world’s most threatened ecosystems, with more than one third of species in these biodiversity hotspots at risk of extinction^{3,4}. This threat may be underestimated because biodiversity assessments have focused on perennial rivers and streams (hereafter PRs), while non-perennial rivers (hereafter NPRs)—which alternate between flowing and dry conditions—have been largely overlooked⁵. NPRs represent a substantial fraction of the global river network^{6,7} and their integration into assessments could therefore fundamentally alter understanding and conservation strategies related to river biodiversity.

Characterizing biodiversity in NPRs is particularly challenging because shifts between flowing and dry conditions lead to strong temporal and spatial variability in biological communities⁸. Despite this complexity, NPRs are often documented species-poor compared to PRs^{9,10} and are frequently excluded from ecological assessment and conservation programs^{11–13}. However, this

characterization is largely driven by methodological, taxonomic and hydrological-phase-related biases. In particular, NPRs have been studied predominantly when flowing and with a strong focus on aquatic macroinvertebrates^{10,14}, using sampling methods suitable for flowing but not dry conditions. This narrow focus has reinforced the view that dry riverbeds represent degraded aquatic habitats or transitional, terrestrialized systems¹⁵. However, increasing evidence suggests that NPRs can contribute substantially to regional biodiversity due to strong spatiotemporal variability in community composition and the presence of taxa adapted to drying–wetting cycles^{16,17}. In particular, dry riverbeds have recently been recognized as supporting high biodiversity^{18–20}. These findings suggest that NPR communities may not simply represent subsets or transitional stages of aquatic and terrestrial communities, but instead include distinct community configurations associated with non-perennial conditions (Fig. 1).

A typical key limitation of previous studies is the assessment of NPR hydrological phases in isolation, and from either an aquatic or a terrestrial perspective. As a result, the full biodiversity of NPRs within the aquatic–terrestrial continuum remains poorly resolved. Understanding biodiversity in NPRs requires an integrated framework that considers their dynamics across both hydrological phases, alongside PRs and adjacent riparian soils as reference states defining an aquatic–terrestrial continuum (Fig. 1). In addition, the focus on a limited set of taxonomic groups, particularly macroinvertebrates, leaves the responses of other major components of biodiversity largely unexplored. Addressing these gaps requires both a multi-taxon perspective and a methodology that, unlike conventional, habitat-specific sampling methods, can be consistently applied across habitats and hydrological phases. Environmental DNA (eDNA) metabarcoding provides such an approach, enabling the standardized characterization of biodiversity in multiple taxonomic groups across flowing streams, dry riverbeds and soils within a unified analytical framework^{21,22}.

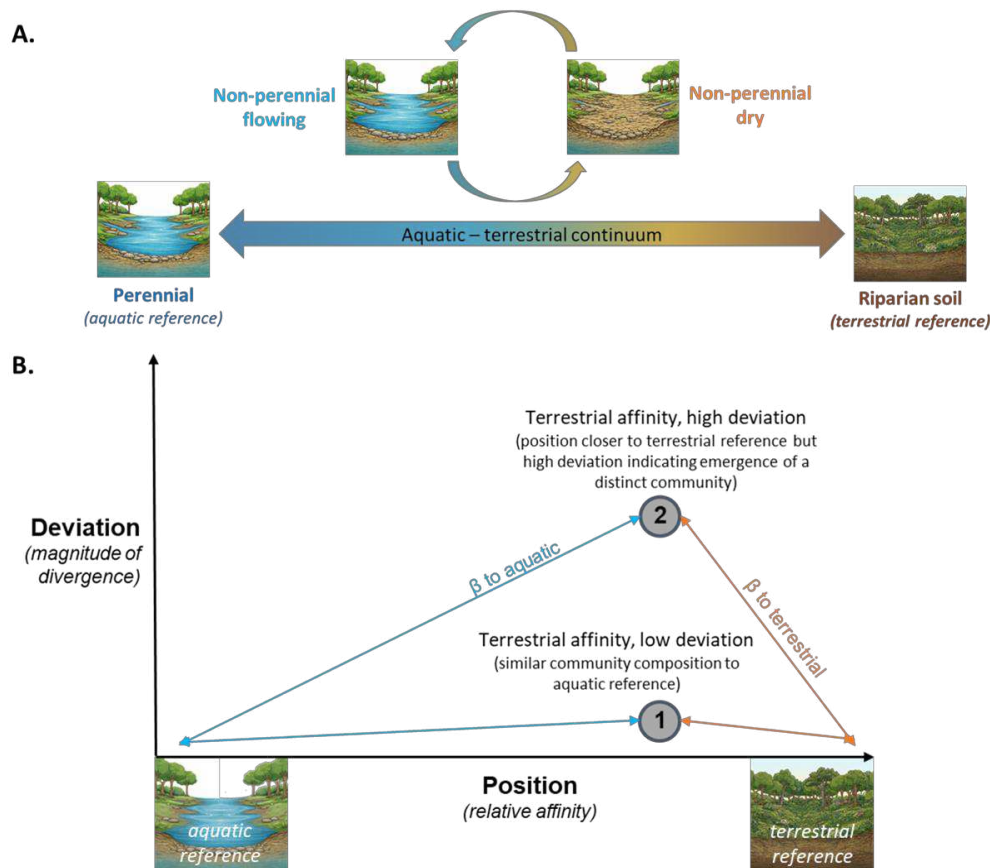
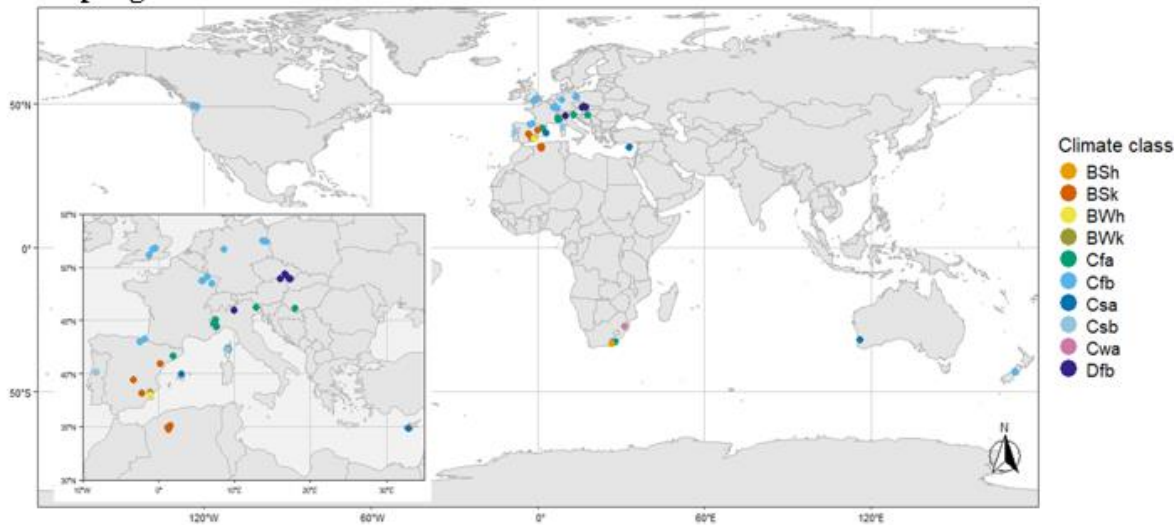


Fig. 1: A. The aquatic-terrestrial continuum. Non-perennial rivers (NPRs) alternate between flowing and dry conditions, whereas perennial rivers and riparian soils represent stable reference states at the aquatic and terrestrial ends of the continuum. **B. Positions of different hypothetical NPR biological communities along the continuum.** Community 1 has a smaller β distance to the terrestrial reference (X-axis) than to the aquatic one, is positioned close to the terrestrial reference and has a low deviation value (Y-axis). Community 2 is also positioned close to the terrestrial reference but has higher β distance to both references resulting in a high deviation value, suggesting a distinct community composition. Together, position and deviation indicate whether NPR communities shift along the aquatic-terrestrial continuum or instead diverge toward alternative community configurations.

We conducted a large-scale study through a collaborative research network to characterize NPR biodiversity across the aquatic-terrestrial continuum and test whether NPR communities transition between aquatic and terrestrial communities or have distinct ecological states. We analysed the taxonomic diversity and composition of seven major taxonomic groups (algae, archaea, bacteria, fungi, meiofauna, invertebrates and protozoa) across 58 sampling sites, each comprising an NPR reach, a PR reach and their respective adjacent riparian soils. The dataset covered 14 countries across 5 continents and 10 Köppen-Geiger climate classes, capturing temperate, continental, semi-arid and arid environments across both hemispheres (Fig. 2). We asked three questions: (1) Does biodiversity differ in NPRs and PRs and their riparian soils?

(2) Do NPR communities change along the aquatic–terrestrial continuum or diverge from it into distinct ecological states? (3) Which environmental factors best explain these biodiversity patterns at large spatial scales? To address these questions, we compared alpha diversity between PR and NPR riverbeds and riparian soils, used beta-diversity metrics to assess the position of NPR communities along and their deviation from the aquatic–terrestrial continuum, and evaluated the influence of abiotic factors on biodiversity patterns.

A. Sampling sites



B. Sampling design

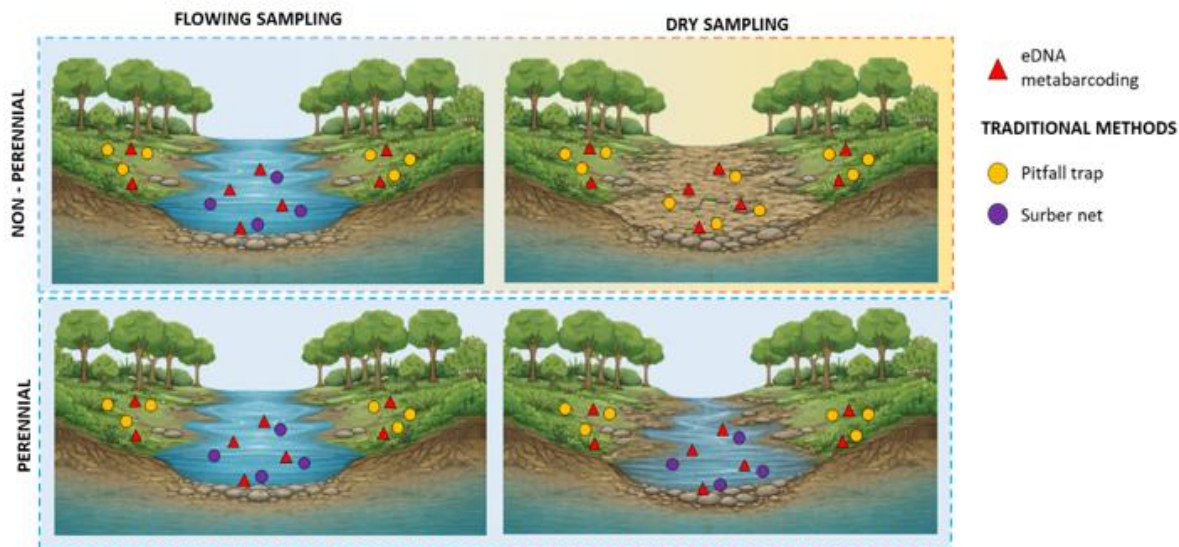


Fig. 2: A. Map of sampling sites coloured according to their Köppen-Geiger climate classification. Each dot corresponds to a sampling site comprising one non-perennial river (NPR) and one perennial river (PR) reach. **B. Overview of the sampling design.** Each NPR-PR reach pair was sampled twice, corresponding to NPR flowing and dry conditions. Riverbed sediment and riparian soil were collected for eDNA metabarcoding; and aquatic and terrestrial invertebrates were sampled using traditional methods.

2. Results

On each NPR and PR, riverbed sediment and adjacent riparian soil were collected twice, during the flowing and dry conditions of NPRs, producing 1,823 eDNA samples (Fig. 2B). eDNA metabarcoding identified 2,434 invertebrate molecular operational taxonomic units (MOTUs) (assigned to 378 families), 281 meiofauna MOTUs (20 families), 942 algae MOTUs (115 families), 1,308 fungi MOTUs (242 families), 1,918 protozoa MOTUs (159 families), 12,189 bacteria MOTUs and 215 archaea MOTUs. Morphological identification of invertebrates sampled by traditional methods, i.e. Surber samples and pitfall traps for flowing and dry conditions, respectively (Fig. 2B), yielded an additional dataset comprising 464 families.

Changing the paradigm: NPRs are not species-poor ecosystems

Contrary to the prevailing view that NPRs represent biodiversity-poor analogues of PRs, our results provide no evidence of systematic biodiversity loss in NPRs. During flowing conditions, taxonomic richness was broadly comparable between NPRs and PRs (Fig. 3A, Supp. Fig. 1). For fungi and protozoa, NPRs contributed more to local biodiversity than PRs, whereas eDNA-based invertebrates, meiofauna and algae did not differ between river types. Only morphologically identified invertebrates exhibited lower richness in NPRs than in PRs (Fig. 3A).

Flowing and dry NPRs (Fig. 3B, Supp. Fig. 1) had strong group-specific differences in richness. The richness of morphologically identified invertebrates, meiofauna and algae was higher when flowing, whereas fungi, protozoa, bacteria, and archaea richness tended to be higher in dry conditions. Comparisons between dry riverbeds and adjacent riparian soils showed contrasting patterns among taxonomic groups (Fig. 3C; Suppl. Fig. 1). Richness was higher in riparian soils for fungi, but higher in dry riverbeds for algae, meiofauna and archaea.

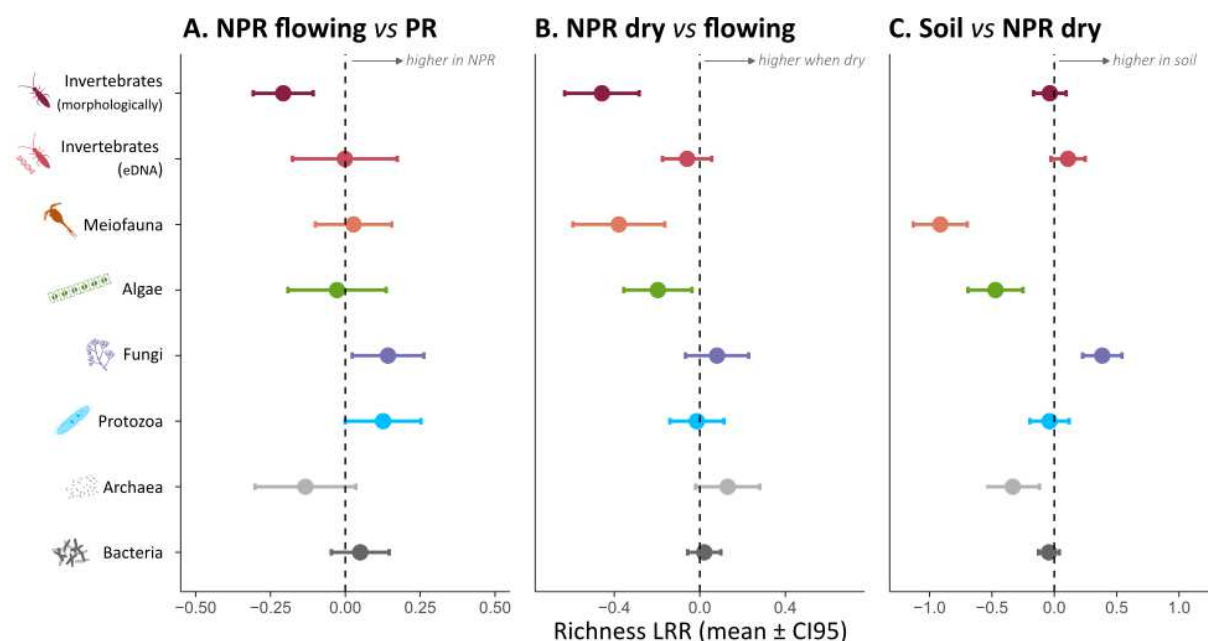


Fig. 3: Differences in taxonomic richness between phases of non-perennial rivers (NPRs), perennial rivers (PRs) and their riparian soils. A. NPRs when flowing compared to PRs. B. Dry compared to flowing NPRs. C. NPR dry riverbeds compared to riparian soils. Points and error bars represent mean and 95% confidence intervals (CI95) for log-response ratios (LRR); dashed vertical lines indicate no effect (LRR = 0). Values >0 and <0 indicate higher and lower richness, respectively, in NPRs (A), dry NPRs (B), and soil (C).

NPR communities are not simple aquatic–terrestrial transition states

To assess whether NPR communities occupied intermediate positions along a continuum from PR to riparian soil communities or represented distinct states, we compared each flowing-phase and each dry-phase NPR community with aquatic (PR) and terrestrial (riparian soil) reference communities (Fig. 1B). Based on Bray-Curtis dissimilarity matrices, we calculated two complementary metrics: (i) position, representing the relative affinity of NPR communities to aquatic versus terrestrial reference states, and (ii) divergence from the continuum, representing the extent to which NPR communities differ from both reference states rather than exhibiting an intermediate composition (Fig. 4). To identify taxa associated with each habitat, we performed indicator taxa analysis (Suppl. Tables 2 and 3).

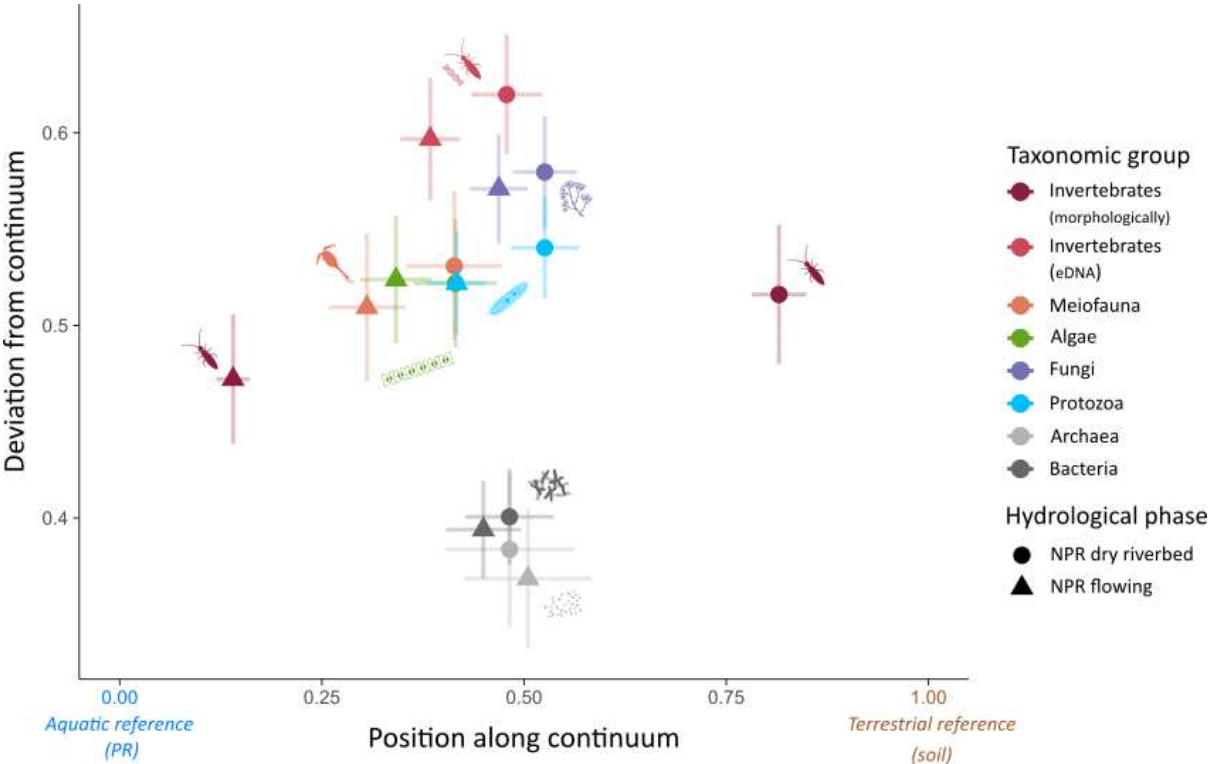
NPR communities occupied distinct positions along the aquatic–terrestrial continuum depending on taxonomic group and hydrological phase. Prokaryotic communities (bacteria and archaea) consistently occupied intermediate positions (~0.5) with low deviation (<0.4),

indicating their proximity to the continuum with an intermediate composition between aquatic and terrestrial reference states. The strong overlap between flowing- and dry-phase communities (Fig. 4) indicated limited compositional change across phases. This community-level stability was also reflected by indicator taxa: most bacterial and archaeal indicators were associated with habitat combinations including both NPR phases and PRs, whereas relatively few taxa were indicative of individual habitats (Suppl. Table 2 and 3).

Fungal communities also occupied intermediate positions along the continuum but showed high deviation (>0.5), with substantial overlap between phases. This pattern suggests distinct community states rather than occurrence at simple transitional positions along the continuum, reflecting compositional reorganization within a shared regional species pool (Suppl. Fig. 4). Most fungal indicator taxa were associated with dry riverbeds and riparian soils, whereas relatively few were indicative of flowing conditions or PRs (Suppl. Table 2).

Invertebrate communities characterized using eDNA metabarcoding also occupied intermediate positions along the continuum in both flowing and dry conditions (Fig. 4) and deviated most from the continuum among all taxonomic groups. Protozoa, algae, and meiofauna occupied similar positions along the continuum, but with lower deviation. For protozoa and meiofauna, the positions of flowing- and dry-phase communities did not overlap along the continuum, indicating a marked compositional turnover between phases (Suppl. Fig. 2). However, their NPR communities were moderately to strongly divergent from both aquatic and terrestrial reference states. Most taxa were shared across habitats (Suppl. Fig. 4), and this deviation thus reflects changes in community composition rather than distinct species pools. Hydrological transitions thus reorganize NPR communities rather than shifting them between aquatic and terrestrial reference states. Protozoan indicator taxa were dominated by ciliates across most habitat combinations, accompanied by cercozoans and amoeboid taxa in dry riverbeds and

235 riparian soils. Most algal indicator taxa for flowing NPR and PR were diatoms
 236 (Bacillariophyta), whereas dry riverbeds and riparian soil habitats contained a greater
 237 proportion of chrysophytes and other flagellated algal groups. For meiofauna, gastrotrichs and
 238 rotifers were strongly represented across NPR and PR habitats, whereas tardigrades were more
 239 frequently associated with riparian soils (Suppl. Tables 2, 3).



240 **Fig. 4: Position and deviation of non-perennial river (NPR) biological communities along the aquatic-**
 241 **terrestrial continuum.** Position and deviation were calculated from Bray-Curtis dissimilarity distances between
 242 NPR communities sampled in flowing and dry conditions, and two reference states: perennial rivers (PRs) as the
 243 aquatic reference and riparian soils as the terrestrial reference. Points and error bars represent mean and 95%
 244 confidence intervals.
 245

246 Morphologically identified invertebrates showed a fundamentally different pattern, with the
 247 community completely shifting from aquatic positions (~0.2) during flowing conditions to
 248 terrestrial positions during dry conditions (~0.8) (Fig. 4), closely tracking the aquatic–terrestrial
 249 continuum. Their low deviation (<0.5) is consistent with replacement of aquatic communities
 250 by terrestrial ones, as also reflected by indicator taxa. Flowing conditions were characterized
 251 by lotic taxa such as Chironomidae, Baetidae, Hydropsychidae and Gammaridae, whereas dry

riverbeds were dominated by terrestrial taxa including Formicidae, Carabidae, Staphylinidae and Araneae (Suppl. Table 3).

Climatic variables drive the divergence from the aquatic-terrestrial continuum

Spanning a wide range of climatic conditions and capturing NPR communities in both hydrological phases, our study identified abiotic factors associated with changes in community composition along the aquatic–terrestrial continuum. Across most taxonomic groups and in both flowing and dry phases, environmental conditions consistently influenced how strongly NPR communities deviated from the continuum from aquatic to terrestrial reference communities, and, to a lesser extent, where they occurred along the continuum. Temperature and precipitation, rather than hydrological variables, explained most of the variation in community-level deviation (Fig. 5, Supp. Tables 4, 5).

Higher temperatures and lower precipitation were associated with greater community deviation from aquatic and terrestrial reference communities in both hydrological phases. This pattern was significant at the community level (Suppl. Table 4) and supported by taxon-specific analyses (Fig. 5, Suppl. Table 5). In contrast, these climatic variables did not drive consistent community shifts along the aquatic–terrestrial continuum. Meiofauna and algae were the only groups to shift towards more terrestrial community positions as the temperature increased (Fig. 5, Suppl. Tables 4, 5). Overall, these results indicate that more arid conditions (higher temperatures and lower precipitation) are associated with increased deviation of NPR communities from aquatic and terrestrial reference states.

Drying–wetting hydrological dynamics played a secondary role in structuring NPR communities. During flowing conditions, time since wetting was associated with a gradual compositional shift towards PR communities and a reduction in deviation from the continuum.

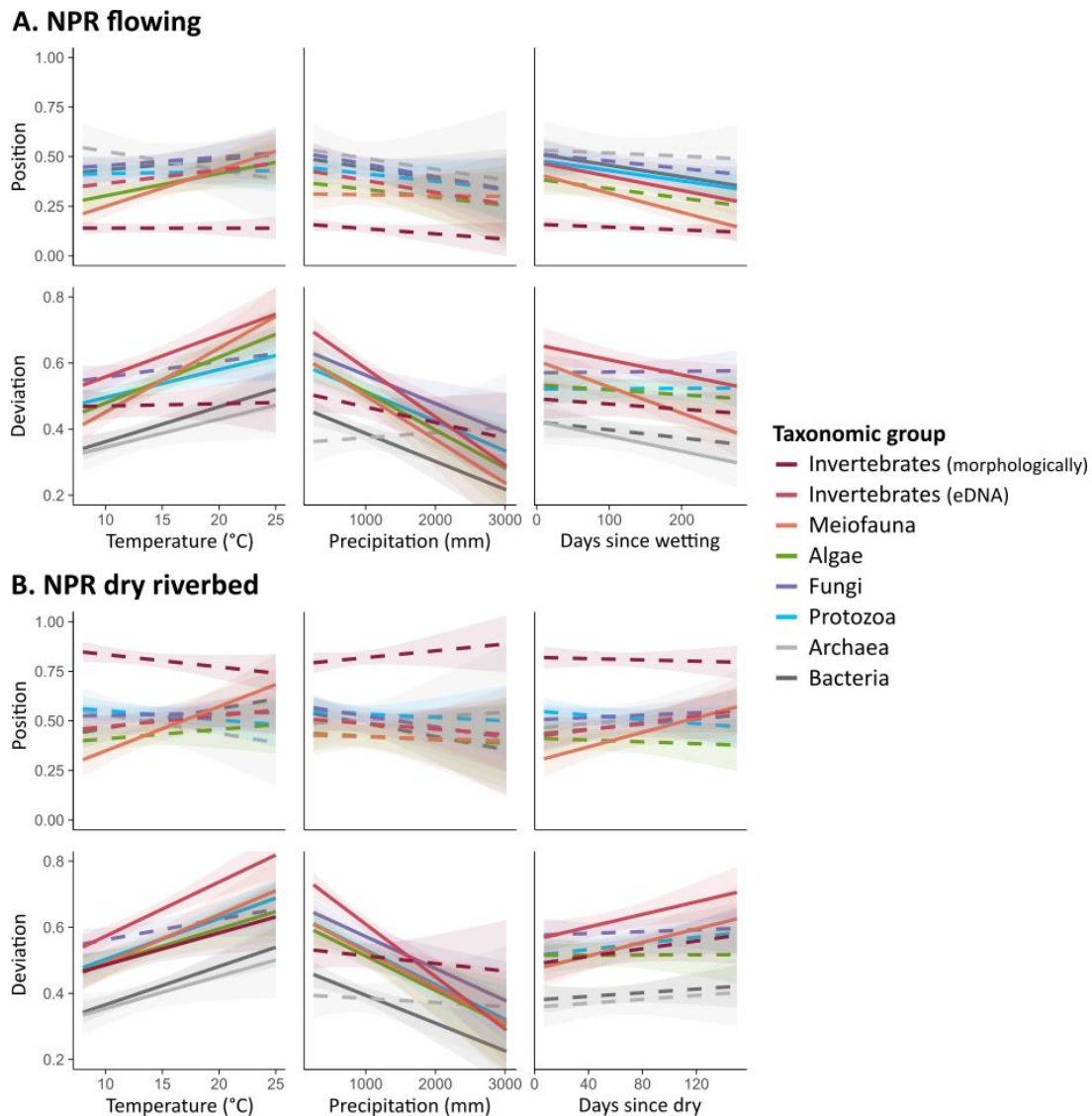


Fig. 5. Effects of climatic and hydrological variables on the position of non-perennial river (NPR) communities along the aquatic-terrestrial continuum. Relationships between climatic variables (mean annual temperature and precipitation) and hydrological variables (time since wetting for flowing-phase communities and time since drying for dry-phase communities) with community position along and deviation from the aquatic-terrestrial continuum. Continuum position values closer to 0 and 1 indicate aquatic-like and terrestrial-like communities, respectively. Relationships are shown separately for NPR communities during flowing (A) and dry (B) phases. Solid lines indicate significant relationships based on taxon-specific linear models ($p < 0.05$).

Community-level effects were significant (Suppl. Table 4), with taxon-specific responses in morphologically identified invertebrates, meiofauna, protozoa and bacteria (Fig. 5, Suppl. Table 5). In contrast, during the dry phase, time since drying was not associated with a directional shift towards terrestrial reference communities (Suppl. Table 4). Taxon-specific responses differed among taxonomic groups, with significant effects only for meiofauna (Suppl. Table 5). This lack of consistent directional response suggests that dry-phase communities did

not progressively converge towards terrestrial analogues, but instead remained in relatively stable states. The proportion of NPR reaches in the river network and the distance to the closest PR had limited influence on NPR communities. However, in flowing conditions, an increasing proportion of NPR within the network was associated with shifts towards more terrestrial-like positions along the continuum (Suppl. Tables 4, 5).

3. Discussion

Our study challenges the prevailing view of non-perennial rivers (NPRs) as biodiversity-poor versions of perennial rivers (PRs)^{9,10,23,24}. Drying did not result in a simple terrestrialization of riverbeds, supporting conceptual recognition of dry riverbeds as distinct ecological systems¹⁵. We show that NPRs harbour high levels of biodiversity across multiple taxonomic groups. Moreover, they support compositionally distinct communities that emerge with non-perenniality²⁵, promoting high beta diversity in space and time at river-network scales, as reported at the local scale^{26,27}. Together with recent recognition that most river networks comprise both NPRs and PRs^{7,8}, our results support a paradigm shift in river ecology, from viewing NPRs as biodiversity-poor or transitional systems to recognizing them as dynamic ecosystems that support distinct communities. This shift has important consequences for biodiversity conservation and restoration approaches.

Our results based on morphologically identified invertebrates showed reduced biodiversity in NPRs compared to PRs, and during dry conditions, as previously reported^{11,28–30}. In contrast, eDNA-based analyses revealed greater variability in biodiversity patterns across taxonomic groups and habitats. While methodological biases inherent to molecular approaches (e.g. DNA persistence, transport and detection probability) may affect absolute diversity estimates^{31,32}, the strong taxon-specific responses suggest that the observed patterns primarily reflect underlying ecological processes rather than methodological artefacts.

Our findings support a conceptual shift in understanding of NPRs, from a one-dimensional view of the aquatic–terrestrial continuum to a multidimensional framework in which communities are structured by their position along the continuum and their deviation from reference states. This framework captures both NPR community affinity to aquatic or terrestrial systems and the degree to which communities form distinct ecological composition. Responses to flow intermittence varied among taxonomic groups and included persistence across hydrological phases, shifts between aquatic and terrestrial assemblages, and community reorganization into distinct ecological states. Prokaryotic communities largely persisted across conditions, showing stable richness and occupying intermediate positions along the continuum. This stability is consistent with the reported limited effects of hydrological phase on bacterial communities^{33,34}, and likely reflects the persistence of prokaryotes under contrasting environmental conditions, facilitated by their small size, broad environmental tolerances, rapid turnover rates, resistance to desiccation, and buffering microhabitats within stream sediments³⁵. Archaeal communities also remained stable across flowing and dry conditions, with indicator taxa including representatives of functional groups such as methanogenic and ammonia-oxidizing lineages. Similar groups commonly occur in soils and lake sediments exposed to repeated drying–wetting cycles^{36,37}, suggesting that sediments maintain functionally diverse microbial assemblages that can sustain multiple biogeochemical pathways under contrasting hydrological phases.

Morphologically identified invertebrates showed a clear directional replacement following drying. These communities closely tracked hydrological phases, shifting from aquatic-like assemblages during flowing to terrestrial-like assemblages during dry conditions. Typical lotic taxa such as mayflies, caddisflies, and amphipods (Buffagni et al. 2009) were replaced by terrestrial predators and organic matter decomposers, including ants, ground beetles and spiders³⁸. This pattern is consistent with documented direct exposure to desiccation stress and rapid colonization by terrestrial taxa^{19,25}. However, rather than representing the dominant

response across taxa, morphologically identified invertebrates were the only taxonomic group that fully conformed to the traditional conceptualization of NPR communities shifting from aquatic to terrestrial states.

Other groups, including meiofauna, protozoa, algae and fungi, did not follow simple directional transitions. The absence of comparable shifts in these groups, which are associated with sediments and interstitial habitats, indicates habitat use as major determinant of biological responses to drying. These habitats can retain moisture and accumulate organic matter during dry periods^{38–40}, creating local environmental conditions that differ from both PRs and surrounding riparian soils²⁵, and thereby decoupling community dynamics from stream hydrology. This explains why many communities strongly deviated from both aquatic and terrestrial reference states: NPR eDNA-based invertebrates, meiofauna, protozoa, algae and fungi were neither replaced aquatic communities nor were fully replaced by terrestrial ones, but instead reorganised into distinct ecological states. Algal communities shifted from diatom-dominated assemblages in flowing conditions to assemblages associated with moist sediments during dry phases. Similarly, protozoan communities remained largely dominated by ciliates associated with biofilms and humid sediments. Fungal richness was higher in dry riverbeds than during flowing conditions, and even higher in riparian soils. Consistent with this pattern, relatively few taxa were indicative of PRs or exclusively flowing habitats, whereas many were associated with dry riverbeds and soils. Fungi play a central role in leaf litter decomposition in both aquatic and terrestrial ecosystems, and dry riverbeds can accumulate substantial amounts of organic matter during periods without flow³⁹. Consequently, fungal communities may be less constrained by the presence of surface water than many aquatic organisms and may even benefit from environmental conditions associated with dry phases. In particular, chytrid fungi, which can tolerate desiccation and inhabit both aquatic and terrestrial environments, occurred across NPR habitats⁴¹.

Temperature and precipitation emerged as the strongest predictors of community deviation from the continuum. Warmer and drier conditions were associated with increased ecological differentiation of NPR communities, highlighting that climatic context modulates both the extent of intermittence and its ecological consequences. Under increasingly arid conditions, NPR dry-phase habitats may become more different from those in both flowing streams and surrounding soils, generating the observed higher deviation from the aquatic–terrestrial continuum. Antecedent hydrological conditions were important for a few groups, particularly meiofauna and invertebrates, suggesting that metazoans may be more directly constrained by the duration of sufficiently moist conditions within sediments than microorganisms.

Overall, these results show that community composition in NPRs is not simply transitional along the aquatic–terrestrial continuum, but is structured by habitat-specific persistence and assembly processes operating within a shared regional species pool. Instead, NPRs are multifaceted systems in which different taxonomic groups follow distinct response pathways to flow intermittence. Whereas microbial communities persist across hydrological phases, sediment-associated taxa reorganize within semi-stable habitats, and warmer and drier conditions amplify deviation from both PR communities and soils. Given the increasing global extent of non-perennial systems under climate change, these findings have direct implications for biodiversity monitoring and conservation. Focusing only on NPR when flowing or on specific taxa substantially underestimates their ecological role in maintaining regional biodiversity. Our findings therefore call for a re-evaluation of how biodiversity is conceptualized and monitored in river networks globally.

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ONLINE METHODS

Reach selection and environmental variables

Samples were collected between 2020 and 2022 by partners of the 1000 Intermittent Rivers Project (IRP), an international network of non-perennial river researchers⁴², (<https://1000irp.hub.inrae.fr/>). The experiment was performed in 58 pairs of rivers (Fig. 2A, Suppl. Table 1) distributed across 14 countries, five continents, and 10 Köppen-Geiger climate classes, covering a latitudinal range from 43.2913°S to 52.4988°N.

Each pair (hereafter, a sampling site) consisted of one perennial river (PR) and one non-perennial river (NPR) reach, the latter experiencing drying. The paired reaches were selected to maximize the comparability of their substrate, channel width, topography, as well as exposure to human impacts. For each reach, eight environmental variables were determined (from local and regional agencies, Google Earth, and the Global Watersheds database

<https://www.hydrosheds.org/>): 1) Strahler stream order, 2) stream length (km), 3) distance to the main downstream confluence (km), 4) catchment area (km²), 5) percentage of non-perennial reaches in the network, 6) Köppen-Geiger climate class, 7) mean annual precipitation (mm), and 8) mean annual temperature (°C).

On each reach, a stretch corresponding to 10× the mean width of the active channel was selected and five environmental variables measured: 1) active channel width (m), 2) riverbed substrate composition (visual estimation of percentage cover of silt/ sand/ gravel/ cobble/ boulder/ bedrock), 3) percentage riparian cover (estimated visually), 4) type of riparian vegetation (absent/herbs and grasses/shrubs/trees) and 5) dominant riparian tree species. For NPRs, we also recorded: 1) distance to the closest PR reach (km), and 2) average number of dry days per year (<30, 30–90, >90). The distance to the closest PR reach and the average number of dry days per year were estimated using a combination of GIS-based information, field observations, local knowledge or previous studies and/or hydrological data from gauges or data loggers from the study year.

The biodiversity of seven major taxonomic groups (see sections below) was characterized twice in each reach: once when both reaches were flowing and once when the NPR was dry. In both sampling times, both the riverbed and adjacent riparian soil were sampled. The number of days between the onset of drying in the NPR and the date of dry sampling, and between flow resumption and the date of the flowing sampling were recorded.

Environmental DNA metabarcoding

Sediment and soil sampling. Biodiversity in riverbed sediment and riparian soil was characterized using environmental DNA (eDNA) metabarcoding. This research complied with

all relevant ethical regulations, and the samples were collected and exported with the necessary permissions.

The riparian zone was defined as the area extending from the edge of the high-water channel to the edge of terrestrial vegetation, characterized by the presence of a distinctive riparian vegetation and substrate type. For each reach and each sampling time, four sediment samples were collected from the riverbed (two each in habitats forming riffles and pools in flowing conditions) and four soil samples were collected in the adjacent riparian soil (two on each side of the channel). Each sample consisted of a composite sample of ~2 L in volume, taken at 0–10 cm depth within a circular area with 1 m radius and sieved to retain the fraction <5 mm. Samples were stored in 50-ml Falcon tubes at -20°C until being shipped to the Laboratoire d'Ecologie Alpine (Grenoble, France) for analysis.

eDNA extraction. Extracellular DNA from each sample was extracted using the NucleoSpin® Soil kit (Macherey-Nagel) following an adapted protocol²². We weighed 15 g of homogenized soil/sediment (to reduce spatial heterogeneity), added 20 ml of saturated Na₂HPO₄ buffer (0.12 M, pH 8.0), and shook the mixture for 15 min at 200 rpm (orbital shaker). We placed 2 ml of the resulting mixture in a 2-ml microcentrifuge tube before centrifugation at 11,000 RPM for 5 min. We recovered 400 µl of the supernatant in a new microcentrifuge tube and added 250 µl of SB buffer. The subsequent protocol steps were performed following the manufacturer handbook, with a final elution step in 100-µl SE buffer. During extraction, reagents used were saved and separately extracted in a second DNA extraction session (extraction controls). Several samples were randomly selected and extracted in an independent DNA extraction session, resulting in two independent DNA extracts for these samples.

Amplification. All extracts were organized in 96-well plates before amplification. Controls comprised: PCR negative controls (no template, n = 178), PCR positive controls (Bact03:

456 equimolar commercial mock community; Euka02: DNA from octopus, seal and cuttlefish,
457 diluted to mimic sample concentrations; n = 128) and sequencing controls/empty wells (no
458 primer, no template, n = 435). Plates were amplified in triplicate using two sets of tagged
459 markers targeting the 16S gene of bacteria and archaea, Bact03²², and the 18S gene of
460 Eukaryota, Euka02⁴³. The associated tag sequences were designed using the oligoTag function
461 in the OBITools package⁴⁴. The amplification mix contained: 1× Mix Applied Biosystems™
462 Master Mix AmpliTaq Gold™ 360 [composed of 1 U of AmpliTaq Gold Polymerase (Applied
463 Biosystems), 2 mM of MgCl₂ and 0.2 mM of each dNTPs], 0.5 µM of each tagged forward and
464 reverse primer and 0.0032 mg of bovine serum albumin (Roche Diagnostics) in a final reaction
465 volume of 20 µl including 2 µl of extracted DNA. The PCR program comprised an initial
466 denaturation step of 10 min at 95°C, followed by 45 cycles (Euka02) and 35 cycles (Bact03) of
467 30 s at 95°C, annealing for 30 s at 45°C (Euka02) and 52°C (Bact03) and an elongation step of
468 90 s for Bact03 and 60 s for Euka02 at 72°C, with a final elongation step at 72°C for 7 min.

469 Sequencing and data curation. PCR products were pooled according to their 3' end primer tags
470 to prevent the repetition of tag combinations (as all tagged primers were used 8 times) resulting
471 in two sets (one for each primer) of 8 pooled PCR products. Pooled DNA purification and
472 library preparation were performed at Fasteris facilities (Geneva, Switzerland) using the
473 metafast PCR-free protocol ([www.fasteris.com/en-](http://www.fasteris.com/en-us/NGS/DNAsequencing/Metabarcoding/Metagenomics-16S-18S-ITS-or-custom-PCR-amplicons)
474 [us/NGS/DNAsequencing/Metabarcoding/Metagenomics-16S-18S-ITS-or-custom-PCR-](http://www.fasteris.com/en-us/NGS/DNAsequencing/Metabarcoding/Metagenomics-16S-18S-ITS-or-custom-PCR-amplicons)
475 [amplicons](http://www.fasteris.com/en-us/NGS/DNAsequencing/Metabarcoding/Metagenomics-16S-18S-ITS-or-custom-PCR-amplicons)). A total of 16 libraries were prepared, then sequenced on an Aviti HO (2×300 bp,
476 Element Biosciences) for the eight Bact03 libraries and on an Aviti Excl. 500M (2×150 bp,
477 Element Biosciences) for the eight Euka02 libraries. Sequencing data were then curated using
478 OBITools and the R package metabar⁴⁵. Briefly, paired-end reads were assembled then
479 assigned to their respective samples/marker before being dereplicated. Sequences shorter than
480 the expected amplicon size and singletons were removed before using the obiclean command

to remove PCR errors. Molecular operational taxonomic units (MOTUs) overrepresented in extraction controls relative to sample extracts and detected in PCR negative controls were considered as contaminants and removed. PCR replicates with a number of reads and MOTU lower than or similar to PCR negative controls were considered as dysfunctional PCRs and removed³². Aberrant PCR replicates, identified based on their MOTU composition similarity with replicates from the same sample, were removed. Each MOTU was assigned to a taxonomic clade with the obitag program⁴⁴, using a set of reference databases extracted with the obipcr software⁴⁶ from Genbank database release 259. MOTUs were clustered at 97% similarity using the Sumacust algorithm⁴⁷. The abundance of each MOTU was defined as the sum of read abundances of sequences belonging to it. Each MOTU was represented by its most abundant sequence. Following quality filtering, retained PCR replicates were pooled for each sample. At this step, as a final quality check, samples that were extracted twice were checked for their similarity and pooled (Suppl. Tables 6 and 7). For further analysis, only taxonomic annotations with >95% identities were retained. Samples with < 1000 reads were discarded.

Traditional invertebrate sampling and morphological identification

Riverbed sediment and riparian soil invertebrate communities were also characterized by traditional methods (hereafter morphology-based).

Riverbed invertebrates. For PR reaches on both sampling dates and for NPRs reaches in flowing conditions, four Surber samples (each 0.1 m² area, mesh size 250 µm) were collected (two in riffle habitats and two in pool habitats) by vigorous manual disturbance of the substrate within the frame for 1 min. For NPR reaches in dry conditions, riverbed invertebrates were sampled with four pitfall traps per reach, as described below for riparian communities. Each sample was preserved in 70% ethanol.

504 Riparian soil and NPR dry riverbed invertebrates. For each reach type (PR and NPR) and
505 sampling date, three locations were established in the riparian zone on each bank, $\geq 2\times$ the mean
506 channel width apart and spaced perpendicular to the channel. At each location, one pitfall trap
507 was installed. Pitfall traps consisted of plastic containers (~80 mm height, ~80 mm diameter)
508 inserted into the substrate, 3/4-filled with ethylene glycol and with a plastic cover to prevent
509 entrance of rain, leaf litter and/or other material. After seven days, all traps were collected,
510 pooled and preserved in 70% ethanol.

511 All Surber-sampled and pitfall trapped invertebrates were identified by each partner institution
512 to the lowest taxonomic resolution possible, and at least to family level.

513 **Data analysis**

514 Only sites with data from both habitats (riverbed sediment and riparian soil), both reach types
515 (PR and NPR) and both sampling dates were analysed (i.e. 58 sites). All morphology-based
516 taxonomic data were analysed at family level, with taxa not reaching this level being excluded,
517 and taxa below this level grouped at family level. Both morphology-based data (number of
518 individuals per family) and eDNA metabarcoding data (number of reads per MOTU) were
519 transformed to relative abundance within each sample.

520 All analyses were performed separately for each main taxonomic group: invertebrates (taxa
521 belonging to the phyla Annelida, Arthropoda, Mollusca, Nematoda, Nematomorpha,
522 Platyhelminthes, Porifera; analysed using both eDNA metabarcoding and morphology-based
523 data); meiofauna (phyla: Bryozoa, Cnidaria, Gastrotricha, Micrognathozoa, Rotifera,
524 Tardigrada); algae (taxa belonging to the classes: Bacillariophyceae, Bangiophyceae,
525 Charophyceae, Chlorophyceae, Chrysophyceae, Coleochaetophyceae, Coscinodiscophyceae,
526 Cryptophyceae, Dinophyceae, Eustigmatophyceae, Florideophyceae, Fragilariophyceae,

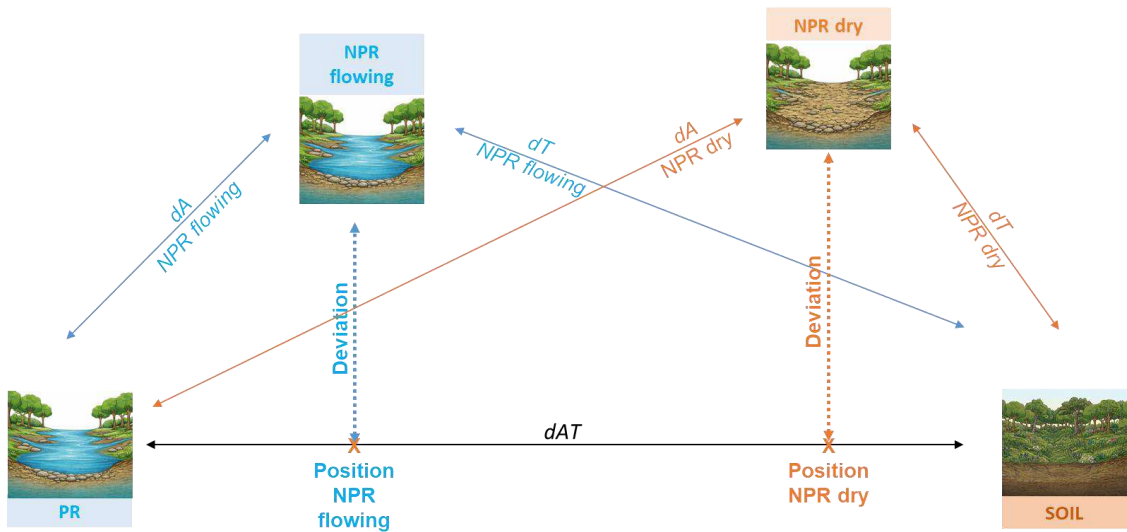
527 Glaucocystophyceae, Mamiellophyceae, Mediophyceae, Mesostigmatophyceae,
528 Nephroselmidophyceae, Phaeophyceae, Phaeosacciophyceae, Phaeothamniophyceae,
529 Pyramimonadophyceae, Synurophyceae, Trebouxiophyceae, Ulvophyceae, Xanthophyceae,
530 Zygnemophyceae); fungi (phyla: Aphelidiomycota, Ascomycota, Basidiomycota,
531 Chytridiomycota, Olpidiomycota, Sanchytriomycota); protozoa (phyla: Apicomplexa, Bigyra,
532 Cercozoa, Choanozoa, Ciliophora, Discosea, Endomyxa, Euglenozoa, Evosea, Heliozoa,
533 Nibbleridia, Tubulinea); bacteria and archaea.

534 For each taxonomic group, reach type (PR and NPR), sampling date (flowing and dry) and
535 habitat (riverbed and riparian soil), we calculated three alpha diversity metrics (taxonomic
536 richness, Shannon index, and Simpson index; *specnumber* and *diversity* functions in the R
537 package *vegan*⁴⁸).

538 To address **question 1**, we compared the alpha diversity of flowing NPR and PR reaches. We
539 calculated the log-response-ratio (LRR) for each taxonomic group and metric as: $\log (NPR$
540 $diversity / PR\ diversity)$. We compared NPR alpha diversity during flowing and dry conditions,
541 and that of NPR dry riverbeds with the adjacent riparian soil. Each NPR–PR site pair was
542 compared and means and 95% confidence intervals calculated. Differences were considered
543 significant if the confidence interval did not overlap with zero.

544 To address **question 2**, we compared the composition of NPR communities relative to aquatic
545 and terrestrial reference communities. Specifically, for each NPR reach, we defined its aquatic
546 and terrestrial references using the paired PR reach and adjacent riparian soil, respectively. We
547 constructed beta-diversity matrices using Bray–Curtis dissimilarity (based on Hellinger-
548 transformed data, *betapart* R package⁴⁹, function *bray.part*) and Jaccard dissimilarity
549 (*beta.pair*, *index.family* = "jaccard"). From these matrices, we derived two complementary

metrics describing NPR community composition relative to the aquatic and terrestrial references as follows:



First, position along the aquatic–terrestrial continuum was calculated as the orthogonal projection of each NPR community onto the line connecting aquatic and terrestrial references in a dissimilarity space. This metric reflects the relative similarity of NPR communities to aquatic and terrestrial reference communities:

$$Position = \frac{dA^2 + dAT^2 - dT^2}{2dAT^2}$$

Where:

- dA = dissimilarity between the (flowing or dry-phase) NPR community and the aquatic reference community
- dT = dissimilarity between the (flowing or dry-phase) NPR community and the terrestrial reference community
- dAT = dissimilarity between aquatic and terrestrial reference communities

Values closer to 0 and 1 indicate greater compositional similarity to aquatic and terrestrial reference communities, respectively. Values around 0.5 indicate that NPR communities are compositionally intermediate relative to both reference states, reflecting comparable similarity (or dissimilarity) to each reference.

Second, deviation from the continuum was calculated as the perpendicular distance of each NPR sample to the reference line. This metric quantifies whether the observed community composition can be represented as a linear combination of aquatic and terrestrial communities:

$$Deviation = \sqrt{dA^2 - (Position * dAT)^2}$$

Low deviation values indicate NPR communities close to the continuum, which can be approximated as proportional combinations of aquatic and terrestrial reference communities. In contrast, high deviation values indicate that communities cannot be explained as such combinations, instead reflecting communities that are compositionally distinct from both references. Together, the position and deviation metrics indicate the extent to which variation in NPR communities reflects changes along the continuum between aquatic and terrestrial states, or whether they instead diverge from both references, forming alternative community configurations.

Additionally, for each taxonomic group, Venn diagrams (*venn.diagram* function of the VennDiagram R package⁵⁰) were used to quantify the number of morphologically identified families or eDNA MOTUs shared among flowing NPRs, dry NPRs, PRs and/or riparian soil (hereafter habitats) and those occurring exclusively in one of them. To further identify taxa associated with specific habitats or habitat combinations, we performed an indicator taxa analysis (IndVal.g index, *multipatt* function of the indicpecies R package⁵¹). Taxa were considered indicative when $p < 0.05$ and IndVal scores were > 0.5 ⁵².

To address **question 3**, we tested whether the differences in position and deviation of NPR communities along the aquatic–terrestrial continuum were explained by hydrological variables (time since flow resumption or onset of drying, NPR proportion of river network, and distance to the nearest PR reach) or by climatic conditions (average annual precipitation and temperature). To analyse community level patterns, we first fitted linear mixed-effects models (*lmer* function, lme4 R package⁵³) with position and deviation as response variables, each climatic or hydrological predictor and its interaction with taxonomic group as fixed effects, and sampling site as a random intercept to account for non-independence among groups from sites sharing the same environmental conditions. To identify taxon-specific responses, we fitted separate linear models (*lm* function) for each taxonomic group. Statistical significance was assessed at $p < 0.05$. All statistical analyses were performed in R version 2025.05.0+496⁵⁴.

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722

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