

FORUM**Uncovering the complete biodiversity structure in spatial networks – the example of riverine systems**

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Abstract

Uncovering biodiversity as an inherent feature of ecosystems and understanding its effects on ecosystem processes is one of the most central goals of ecology. Studying organisms' occurrence and biodiversity patterns in natural ecosystems has spurred the discovery of foundational ecological rules, such as the species-area relationship, and is of general scientific interest. Recent global changes add relevance and urgency to understanding the occurrence and diversity of organisms, and their respective roles in ecosystem processes. While information on ecosystem properties and abiotic environmental conditions are now available at unprecedented, highly-resolved spatial and temporal scales, the most fundamental variable – biodiversity itself – is still often studied in a local perspective, and generally not available at a wide taxonomic breadth, high temporal scale and spatial coverage. This is limiting the capacity and impact of ecology as a field of science. In this forum article, we propose that complete biodiversity assessments should be inclusive across taxonomic and functional groups, across space, and across time to better understand emergent properties, such as ecosystem functioning. We use riverine ecosystems as a case example because they are among the most biodiverse ecosystems worldwide, but are also highly threatened, such that an in-depth understanding of these systems is critically needed. Furthermore, their inherent spatial structure requires a multiscale perspective and consideration of spatial autocorrelation structures commonly ignored in biodiversity-ecosystem functioning studies. We show how recent methodological advances in environmental DNA (eDNA) provide novel opportunities to uncover broad biodiversity and link it to ecosystem processes, with the potential to revolutionize ecology and biodiversity sciences. We then outline a roadmap for using this technique to assess biodiversity in a complete and inclusive manner. Our proposed approach will help to get an understanding of biodiversity and associated ecosystem processes at spatial scales relevant for landscape ecology and environmental managers.

Why improve biodiversity data?

Uncovering biodiversity and understanding its effects on ecosystem processes is one of the most central goals of ecology (Cardinale et al. 2012). Current global pressures, such as climate change, invasive species, environmental pollution, or habitat loss add urgency to the goal of understanding fundamental features of organisms' distributions and their respective roles in ecosystem processes (Urban et al. 2016). Information on many ecosystem properties and abiotic environmental conditions, such as temperature, productivity, biomass, or vegetation type, are becoming available at unprecedented spatio-temporal scales (Anderson 2018, Jetz et al. 2016). However, biodiversity itself is still understudied and often not available at relevant resolutions with respect to taxonomic and functional breadth, temporal and spatial coverage. This is seriously limiting the capacity and impact of ecology as a field of science.

To move forward, the fields of ecology and biogeography must be able to understand and describe the state of a system, but also recognize the complex dynamics within. This will require more complete and more resolved biodiversity data. Firstly, for all axes of complexity (taxa, space and time; Fig. 1), measuring at a higher resolution or at multiple levels can provide a fundamentally different understanding than measuring at one level or resolution (Chase et al. 2018, McGlinn et al. 2019). Even more so, looking at only some of these facets, we may get things wrong or miss important parts (Levin, 2009): only by looking at multiple species (versus looking at a single one) can we study species interactions; only by studying more than one patch can we understand if metapopulation dynamics are driving a system (Adler and Lauenroth 2003, Altermatt et al. 2008, Bannar-Martin et al. 2018, Chase et al. 2019); and only by including multiple time points can we resolve temporal trajectories, transient dynamics (Hastings et al. 2018) or stability components of systems (such as

variability in ecosystem functions (Wang and Loreau 2014)). Secondly, there are aspects that can only be understood with highly resolved data along these three axes, for example the scaling of biodiversity across space and time (Rosenzweig 1995; Adler and Lauenroth 2003), and how such scaling changes across taxa and trophic levels (Holt et al. 1999). This is especially needed in the context of global changes, where a more mechanistic understanding of the spatial and temporal dynamics of biodiversity loss is critically needed. Thirdly, the sheer fact of having more measurements can improve inference into causal relationships (Sugihara et al. 2012), for example when understanding predator-prey dynamics or making predictions about the future (Petchey et al. 2015). Altogether, this justifies a more complete assessment and understanding of biodiversity, which is increasingly urgent in a time of growing global change and ecological uncertainty.

Understanding the processes, mechanisms and factors underlying biodiversity, loss of biodiversity, and associations with ecosystem functions is crucially needed and relevant for all ecosystems worldwide. It may, however, be most urgent in freshwater riverine ecosystems (Darwall et al. 2018). Freshwater ecosystems are, relative to their area, among the most biodiverse ecosystems worldwide (Dudgeon et al. 2006, Vorosmarty et al. 2010), supporting over 10 % of all known species, and having a large economic and societal relevance for mankind. However, they are also among the most threatened by global pressures (WWF 2018) and show the largest loss of biodiversity and associated ecosystem functions (Darwall et al. 2018, Vorosmarty et al. 2010). Still, information on biodiversity in freshwater riverine systems is taxonomically, spatially, and temporally scattered, and pressing questions of conservation biology remain understudied in freshwater compared to other ecosystems (Jucker et al. 2018). While recent technological advances in remote sensing are suited to study biodiversity variables in forest or grassland ecosystems, alternative technologies are needed in freshwater systems (Turak et al. 2017), particularly in rivers, due to the submerged occurrence

of organisms, their specific spatial network structure, and the directional transport of water (Altermatt 2013, Isaak et al. 2014).

Biodiversity in river ecosystems is often only measured for a few target groups, such as fish, diatoms, or macroinvertebrates (e.g., Barbour et al. 1999, Heino et al. 2015), and it is largely unknown if and how biodiversity patterns compare across these different taxonomic groups. Even within some of the most commonly used indicator taxa, aquatic macroinvertebrates, it has recently been demonstrated that findings from one taxonomic group cannot be transferred to others (Darwall et al. 2011, Seymour et al. 2016). The use of a subset of organisms can also lead to biases with respect to the patterns as well as the fundamental underlying processes, as the diversity patterns observed are not universal across taxonomic/functional groups, and may depend on the environmental state. For example, local species richness (α -diversity) in riverine ecosystems has been shown to increase with downstream position for some taxonomic groups, such as fish or macroinvertebrates (e.g., Altermatt et al. 2013, Muneeppeerakul et al. 2008), while completely reversed patterns were found in other taxa, such as bacteria or amphibians (Besemer et al. 2013, Grant et al. 2010). Recent experimental and theoretical work linked these seemingly contradicting patterns to the amount and occurrence of environmental disturbances (Harvey et al. 2018). The restriction to a few taxonomic groups also hinders a complete understanding of biodiversity and its role in ecosystem processes, including primary production, nutrient and carbon turnover, or decomposition. Thus, there is a great need to better understand the distribution of biodiversity, and how it is changing across major ecosystems, such as riverine systems.

In this forum article, we develop a roadmap on how to use eDNA metabarcoding to assess organismal biodiversity of river basins in a more inclusive (i.e. with respect to range of taxa included), temporally resolved, and spatially explicit perspective. We term this consideration of taxonomic, spatial and temporal inclusivity “complete biodiversity” (Fig. 1),

which better allows the study of emergent properties, such as functioning of ecosystems, and show how this can address and answer major questions in riverine systems and beyond.

Riverine networks

Riverine networks are characterized by a specific, but universal spatial structure that is shaped by general hydrological and erosional forces. As such, riverine networks generally branch in a fractal pattern and produce a space-filling network (Rodriguez-Iturbe and Rinaldo 1997). This results in a spatial distribution of habitat patches, each connected to any other patch by exactly one path along the network, a biased distribution of habitat patch sizes with a predominance of small streams to large streams (≥ 70 % of total stream length being small to very small streams), and a unidirectional transport of materials along the water flow.

Over the last several decades, our understanding about how this riverine network structure controls abiotic and biotic conditions has become more nuanced. A classic framework to consider riverine diversity and ecosystem function is the River Continuum Concept (Vannote et al. 1980), which posits that the relative importance of terrestrial inputs and light availability leads to differing conditions and resource types from upstream to downstream, resulting in characteristically different communities performing different functions. While it is a simplification, overlooking several important aspects of river ecology, thinking of rivers as a continuum is nevertheless useful. A key tenet of this framework is that resources flow downstream, processed by biological communities along the way.

Downstream flow is essential in defining river conditions, even in highly charismatic and atypical contexts: for instance, the accumulation of hippopotamus-borne carbon and nitrogen subsidies with downstream distance in a large African river (Subalasky et al. 2018). However, this is not the only important way that river networks shape the communities and processes they contain, and many organisms are not hindered by flow directionality in their distribution.

Network structure also has important implications for the food web structure, energy flow, and their relationship (Power and Dietrich 2002). For example, removal of species results in different responses of the food-web, that is, new guilds dominating, when done in headwater or mainstem reaches (Power and Dietrich 2002). Headwaters are also less productive, therefore the uptake and excretion of food has, per individual organism, a greater per biomass effect on local flows of energy and material cycling in headwaters compared to downstream reaches. More recently, considerable interest has been paid to the ways that spatial network structure itself, paired with dispersal limitation, can generate and maintain biodiversity patterns (Altermatt and Fronhofer 2018, Grant et al. 2007, Muneeppeerakul et al. 2008). This can occur even in the absence of environmental heterogeneity through the network (Carrara et al. 2012, Seymour et al. 2015), with direct effects on metacommunity dynamics.

Ecology has long acknowledged the importance of space in determining biodiversity patterns (Anderson 2018, Levin 1992): across all types of ecosystems, it is natural to assume that abiotic conditions are more similar in patches that are close to one another than they are in far-apart patches. Environmental conditions are an important determinant of community composition and this implies that communities too are more similar in near patches than far patches. Additionally, the vast majority of organisms are dispersal-limited at some distance, providing another mechanism by which community dissimilarity should increase with distance. When we seek to understand complete biodiversity across scales, this must be done across different spatial scales, and the spatial effects and dependencies must be adequately considered (Legendre and Fortin 1989). While most classical statistical techniques assume the independence of samples, specific spatial statistics have been developed to incorporate spatial autocorrelation into models when this assumption of independence is violated (Fortin and Dale 2005).

In riverine networks, the relationship between spatial location and biodiversity is highly pronounced, even more than in many other ecosystems, because both habitats and dispersal routes are often limited to the water channels themselves (Altermatt 2013, Grant et al. 2007). Thus, spatial autocorrelation along a grid-like two-dimensional landscape in Cartesian space, does not capture the spatial dependencies organisms perceive, and topological distances and respective spatial autocorrelations should be considered. Consequently, using overly simple Cartesian models, which assume that riverine biodiversity is distributed uniformly or randomly in space throughout the network, will in most cases lead to incorrect conclusions and predictions.

Complete Biodiversity

The study of diversity patterns and ecosystem properties in riverine networks has a long tradition, but has arguably only modestly contributed to general ecological theory (Fisher 1997). There are at least two possible reasons: i) Ecological processes may follow different rules in riverine ecosystems compared to other ecosystems; ii) patterns and processes have been studied in riverine ecosystems at scales that were too system-specific, thereby hindering generalization. We would argue for the latter, indicating that an appropriate study of patterns and processes should not only allow a better understanding of riverine ecosystems, but could also be informative on general ecological dynamics.

Thus, what would a complete biodiversity assessment in riverine systems look like? We postulate that it should be inclusive across taxonomic and functional diversity, space, and time, in order to get a better understanding of emergent ecosystem functioning (Fig. 1). Recent advances in molecular methods, computational technologies, and an increased awareness, not only for the state of biodiversity, but also the subsequent functioning of ecosystems bring such a complete assessment within our reach. Such an integration would

help to better plan freshwater biological monitoring (Jackson et al. 2016, Pawlowski et al. 2018), to better answer general questions in biodiversity research, and to bridge different fields and approaches to get an enhanced understanding of freshwater ecosystems in general (Bush et al. 2017). Finally, it would also improve forecasting freshwater biodiversity under global change (Urban et al. 2016).

Inclusive across taxa

Riverine ecosystems are characterized by a very high diversity of organisms across many taxonomic groups, ranging from bacteria to aquatic plants, invertebrates and vertebrates. All of these groups play critical roles in ecosystem functioning. For example, bacteria and other microbial organisms are critical constituents of stream biofilms. They drive crucial ecosystem processes, such as organic matter cycling, ecosystem respiration and even primary production (Battin et al. 2016), and link terrestrial subsidies to aquatic food webs. Recent sequencing technologies have led to taxonomically highly resolved community data (Besemer et al. 2013, Savio et al. 2015), and revealed their central role for global biogeochemical fluxes (Battin et al. 2016). Similarly, aquatic invertebrates are highly diverse, including aquatic key groups such as molluscs, insects, or crustaceans (Heino et al. 2015). These organisms have central roles in food webs, as they link terrestrial biomass input and aquatic primary production (often in biofilms) to higher trophic levels: aquatic invertebrates filter, graze, scrape and scratch on these resources, and are themselves among the most important food resource for higher trophic orders, such as fish or amphibians. Finally, vertebrates are often at the top of aquatic food chains, exerting top-down control and trophic cascades on communities. This diversity of vertebrates in freshwater systems is not only high but also strongly spatially structured and severely threatened (Abell et al. 2008).

A major shortcoming of past biodiversity work in rivers is its general focus on a few restricted indicator groups (such as diatoms, or mayflies, stoneflies, and caddisflies [EPT], or fish) that are well-studied from an ecological point of view and that are known to react to specific drivers of environmental change (Barbour et al. 1999), but whose relevance and representativeness for other taxonomic groups have not always been established. However, any “complete” assessment of biodiversity should be inclusive beyond these classic indicator groups, link diversity of different taxonomic groups in a coherent manner, and allow inference on ecological dynamics. In that context, the diversity of sampling and assessment methods, each optimized for their respective focal groups, from diatoms to invertebrates to fish (Barbour et al. 1999), may hinder the unification of diversity data. For example, microbial communities in aquatic biofilms are characterized by scraping and sampling a small portion of biofilm from a rock, while aquatic invertebrates are collected by kick-net sampling and fish communities are characterized by electrofishing. The comparison is especially limited by the different sampling error rates of the different methods: to make “apple and oranges comparable”, one needs to agree on common criteria, common measures, and standardized methods. Specifically, a comparison of biodiversity assessment methods assumes comparable sampling efforts across methods (Gotelli and Colwell 2001), which can be achieved by calculating species accumulation curves (with increasing sampling intensity). Such knowledge is rarely established, and thus the comparison is generally not given. An inclusive measure of biodiversity therefore must both cover all ecologically relevant groups and give a general overview of the diversity across all taxonomic groups. Importantly, however, our proposed direction towards a more complete assessment should also be complemented by a more in-depth study of the (aut)ecology of the same taxa: new genetic tools will give insights into the diversity of groups hitherto largely ignored, but the true value

will only emerge if this is complemented with sufficient information on the respective ecological context.

Inclusive across space

Biodiversity patterns at one scale can be shaped by ecological processes operating at multiple scales (Levin 1992), and in a riverine network, abiotic parameters as well as community structure in a downstream patch are intuitively affected by the ones upstream (Vannote et al. 1980). Additionally, conclusions about biodiversity made from one spatial scale do not necessarily extend to others. A consistent approach to monitor biodiversity across scales is key to uncovering patterns of biodiversity changes across scales and their underlying drivers. In riverine ecosystems, spatially explicit approaches, linking local-scale dynamics to the network, become ever more feasible due to the availability of highly resolved, spatially explicit environmental variables (e.g., Domisch et al. 2015). Such data may also offer a great opportunity to apply the metacommunity framework, given the dispersal network is clearly defined (Altermatt 2013).

An across-scale monitoring program of biodiversity is also key to understanding the functional consequences of biodiversity changes. Research on biodiversity and ecosystem functioning has been a major topic in ecological studies, which greatly advanced our understanding of the impact of biodiversity loss (for a review see Tilman et al. 2014). These studies have mostly been conducted at local scales, and it is unclear whether conclusions from these small-scale studies can be extrapolated to landscape scales shaped by different land-use practices and at which scale management ideally occurs. Recent studies have attempted to fill this gap by developing new theories (Wang and Loreau 2014, Wang and Loreau 2016) and analyzing datasets that cover large spatial scales (Oehri et al. 2017). These datasets represent spatial scales that are much larger than field experiments, and are collected in very different

ways (e.g., remote sensing) from the experiments. Therefore, a consistent approach to monitor biodiversity and ecosystem functioning across scales is key to scaling up previous knowledge on links between biodiversity and ecosystem functioning for real-world applications.

Inclusive across time

The study of many ecosystems, including riverine ecosystems, has been driven by an equilibrium notion, or the assumption of ecosystems being in a climax state. However, ecosystems are under constant change, be it species turn-over or directed changes of ecological variables, especially in the context of global change. The speed and magnitude of temporal community fluctuations can be huge in freshwater ecosystems. For example, these changes have been exemplified by complete community shifts due to biological invasions of aquatic invertebrates in major rivers within a few years (e.g., Van den Brink and Van der Velde 1991), or continental-scale effects of environmental perturbations and pollution on stream ecosystem functioning (e.g., Woodward et al. 2012).

Thus, an understanding of an aquatic ecological system must be based on data that adequately reflect and capture such temporal dynamics. However, the vast majority of studies on ecological patterns and biodiversity in riverine ecosystems are still based on a single time point, or on time series with a short duration and inadequate frequency. The most important aspects are to cover time scales and frequencies that are ecologically appropriate. This is obviously different for various groups of organisms and must be considered. For example, microbial dynamics occur at the timescale of hours to days, while dynamics of longer lived-vertebrates could occur at timescales of months to years. While monitoring of tree diversity and population-based community composition in forests would be deemed infeasible at timescales of either sampling at hourly intervals or only sampling every couple of thousand years, yet analogous sampling is commonly done in riverine systems: key short-lived

organisms such as microbes (cyanobacteria, diatoms) or invertebrates with generation times of days to months, are in many well-funded and large monitoring schemes only looked at every couple of years (e.g., Kunz et al. 2016), which is equivalent to dozens to hundreds of generations apart. Having such a sampling scheme may be better than sampling without any temporal replication but is still far below an ideal sampling that covers the different temporal scales of various groups of organisms at respective rates. A possible way to improve this is to have multiple temporal sampling frequencies overlaid, such that both short- and long-term dynamics are considered.

eDNA to assess biodiversity

Environmental DNA (eDNA) is seen as a promising technological advance that could revolutionize ecology and biodiversity sciences, especially in aquatic ecosystems (e.g., Bohmann et al. 2014, Jackson et al. 2016, Taberlet et al. 2018). Environmental DNA is DNA directly extracted from environmental samples (e.g. soil, sediment, water or air). The captured DNA may originate from whole organisms (for micro-organisms, such as algae or rotifers), but in its purest form describes the DNA shed from an organism in the form of faeces, mucus, skin cells, organelles, gametes or even extracellular DNA (Taberlet et al. 2018, Zhang et al. 2018). Thus, it can consist of free DNA (strict sense) or DNA still locked in cells or organelles (wider sense) (Cristescu and Hebert 2018, Deiner et al. 2017a). As such, the method is non-invasive, potentially scalable to a very large number of samples, and has a strongly diminishing cost per sample with increasing number of samples. The use and application of eDNA in ecological research is very recent but has already gained a great momentum. Environmental DNA metabarcoding is particularly suitable for measuring complete biodiversity in riverine or other aquatic ecosystems (e.g., Bohmann et al. 2014, Deiner et al. 2017a, Pfrender et al. 2010) due to i) a relative short persistence of DNA in the

water column, making it a highly contemporary method (Barnes et al. 2014, Deiner & Altermatt 2014), ii) the ease of sampling, which can be easily automated based on sampling procedures for water chemistry, and iii) the downstream transport, which allows a spatial integration of the biodiversity information. However, despite the many promises of the method, challenges also lie ahead.

The application of eDNA initially focused on surveying individual target species (e.g., Lodge et al. 2012, Thomsen et al. 2012), certain communities (zooplanktons, diatoms) (Yang et al. 2017; Apothéloz-Perret-Gentil et al., 2017), or on the study of complete diversity at a few individual locations (Deiner et al. 2016, Li et al. 2018), but largely has not yet addressed fundamental ecological questions (Balint et al. 2018). In parallel to these rapid technical advances and first applications, a large number of reviews and opinion articles have been published over the last few years, outlying the potential of the technique to revolutionize biodiversity and conservation study at local scales (e.g., Bohmann et al. 2014, Creer et al. 2016, Deiner et al. 2017a, Lodge et al. 2012, Pfrender et al. 2010, Taberlet et al. 2018). eDNA metabarcoding has been prominently suggested as a powerful method for improving environmental management and implementation of environmental laws due to its high sensitivity in detecting species and general applicability (Jackson et al. 2016). Compared to classic morphology-based bioassessments, it is non-invasive, and gives an increased taxonomic precision, and is less labor-intensive (Pfrender et al. 2010). A number of key studies have established the use of eDNA in ecology, and it has been identified for its potential for a broad-scale biodiversity monitoring for animal and plants (for historic overview see Taberlet et al. 2018). However, studies are often motivated by a conservation perspective and/or focus at a localized scale and have not been properly linked to recent advances in the fields of biodiversity sciences and spatial ecology (Joly et al. 2014).

eDNA - Inclusive across taxonomic and functional diversity

Many studies have explored this novel technique by comparing it to traditional sampling methods, for example electrofishing (e.g., Olds et al. 2016) or kick-net sampling (Hajibabaei et al. 2019, Mächler et al. 2019) and find comparable or increased richness with eDNA monitoring when compared to these traditional methods (for an extensive review see Deiner et al. 2017a). The focus of these studies has often been restricted either on the detection of eukaryotes (Deiner et al. 2015, Macher et al. 2018) or more specifically, a group of fish or amphibian species (e.g., Hänfling et al. 2016, Shaw et al. 2016). Assessment is also often only done at presence/absence levels due to the variability in biomass and sequence numbers generated by high-throughput sequencing. However, eDNA has the potential to revolutionize biodiversity assessment with the ability to sample broad biodiversity in one stroke. Recent work suggests that the use of multiple markers could be the key to efficiently detect a broad taxonomic diversity (e.g., Cannon et al. 2016). Barcoding regions are well defined for some taxonomic groups (fungi, bacteria), while others are still under debate, such as for eukaryotes (e.g., Elbrecht et al. 2016) or plants (e.g., Fahner et al. 2016), because these regions often span across a large phylogenetic branch and do not always perform equally well for all the involved taxonomic subgroups. The best example may be the cytochrome oxidase I (COI) region, which is a common barcoding region used for eukaryotic diversity (Hebert et al. 2003). However, due to the poorly conserved region there is often primer bias (Elbrecht and Leese 2015) and identification to species-level is limited to some major taxonomic groups. These aspects hinder the equal amplification and thus detection of all targeted taxonomic groups in the same sample. A necessary condition is thus to have adequate barcoding regions for all taxonomic groups, to ensure equal biodiversity coverage from relatively low numbers of water samples. Such barcoding regions exist (Pawlowski et al. 2012). They are, however not universal for all organisms, and currently the taxonomic assignment is mostly restricted

by the lack of complete and adequate reference databases. Important ways forward are thus: i) the design or optimisation of primers (both their specificity but also generality) (Elbrecht and Leese 2016, Macher et al. 2018), ii) to complement and fill the respective databases (Blackman et al. 2019, Weigand et al. 2019), and iii) to possibly think of whole-mitochondrial sequencing based on eDNA samples (Deiner et al. 2017b), in order to combine data of multiple markers from the same organism. All three areas are under ongoing research and major progress is being made. We can therefore expect current hurdles to be overcome within a few years from now.

Importantly, the approach of eDNA-based diversity assessment is not necessarily a 1:1 substitution for classic existing approaches, but should rather serve as a complement which extends beyond current limitations. For example, it is well known that the classic sample processing and taxonomic identification of macroinvertebrates can be associated with considerable error (Haase et al. 2006), and that there are constraints imposed (e.g., taxa looked at, methods used) that would preferably be avoided with the new approaches. Rather than focusing on the shortcomings of new methods in areas that current methods handle well, the focus should be on the strengths of the new methods in areas that current methods address imperfectly, such that the overall toolbox of methods gets us closer to measuring complete biodiversity. Current challenges for eDNA are already the focus of research and likely to be overcome: such as inferring organismal abundance (Hänfling et al., 2016), or localizing and extrapolating the eDNA signal in space and time (Carraro et al. 2018).

eDNA - Inclusion across space

River systems act as a “conveyor belt” (Deiner et al. 2016) for biological information. Therefore, sampling eDNA from a catchment offers the chance to detect biodiversity on a greater spatial scale than previous methods which focused on a single point (i.e. kick-net

sampling) or a short stretch (i.e. electrofishing or macrophyte surveys). Several studies have estimated the transport distance in rivers for eDNA of single species and results vary from 0.25 to 12 km (Deiner and Altermatt 2014, Jane et al. 2015). However, it is unclear what other factors than flow, such as sedimentation or degradation, drive the transport in the system. Although eDNA is a promising opportunity to detect broad diversity, its origin, state, persistence, and transport in the environment are not yet fully understood (e.g., Strickler et al. 2015). However, progress has already been made from hydrological models on how to make probabilistic predictions on the origin and transportation of eDNA (Carraro et al. 2018).

A particular advantage of using eDNA sampling is its simplicity compared to other sampling approaches. Environmental DNA collection is quick and easy due to the nature of the sample collected: water, sediment or soil, rather than collection of specimens, and sampling needs only minimal training. This will allow monitoring strategies to increase in sample number, allowing for a far more intense collection of data, and thus recovering a better view of spatial patterns of biodiversity.

eDNA - Inclusive across time

The use of eDNA to track long-term temporal dynamics is most obvious in the reconstruction of past communities (decades to centuries), for example from sediment cores (Balint et al. 2018, Monchamp et al. 2018). In the water column, however, it has a relatively short persistence time of days to maximally 1–2 weeks (Thomsen et al. 2012), which ensures a contemporary community estimate. Although many studies have demonstrated a greater sensitivity, or an increased number of taxa detected using eDNA, it can be highly variable depending on the target taxa. We therefore need to understand this variation, which can occur not only within taxa groups but also across seasonal changes, with some species DNA

production increasing during moulting or breeding seasons only (e.g., Bylemans et al. 2017, Dunn et al. 2017).

Will a complete biodiversity assessment increase our understanding of ecosystem functioning?

Classic approaches of biomonitoring generally assess biodiversity, and then, indirectly, link this to ecosystem functions, such as primary production or decomposition. Novel approaches in ecogenomics, however, may allow to measure diversity and functions at the same time, and in a direct manner. The approach of these eDNA-based technologies, including metabarcoding, metagenomics, and metatranscriptomics, is to analyse the occurrence and expression of functional genes, and to analyze phylogenetic, functional, and metabolic diversity of organisms and their respective expressions within natural communities.

As such, ecosystem functioning and services as emergent properties of ecological systems can be inferred not only through inspections of species inventories, but also via the direct count (read abundance) of distinct functional genes at the ecosystem level (Taberlet et al. 2018). For example, by evaluating the relative read abundance of protein-coding genes in a community, metatranscriptome analyses gives a direct insight into nitrogen cycling, a key ecosystem function (Zheng et al. 2017). These approaches also allow us to look at the diversity and respective functions carried out by microorganisms simultaneously. Responses to environmental change, such as nutrient enrichment, can be assessed at the functional level, and then, using barcode markers, these functions can be linked to specific taxa (Grossmann et al. 2016). Together with emerging or existing bioinformatic approaches (Keck et al. 2017), these metagenomic and metatranscriptomic data can be linked with data on environmental properties, either sensed in-situ or by remote sensing, in order to link environmental states

and functions to the underlying drivers (i.e. environmental drivers) and respective biological processes (i.e. gene expression).

The unique spatial network structure of rivers requires specific tools

A major step forward for a better understanding of biodiversity is the ability of upscaling site-specific measurements and knowledge to the network level. This must be done in a spatially explicit perspective, which is non-trivial in dendritic riverine networks. To account for the unique structure of river networks, new statistical frameworks have arisen to either account for spatial autocorrelation, so that estimates of the relationships determining biodiversity or ecosystem function are unbiased, or to explicitly measure the contribution of spatial relationships in determining these responses (e.g., Hocking et al. 2018, Ver Hoef et al. 2014) (for a comparison of methods, see Holthuijzen 2017). Methods such as Spatial Stream Network Models (SSNM's) incorporate spatial covariance structures that make sense for riverine networks, and allow the incorporation of both Euclidean and network distance matrices, as well as flow directionality, which can be seen as an analogous approach to phylogenetic comparative methods, analyzing phylogenetic trees and incorporating their inherent structure in the analysis (Felsenstein 1985). These new methods can first facilitate identification and description of the spatial patterns in datasets, whether the response variable is an abiotic condition such as temperature, a single-species or complete biodiversity measure, or an ecosystem function. These methods can also be used in spatial regression analyses, to produce parameter estimates for the relationships between predictor and response variables, which account for spatial covariance (Ver Hoef et al. 2014). Finally, they can be used to partition the variance in metrics such as biodiversity and ecosystem functions into those attributable to predictor variables (typically environmental variables, or perhaps biodiversity) or to other spatial aspects. Use of such statistical techniques has already led to important

insights about controls on water chemistry (e.g., Brennan et al. 2016), bacterial contamination (e.g., Holcomb et al. 2018), the relationship between abiotic conditions and species habitat (e.g., Isaak et al. 2009), and species abundances through networks (e.g., Hocking et al. 2018). These approaches also provide a way to match highly-resolved environmental data with biotic responses for which only local data is available, combining them to make catchment- and reach-scale predictions (Isaak et al. 2014). However, they have rarely, if ever, been applied to complete biodiversity measurements, thus we find that matching these could represent a major step forward in our understanding of biodiversity.

Most studies of biodiversity and ecosystem function have been conducted at local scales (examples: grassland), and linking landscape- or continental-scale biodiversity to functions is only at its infancy (Oehri et al. 2017). As such, the importance of spatial relationships in determining biodiversity, ecosystem function, or the relationship between the two has largely been neglected. Throughout ecology, we need to begin examining the relationships across scales while considering biodiversity and ecosystem function if we want to truly understand it. Riverine networks are a logical place to start because the spatial connections between local sampling sites are intuitive. We can improve our understanding of complete biodiversity and its relationship to ecosystem processes in river networks by accounting for space in two steps. First, sampling designs should be optimized with respect to network location, so that spatial structures can be detected and the influence of important features such as confluences are examined (Som et al. 2014) (Fig. 2). Choosing the wrong sampling design – placing points too close together, too far apart, with equal spacing between them, or without regard for natural and man-made features such as confluences and dams – could lead to unnecessary redundancy in sampling effort, or else failure to detect interesting environmental variation (Jackson et al. 2015). Then, after sampling is completed, data should

be analyzed in a framework that accounts for the specific types of spatial dependencies typical of riverine networks (Isaak et al. 2014).

Challenges ahead and roadmap

Current monitoring methods do not address biodiversity assessment across spatial networks such as rivers. It is therefore crucial to explore alternative methods to fill this knowledge gap. Here, we have proposed the use and potential application of eDNA-based monitoring tools, which encompasses assessment of biodiversity across taxa, space, and time (Fig. 3) to better understand emergent properties, such as ecosystem function. These methods are highly promising, and could cover both genetic composition and species traits in the future.

Research on the use of eDNA methods has focused primarily on method development and application in a wide range of habitats. However, to further develop the use of this method for complete biodiversity assessment, a number of uncertainties must be addressed. First, the nature of a spatial network infers the dispersal of information. Applied to eDNA within a river system context, this means information is being transported through the catchment downstream. As we have discussed, this is a particularly important issue when aiming to identify biodiversity hotspots using a method which provides information from a greater spatial scale than used previously. A further understanding of the processes (flow dynamics) influencing the availability of that information (e.g., the detection of species) must also be explored in greater detail, such as using hydrological tracers to identify the effects of discharge, flow speed and dilution on transport and the detection of DNA. Second, as with most established biodiversity monitoring approaches, abundances are often crucial in assigning value or ecological assessment to a community (Balmer 2002). The requirement of abundance values over presence/absence detection has often been noted as a primary

limitation of the eDNA metabarcoding method. Abundance information using eDNA is often limited to using single target species and qPCR or ddPCR approaches. However, a study by Hänfling et al. (2016) demonstrated a correlation between NGS read number and rank abundance of fish communities, therefore exact figures of abundance or biomass may not be possible with eDNA but rank abundance or site occupancy modelling should be seen as an encouraging alternative method (Doi et al. 2019). Third, the assessment of species interactions needs to be better resolved, or limitations identified. eDNA can tell us what species are there, but it is far from accepted (or may even be impossible) to infer from such data on how they interact with each other (Barner et al. 2018, Morueta-Holme et al. 2016). In particular, if and how to build up a food web from eDNA is strongly debated, since this would depend on co-occurrence assumptions and co-occurrence data, which by themselves are debated to be sufficient for reconstructing interactions (Barner et al. 2018, Pellissier et al. 2018). Lastly, the use of eDNA for functional understanding of an ecosystem requires the greatest development, but is the most promising aspect of this new tool in terms of gaining a greater insight into biodiversity and ecosystem processes with a river catchment. Studies therefore should fully explore the potential of NGS data to include ecosystem understanding and it is hoped that focus now be directed at the opportunities this new form of data provides.

Overall, we see great promises of novel, eDNA-based approaches to tackle the state, change and function of biodiversity in natural ecosystems, and in particular in spatially highly structured systems such as riverine networks. Application and integration of these tools across a wide range of taxonomic groups, across spatial and temporal scale, and applied to different ecosystem functions will be essential to get a better understanding of aquatic ecosystems. Such an appropriate inclusion of patterns and processes will not only be informative for general ecological dynamics, but will also improve the applied understanding of riverine ecosystems, upon whose functions and services we eventually all depend.

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Alternative Viewpoints

In this article, we argue for biodiversity assessment to be complete and inclusive across taxonomic and functional groups, across space and time. We then identify how recent advances in molecular methods may give us the tools to do so. We acknowledge that there are alternative viewpoints with respect to extensively measuring biodiversity: from a parsimony perspective, one could also argue that one should aim to measure as little as possible, that is, the minimum amount necessary to understand a pattern and processes leading to it. This, however, assumes that one measures the “right” thing, and additionally that one can recognize when a system is sufficiently understood/described. In an ideal world, one would know *a priori* which are the important organisms and scales to measure, and only then do so. However, reality is that we often don’t know these aspects at the outset, and many past measurement and assessment approaches have been driven (and limited) by the tools available at the time. Measuring extensively also gives more robustness in the sense of being prepared for when new drivers emerge. We feel such a debate may have an analogy in statistical model selection with many parameters: Should one start with the full model including all parameters and their interactions, and simplify to “the best” model? Or start with a simple model, and incrementally add parameters and interactions until “the best” model is found? It is well known that these two approaches can, but do not have to, lead to the same endpoint. In the former case one may lack parsimony, while in the latter case one may miss important drivers. We feel that in a world facing many environmental changes and unprecedented losses of diversity, the risk of knowing “too much” is worth taking, while the risk of knowing “too little” is not.

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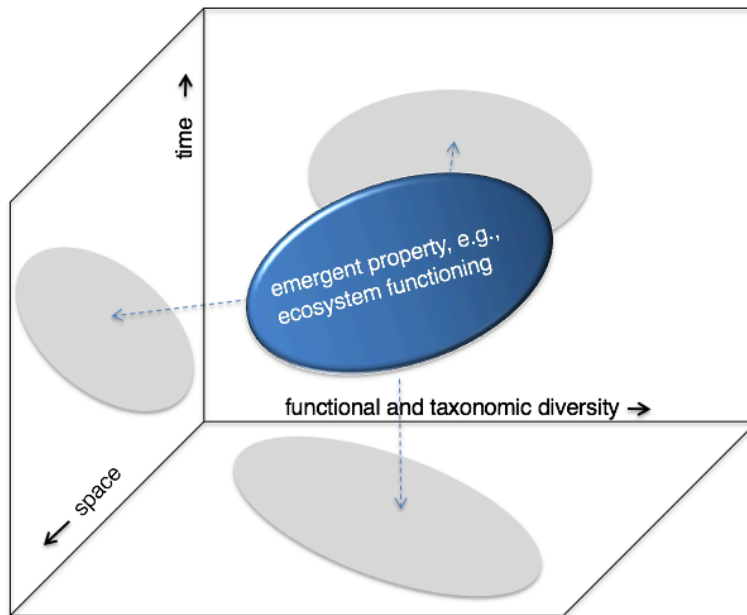
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970 **Figures**

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973 Figure 1. Proposed axes of an assessment of complete biodiversity, covering diversity of taxa
974 and functional groups, space, and time (their respective pairwise dependencies projected by
975 dashed lines). We propose that only an adequate consideration of these three axes will enable
976 a coherent understanding of emergent properties in this multidimensional space, such as
977 ecosystem functioning (illustrated here).

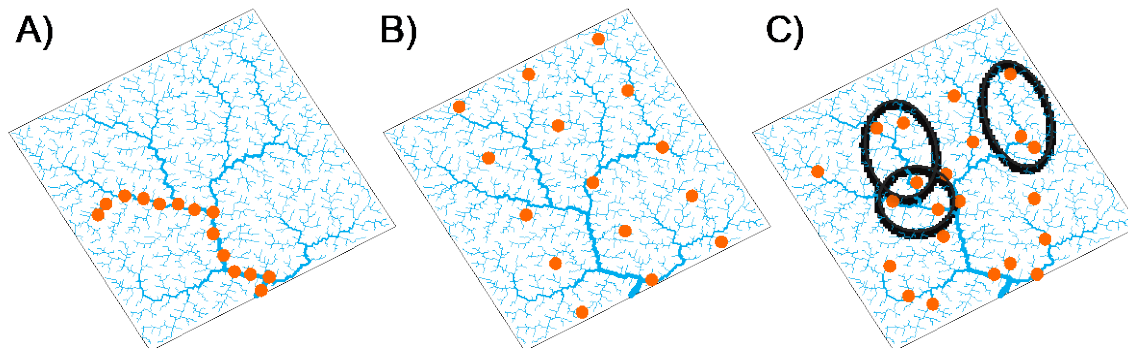


Fig. 2. Various schematic sampling schemes applied to riverine networks commonly applied to aquatic biodiversity monitoring and aquatic ecology studies: A) sampling scheme representing and covering a linear longitudinal transect in a riverine network, following the River Continuum Concept approach. Such an approach may allow tracking longitudinal environmental changes, but is not adequately representing the network. B) Grid-like network with overall randomized position across the network. This approach is adequately covering the different stream and river size classes, but is not able to capture it in a spatially adequate perspective that preserves/follows the inherent network structure. C). Sampling scheme designed to adequately reflect the network structure and capture confluences and respective headwater contributions. Such a scheme captures individual contributing streams and subsequent downstream confluences (exemplified in three cases by black ovals), thereby capturing the hierarchical structure, and allowing a spatial reconstruction of diversity. Network illustration extracted from Carrara et al. (2012).

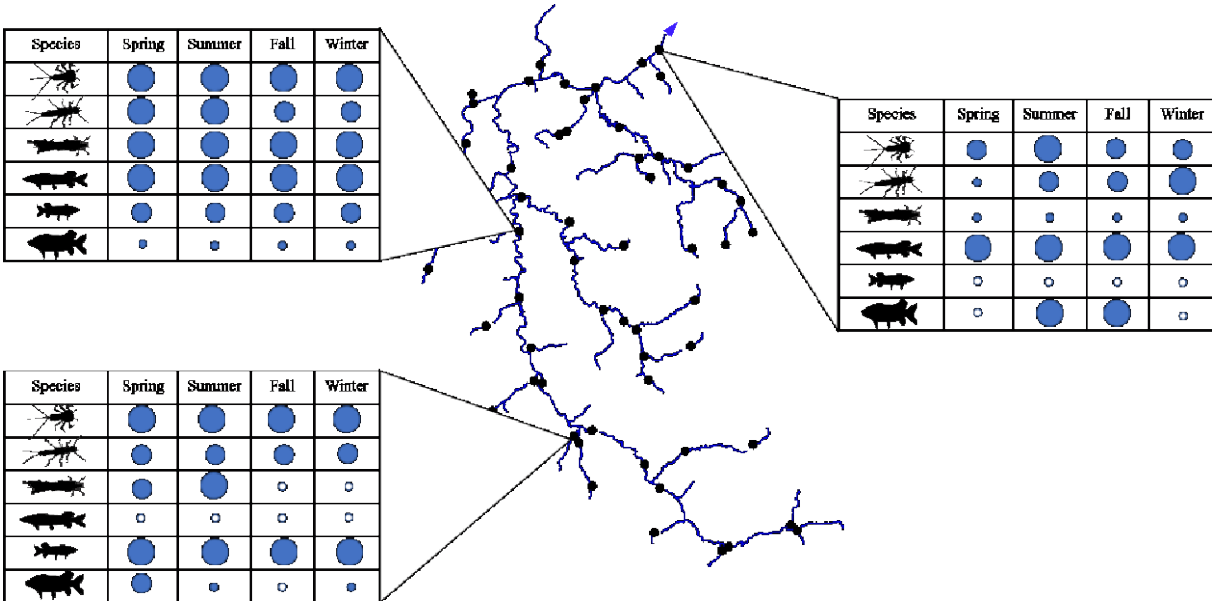


Fig. 3. eDNA sampling allows an integration across time, space and taxonomic (species) groups. Large circles - high read number; Mid-sized circles – medium read number; Small circles - low read number; empty circles – species not present. Samples taken across the different seasons spring, summer, fall, and winter.