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Dissecting biodiversity: assessing the taxonomic, functional and phylogenetic structure of an insect metacommunity in a river network using morphological and metabarcoding data

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Abstract

Most empirical metacommunity studies rely solely on morphological identification of taxa, precluding the species-level identification of several biotic groups, which can influence the characterization of metacommunities. DNA metabarcoding enables inference of species and even intraspecific diversity from community samples but has rarely been used to infer metacommunity structure. Here, we combined morphology and metabarcoding to improve the characterization of an insect metacommunity at different identification levels. We included measures of taxonomic, functional and phylogenetic richness, and we evaluated drivers affecting metacommunity structure (i.e., environmental filtering and dispersal). Communities were sampled from an area that included nine perennial, two near-perennial and two intermittent sites in a river network characterized by high hydrological variability. We identified organisms to a mixed (family to species) taxonomic level using morphology, and to operational taxonomic unit (OTU) and haplotype levels using metabarcoding of the mitochondrial cytochrome c oxidase gene. Diptera and Ephemeroptera showed the greatest increases in taxonomic and phylogenetic richness but not biological trait richness with increasing taxonomic resolution. The joint effect of environmental filtering and dispersal was more important than their individual effects in shaping metacommunity structure at all identification levels. Mixed-level and OTU-level identification were more effective than family and haplotype in characterizing the drivers of metacommunity structure. We demonstrate that the greater taxonomic resolution enabled by metabarcoding could improve understanding of metacommunities within river networks, thus enhancing our capacity to predict ecological responses in ecosystems adapting to global change.

Keywords: *Exact sequence variant*, *IRES*, *taxonomic sufficiency*, *habitat filtering*, *taxonomic surrogacy*

Introduction

Quantifying biodiversity is essential to understand how it is shaped in space and time, and this is even more important in a context of ongoing global change and biodiversity loss. Metacommunity theory provides a framework in which to explore the factors structuring biological communities (Leibold & Chase 2018). Dispersal and environmental filtering play key roles in assembling metacommunities and, to date, their contributions have

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typically been quantified using morphologically identified taxa. However, for smaller, less conspicuous and taxonomically complex biotic groups such as most of the invertebrates, algae and protists, morphological approaches can preclude species-level identification and often result in mixed-level identification being used as a surrogate for inferring species-level patterns (Jackson et al. 2014; Zimmermann et al. 2015; Gauthier et al. 2020a). However, taxonomic levels coarser than species can alter the characterization of assembly processes because of differences in species response to environment and dispersal (Heino & Soininen 2007; Laini et al. 2020).

Mixed-level taxonomic identification is typically done to the "lowest practical taxonomic" level (Guerold 2000; Jones 2008; Filstrup et al. 2014) because of (i) the scarcity of taxonomic experts, (ii) the time needed to identify to species level compared to coarser levels, and (iii) difficulties in identifying morphologically complex and cryptic taxa, early instar insects and damaged specimens (Jones 2008; Gauthier et al. 2020a). Mixed-level taxonomic identification can complicate data analysis if ambiguous parent–child pairs (e.g., Baetidae and *Baetis*) are present, and the methods used to resolve this issue can alter the results obtained (Cuffney et al. 2007; Meredith et al. 2019). The effectiveness of coarser taxonomic levels as surrogates for species-level identification depends on multiple factors, including the ratio of species to coarser taxonomic levels and the distribution of species numbers among these coarser levels (Jones 2008; Rosser 2017). The influence of these factors and their interactions is context dependent, preventing generalizations about the use of coarser taxonomic levels to characterize metacommunity structure and infer related processes such as dispersal and environmental filtering (Heino & Soininen 2007; Angiolini et al. 2017; Laini et al. 2020).

Dispersal is influenced by species-specific physiological, morphological and behavioral adaptations, density-dependent processes, and geographic and environmental constraints (Clobert et al. 2012; Heino et al. 2017). This complexity introduces variability within taxonomic levels; for example, dispersal-related adaptations can vary within insect genera (Sarremejane et al. 2020a). Community responses to abiotic variables can also differ depending on taxonomic resolution (Heino & Soininen 2007; Vilmi et al. 2016), and although niche conservatism is expected among congeneric species (Wiens & Graham 2005), minor differences can influence metacommunity structure. For example, the response of aquatic invertebrate juveniles to drying in intermittent water bodies differs among congeneric species within the true fly genus *Polypedilum* (Diptera, Gusev et al. 2014) and the stonefly genus *Taeniopteryx* (Plecoptera, López-Rodríguez & de Figueroa 2006). Moreover, cryptic species and congeners within species complexes can differ markedly in their tolerance of environmental stressors (Sturmbauer et al. 1999; Eisenring et al. 2016; Macher et al. 2016). Methods that provide species-level or even intraspecific information for whole communities could thus advance understanding of metacommunity processes (Tesson & Edelaar 2013; Gounand et al. 2018; Gauthier et al. 2020a).

DNA metabarcoding provides high-resolution taxa lists from bulk samples (e.g. samples containing invertebrates collected from the investigated sites) by targeting specific DNA regions (Taberlet et al. 2012; Yu et al. 2012). Metabarcoding can reach or exceed the taxonomic level achieved by morphological identification, despite some discrepancies due to primer bias and rare species (Hajibabaei et al. 2011; Elbrecht & Leese 2015, 2017; Kuntke et al. 2020). The taxonomic resolution obtained with metabarcoding depends on the targeted region, as well as the completeness of the reference database against which sequences are compared (Elbrecht et al. 2017; Meyer et al. 2021). Genetic information can be grouped into operational taxonomic units (OTUs) (Floyd et al. 2002; Rognes et al. 2016), a commonly used classifier of biodiversity that frequently aligns to the biological species concept (Blaxter et al. 2005). Furthermore, intraspecific diversity can be described using "exact sequence variants", such as haplotypes for the mitochondrial cytochrome c oxidase (COI) gene, which have the potential to further improve the characterization of metacommunity patterns (Turon et al. 2020; Zizka et al. 2020; Antich et al. 2021). In addition, phylogenetic relationships inferred from DNA-based methods can enhance the detection of metacommunity assembly processes (Vamosi et al. 2009; Hill et al. 2019; Li et al. 2021).

We studied metacommunity patterns in a small river network using morphological and DNA metabarcoding data, and compared measures of (i) taxonomic richness, (ii) phylogenetic composition and (iii) biological traits inferred through the two approaches. We selected aquatic insects, which are abundant, biodiverse, and include taxa that have diverse biological trait profiles (*sensu* Tachet et al. 2010) and which are often difficult to identify at species level (Jones 2008; Heino et al. 2017). The taxonomic identification levels typically achieved vary considerably among insect orders and families, and coarser taxonomic levels are frequently used as surrogates for species (Jones 2008).

First, we predicted that increasing taxonomic resolution would alter estimates of the overall taxonomic, functional, and phylogenetic richness of different insect orders due to greater characterization of species-level and intraspecific diversity; and second we predicted that the inferred importance of local environmental conditions and dispersal in structuring metacommunities would increase with taxonomic resolution.

Methods

Field sampling, environmental variables and morphological identification

Sampling campaigns were conducted from the 17th to 24th of April 2018 at 13 sites on the River Ceno and five of its tributaries, northern Italy (9.65°E, 44.6°N). Two sites are intermittent and dry annually, two are near-perennial and dry only rarely, and nine are perennial (Figure 1). Due to the high variability of hydrogeological features in this area, the targeted river network provides a natural laboratory for testing patterns that shape ecological dynamics at broader regional scales. Intermittent and near-perennial sites dried completely during summer 2017, whereas only intermittent sites dried in 2018, as inferred from temperature data loggers (HOBO® model UA-002-08, Onset Computer Corporation, MA, USA). Insects were sampled from cobble-dominated (grain size 6–20 cm) riffle mesohabitats to minimize variability introduced by taxa with different habitat preferences. Samples were collected using a Surber net (0.05 m^2; 500 µm mesh), sorted in situ and organisms preserved in 99% ethanol. The ethanol was replaced once <24 h after collection and samples stored at 4°C. Organisms were counted and identified morphologically to a mixed taxonomic level, from species to family (Table SI).

Metabarcoding

Metabarcoding analysis was performed following Elbrecht and Leese (2015) but using a two-step PCR protocol as described in Zizka et al. (2019). Each sample was dried overnight at room temperature, then ground with an IKA® Ultra Turrax® Tube Drive Control (Staufen, Germany) for 30 min at 4000 rpm. DNA was extracted with a modified salt extraction protocol (Sunnucks & Hales 1996; Elbrecht et al. 2017) and RNA digested with RNase A (10 mg mL^{-1}) to improve downstream analysis steps. DNA was purified using a NucleoSpin® Gel and PCR cleanup kit (MachereyNagel, Germany). DNA sample concentration was equilibrated to 25 ng μL^{-1} and the first PCR step (20 cycles) performed using the

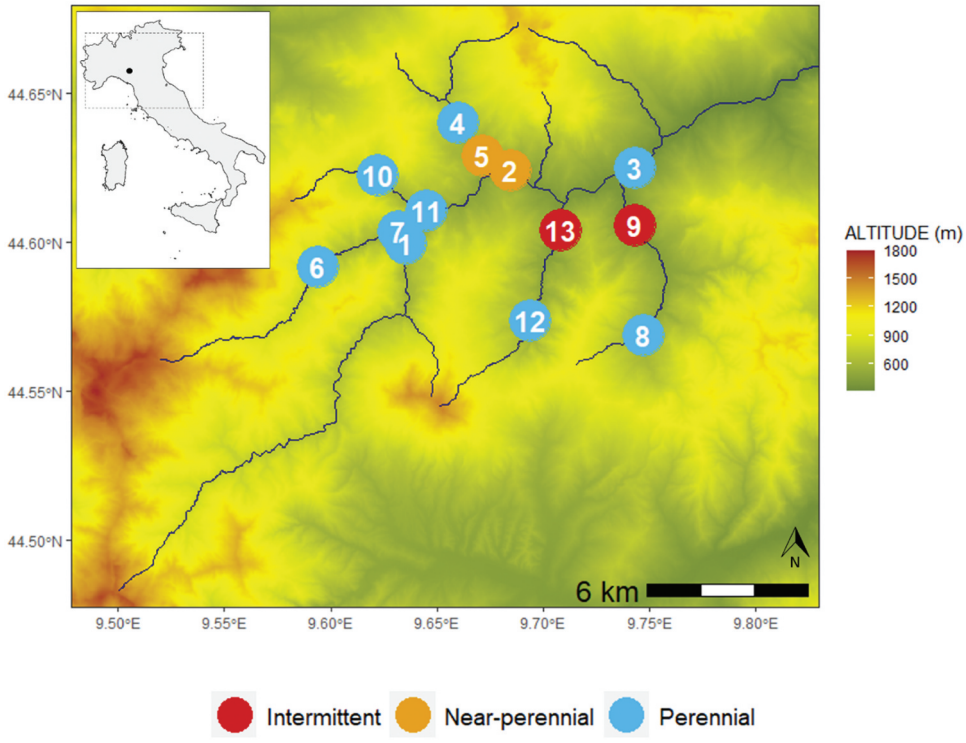

Figure 1. Location of the 13 sampling sites on the River Ceno and its tributaries, indicating sites with perennial, intermittent and near-perennial flow regimes, and the river location in northern Italy (see inset).

BF2 + BR2 primers (Elbrecht & Leese 2017) and the Qiagen Multiplex PCR Plus kit in 25 μL reactions. Two replicate samples were multiplexed following the tagging strategy described in Elbrecht and Steinke (2019) during the second PCR (15 cycles), also in 25 μL reaction volumes. The final DNA barcode library was prepared by pooling an equal quantity of amplicons for each sample, which was then purified using 0.76 × SPRIselect (Beckman Coulter, Germany). The library was sequenced using an Illumina HiSeq Rapid Run 2 × 250 bp (Illumina, CA, USA) as part of a library containing 288 samples (by Eurofins, Constance, Germany). Illumina reads have been deposited in the SRA database under the accession number PRJNA943200.

Metabarcoding data were analysed with JAMP version 0.69 (https://github.com/VascoElbrecht/JAMP), an R-based metabarcoding pipeline that integrates functions of USEARCH 11.0.667 (Edgar 2010) and VSEARCH 2.10.4 (Rognes et al. 2016). The sequencing files were demultiplexed according to the sample tags and quality checked using FastQC v0.11.9. Paired-end reads were merged and reverse complements built where needed. Primers were trimmed with Cutadapt (Martin 2011) and sequences filtered according to the maximum expected error (Edgar & Flyvbjerg 2015), after which only the sequences ± 10 bp of the expected length (421 bp) were retained. The number of reads per sample was subset to the lowest number of reads found in our samples (n = 351,600) due to differences in sequencing depths. OTU clustering was done using USEARCH with a clustering threshold of 97% similarity and reads including singletons matched against OTUs to generate an OTU table. OTUs with read abundance <0.01% and those present in only one replicate sample were discarded prior to data analysis. Haplotypes were inferred using the unoise3 algorithm (Edgar 2016) following the denoising approach described in Elbrecht et al. (2018).

Data analysis

Functional and phylogenetic trees

Trait trees for families, mixed-level taxa, OTUs and haplotypes were built with complete linkage clustering on a distance matrix calculated using the biological traits of Tachet et al. (2010): dispersal, feeding habit, preferred food, life cycle duration, locomotion and substrate relation, resistance forms, respiration, maximum potential size, aquatic stages and voltinism. Each trait includes multiple categories (e.g., different body sizes) and each taxon can show an affinity for more than one category. We standardized the trait matrix as proportional affinities for each category within each trait. We averaged traits for each taxon when using family-level data. We used the mixed-variables coefficient to calculate an among-taxa distance matrix (Pavoine et al. 2009). Traits of the nearest taxon in the taxonomy were assigned to OTUs and haplotypes, resulting in the assignment of the same traits to more than one OTU or haplotype.

Phylogenetic trees were built for OTU and haplotype sequences, using phylogenetic placement to position new sequences on an existing reference tree (Matsen et al. 2010; Berger et al. 2011). Since COI data alone cannot create a robust backbone phylogeny, the reference phylogenetic tree was built using concatenated alignments of small and large subunit (SSU and LSU, respectively) ribosomal RNA gene sequences from the SILVA database (Quast et al. 2013) as well as COI sequences from the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/). The SILVA database was filtered to retain only freshwater species, as classified by the freshwaterecology.info database (Schmidt-Kloiber & Hering 2015). COI sequences were retrieved with the R package rentrez (Winter 2017) and aligned separately for each order with MUSCLE using default settings (Edgar 2004). Alignments were inspected visually and misaligned sequences removed. We retained only sequences belonging to species in SSU or LSU alignments, and randomly selected one representative sequence for each species. Poorly aligned positions and divergent regions of SSU, LSU and COI alignments were identified and removed with GBlocks (Castresana 2000). The three alignments were concatenated, and a phylogenetic tree was inferred using a general time reversible model (GTRCAT), with automated, rapid bootstrapping analysis (autoMR) used to search for the best scoring maximum likelihood tree in one program run with RaxML version 8 (Stamatakis 2014). Analysis was partitioned to estimate empirical base frequencies and evolutionary rates separately for SSU, LSU and COI. For COI, we inferred distinct model parameters jointly for all first and second codon positions and separately for the third position. Metabarcoding sequences were aligned with the reference sequence alignment using MAFFT (Katoh et al. 2002) and phylogenetic placement was performed with the evolutionary placement algorithm (Berger et al. 2011).

Taxonomic, functional and phylogenetic richness of insect orders at different identification levels

We explored the richness of different orders at family and mixed levels determined with morphology and OTU and haplotype resolutions determined with metabarcoding. Taxonomic and functional richness were calculated for all four identification levels, and phylogenetic richness was calculated for OTU and haplotype levels. Functional and phylogenetic richness were calculated as the sum of the branch lengths of a trait and phylogenetic tree, respectively, connecting all species (Faith 1992; Petchey & Gaston 2002). Developed to measure diversity, this approach also provides a functional and phylogenetic generalization of taxonomic richness (Chao et al. 2014).

Drivers of metacommunity structure

We inferred the importance of drivers of metacommunity structure using the metacommunity assembly by trait selection approach (mCATS, Brown et al. 2018), which simulates the selection of communities from the regional species pool under dispersal, trait selection and dispersal–trait selection scenarios. We used trait selection as a measure of environmental filtering and extended this approach to simulate phylogenetic selection. For each site, the dispersal scenario was simulated by randomly selecting taxa from the regional pool with the probability of selection set as inversely proportional to three spatial distances (Euclidean, network and least-cost) to other sites. Least-cost distances were calculated as the path of least resistance to movement, where landscape resistance was estimated using a 10 × 10 m digital terrain model. Least-cost distances assume overland dispersal and can better reflect the role of overland dispersal in structuring invertebrate metacommunities (Tonkin et al. 2018). Since all spatial distances were highly correlated (Mantel test: $r \geq 0.90$, $p < 0.001$) we only present the results obtained with least-cost distances. For the trait selection scenario, taxa were iteratively selected from the regional pool until the community mean trait/phylogenetic distance and skewness ranged between ±2.5% of the observed value. For the dispersal-trait model, we combined occurrences from the dispersal and trait selection scenarios.

For each scenario, 10,000 simulations were performed, with the number of taxa kept constant for each site. For each simulation, the Jaccard similarity between the simulated and observed communities was calculated (García-Girón et al. 2019) and presented as standardized effect sizes (SES), using:

$$SES = \frac{(\mu_{mod} - \mu_{null})}{\sigma_{null}}$$

where μ_{mod} is the mean similarity of the simulated communities (dispersal, trait selection and joint dispersal-trait selection), and μ_{null} and σ_{null} are the mean and SD of the similarity from a random scenario built by randomly selecting taxa from the regional pool irrespective of spatial distances.

The effect of different scenarios (dispersal, trait selection, joint dispersal–trait selection) and taxonomic resolutions (family, mixed-level, OTU, haplotype) on SES was assessed with linear mixed-effects models (LMM). LMM included scenario, taxonomic resolution and their interaction as fixed effects and site as a random effect. To control for heteroscedastic residuals, scenario was used as a grouping factor to correct the model variance structure and SES were square-root transformed. The significance of the fixed effects was evaluated with Wald's Chi-square tests. Pairwise differences between estimated marginal means were then compared using the Tukey adjustment for multiple comparisons. We report pairwise comparisons by referring to scenarios including functional or phylogenetic information as e.g., OTU-traits or haplotype-phylo, respectively, and to the taxonomic level alone to refer to both information types for OTU and haplotypes.

Analyses were performed in R (R Core Team 2020) using the packages ape (Paradis & Schliep 2019), biomonitoR (Laini et al. 2022), emmeans (Lenth et al. 2020), gdistance (van Etten 2017), nlme (Pinheiro et al. 2020), picante (Kembel et al. 2010), raster (Hijmans et al. 2020) and vegan (Oksanen et al. 2019), with dplyr (Wickham et al. 2020) and tidyr (Wickham 2020) used to manage data and ggplot2 (Wickham 2009) to plot results.

Results

Morphology vs DNA metabarcoding and metacommunity structure

We identified 64 mixed-level taxa in 41 families using morphology, and 222 OTUs and 537 haplotypes using metabarcoding (Figures S1, S2). Haplotypes belonged to 97 OTUs due to the stricter quality filtering (denoising) used to infer haplotypes (Antich et al. 2021). Metabarcoding was effective in detecting taxa identified with morphological identification (usually at family/subfamily/genus level), consistently increasing the taxonomic resolution (e.g., Athericidae to *Atherix marginata*, *Torleya* to *Torleya major*, etc.). Of the identifications based on metabarcoding approaches, 54% (66 hits out of 123 total metabarcoding taxonomic assignments) belonged to the species level (Figure 2, Table SII).

Overall, the order Diptera contributed the most families, OTUs and haplotypes, whereas Plecoptera and Diptera had the most mixed-level taxa. Diptera also contributed most to family, mixed-level taxa and OTU functional richness, whereas Ephemeroptera had the highest values for phylogenetic richness of OTUs and haplotypes, and Plecoptera for the trait richness of haplotypes (Figure 3).

Drivers of metacommunity structure

The standardized effect size (SES) obtained with the mCATS approach varied depending on taxonomic resolution ($\chi^2_{(5)}$ = 104.5, p < 0.001), scenario of metacommunity assembly ($\chi^2_{(2)}$ = 639.6, p < 0.001) and their interaction ($\chi^2_{(10)}$ = 98.8, p < 0.001), indicating that the importance of environmental filtering, dispersal and their interaction differed depending on taxonomic resolution. SES > 2 indicate approximate significance (two-tailed test; Ulrich & Gotelli 2007) and thus, in our study, the proximity of SES to 2 in scenarios with higher SES (e.g., joint scenarios for OTUs; Figure 4c–d) indicated a weak effect in most of the taxonomic levels.

Although the effect was weak, significant differences were found among scenarios within the same taxonomic resolution and among taxonomic resolutions within the same scenario. For the

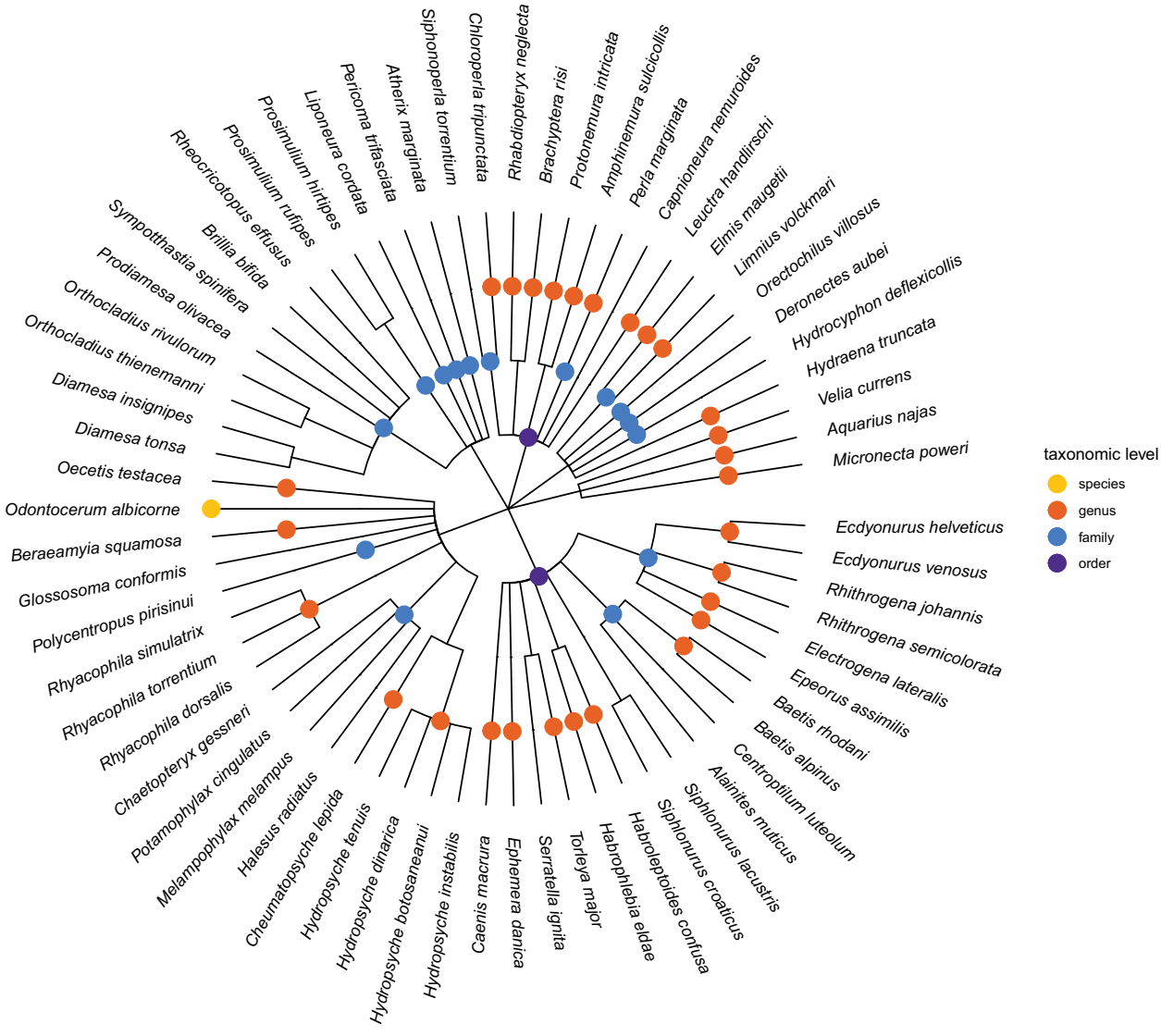

Figure 2. Species identified with metabarcoding and the corresponding taxonomic level used for morphological identification. Two or more species identified with metabarcoding can be assigned to more than one taxon identified with morphology because of missing diagnostic characters of early instars and damaged specimens (e.g. *Baetis alpinus* and *Baetis rhodani* with metabarcoding, *Baetis* and Baetidae with morphological identification). Coloured dots represent taxa identified with morphology.

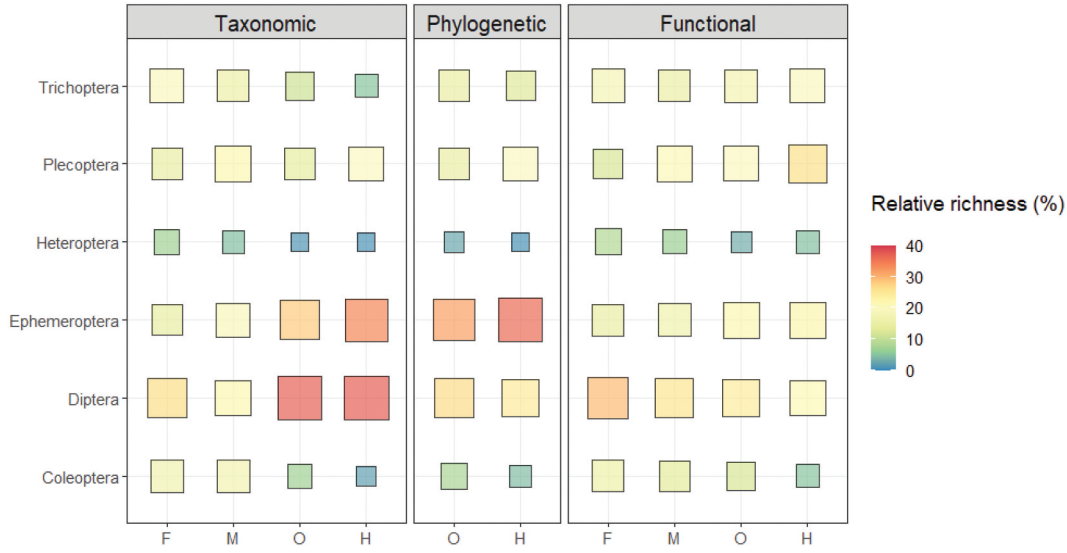

Figure 3. Taxonomic, phylogenetic and functional richness of six aquatic insect orders, as measured at family (F) and mixed taxa (M) level using morphology, and as operational taxonomic units (O) and haplotypes (H) using metabarcoding. Square size and colors are proportional to relative richness, calculated for all recorded taxa.

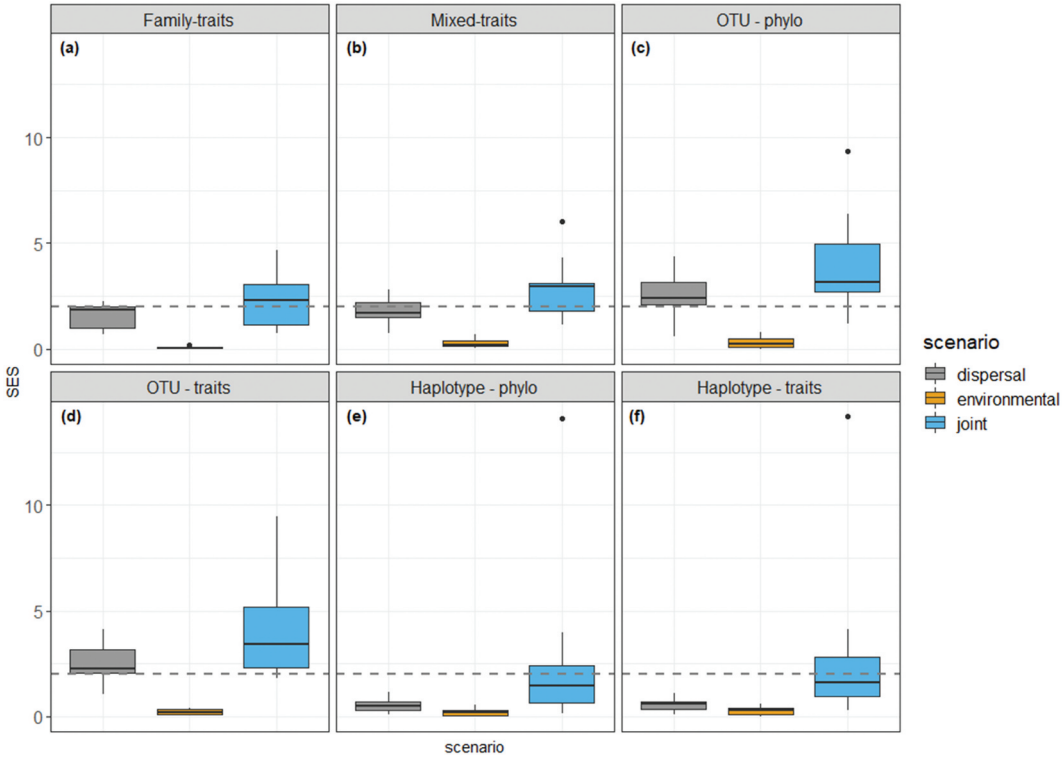

Figure 4. Simulated community selection for dispersal, environmental filtering and joint scenarios at (a) family, (b) mixed, (c, d) OTU and (e, f) haplotype levels, using phylogenetic (c, e) and functional (trait-related) information (d, f). The dashed line at 2 indicates an approximate significant effect of the scenario on metacommunity composition (two-tailed test).

dispersal scenario, mean SES were higher for OTU than for family (Wald's Chi-square test, $p < 0.01$), and haplotype SES were lower than for other levels ($p < 0.001$; Figure 4). For the trait selection scenario, family-level SES were lower than OTU-phylo ($p < 0.05$). For the joint dispersal–trait selection scenario, SES were comparable among all taxonomic levels, except OTU-phylo was greater than haplotype-phylo ($p < 0.05$) and OTU-traits greater than haplotype-traits ($p < 0.05$; Figure 4). Within taxonomic levels, SES for the trait selection scenario were generally lower than for dispersal and joint scenarios ($p < 0.001$), except in the comparison between dispersal and environmental scenarios within haplotype-phylo ($p < 0.01$) and haplotype-traits ($p < 0.05$). For the dispersal and joint scenarios, SES were comparable for family, mixed and OTU levels ($p > 0.05$). SES were higher for the joint than the dispersal scenario for haplotype-phylo ($p < 0.01$) and haplotype-traits ($p < 0.001$).

Discussion

Variability in the taxonomic resolution of a biological dataset can affect the detection of processes driving metacommunity structure, because coarser taxonomic levels may not effectively represent species-level or intraspecific patterns (Heino & Soininen 2007). By identifying organisms to multiple levels using morphological and metabarcoding approaches, we show how taxonomic resolution affects the fundamental properties and inferred drivers of taxonomic, functional (trait-based) and phylogenetic metacommunity structure. OTUs and haplotypes most effectively characterized the richness of species-rich families with complex diagnostic characteristics such as Heptageniidae and Chironomidae, influencing our capacity to infer metacommunity structure. Across identification levels, the joint effect of dispersal and environmental filtering had the greatest influence on metacommunity structure. However, the taxonomic levels achieved by metabarcoding either increased (OTU) or decreased (haplotype) the inferred importance of dispersal compared to morphological identification. As cost-effective methods to quantify biodiversity using DNA rapidly increase (Taberlet et al. 2012; Deiner et al. 2017), our results highlight the potential of the finer-level taxonomic resolution achieved by metabarcoding to enhance characterization of metacommunity structure in dynamic river networks.

Taxonomic, functional and phylogenetic richness of insect orders at different identification levels

Insect orders contributed differently to the overall taxonomic, functional, and phylogenetic richness among taxonomic levels, partly supporting our first prediction. Ephemeroptera and Diptera increased, due to notable OTU and haplotype richness in the Heptageniidae and Chironomidae, respectively. Heptageniidae is the mayfly family with the highest species richness in Europe (Schmidt-Kloiber & Hering 2015) and its genera *Electrogena* and *Rhithrogena*, found frequently in our samples, have ambiguous taxonomy and include cryptic species (Vuataz et al. 2016; Polášek et al. 2018; Tenchini et al. 2018). The European Chironomidae comprise >1000 species in nearly 200 genera (Serra et al. 2016) but are usually identified to coarse taxonomic levels due to their complex diagnostic characteristics and despite their species-specific responses to environmental variability (Milošević et al. 2013; Cañedo-Argüelles et al. 2016; Beermann et al. 2018). In contrast, Plecoptera relative richness was stable across taxonomic levels, and the relative taxonomic richness of Trichoptera, Coleoptera and Heterop-tera decreased from family to haplotype, despite including species with complex taxonomy.

Our results for the mixed morphological identification level are partially driven by the taxonomic resolution. Subfamily and family-level identification of families including Chironomidae and Simuliidae probably reduced taxonomic richness estimates of Diptera. Moreover, treating haplotypes as distinct entities inflated taxonomic richness estimates at this level, because many haplotypes represent intraspecific variants (537 haplotypes but only 97 OTUs). Our results thus indicate that the potential of metabarcoding to contribute to a finer description of how biodiversity varies among insect orders and is particularly high for morphologically difficult to identify and at the same time species-rich taxa.

Phylogenetic richness showed similar patterns to taxonomic richness. A strong, positive relationship between these richness metrics is expected in regions with large, diverse species pools and when evolutionarily distinct species with narrow geographic distribution are lacking (Tucker et al. 2012; Tucker & Cadotte 2013). In our study, nearly all recorded orders occurred at each site, facilitating observation of this relationship. The major contribution of Ephemeroptera and Diptera to phylogenetic richness found in this study likely reflects their regional richness and also their higher rates of molecular evolution compared to other orders (Welch et al. 2008; Elliott et al. 2018).

Patterns of functional richness are driven in part by evolutionary history. For example, we recorded both major lineages of Plecoptera, Systellognatha and Euholognatha, which differ in traits including preferred food, feeding strategy, size and lifespan (Tachet et al. 2010; Tierno de Figueroa & López-Rodríguez 2019). However, trait richness did not closely match phylogenetic richness patterns. We likely underestimated any increase in trait richness associated with increasing taxonomic resolution (Schmera et al. 2017), because of the coarse taxonomic level at which traits were assigned to some groups, e.g., subfamily (Chironomidae) or family (Athericidae; Tachet et al. 2010). Using genus or species-level trait information could enhance assessment of trait richness, due to intra-family and intrageneric differences in biological traits (Waringer et al. 2013; Serra et al. 2016; Sarremejane et al. 2020a).

Effects of taxonomic resolution on community assembly processes

We identified the joint effects of environmental filtering and dispersal as the best predictor of insect community composition. The spatial proximity of colonist sources determines the pool of taxa that can disperse to a site, whereas local environmental filtering selects taxa according to their biological and ecological traits. Macroinvertebrate communities identified to a mixed (family to species) level using a morphological approach thus appear to be structured by both environmental filtering (Robinson et al. 2014; Li et al. 2020) and dispersal (Downes & Lancaster 2018; Gauthier et al. 2020b). These differences highlight dispersal and niche-based processes as context-dependent influences on metacommunity structure (Tonkin et al. 2016) that vary according to the spatial scale studied (Viana & Chase 2019). Contrary to previous research at species-to-family levels (Martin et al. 2016), our results indicate that differences can be attributed to taxonomic resolution as well as spatial and environmental context.

The inferred importance of dispersal in structuring local communities was weaker for haplotype than other taxonomic levels. This may reflect the method used to infer haplotypes from metabarcoding data, which is less effective in characterizing overall metacommunity diversity than the OTU level, due to the stricter filtering step (denoising) during sequence selection (Elbrecht et al. 2018). This finding may also reflect an inherent sampling bias, because few specimens (e.g. one individual for *Velia currens*) were collected for some taxa and thus intraspecific variation among sites may reflect stochastic effects (Zizka et al. 2020). However, small-scale (10–100 km) differentiation in haplotype frequencies can definitely occur between populations of one aquatic insect species, with some (typically rare at the global analysis level) haplotypes restricted to few sites (Hughes et al. 2003; Zickovich & Bohonak 2007; Elbrecht et al. 2014). Such a real population subdivision, but also stochastic selections from a diverse pool of COI haplotypes, may thus have contributed to the observed within-species dissimilarity. As such, it is a logical consequence that dispersal-limited arthropods can have a lower inferred dispersal of haplotypes compared to coarser taxonomic levels (Arribas et al. 2021).

We inferred weak effects of environmental filtering and/or dispersal on metacommunity structure. The weak effects of local environmental factors in predicting metacommunity structure and near-random structural patterns identified by metabarcoding data may indicate that stochastic processes influenced community assembly at fine taxonomic resolutions (Bush et al. 2020), as can also occur at mixed morphological levels (Sarremejane et al. 2020b). Moreover, our representation of ecological patterns with metabarcoding may have been influenced by the omission of rare and scarce taxa, especially at haplotype level due to methodological limitations (Zizka et al. 2020). Lastly, our limited number of samples could have constrained the characterization of the species pool and of the overall metacommunity structure.

Conclusions

Our study highlights the complex interplay between taxonomic resolution, functional and phylogenetic information, and how these factors influence the inference of metacommunity structure. Our results indicate that an increase in taxonomic resolution can improve estimates of the taxonomic, functional and phylogenetic richness of the main aquatic insect orders. In addition, the importance of dispersal increased from family to haplotypes in our insect metacommunity. Accurate richness estimates can thus advance insights achieved at a coarser taxonomic resolution by revealing species-level and intraspecific dynamics. Our study contributes to an increasing body of evidence demonstrating that genetic methods can support effective quantification of biodiversity and the factors driving metacommunity structure in dynamic ecosystems including river networks, which could inform predictions of metacommunity responses to global change.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

Metabarcoding data have been deposited in the SRA database under the accession number PRJNA943200, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA943200/. The data used for this work are available from the corresponding author on reasonable request.

Supplementary material

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References

Angiolini C, Nucci A, Landi M, Bacchetta G. 2017. What drives riparian plant taxa and assemblages in Mediterranean rivers? Aquatic Sciences 79:371–384. DOI: 10.1007/s00027-016-0503-2.

Antich A, Palacin C, Wangensteen OS et al. 2021. To denoise or to cluster, that is not the question: Optimizing pipelines for COI metabarcoding and metaphylogeography. BMC Bioinformatics 22:177. DOI: 10.1186/s12859-021-04115-6.

Arribas P, Andújar C, Salces-Castellano A et al. 2021. The limited spatial scale of dispersal in soil arthropods revealed with whole-community haplotype-level metabarcoding. Molecular Ecology 30:48–61. DOI: 10.1111/mec.15591.

Beermann AJ, Zizka VM, Elbrecht V et al. 2018. DNA metabarcoding reveals the complex and hidden responses of chironomids to multiple stressors. Environmental Sciences Europe 30:26. DOI: 10.1186/s12302-018-0157-x.

Berger SA, Krompass D, Stamatakis A. 2011. Performance, accuracy, and web server for evolutionary placement of short sequence reads under maximum likelihood. Systematic Biology 60:291–302. DOI: 10.1093/sysbio/syr010.

Blaxter M, Mann J, Chapman T et al. 2005. Defining operational taxonomic units using DNA barcode data. Philosophical Transactions of the Royal Society B: Biological Sciences 360:1935–1943. DOI: 10.1098/rstb.2005.1725.

Brown LE, Khamis K, Wilkes M et al. 2018. Functional diversity and community assembly of river invertebrates show globally consistent responses to decreasing glacier cover. Nature Ecology & Evolution 2:325–333. DOI: 10.1038/s41559-017-0426-x.

Bush A, Monk WA, Compson ZG et al. 2020. DNA metabarcoding reveals metacommunity dynamics in a threatened boreal wetland wilderness. Proceedings of the National Academy of Sciences 117:8539–8545. DOI: 10.1073/pnas.1918741117.

Cañedo-Argüelles M, Bogan MT, Lytle DA et al. 2016. Are Chironomidae (Diptera) good indicators of water scarcity? Dryland streams as a case study. Ecological Indicators 71:155–162. DOI: 10.1016/j.ecolind.2016.07.002.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17:540–552. DOI: 10.1093/oxfordjournals.molbev.a026334.

Chao A, Chiu CH, Jost L. 2014. Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through Hill numbers. Annual Review of Ecology, Evolution, and Systematics 45:297–324. DOI: 10.1146/annurev-ecolsys-120213-091540.

Clobert J, Baguette M, Benton TG, Bullock JM. 2012. Dispersal ecology and evolution. Oxford: Oxford University Press.

Cuffney TF, Bilger MD, Haigler AM. 2007. Ambiguous taxa: Effects on the characterization and interpretation of invertebrate assemblages. Journal of the North American Benthological Society 26:286–307. DOI: 10.1899/0887-3593(2007)26[286:ATEOTC]2.0.CO;2.

Deiner K, Bik HM, Mächler E et al. 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Molecular Ecology 26:5872–5895. DOI: 10.1111/mec.14350.

Downes BJ, Lancaster J. 2018. Itinerant, nomad or invader? A field experiment sheds light on the characteristics of successful dispersers and colonists. Freshwater Biology 63:1394–1406. DOI: 10.1111/fwb.13167.

Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797. DOI: 10.1093/nar/gkh340.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461. DOI: 10.1093/bioinformatics/btq461.

Edgar RC. 2016. UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv 081257. DOI: 10.1101/081257.

Edgar RC, Flyvbjerg H. 2015. Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics 31:3476–3482. DOI: 10.1093/bioinformatics/btv401.

Eisenring M, Altermatt F, Westram AM, Jokela J. 2016. Habitat requirements and ecological niche of two cryptic amphipod species at landscape and local scales. Ecosphere 7:e01319. DOI: 10.1002/ecs2.1319.

Elbrecht V, Feld CK, Gies M et al. 2014. Genetic diversity and dispersal potential of the stonefly *Dinocras cephalotes* in a central European low mountain range. Freshwater Science 33:181–192. DOI: 10.1086/674536.

Elbrecht V, Leese F. 2015. Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass—Sequence relationships with an innovative metabarcoding protocol. PLoS One 10:e0130324. DOI: 10.1371/journal.pone.0130324.

Elbrecht V, Leese F. 2017. Validation and development of COI metabarcoding primers for freshwater macroinvertebrate bioassessment. Front Environ Sci 5:11. DOI: 10.3389/fenvs.2017.00011.

Elbrecht V, Peinert B, Leese F. 2017. Sorting things out: Assessing effects of unequal specimen biomass on DNA metabarcoding. Ecology and Evolution 7:6918–6926. DOI: 10.1002/ece3.3192.

Elbrecht V, Steinke D. 2019. Scaling up DNA metabarcoding for freshwater macrozoobenthos monitoring. Freshw Biol 64:380–387. DOI: 10.1111/fwb.13220.

Elbrecht V, Vamos EE, Steinke D, Leese F. 2018. Estimating intraspecific genetic diversity from community DNA metabarcoding data. PeerJ 6:e4644. DOI: 10.7717/peerj.4644.

Elliott MJ, Knerr NJ, Schmidt-Lebuhn AN. 2018. Choice between phylogram and chronogram can have a dramatic impact on the location of phylogenetic diversity hotspots. Journal of Biogeography 45:2190–2201. DOI: 10.1111/jbi.13399.

Faith DP. 1992. Conservation evaluation and phylogenetic diversity. Biological Conservation 61:1–10. DOI: 10.1016/0006-3207(92)91201-3.

Filstrup CT, Hillebrand H, Heathcote AJ et al. 2014. Cyanobacteria dominance influences resource use efficiency and community turnover in phytoplankton and zooplankton communities. Ecology Letters 17:464–474. DOI: 10.1111/ele.12246.

Floyd R, Abebe E, Papert A, Blaxter M. 2002. Molecular barcodes for soil nematode identification. Molecular Ecology 11:839–850. DOI: 10.1046/j.1365-294X.2002.01485.x.

García-Girón J, Wilkes M, Fernández-Aláez M, Fernández-Aláez C. 2019. Processes structuring macrophyte metacommunities in Mediterranean ponds: Combining novel methods to disentangle the role of dispersal limitation, species sorting and spatial scales. Journal of Biogeography 46:646–656. DOI: 10.1111/jbi.13516.

Gauthier M, Konecny-Dupré L, Nguyen A et al. 2020a. Enhancing DNA metabarcoding performance and applicability with bait capture enrichment and DNA from conservative ethanol. Molecular Ecology Resources 20:79–96. DOI: 10.1111/1755-0998.13088.

Gauthier M, Launay B, Goff GL et al. 2020b. Fragmentation promotes the role of dispersal in determining 10 intermittent headwater stream metacommunities. Freshwater Biology 65:2169–2185. DOI: 10.1111/fwb.13611.

Gounand I, Harvey E, Little CJ, Altermatt F. 2018. Meta-ecosystems 2.0: Rooting the theory into the field. Trends in Ecology & Evolution 33:36–46. DOI: 10.1016/j.tree.2017.10.006.

Guerold F. 2000. Influence of taxonomic determination level on several community indices. Water Research 34:487–492. DOI: 10.1016/S0043-1354(99)00165-7.

Gusev O, Suetsugu Y, Cornette R et al. 2014. Comparative genome sequencing reveals genomic signature of extreme desiccation tolerance in the anhydrobiotic midge. Nature Communications 5:4784. DOI: 10.1038/ncomms5784.

Hajibabaei M, Shokralla S, Zhou X et al. 2011. Environmental barcoding: A next-generation sequencing approach for biomonitoring applications using river benthos. PLoS One 6:e17497. DOI: 10.1371/journal.pone.0017497.

Heino J, Alahuhta J, Ala-Hulkko T et al. 2017. Integrating dispersal proxies in ecological and environmental research in the freshwater realm. Environmental Reviews 25:334–349. DOI: 10.1139/er-2016-0110.

Heino J, Soininen J. 2007. Are higher taxa adequate surrogates for species-level assemblage patterns and species richness in stream organisms? Biological Conservation 137:78–89. DOI: 10.1016/j.biocon.2007.01.017.

Hijmans RJ, van EJ, Sumner M et al. 2020. Raster: Geographic data analysis and modeling. Version 3.3-13. Available: https://CRAN.R-project.org/package=raster.

Hill MJ, Heino J, White JC et al. 2019. Environmental factors are primary determinants of different facets of pond macroinvertebrate alpha and beta diversity in a human-modified landscape. Biological Conservation 237:348–357. DOI: 10.1016/j.biocon.2019.07.015.

Hughes JM, Mather PB, Hillyer MJ et al. 2003. Genetic structure in a montane mayfly *Baetis bicaudatus* (Ephemeroptera: Baetidae), from the Rocky Mountains, Colorado. Freshwater Biology 48:2149–2162. DOI: 10.1046/j.1365-2427.2003.01150.x.

Jackson JK, Battle JM, White BP et al. 2014. Cryptic biodiversity in streams: A comparison of macroinvertebrate communities based on morphological and DNA barcode identifications. Freshwater Science 33:312–324. DOI: 10.1086/675225.

Jones FC. 2008. Taxonomic sufficiency: The influence of taxonomic resolution on freshwater bioassessments using benthic macroinvertebrates. Environmental Reviews 16:45–69. DOI: 10.1139/A07-010.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30:3059–3066. DOI: 10.1093/nar/gkf436.

Kembel SW, Cowan PD, Helmus MR et al. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463–1464. DOI: 10.1093/bioinformatics/btq166.

Kuntke F, de Jonge N, Hesselsøe M, Lund Nielsen J. 2020. Stream water quality assessment by metabarcoding of invertebrates. Ecological Indicators 111:105982. DOI: 10.1016/j.ecolind.2019.105982.

Laini A, Beermann AJ, Bolpagni R et al. 2020. Exploring the potential of metabarcoding to disentangle macroinvertebrate community dynamics in intermittent streams. Metabarcoding and Metagenomics 4:e51433. DOI: 10.3897/mbmg.4.51433.

Laini A, Guareschi S, Bolpagni R, Burgazzi G, Bruno D, Gutiérrez-Cánovas C, Miranda R, Mondy C, Várbíró G, Cancellario T. 2022. biomonitoR: An R package for managing ecological data and calculating biomonitoring indices. PeerJ 10:e14183. DOI: 10.7717/peerj.14183.

Leibold MA, Chase JM. 2018. Metacommunity ecology. Princeton, NJ: Princeton University Press.

Lenth R, Buerkner P, Herve M et al. 2020 emmeans: Estimated marginal means, aka least-squares means. Version 1.5.0. Available: https://CRAN.R-project.org/package=emmeans.

Li Z, Heino J, Liu Z et al. 2021. The drivers of multiple dimensions of stream macroinvertebrate beta diversity across a large montane landscape. Limnology and Oceanography 66:226–236. DOI: 10.1002/lno.11599.

Li Z, Xing Y, Liu Z et al. 2020. Seasonal changes in metacommunity assembly mechanisms of benthic macroinvertebrates in a subtropical river basin. Science of the Total Environment 729:139046. DOI: 10.1016/j.scitotenv.2020.139046.

López-Rodríguez MJ, de Figueroa JMT. 2006. Life cycle and nymphal feeding of *Rhabdiopteryx christinae* Theischinger 1975 (Plecoptera: Taeniopterygidae). Annales de la Société entomologique de France (N.S.) 42(1):57–61. DOI:10.1080/00379271.2006.10697449.

Macher JN, Salis RK, Blakemore KS et al. 2016. Multiple-stressor effects on stream invertebrates: DNA barcoding reveals contrasting responses of cryptic mayfly species. Ecological Indicators 61:159–169. DOI: 10.1016/j.ecolind.2015.08.024.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12. DOI: 10.14806/ej.17.1.200.

Martin GK, Adamowicz SJ, Cottenie K, 2016. Taxonomic resolution based on DNA barcoding affects environmental signal in metacommunity structure. Freshwater Science 35(2):701–711. DOI: 10.1086/686260.

Matsen FA, Kodner RB, Armbrust EV. 2010. pplacer: Linear time maximum-likelihood and Bayesian phylogenetic placement of sequences onto a fixed reference tree. BMC Bioinformatics 11:538. DOI: 10.1186/1471-2105-11-538.

Meredith CS, Trebitz AS, Hoffman JC. 2019. Resolving taxonomic ambiguities: Effects on rarity, projected richness, and indices in macroinvertebrate datasets. Ecological Indicators 98:137–148. DOI: 10.1016/j.ecolind.2018.10.047.

Meyer A, Boyer F, Valentini A et al. 2021. Morphological vs. DNA metabarcoding approaches for the evaluation of stream ecological status with benthic invertebrates: Testing different combinations of markers and strategies of data filtering. Molecular Ecology 30:3203–3220. DOI: 10.1111/mec.15723.

Milošević D, Simić V, Stojković M et al. 2013. Spatio-temporal pattern of the Chironomidae community: Toward the use of non-biting midges in bioassessment programs. Aquatic Ecology 47:37–55. DOI: 10.1007/s10452-012-9423-y.

Oksanen J, Blanchet FG, Friendly M et al. 2019. Vegan: Community ecology package. Version 2.5-4. Available: https://CRAN.R-project.org/package=vegan.

Paradis E, Schliep K. 2019. ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526–528. DOI: 10.1093/bioinformatics/bty633.

Pavoine S, Vallet J, Dufour A-B et al. 2009. On the challenge of treating various types of variables: Application for improving the measurement of functional diversity. Oikos 118:391–402. DOI: 10.1111/j.1600-0706.2008.16668.x.

Petchey OL, Gaston KJ. 2002. Functional diversity (FD), species richness and community composition. Ecology Letters 5:402–411. DOI: 10.1046/j.1461-0248.2002.00339.x.

Pinheiro J, Bates D, DebRoy S et al. 2020. nlme: Linear and nonlinear mixed effects models. Version 3.1-149. Available: https://CRAN.R-project.org/package=nlme.

Polášek M, Godunko RJ, Rutschmann S et al. 2018. Integrative taxonomy of genus Electrogena (Ephemeroptera: Heptageniidae): The role of innovative morphological analyses for species delimitation. Arthropod Systematics & Phylogeny 76:449–462.

Quast C, Pruesse E, Yilmaz P et al. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Research 41:D590–D596. DOI: 10.1093/nar/gks1219.

R Core Team. 2020. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Robinson CT, Schuwirth N, Baumgartner S, Stamm C. 2014. Spatial relationships between land-use, habitat, water quality and lotic macroinvertebrates in two Swiss catchments. Aquatic Sciences 76:375–392. DOI: 10.1007/s00027-014-0341-z.

Rognes T, Flouri T, Nichols B et al. 2016. VSEARCH: A versatile open source tool for metagenomics. PeerJ 4: e2584. DOI: 10.7717/peerj.2584.

Rosser N. 2017. Shortcuts in biodiversity research: What determines the performance of higher taxa as surrogates for species? Ecology and Evolution 7:2595–2603. DOI: 10.1002/ece3.2736.

Sarremejane R, Cid N, Stubbington R et al. 2020a. DISPERSE, a trait database to assess the dispersal potential of European aquatic macroinvertebrates. Scientific Data 7:386. DOI: 10.1038/s41597-020-00732-7.

Sarremejane R, England J, Sefton CE et al. 2020b. Local and regional drivers influence how aquatic community diversity, resistance and resilience vary in response to drying. Oikos 129:1877–1890. DOI: 10.1111/oik.07645.

Schmera D, Heino J, Podani J et al. 2017. Functional diversity: A review of methodology and current knowledge in freshwater macroinvertebrate research. Hydrobiologia 787:27–44. DOI: 10.1007/s10750-016-2974-5.

Schmidt-Kloiber A, Hering D. 2015. www.freshwaterecology.info – An online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. Ecological Indicators 53:271–282. DOI: 10.1016/j.ecolind.2015.02.007.

Serra SR, Cobo F, Graça MA et al. 2016. Synthesising the trait information of European Chironomidae (Insecta: Diptera): Towards a new database. Ecological Indicators 61:282–292. DOI: 10.1016/j.ecolind.2015.09.028.

Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. DOI: 10.1093/bioinformatics/btu033.

Sturmbauer C, Opadiya GB, Niederstätter H et al. 1999. Mitochondrial DNA reveals cryptic oligochaete species differing in cadmium resistance. Molecular Biology and Evolution 16:967–974. DOI: 10.1093/oxfordjournals.molbev.a026186.

Sunnucks P, Hales DF. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). Molecular Biology and Evolution 13:510–524. DOI: 10.1093/oxfordjournals.molbev.a025612.

Taberlet P, Coissac E, Pompanon F et al. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular Ecology 21:2045–2050. DOI: 10.1111/j.1365-294X.2012.05470.x.

Tachet H, Richoux P, Bournaud M, Usseglio-Polatera P. 2010. Invertébrés d'eau douce: Systématique, biologie, écologie. Paris: CNRS Éditions.

Tenchini R, Cardoni S, Piredda R, Simeone MC, Belfiore C. 2018. DNA barcoding and faunistic criteria for a revised taxonomy of Italian Ephemeroptera. The European Zoological Journal 85:253–266. DOI: 10.1080/24750263.2018.1480732.

Tesson SV, Edelaar P. 2013. Dispersal in a changing world: Opportunities, insights and challenges. Movement Ecology 1:10. DOI: 10.1186/2051-3933-1-10.

Tierno De Figueroa JM, López-Rodríguez MJ. 2019. Trophic ecology of Plecoptera (Insecta): A review. The European Zoological Journal 86:79–102. DOI: 10.1080/24750263.2019.1592251.

Tonkin JD, Altermatt F, Finn DS et al. 2018. The role of dispersal in river network metacommunities: Patterns, processes, and pathways. Freshwater Biology 63:141–163. DOI: 10.1111/fwb.13037.

Tonkin JD, Heino J, Sundermann A et al. 2016. Context dependency in biodiversity patterns of central German stream metacommunities. Freshwater Biology 61:607–620. DOI: 10.1111/fwb.12728.

Tucker CM, Cadotte MW. 2013. Unifying measures of biodiversity: Understanding when richness and phylogenetic diversity should be congruent. Diversity and Distributions 19:845–854. DOI: 10.1111/ddi.12087.

Tucker CM, Cadotte MW, Davies TJ, Rebelo TG. 2012. Incorporating geographical and evolutionary rarity into conservation prioritization. Conservation Biology 26:593–601. DOI: 10.1111/j.1523-1739.2012.01845.x.

Turon X, Antich A, Palacín C et al. 2020. From metabarcoding to metaphylogeography: Separating the wheat from the chaff. Ecological Applications 30:e02036. DOI: 10.1002/eap.2036.

Ulrich W, Gotelli NJ. 2007. Null model analysis of species nestedness patterns. Ecology 88:1824–1831. DOI: 10.1890/06-1208.1.

Vamosi SM, Heard SB, Vamosi JC, Webb CO. 2009. Emerging patterns in the comparative analysis of phylogenetic community structure. Molecular Ecology 18:572–592. DOI: 10.1111/j.1365-294X.2008.04001.x.

van Etten J. 2017. R package gdistance: Distances and routes on geographical grids. Journal of Statistical Software 76:1–21. DOI: 10.18637/jss.v076.i13.

Viana DS, Chase JM. 2019. Spatial scale modulates the inference of metacommunity assembly processes. Ecology 100:e02576. DOI: 10.1002/ecy.2576.

Vilmi A, Karjalainen SM, Nokela T et al. 2016. Unravelling the drivers of aquatic communities using disparate organismal groups and different taxonomic levels. Ecological Indicators 60:108–118. DOI: 10.1016/j.ecolind.2015.06.023.

Vuataz L, Rutschmann S, Monaghan MT et al. 2016. Molecular phylogeny and timing of diversification in Alpine Rhithrogena (Ephemeroptera: Heptageniidae). BMC Evolutionary Biology 16:1–16. DOI: 10.1186/s12862-016-0758-1.

Waringer J, Graf W, Malicky H. 2013. Problems associated with extrapolating ecological traits to higher-than-species level exemplified in the description of the larvae of *Potamophylax haidukorum* Malicky, 1999, *Potamophylax winneguthi* (Klapálek, 1902) and *Melampophylax austriacus* Malicky, 1990. Limnologica 43:441–450. DOI: 10.1016/j.limno.2013.02.001.

Welch JJ, Bininda-Emonds OR, Bromham L. 2008. Correlates of substitution rate variation in mammalian protein-coding sequences. BMC Evolutionary Biology 8:53. DOI: 10.1186/1471-2148-8-53.

Wickham H. 2009. ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag.

Wickham H. 2020. tidyr: Tidy messy data. Version 1.1.2. Available: https://CRAN.R-project.org/package=tidyr.

Wickham H, François R, Henry L et al. 2020. dplyr: A grammar of data manipulation. Version 1.0.2. Available: https://CRAN.R-project.org/package=dplyr.

Wiens JJ, Graham CH. 2005. Niche conservatism: Integrating evolution, ecology, and conservation biology. Annual Review of Ecology, Evolution, and Systematics 36:519–539. DOI: 10.1146/annurev.ecolsys.36.102803.095431.

Winter DJ. 2017. rentrez: An R package for the NCBI eUtils API. The R Journal 9:520–526. DOI: 10.32614/RJ-2017-058.

Yu DW, Ji Y, Emerson BC et al. 2012. Biodiversity soup: Metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. Methods in Ecology and Evolution 3:613–623. DOI: 10.1111/j.2041-210X.2012.00198.x.

Zickovich JM, Bohonak AJ. 2007. Dispersal ability and genetic structure in aquatic invertebrates: A comparative study in southern California streams and reservoirs. Freshwater Biology 52:1982–1996. DOI: 10.1111/j.1365-2427.2007.01822.x.

Zimmermann J, Glöckner G, Jahn R et al. 2015. Metabarcoding vs. morphological identification to assess diatom diversity in environmental studies. Molecular Ecology Resources 15:526–542. DOI: 10.1111/1755-0998.12336.

Zizka VMA, Elbrecht V, Macher JN, Leese F. 2019. Assessing the influence of sample tagging and library preparation on DNA metabarcoding. Molecular Ecology Resources 19 (4):893–899. DOI:10.1111/1755-0998.13018.

Zizka VMA, Weiss M, Leese F. 2020. Can metabarcoding resolve intraspecific genetic diversity changes to environmental stressors? A test case using river macrozoobenthos. Metabarcoding and Metagenomics 4:e51925. DOI: 10.3897/mbmg.4.51925.