# 1  Assessing different components of biodiversity across a river

# 2  network using eDNA

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## 19  **Abstract**

20  1.  Assessing individual components of biodiversity, such as local or regional taxon richness, and

21      differences in community composition is a long-standing challenge in ecology. It is especially

22      relevant in spatially structured and diverse ecosystems. Environmental DNA (eDNA) has been

23      suggested as a novel technique to accurately measure biodiversity. However, we do not yet

24      fully understand the comparability of eDNA-based assessments to previously used approaches.

25  2.  We sampled may-, stone-, and caddisfly genera with contemporary eDNA and kicknet methods

26      at 61 sites distributed over a large river network, allowing a comparison of various diversity

27      measures from the catchment to site levels and providing insights into how these measures

28      relate to network properties. We extended our survey data with historical records of total

29      diversity at the catchment level.

30  3.  At the catchment scale, eDNA and kicknet detected similar proportions of the overall and

31      cumulative historically documented species richness (gamma diversity), namely 42% and 46%,

32      respectively. We further found a good overlap (62%) between the two contemporary methods at

33      the regional scale.

34  4.  At the local scale, we found highly congruent values of local taxon richness (alpha diversity)

35      between eDNA and kicknet. Richness of eDNA was positively related with discharge, a

36      descriptor of network position, while kicknet was not.

37  5.  Beta diversity between sites was similar for the two contemporary methods. Contrary to our

38      expectation, however, beta diversity was driven by species replacement and not by nestedness.

39  6.  Although optimization of eDNA approaches is still needed, our results indicate that this novel

40      technique can capture extensive aspects of gamma diversity, proving its potential utility as a

41      new tool for large sampling campaigns across hitherto understudied complete river catchments,

42    requiring less time and becoming more cost-efficient than classical approaches. Overall, the

43    richness estimated with the two contemporary methods is similar at both local and regional

44    scale but community composition is differently assessed with the two methods at individual

45    sites and becomes more similar with higher discharge.

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## Keywords (eight words or short phrases)

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## Introduction

51

52    Quantifying biodiversity accurately is a long-standing challenge of primary importance in ecology

53    (Dornelas et al., 2013, Gotelli and Colwell, 2001, Whittaker, 1972). On the one hand, there is a

54    fundamental interest to understand the distribution of diversity in time and space and the mechanistic

55    drivers from local to regional scales (e.g., Gaston, 2000, Gotelli and Colwell, 2011, Koleff et al., 2003).

56    On the other hand, the state of ecosystems is inherently linked to biodiversity (Pennekamp et al., 2018)

57    and the current loss of biodiversity has potentially large negative consequences on the functions and

58    services of ecosystems (Chapin et al., 2000, Isbell et al., 2017). This is especially relevant for freshwater

59    habitats because they provide crucial ecosystem services such as drinking water, food security, or

60    recreational value to humanity (Cardinale et al., 2012, Dudgeon et al., 2006, Postel and Carpenter, 1997).

61    In river systems, may-, stone-, and caddisflies (Ephemeroptera, Plecoptera, and Trichoptera;

62    thereafter abbreviated as EPT) are often used as indicators due to their sensitivity to environmental

63    change, their different preferences for ecological niches, and their relatively well-known taxonomy

64    (Schmidt-Kloiber and Hering, 2015). Presence or absence of certain EPT taxa or their overall richness is

65    highly informative and can be tightly linked to habitat quality. Importantly, they describe not only the

66    current state of a water body but also integrate its changes over time. Thus, EPT are at the heart of many

67    freshwater quality assessments around the world and are included in many regulatory frameworks, such as

68    the Water Framework Directive (Directive 2000/60/EC, but see also Borja et al., 2009), or the Canadian

69    Aquatic Biomonitoring Network (CABIN, Reynoldson et al., 2003).

70          Classically, EPT are collected with a standardized kicknet method (Barbour et al., 1999). Taxa are

71    then identified under a dissecting microscope, which is time-consuming and therefore costly. Taxon

72    richness is the most fundamental approach to estimate biodiversity and is still widely used. Even though

73    the number of taxa is a convincingly intuitive proxy of biodiversity and the basis of many fundamental

74    concepts in ecology, it is a difficult variable to measure accurately (Gotelli and Colwell, 2011, Purvis and

75    Hector, 2000). Diversity can be further divided into different components, such as local richness, regional

76    richness and between-site dissimilarity (also known as alpha, gamma, and beta diversity). The emerging

77    technique of environmental DNA (eDNA) metabarcoding is expected to become a complementary or even

78    replacement method (for example see Baird and Hajibabaei, 2012, Deiner et al., 2017, Lawson Handley,

79    2015). About a decade ago, the first study was published on the detection of species through DNA in an

80    environmental sample (Ficetola et al., 2008). With the implementation of high throughput sequencing,

81    which allowed not only detection of single species but whole communities, eDNA metabarcoding has

82    been proposed to revolutionize biodiversity assessments (Bohmann et al., 2014, Lawson Handley, 2015,

83    Shokralla et al., 2012). As with every novel technique, eDNA metabarcoding creates new opportunities

84    but also challenges, especially in terms of recognizing what information it provides and how it compares

85    to previously implemented and established methodologies.

86          Comparisons between eDNA and traditional methods have hitherto mostly focused on either local

87    or regional richness comparisons. In river systems, previous studies have generally detected higher taxon

88    richness with eDNA than kicknet approaches (Civade et al., 2016, Olds et al., 2016, Valentini et al.,

89    2016). Those results were likely due to eDNA at one location integrating taxon information from upstream

90    reaches via downstream transportation of eDNA (Deiner and Altermatt, 2014, Deiner et al., 2016, Pont et

91   al., 2018). This suggests that standard techniques represent a more accurate local estimate while eDNA

92   integrates information across space. In that context, any comparison of the two techniques will be

93   influenced by the scale of the study. However, we still do not know if findings are directly comparable,

94   complementary, or different across different spatial scales and across different components of

95   biodiversity, due to the specific properties of kicknet and eDNA sampling. A detailed understanding is

96   needed to make decisions on how to sample biodiversity in complex landscapes and to compare both local

97   and regional measures. This is particularly relevant in river landscapes, where the typical underlying

98   dendritic network structure is known to affect biodiversity (Altermatt, 2013, Altermatt et al., 2013, Carrara

99   et al., 2012, Harvey et al., 2018, Tonkin et al., 2018).

100   In our study, we compared different measures of biodiversity of EPT sampled classically (i.e., by

101   kicknet) or by eDNA, using a spatially structured approach that representatively covered a river network

102   in a 740 km$^2$ catchment. We analyzed how these two different approaches capture the facets of

103   biodiversity at the level of alpha (local site), beta (between sites), and gamma diversity (catchment level).

104   Gamma diversity information was supplemented with all historically available data. Given that taxon

105   richness is among the most-commonly studied biodiversity variables, we discuss the design of biodiversity

106   monitoring with eDNA in dendritic river networks.

107

## Material & Methods

109   We studied a river network in a 740 km$^2$ catchment containing 61 sampling sites in the upper river

110   Thur in north-eastern Switzerland (Fig.1, Table S1). The catchment comprises three main river-stems:

111   Thur, Glatt, and Necker, the latter two draining into the Thur. In a sampling campaign conducted from

112   June 11 to June 22 2016, we collected eDNA and benthic invertebrate kicknet samples, with the two

113   sampling methods performed at each site within a two-day window. To characterize the position of sites in

114   the network, we extracted stream order, catchment area and the mean annual discharge data for each site

115   from existing databases (BAFU, 2013, BAFU, 2014, Faundler et al., 2013).

116    *Historical data*

117         We obtained long-term biodiversity data on gamma diversity in our catchment from the Centre

118    Suisse de la Cartographie de la Faune (CSCF). These data include all EPT species ever recorded in the

119    whole catchment over the time-period 1981–2016. The data are of various sampling origins, but of high

120    quality, thus giving a highly robust and reliable cumulative estimate of EPT genus richness (and the

121    respective EPT genus identity) at the whole catchment scale. The cumulative data consist of 3,467

122    individual records based on observations of the species in an area of the catchment, which we then

123    converted into genus richness.

124    *Contemporary kicknet data*

125         We collected benthic macroinvertebrates based on three-minute kicknet sampling applied to three

126    microhabitats present at a given site (Barbour et al., 1999). Leaves and debris were removed from the

127    sample and the remaining material was pooled and stored in 96% molecular grade ethanol. In the lab, all

128    EPT individuals were identified with a microscope to species level. A few taxa, only present as early

129    instar larva or containing cryptic species, were grouped in pre-defined complexes, subsequently treated at

130    the genus. We could not assess EPT taxa at one site due to the loss of a sample.

131    *eDNA filtration in the field*

132         At each site, we sampled three times 250 mL of river water, each on a separate filter (for detailed

133    description see Mächler et al., 2018). We collected eDNA samples about 5–10 meters upstream of the

134    kicknet sampling to minimize cross-contamination. Samples were stored in a Styrofoam box equipped

135    with cooling elements until we came back from the field (no longer than 9 h). Thereafter, they were stored

136    at -20 °C until further processing. On each field day, we performed a replicated filter control (FC) that was

137    filtered in the field before any sampling site was visited in order to check if the reusable material was

138    clean. In total, we generated 11 filter controls, each consisting of three replicates. Operational taxonomic

139    units found in at least two replicates of a filter control from the same date were removed from all samples

140    for the further analysis. Further information on filtration, eDNA facilities, and material preparation can be

141    found in the supplementary file.

142    *Extraction and library preparation*

143         Detailed information about the extraction and library preparation can be found in the

144    supplementary file. In short, we used the DNeasy Blood and Tissue kit (Qiagen GmbH, Hilden, Germany)

145    to extract the DNA from eDNA samples, also including extraction controls (EC). We used an Illumina

146    MiSeq dual-barcoded two-step PCR amplicon sequencing protocol. The first PCR was performed with

147    modified primers that contained an adaptor-specific tail, a heterogeneity spacer, and the amplicon target

148    site (see Table S2). On each of the PCR plates, we implemented one negative (NC) and one positive PCR

149    control (PC). The negative control consisted of 5 μL sigma water and the positive control consisted of 4

150    μL sample and 1 μL (0.01 ng/μL) artificial dummy DNA (a randomly generated double-stranded DNA

151    sequence that was 313 bp in length and matched primer region, see supplementary information). We then

152    pooled the three PCR replicates per sample and cleaned it with SPRI beads. For the second PCR, we used

153    the Nextera XT Index kit v2 (Illumina California, USA) to index each sample and cleaned afterwards the

154    index reaction with SPRI beads. We quantified each sample with the Spark 10M Multimode Microplate

155    Reader (Tecan Group Ltd., Männedorf, Switzerland) and pooled them in equimolar parts into a final pool

156    that we cleaned with SPRI beads. All controls (FC, EC, PC, NC) were run alongside the samples and were

157    pooled according to their concentrations. Controls that were too low to quantify were pooled into the

158    second lowest concentrated pool with 10 μL, equal to the volume of the lowest sample in the respective

159    pool. The libraries were added at 16 pM concentration and PhiX control was added at a 10%

160    concentration. A paired-end (2x300 nt) sequencing was performed on an Illumina MiSeq (MiSeq Reagent

161    kit v3, 300 cycles) following the manufacture's run protocols (Illumina, California, USA). To increase the

162    sequencing depth, a second run of the same pooled libraries was conducted.

163

164    *Bioinformatic data processing*

165        After the two successful Illumina MiSeq runs, the data was demultiplexed and the quality of the

166     reads was checked with FastQC (Andrews, 2010). Raw reads were end-trimmed (usearch v10.0.240,

167     R1:30nt, R2:50nt) and merged with an overlap of min 15 bp max 300 bp (Flash, v1.2.11). Next, the primer

168     sites were removed (full length, no mismatch allowed (cutadapt v1.12)) and thereafter, the data was

169     quality filtered (prinseq-lite v0.20.4) using the following parameters: size range (100–500), GC range (30–

170     70), mean quality (20), and low complexity filter dust (30). In a next step, UNOISE3 (usearch v10.0.240)

171     was used to determine amplicon sequence variants (zero-radius OTUs, thereafter called ZOTUs).

172     UNOISE3 has a build-in error-correction to reduce the influence of sequencing errors (Edgar, 2016). An

173     additional clustering at 99% sequence identity was performed to reduce sequence diversity and to account

174     for possible amplification errors in the first PCR. The resulting ZOTUs (zero-radius OTUs, thereafter

175     called ZOTUs) were checked for stop codons using the invertebrate mitochondrial code, to ensure an

176     intact open reading frame. This resulted in 27 M reads corresponding to 11,313 ZOTUS (Table S3). As a

177     final step, the ZOTUs were assigned to taxa (blast 2.3.0 and usearch v10.0.240, tax filter = 0.9).

178     *Statistics*

179        Our analysis was based on the following strategy: First, we confirmed that the two Illumina runs

180     could be combined and second we cleaned the sequencing data and selected only ZOTUs that were

181     assigned to an EPT order (see detailed information in the supplementary file). For individual sites,

182     ZOTUS were only counted if they were present in at least two of the three independent replicates (Fig.

183     S1), which is a highly stringent assumption and conservative with respect to detection of taxa. Thereafter,

184     we were able to analyze various diversity measures to identify similarities and differences between eDNA

185     and kicknet sampling approaches: (i) gamma, (ii) alpha, (iii) beta diversity, and (iv) between method

186     community dissimilarity. All statistical analyses were performed using R (R Core Team, version 3.4.4).

187     Gamma diversity

188        We used the R package 'venneuler' (Wilkinson and Urbanek, 2011) to draw Venn diagrams for

189      gamma richness of historic, eDNA and kicknet data. We used the R package 'vegan' (Oksanen et al.,

190      2011) to calculate taxa accumulation curves of the two methods over the whole catchment.

191      Alpha diversity

192        To compare diversity estimates delivered by the two methods, we used the R packages 'phyloseq'

193      (McMurdie and Holmes, 2013, version 1.22.3) to calculate richness. With a Pearson's correlation test we

194      identified if richness measures at sampling sites correlate between the two contemporary methods. We

195      then tested if genus richness of the two methods increased with discharge, using a linear model.

196      Beta diversity

197        We used Sørensen dissimilarity as a measure of beta-diversity, based on presence/absence data.

198      With the R package 'betapart' (Baselga et al., 2017) we calculated Sørensen dissimilarity, which can be

199      further partitioned into nestedness and turnover components, allowing us to distinguish dissimilarity

200      arising through embedment (i.e. loss) or replacement of species (Baselga, 2010, Baselga et al., 2017). To

201      observe how beta diversity measures relate to stream distance, we extracted distances between sites and

202      nodes from GIS data (swisstopo) and added these as edge weights. We then constructed the adjacency

203      matrix representing our fluvial network consisting of edges and vertices with the R package 'igraph'

204      (Csardi and Nepusz, 2006, version 1.2.1) to calculate stream distance between flow-connected sampling

205      points. We used linear models to test if distance and the difference in stream order of the compared sites

206      explain patterns in the beta diversity measures. We compared models including a null model, models

207      containing only one of the explanatory variables, or both variables (with and without interaction), and

208      performed model averaging in order to calculate relative variable importance (R package 'MuMIn',

209      Barton, 2009, version 1.40.4). We additionally calculated Sørensen dissimilarity and checked for

210      differences among stream orders. First, we tested for heterogeneous variance with a Bartlett test, and if it

211      was significant, we performed a Kruskal-Wallis test to identify whether there was at least one difference

212   in means. If this was true, then we followed a multiple mean comparison post-hoc test on rank sums (R

213   package 'pgirmess', Giraudoux, 2018, version 1.6.9).

214   Between-method community dissimilarity

215       We also calculated Sørensen dissimilarity and its two components, nestedness and turnover,

216   between eDNA and kicknet samples for each individual site to detect discrepancies in community

217   composition between the two contemporary methods.

218

# Results

220   *Gamma diversity*

221       At the regional scale (i.e., the whole catchment level), 96 different EPT genera were historically

222   documented. We found 47 EPT genera with our kicknet samples and 42 genera with our eDNA samples,

223   reflecting 46% and 42% of the historically established taxa richness, respectively (Fig. 2, Table S4). A

224   high proportion of these sampled taxa were already present in the historic records (94% and 95%

225   respectively). Thirty-six of these EPT genera were detected with both the kicknet and the eDNA method,

226   reflecting a 62% overlap of the two methods. Both methods also detected a similar additional proportion

227   of taxa found by one method only, but present in the historic dataset (Fig. 2). Finally, we found four

228   genera with eDNA, kicknet sampling, or both approaches that were not previously listed in the historic

229   data. All of these four genera occur at the border of our studied catchment and rare, single appearances

230   within the catchment are possible. Separate taxon accumulation curves (accumulating gamma richness

231   with number of sites included) for the kicknet and the eDNA methods were qualitatively similar (Fig.

232   S2A), based on visual comparison of the curves and their 95% confidence band. This pattern remained

233   similar even if sites were accumulated in the order from the most downstream to the most upstream sites

234   (Fig. S2B).

235    *Alpha diversity*

236          At the local scale, the two contemporary methods revealed similar genus richness (eDNA $M =$

237    10.22, $SD = 4.2$; kicknet $M = 10.47$, $SD = 3.1$), and the detected richness values were positively correlated

238    ($r(58) = 0.42$, $P < 0.001$; Fig. 3). As eDNA is transported through the river network, we expected a

239    positive dependency of the detected richness of eDNA on increasing discharge (i.e., in more downstream

240    sites), which we do not expect for kicknet samples. Indeed, we found a positive relationship between

241    genus richness and discharge for eDNA ($\beta = 0.537$, $t(58) = 2.848$, $p = 0.006$) but not for kicknet ($\beta =$

242    0.073, $t(58) = 0.495$, $p = 0.62$), however, the adjusted $R^2$ was relatively low (eDNA $R^2 = 0.101$, Fig. 4).

243

244    *Beta diversity*

245          Overall, we found comparable Sørensen dissimilarity between flow-connected sites for eDNA

246    and kicknet. For both methods, the turnover component contributed more to the dissimilarity than

247    nestedness-related components (Fig. 5, see Table S5 for information on detected genera per site), which

248    was more pronounced for eDNA than kicknet. The analysis for the relative importance of variables

249    showed that differences in stream order were generally more important for all beta diversity measures,

250    however, for turnover of both eDNA and kicknet, pairwise distance between sites showed only partially

251    lower importance compared to the differences in stream order (Table S6). Both methods showed

252    significant differences in mean Sørensen dissimilarity among stream orders, and we found significant

253    group differences for the post-hoc mean comparisons in stream orders (Fig. S3, Table S7).

254

255    *Between-method community dissimilarity*

256          We found an intermediate discrepancy in the community compositions described by the two

257    contemporary methods ($M = 0.48$, $SD = 0.14$, Fig. S4), and partitioning this difference into nestedness and

258    turnover components indicates that turnover contributes more to the differences of detected EPT genera.

259

260    # Discussion

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261     We compared different measures of biodiversity of EPT sampled by kicknet versus by eDNA, using

262     a spatially structured approach and covering a major river network. Using a unique historically assembled

263     overview of cumulative, "true" gamma diversity within the study region, we were able to put our

264     contemporary samples in a historic context. We found a quantitatively similar overlap between each

265     contemporary approach and historic gamma diversity, in accordance with other studies comparing eDNA

266     with long-term data in freshwater systems (e.g., Hänfling et al., 2016, Valentini et al., 2016). Given that

267     the historic data set covers several decades (1981–2016), we do not expect that all taxa are still present in

268     the catchment at the time of this study (2016) due to changes in distribution or local extinctions, and we

269     would also expect some new taxa to appear. Thus, the observed 42% overlap between a single snapshot

270     sampling campaign and the gamma diversity obtained by cumulative sampling efforts over almost four

271     decades is relatively high. Surprisingly, taxon accumulation curves for eDNA and kicknet sampling were

272     not significantly different, although we expected that downstream transport of DNA would contribute to a

273     faster increase and saturation of genera for eDNA compared to kicknet sampling. Our approach suggests

274     that a single eDNA sampling campaign may cover large parts of historically detected gamma diversity.

275     This indicates that eDNA could be used as a new tool for rapid network-level richness analyses and

276     biodiversity assessments. Such systematic BioBlitz sampling campaigns (Lundmark, 2003, Laforest et al.,

277     2013) across hitherto understudied complete river catchments may be a promising avenue, since

278     minimally trained people without any taxonomic expertise can collect great parts of regional or even

279     landscape richness in a rapid time frame with this method.

280     We also found a reasonable congruency in local alpha diversity of the two contemporary methods

281     and identified a new dependency of eDNA estimates on discharge level. Overall, local richness estimates

282     of eDNA and kicknet sampling are highly comparable. This comparability may be strengthened by our

283     highly stringent inclusion criteria for eDNA estimates which are more conservative than those used in

284     previous studies that detected higher richness with eDNA compared to traditional methods (e.g., Deiner et

285     al., 2016, Valentini et al., 2016). In addition, we used a barcoding primer targeting a broad taxonomic

286     range, which may also have a lower detection rate for specific taxonomic groups. In near future, the

287    forthcoming design and use of EPT specific primers or the completion of EPT sequence references should

288    reduce the current drawbacks of eDNA methods regarding taxonomic identification. For eDNA we found

289    a positive relationship between richness and discharge, which was not the case for kicknet data and could

290    be attributed to eDNA integrating biodiversity detection across space due to downstream transport (Deiner

291    et al., 2016, Li et al., 2018, Pont et al., 2018). Alternatively, richness indeed increased with discharge (i.e.

292    downstream), but the slightly sub-optimal sampling period (summer) and the simplified kicknet protocol

293    we used was an inappropriate technique for sampling larger streams and thus obscured the relationship.

294    Both aspects reduce detection of genera with the traditional methods, as some species might be missed due

295    to reduced sampling effort or immature larval stages hampering identification. Surprisingly, the $7^{th}$ stream

296    order sites diverge from this pattern, potentially because we could only access part of these wide river

297    cross-sections, and sampling was restricted to the river edge where less mixing occurs, where more

298    extensive sampling may be needed with eDNA (Bylemans et al., 2018). Also, recent studies indicate that

299    eDNA is not evenly distributed in the water column (Macher and Leese, 2017), and it is still unclear how

300    spatial variance in structures, such as riffles and ponds, are affecting the mixing of the water column and

301    thus the equal detection of eDNA along the water column. We speculate that only in smaller streams ($1^{st}$

302    to $5^{th}$ order) one or two samples from the edge or in the center adequately reflect the eDNA distribution

303    across the river transect, while in larger rivers multiple samples across the cross-section may be

304    recommended.

305        We found that pairwise beta diversity (Sørensen dissimilarity) at the local scale was similarly

306    assessed by the two methods, which is congruent to findings by Li et al. (2018). Despite differences

307    among sites in Sørensen dissimilarity, the turnover component (i.e., species replacement) contributes more

308    to the dissimilarity than nestedness for both methods but is even more pronounced for eDNA than kicknet.

309    Turnover indicates that species are replaced between the sites and implies that transportation of eDNA is

310    not the main mechanisms driving differences, otherwise nestedness would be expected to be stronger.

311    Barnes & Turner (2016) presented many processes (e.g., degradation, re-suspension, or fragmentation)

312     that influence eDNA in the environment and therefore challenge our mechanistic understanding. Thus, it

313     remains unclear whether the detected differences stem from ecological or methodological variation.

314          Studies on diversity patterns have long focused on a linear view of streams. But it is now

315     increasingly acknowledged that the underlying network structure plays a significant role in shaping

316     species distributions (Carrara et al., 2012, Harvey and Altermatt, 2019, Harvey et al., 2018, Holyoak et al.,

317     2005, Seymour et al., 2015, Tonkin et al., 2018). While experimental lab studies and field surveys have

318     found general patterns of diversity distribution in river landscapes, we do not know if eDNA will lead to

319     similar findings or not. Multiple studies showed that there is higher beta diversity among headwaters

320     compared to downstream reaches (Altermatt et al., 2013, Carrara et al., 2012, Finn et al., 2011). When

321     comparing beta diversity within stream orders we detect differences between groups of stream orders for

322     both methods. These differences are mainly between beta diversity of large stream orders and small

323     stream orders, as expected by theory.

324          Overall, for local composition, we see some discrepancy of the two methods for community

325     composition at specific sites. This discrepancy is driven by turnover, indicating that detection of different

326     species with the two methods differs, and is not due to the integration of eDNA over distance. We see

327     several mechanisms which could cause this difference between the two methods. First, bias introduced in

328     the lab process may have hampered the detection of species that were actually present (e.g., through

329     primer bias (Elbrecht and Leese, 2017), PCR stochasticity (Leray and Knowlton, 2017), sequencing depth,

330     etc.). The design of specific EPT primers may improve comparison and deliver even a better overlap than

331     a universal eukaryotic primer as we used in this study. Second, DNA shedding rates, densities, and

332     activity can differ between genera (Bylemans et al., 2017, de Souza et al., 2016, Sassoubre et al., 2016)

333     and affect the eDNA detection. Also, it remains unknown how habitat preferences of different genera

334     affect detection due to limited mixing or flow of the preferred habitat. Third, it is possible that DNA got

335     re-suspended from sediments through the mixing of the water column or other disturbances (Jerde et al.,

336     2016, Shogren et al., 2017, Shogren et al., 2016), resulting in a signal of locally extinct taxa. However, we

337    have no indication from the historic data that genera detected only with eDNA have been absent in the

338    catchment for a longer time.

339

# Conclusion

341         The distribution of biodiversity and indicator species is of interest to many fields in ecology, from

342    basic research and theory to applied projects of biodiversity conservation and ecosystem assessments. Our

343    work identifies novel opportunities of eDNA as a reliable tool to detect biodiversity patterns, similar to

344    traditional kicknet sampling in riverine networks. Our findings show high robustness of the method,

345    allowing its use for rapid richness analyses and offering the potential to do quick assessments of

346    biodiversity with untrained collectors for BioBlitz campaigns or citizen science projects. However, if the

347    goal is to extend previous biomonitoring or biodiversity datasets with eDNA sampling, the spatial scale

348    must be considered when designing sampling schemes to detect taxon richness.

349

# Authors' contributions

351    EM, CJL, RA, EF, IG, EH, JCW and FA conceived the ideas and designed methodology; EM, CJL, RW,

352    RA, EF, IG, EH, SH, JCW, and FA collected the data; EM, JCL, RW, and JCW analysed the data; EM

353    and FA led the writing of the manuscript. All authors contributed critically to the drafts and gave final

354    approval for publication.

355

# Acknowledgements

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366

## 367    Data accessibility

368    Sequencing data will be publicly available on Dryad after publication.

369

## 370    Supporting information

371    See supplementary file attached to the paper.

372

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546 **Figures**
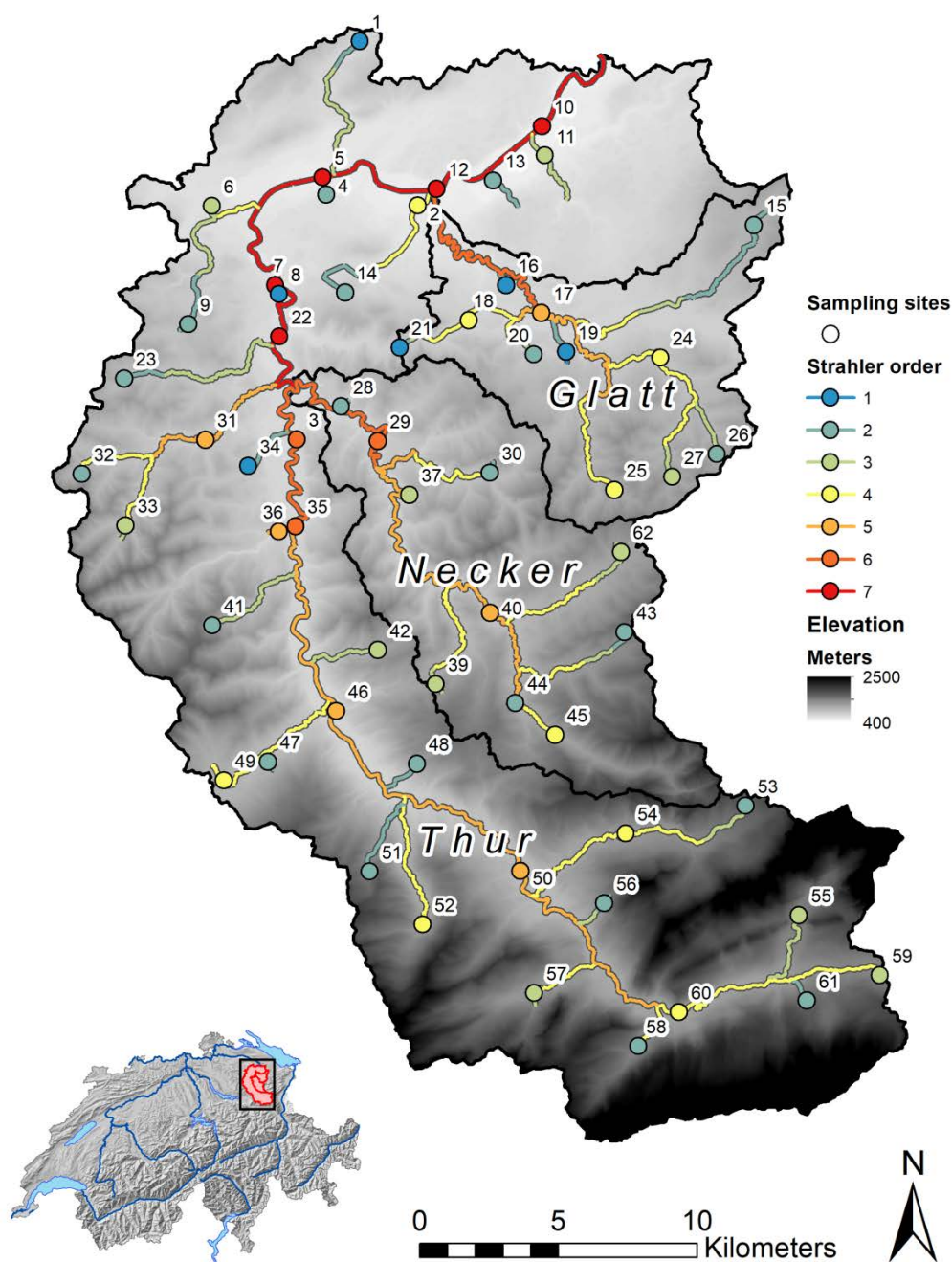


548 **Fig. 1:** Field sites in the river Thur catchment in North-Western Switzerland (insert at bottom
549 right). Colors are coding for stream order (first to seventh order streams: blue, green, yellow,
550 orange, orange-red, and red respectively). Black lines indicate the three major subcatchments:
551 Thur, Necker and Glatt. Data source: swisstopo: VECTOR200 (2017), DEM25 (2003),
552 SWISSTLM3D (2018); BAFU: EZG (2012); Bundesamt für Landestopographie (Art.30 Geo IV):
553 5704 000 000, reproduced by permission of swisstopo / JA100119.

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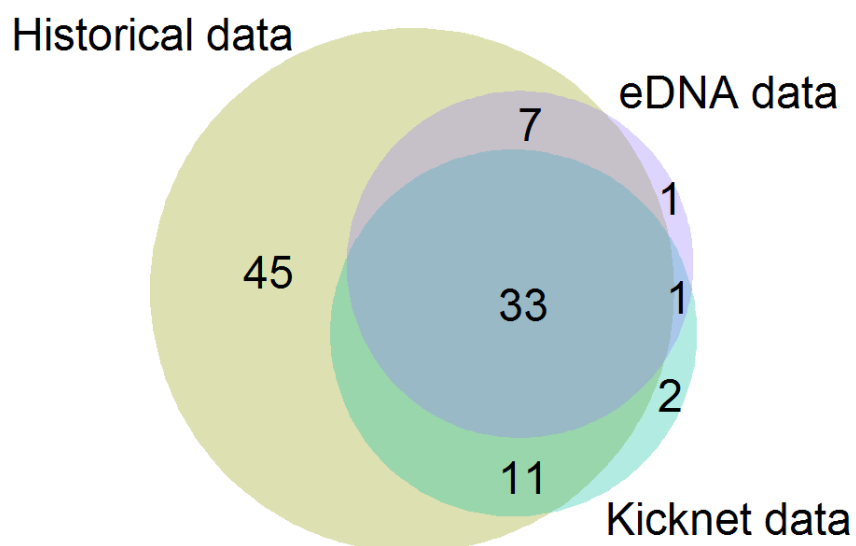
**Fig. 2:** Overlap of EPT genera in the three different datasets over the whole catchment. Bubble size is proportionate to the number of genera detected.

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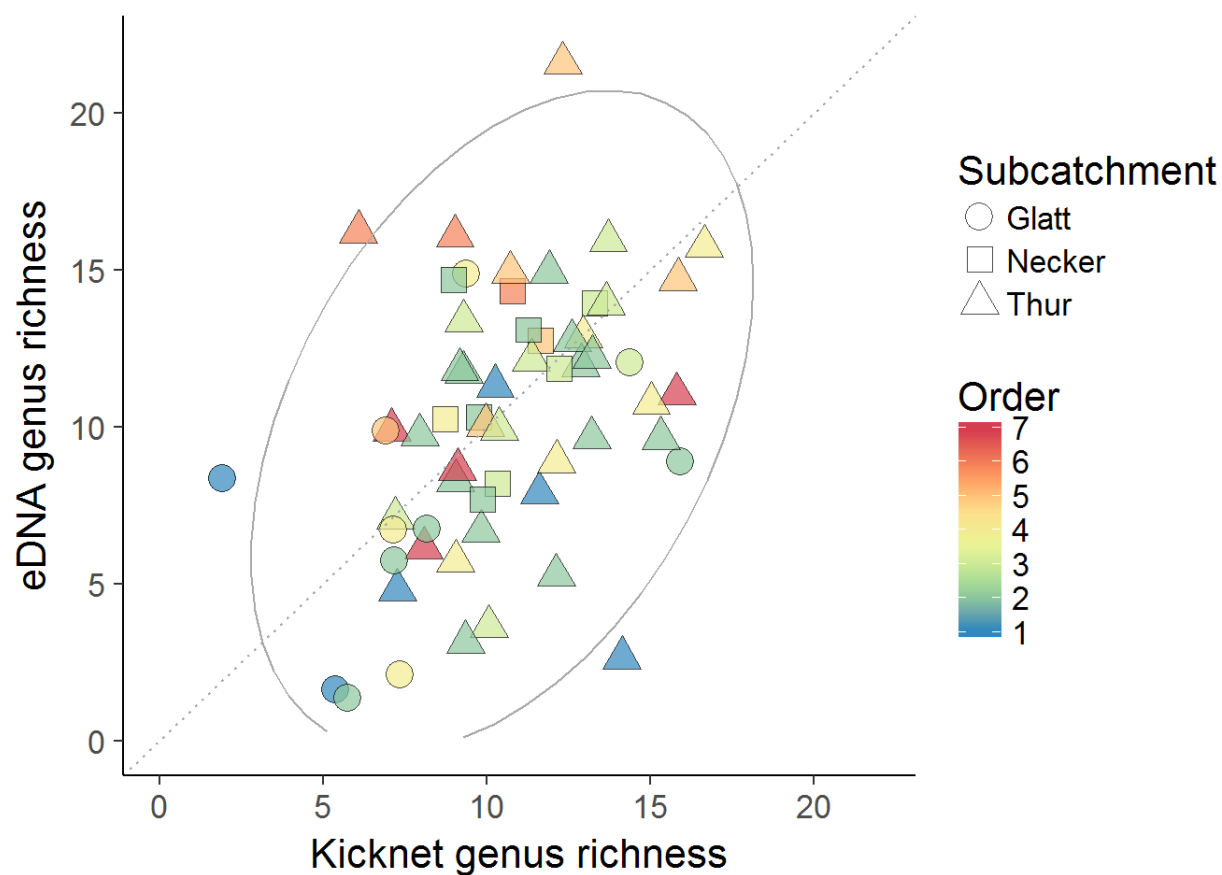
**Fig. 3:** Detected richness of EPT genera comparing kicknet versus eDNA sampling. The grey line indicates the confidence ellipse based on multivariate normal distribution and the dotted line is the 1:1 line. Colors and shapes of the individual data-points are according to stream order and the sampling sites' subcatchment respectively. Points are minimally jittered due to overlapping cases.

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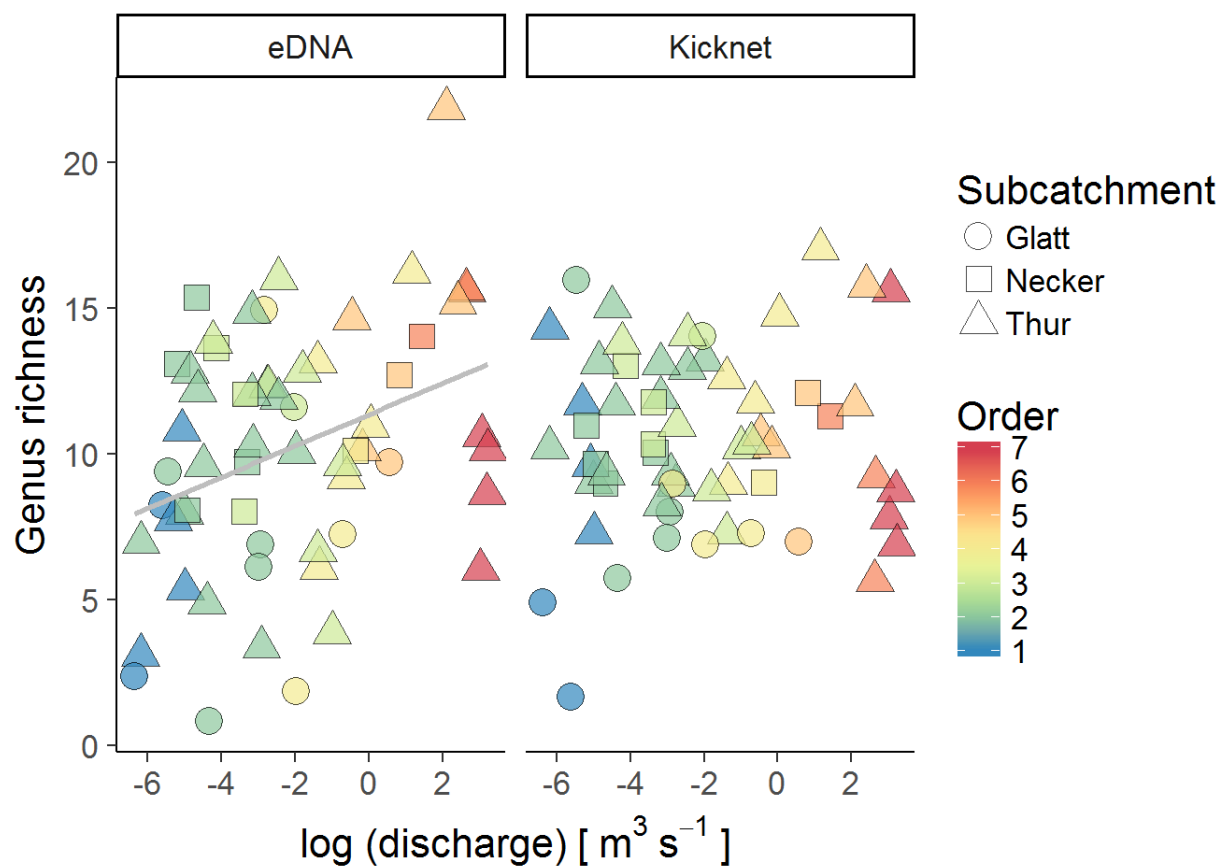
**Fig. 4:** Richness of eDNA and kicknet samples plotted against the logarithmic annual mean discharge. The grey line gives the significant linear regression line for eDNA only due to non-significance for kicknet genus richness. Colors are according to stream order and the shape indicates to what subcatchment the site belongs to. Points are minimally jittered due to overlapping cases.

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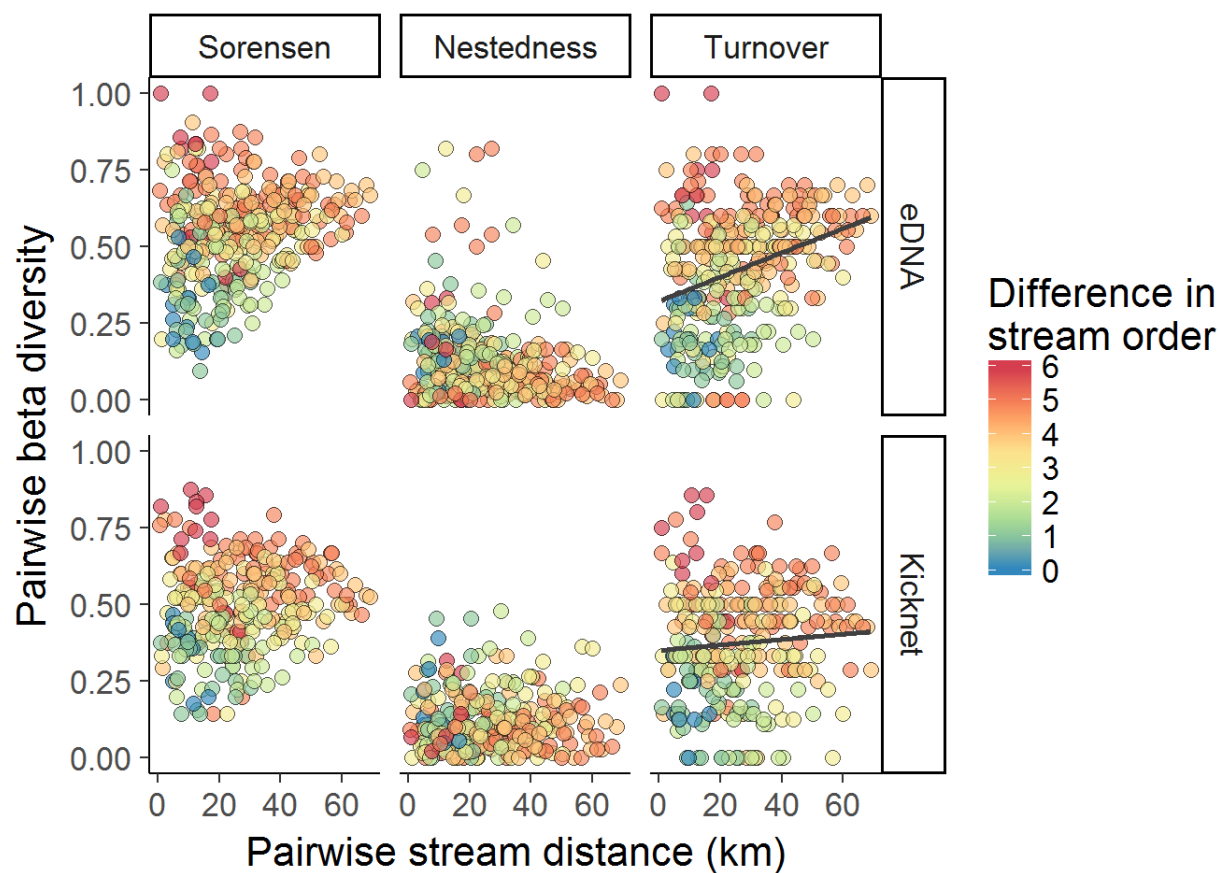
**Fig. 5:** Pairwise beta diversity against pairwise stream distance for flow connected sites only. Beta diversity is calculated based on Sorensen dissimilarity and is split into the two components of nestedness and turnover. The color gradient indicates the difference in stream orders of the pairwise compared sites and solid lines indicate the regression line in cases where stream distance showed relative importance too.