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# Metabarcoding-based assessment of the influence of sediment bypass tunnels on the macroinvertebrate communities in dam-fragmented rivers

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## Abstract

Catchment-based impacts of dams have direct influence on species richness, diversity and community composition of stream insects. Sediment bypass tunnels (SBTs) are guiding structures in reservoirs used to reestablish sediment regimes downstream. Studies monitoring the ecological effects of SBT operation on downstream reaches suggests a positive influence of SBTs on the recovery of riverbed condition and macroinvertebrate community composition in previously degraded channels. In this study, we utilized metabarcoding analysis to estimate species richness and diversity to assess the influence of SBTs on the macroinvertebrate communities in dam-fragmented rivers for both upstream and downstream sites in comparison to dam-fragmented rivers without SBTs and to free-flowing rivers. We detected significant community composition differences among dam-fragmented and free-flowing rivers at higher taxonomic levels. SBTs of two Alpine rivers fragmented by the Pfaffensprung and Solis dams have positively contributed to the recovery of community compositions in the downstream sites. The river fragmented by Egschi dam was negatively affected by habitat fragmentation. We suggest that assessment and development of proper management strategies for SBT operations in reservoirs are required for a positive influence of SBTs on the community composition of dam-fragmented rivers.

Keywords: habitat fragmentation, sediment bypass tunnel, metabarcoding, macroinvertebrate community, biodiversity

## 1 Introduction

Anthropogenic channel modifications such as dam and reservoir construction have damaging effects on riverine ecosystems. Catchment-based processes alter the physical, chemical and biological structure and functions of streams and rivers (Monaghan *et al*. 2005, Nilsson *et al*. 2005, Greathouse *et al*. 2006). Dams serve as physical barriers interrupting the natural river channel, and intercept large amount of sediments (Dai and Liu 2013). Altering the downstream hydrology and the sediment carrying capability of the river causes disturbance on both upstream and downstream reaches, consequently

affecting its inhabitants (Júnior *et al*. 2016). In particular, some stream insects require downstream drift at the juvenile stage to colonize the downstream area, and upstream adult aerial flights to recolonize the upstream site (Caudill 2003, Chaput-Bardy *et al*. 2008). Dams obstruct the downstream dispersal and migration of the juvenile insects. Habitat fragmentation results into a decrease in downstream population size limiting the number of adult insects for upstream flight (Vinson 2001, Hagen *et al*. 2012). Smaller population size and immobility influence the fitness of local populations and increase the risk of extinction (Dijkstra *et al*. 2014). Changes in flow pattern and sediment type, isolation of fragments, and reduced total habitat area caused by dams and its processes have direct influence on species richness, diversity and community composition of freshwater insects.

In countries such as Japan and Switzerland, some dams have guiding structures installed in reservoirs to reestablish sediment regimes downstream. Sediment bypass tunnels (SBTs) routes sediments around the dam into the tail water to reduce sediment accumulation in reservoirs due to bed- and suspended load (Sumi *et al*. 2004, Auel and Boes 2011, Boes *et al*. 2014). A few studies have monitored the effects of SBT operation on the ecosystem of downstream reaches. Kobayashi *et al*. (2016) suggests that riverbed condition and macroinvertebrate community composition in degraded channels recover with years after SBT operation. Although water and sediment flash disturb downstream habitats, Martín *et al*. (2015) reported that SBT operation have positive influence on downstream environment recovery in the long term.

To assess the influence of SBT operation on downstream reaches, the previous studies assessed variation in macroinvertebrate community composition. However, baseline comparison against control habitats were not examined, and assessment was done at the functional feeding and family levels only. Hence, researchers are encouraged to conduct further studies on community composition and taxon-specific responses to enable effective monitoring and development of management strategies for SBT operations. However, constraints on taxonomic expertise, time and cost of surveys are impediments to such analyses. Metabarcoding is a molecular method, a combination of DNA taxonomy and high-throughput sequencing (HTS) used for rapid biodiversity assessment. DNA barcoding alleviate the limits of traditional morphological taxonomy, while HTS enables a rapid screening of taxonomically complex communities. In this study, we utilized metabarcoding analysis to estimate richness and diversity indices to assess the influence of SBTs on the fragmented macroinvertebrate communities in dam-fragmented rivers on both up- and downstream sites in comparison to dam-fragmented rivers without bypass tunnels and free-flowing rivers.

# 2 Materials and methods

## 2.1 Sampling

Sampling was conducted in rivers located in Zurich, Switzerland in August 2014 (see Fig. 1). Three of the seven study rivers were dam-fragmented with operational SBTs i.e. Solis, Egschi and Pfaffensprung dams. Rivers fragmented by dams with SBTs (except for Pfaffensprung) were sampled on three sites: upstream of the reservoir (A), below the dam but upstream of the SBT outlet (B), and downstream of SBT outlet (C). Two rivers fragmented by dams without SBTs i.e. Burvagn and Isenthal dams, and two free-flowing rivers were sampled at both upstream (A) and downstream (B) locations. In total, sixteen study sites were sampled. Qualitative samples of macroinvertebrate larvae were collected via D-flame nets, and immediately preserved in 99.5% ethanol. They were sorted up to family level under a stereomicroscope (x 120).


Fig. 1: Study site: Map of Switzerland showing upstream (A) and downstream (B, C) sampling locations (left). River types: dam-fragmented river (1), dam-fragmented river w/ SBT (2), and free-flowing river (3) (right).

## 2.2 DNA extraction and COI amplification

Samples per site were dried, grounded and homogenized in separate centrifuge tubes. Genomic DNA was extracted via proteinase K digestion using the DNeasy Blood & Tissue Kit (QIAGEN; Hilden, Germany) according to manufacturer's instructions. PCR amplification of the cytochrome oxidase I (COI) region of the mitochondrial DNA was carried out in a total volume of 40 µL using 3 µL of diluted DNA (10X), 20 µL Phusion® High-Fidelity PCR Master Mix with HF Buffer (New England Biolabs), 2 µL each of the forward and reverse primers (10 µM), and 13 µL of PCR-grade water. The PCR primers used are the universal Folmer primers LCO1490 and HCO2198 phosphorylated in the 5' end (Vrijenhoek 1994). PCR amplification and adopter ligation was done following the procedure of Yaegashi and Watanabe (2016). The 16 DNA libraries were pooled, and sequencing was carried out using MiSeq with 300 bp paired-end sequencing.

## 2.3 Data analysis

Paired-end sequencing generated a total of 11.62M raw reads. Reverse reads were discarded due to low quality with lengths ranging from 35–43 bp only. The remaining

5.81M forward reads ranging from 35–301 bp length were treated as unpaired for subsequent analysis. Read quality was checked using FastQC v0.11.5 (Andrews *et al*. 2011). Non-biological sequences i.e. primer and index sequences were trimmed via Trimmomatic v0.36 (Bolger *et al*. 2014). For quality filtering, we followed the UPARSE pipeline (Edgar 2013) implementing the maximum expected error method (maxee) retaining ~50% reads for all sampling sites. Reads >2.0 expected error and <150 bp in length were discarded. Surviving reads were truncated at 150 bp length to obtain globally alignable reads. Then, reads from the 16 sampling sites were pooled and collapsed into unique sequences using a command in USEARCH v9.2.64 (Edgar 2010). Unique reads were clustered into operational taxonomic units (OTUs) with a similarity cut-off value of 97%, subsequently discarding chimeric and singleton sequences from the data set. Taxonomic assignment was generated by querying the OTU representative sequences in BOLD (Ratnasingham and Hebert 2007) and GenBank (Camacho *et al*. 2009). OTUs without matches, or query sequence with taxonomic assignment <97% identity and non-arthropod matches were excluded from subsequent analyses.

The OTU table output from the UPARSE pipeline was used as input data to calculate for diversity indices using the tool QIIME (Caporaso *et al*. 2010) following the eukaryotic diversity analysis protocol developed by Leray and Knowlton (2016) with some modifications. Prior to the estimation of diversity, the OTU table was rarefied to accommodate the differences in sequence depth between sampling sites. A rarefaction analysis is required since non-parametric and parametric estimates are sensitive to sample size (Zhan *et al*. 2014, Leray and Knowlton 2016). The OTU table was subsampled down to the lowest number of sequences. The alpha diversity metric used was Simpson's diversity D. Pairwise compositional dissimilarities or beta diversity were estimated using both quantitative and qualitative metrics. We used Jaccard (based on incidence/presence or absence), and Bray-Curtis (based on relative abundance) to calculate pairwise community distance.

# 3 Results

## 3.1 Processing of reads

After quality filtering, 2.35M (44%) reads were retained and 2.11M (36%) of these reads were mapped into OTUs. Out of 1,222 OTUs detected, BOLD predicted taxonomy for 322 OTUs and GenBank for 417 OTUs with significant matches accounting for 1,908,508 (33%) reads. For this analysis, non-Arthropoda sequences were discarded retaining 734 OTUs with 1,908,379 reads, all identified at the species level (90.24% of the reads mapped to OTUs are Arthropoda sequences). A total of 8 orders, 36 families, 65 genera, and 131 species of Arthropoda were represented. Ephemeroptera was the most prevalent order with 86.7% of reads, followed by Diptera, Plecoptera and Trichoptera with 8.6%, 3.8% and 0.6%, respectively. Species with the most abundant sequences were *Baetis*

*alpinus* (46%) and *Baetis rhodani* (13%). Other species with >2% sequence abundance were *Ecdyonurus venosus* (8%), *Rhithrogena* sp. 28 (5%), *Rhithrogena* sp. 14 (4%), *Rhithrogena* sp. 19 (4%), *Simulium argyreatum* (3%), *Simulium variegatum* (3%) and *Ecdyonurus submontanus* (2%).

## 3.2 Alpha and beta diversity estimates

Simpson's diversity (D) calculated for the 16 sites ranged from 0.80–0.94. Low dominance value equates to a community with high level of diversity. Dam-fragmented rivers have relatively higher D compared to free-flowing river sites, thus having fewer species dominating the community. For dam-fragmented sites with SBT, up- and downstream areas of the river fragmented by Pfaffensprung dam has the lowest D for all study sites, while the estimated D for Egschi dam were relatively higher compared to other dams with SBT and free-flowing river sites (see Fig. 2). Interpreting the estimate of beta diversity using presence or absence data, dissimilarity between the up- and downstream of the SBT outlet of Egschi has the highest value of 6.3, while Burvagn has the lowest with 0.34. Comparing the dissimilarity values of each sites based on the relative abundance of sequences, both dam-fragmented rivers i.e. Burvagn and Isenthal have relatively high level of dissimilarity with values 0.54 and 0.53, respectively, while free-flowing rivers have relatively low dissimilarity values. In the case of dam-fragmented rivers with SBTs, the A and B sites of Pfaffensprung has the lowest dissimilarity value compared to all the study sites. Egschi's A and B dissimilarity was not significantly different from dam-fragmented rivers without SBTs, with highest dissimilarity between its A and C sites (see Fig. 3).

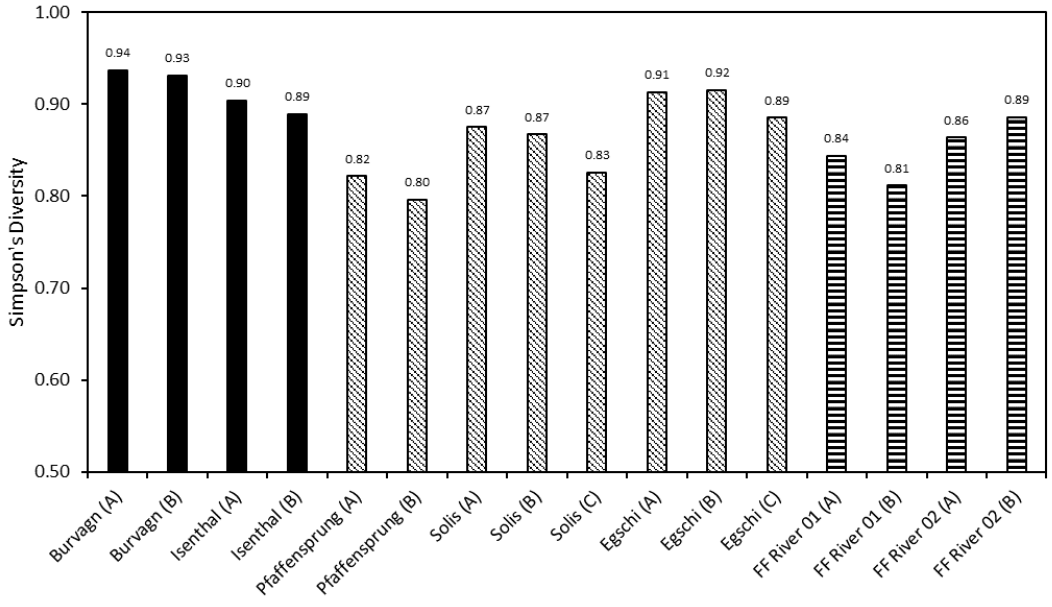


Fig. 2: Estimation of alpha diversity: Simpson's diversity D of the up- and downstream sites of 7 Alpine rivers.

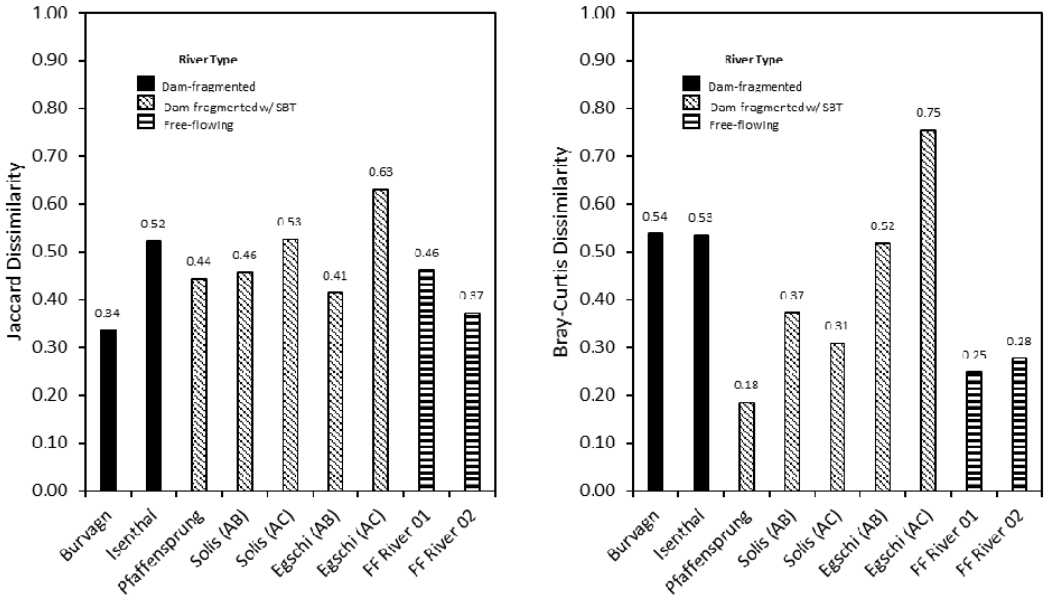


Fig. 3: Estimation of beta diversity: Pairwise compositional dissimilarities between up- and downstream sites of the 7 Alpine rivers. Jaccard - incidence-based dissimilarity (left). Bray-Curtis - sequence abundance-based dissimilarity (right).

# 4 Discussion

Metabarcoding has become a popular tool for assessing community composition and structure. After employing various amplicon read processing strategies, we have identified 131 species from reads sequenced via the MiSeq platform. We were able to recover arthropod sequences up to the species level, and obtain community profiles from COI fragments of 150 bp length to estimate diversity. A handful of studies have evaluated the reliability of metabarcoding for the assessessment of taxonomic composition and diversity of freshwater macroinvertebrate communities has been conducted (Hajibabaei *et al*. 2011, Ji *et al*. 2013, Cristescu 2014, Majaneva *et al*. 2015, Beng *et al*. 2016). It provides a rapid and reliable method of identifying organisms at various taxonomic levels expanding the taxonomic coverage of ecological studies (Leray and Knowlton 2016).

Our data showed that up- and downstream communities of un-fragmented rivers have low dissimilarity values, and fragmented rivers have higher dissimilarity which clearly supports earlier claims on the negative effect of habitat fragmentation on macroinvertebrate community composition. With regards to the influence of SBTs on the fragmented communities of the three experimental rivers, our results showed positive effects of SBTs for Pfaffensprung and Solis dam-fragmented rivers, but not similar for Egschi. Difference in the influence of the SBTs between the dams could be accounted for by the difference in time of SBT operation, and the amount and size of sediments being transported downstream. Kobayashi *et al*. (2016) reported that riverbed conditions and invertebrate communities in degraded channels recover years after SBT operation due to

increased chances of bed material mobility, which is crucial for the reestablishment of the natural invertebrate community. On the other hand, Martín *et al*. (2015), observed that water and sediment scouring or deposition disturbs the sediment respiration, periphyton biomass and macroinvertebrate richness of the downstream sites. Events such as these SBT processes may have direct influence on the community structure downstream but further assessment are needed to directly correlate such events as negative factors.

In summary, we detected significant community composition variation between dam-fragmented and free-flowing rivers at higher taxonomic levels. We report the positive influence of SBTs on the community composition of the downstream sites of two Alpine rivers fragmented by the Pfaffensprung and Solis dams. On the other hand, the river fragmented by Egschi Dam was negatively affected by habitat fragmentation. With this study, we aim to promote metabarcoding as a tool for identifying species and profiling biodiversity of freshwater habitats to develop holistic management strategies for reservoir and SBT operations.

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## References

Andrews, S., Lindenbaum, P., Howard, B., & Ewels, P. (2011). FastQC: High Throughput sequence QC report v. 0.10. 1. Cambridge, UK: Babraham Bioinformatics.

Auel, C., & Boes, R. M. (2011). Sediment bypass tunnel design–review and outlook. Dams and Reservoirs under Changing Challenges, 403-412.

Beng, K. C., Tomlinson, K. W., Shen, X. H., Surget-Groba, Y., Hughes, A. C., Corlett, R. T., & Slik, J. F. (2016). The utility of DNA metabarcoding for studying the response of arthropod diversity and composition to land-use change in the tropics. *Scientific Reports*, 6.

Boes, R. M., Auel, C., Hagmann, M., Albayrak, I., Schleiss, A. J., De Cesare, G., Pfister, M. et al. (2014). Sediment bypass tunnels to mitigate reservoir sedimentation and restore sediment continuity. *Reservoir Sedimentation*, 221-228.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, btu170.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: architecture and applications. *BMC Bioinformatics*, 10(1), 421.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Huttley, G. A., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5), 335-336.

Caudill, C. C. (2003). Measuring dispersal in a metapopulation using stable isotope enrichment: high rates of sex‐biased dispersal between patches in a mayfly metapopulation. *Oikos*, 101(3), 624-630.

Chaput‐Bardy, A., Lemaire, C., Picard, D., & Secondi, J. (2008). In-stream and overland dispersal across a river network influences gene flow in a freshwater insect, *Calopteryx splendens*. *Molecular Ecology*, 17(15), 3496-3505.

Cristescu, M. E. (2014). From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. *Trends in Ecology & Evolution*, 29(10), 566-571.

Dijkstra, K. D. B., Monaghan, M. T., & Pauls, S. U. (2014). Freshwater biodiversity and aquatic insect diversification. *Annual Review of Entomology*, 59, 143-163.

Dai, Z., & Liu, J. T. (2013). Impacts of large dams on downstream fluvial sedimentation: an example of the Three Gorges Dam (TGD) on the Changjiang (Yangtze River). *Journal of Hydrology*, 480, 10-18.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26(19), 2460-2461.

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, 10(10), 996-998.

Greathouse, E. A., Pringle, C. M., McDowell, W. H., & Holmquist, J. G. (2006). Indirect upstream effects of dams: consequences of migratory consumer extirpation in Puerto Rico. *Ecological Applications*, 16(1), 339-352.

Hagen, M., Kissling, W. D., Rasmussen, C., Carstensen, D. W., Dupont, Y. L., Kaiser-Bunbury, C. N., Alves-Dos-Santos, I. et al., (2012). Biodiversity, species interactions and ecological networks in a fragmented world. *Advances in Ecological Research*, 46, 89-120.

Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G. A., & Baird, D. J. (2011). Environmental barcoding: a next-generation sequencing approach for biomonitoring applications using river benthos. *PLoS One*, 6(4), e17497.

Ji, Y., Ashton, L., Pedley, S. M., Edwards, D. P., Tang, Y., Nakamura, A., ... & Larsen, T. H. (2013). Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecology Letters*, 16(10), 1245-1257.

Júnior, S. P., Perbiche‐Neves, G., & Takeda, A. M. (2016). The environmental heterogeneity of sediment determines Chironomidae (Insecta: Diptera) distribution in lotic and lentic habitats in a tropical floodplain. *Insect Conservation and Diversity*, 9(4), 332-341.

Leray, M., & Knowlton, N. (2016). Visualizing Patterns of Marine Eukaryotic Diversity from Metabarcoding Data Using QIIME. *Marine Genomics: Methods and Protocols*, 219-235.

Kobayashi, S., Auel, C., Sumi, T., & Takemon, Y. (2016). Recovery of Riverbed Features and Invertebrate Community in Degraded Channels by Sediment Supply through Bypass Tunnel. Presented in the 12th International Conference on Hydroscience & Engineering for Environmental Resilience, November 6-10, Tainan, Taiwan.

Majaneva, M., Hyytiäinen, K., Varvio, S. L., Nagai, S., & Blomster, J. (2015). Bioinformatic amplicon read processing strategies strongly affect eukaryotic diversity and the taxonomic composition of communities. *PloS One*, 10(6), e0130035.

Martín, E. J., Doering, M., & Robinson, C. T. (2015). Ecological effects of sediment bypass tunnels. *In Proceedings of 1st international workshop on sediment bypass tunnels*, 232, 147-156.

Monaghan, M. T., Robinson, C. T., Spaak, P., & Ward, J. V. (2005). Macroinvertebrate diversity in fragmented Alpine streams: implications for freshwater conservation. *Aquatic Sciences*, 67(4), 454-464.

Nilsson, C., Reidy, C. A., Dynesius, M., & Revenga, C. (2005). Fragmentation and flow regulation of the world's large river systems. *Science*, 308(5720), 405-408.

Ratnasingham, S., & Hebert, P. D. (2007). BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). *Molecular Ecology Notes*, 7(3), 355-364.

Sumi, T., Okano, M., & Takata, Y. (2004). Reservoir sedimentation management with bypass tunnels in Japan. *In Proceedings of the Ninth International Symposium on River Sedimentation*, 2, 1036-1043.

Vinson, M. R. (2001). Long-term dynamics of an invertebrate assemblage downstream from a large dam. *Ecological Applications*, 11(3), 711-730.

Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol*, 3(5), 294-299.

Yaegashi, S. & Watanabe, K. (2016). Multispecies assessments of effects of habitat fragmentations on population genetic structures of stream invertebrates using next-generation sequencing. Presented in the 20th Congress of the Asia Pacific Division of the International Association for Hydro Environment Engineering & Research, August 28-31, Colombo, Sri Lanka.

Zhan, A., He, S., Brown, E. A., Chain, F. J., Therriault, T. W., Abbott, C. L., MacIsaac, H. J., et al. (2014). Reproducibility of pyrosequencing data for biodiversity assessment in complex communities. *Methods in Ecology and Evolution*, 5(9), 881-890.

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