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学 位 論 文 要 約
Dissertation Abstract

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論　　文　　名：次世代淡水バイオモニタリング：DNAメタバーコーディングのコミュニティ・人口ベースの生態学的評価への応用

(Dissertation Title) NEXT-GENERATION FRESHWATER BIOMONITORING: APPLICATIONS OF DNA METABARCODING FOR COMMUNITY AND POPULATION-BASED ECOLOGICAL ASSESSMENT

Freshwater ecosystems face increasing pressures due to habitat degradation, biodiversity loss, increasing rates of extinction, and emerging challenges from anthropogenic stress, e.g., dam impoundment. This warrants reliable, verifiable, and efficient biomonitoring and ecological assessment schemes for the management of freshwater resources and restoration of damaged ecosystems. DNA metabarcoding is a powerful tool at the service of taxonomists and ecologists. However, the development and validation of DNA-based monitoring methods are not trivial and involves many steps from sample collection, laboratory protocols, and bioinformatics. The main goal of this thesis was to explore the application of DNA metabarcoding for freshwater biomonitoring, with a specific focus on river ecosystems impacted by dam-fragmentation that have undergone or are currently undergoing restoration programs by sediment management and augmentation. We presented here the use of both benthic macroinvertebrates and microbial communities as focal groups for bioindicator assessment of freshwater river ecosystems. We also explored the potential of using DNA metabarcoding for haplotype-level inference.

First, we assessed and compared morphology-based datasets against DNA metabarcoding to explore methodological biases and technical limitations, with a specific focus on freshwater macroinvertebrate communities. In the direct comparison of the methods, recovery of samples by incidence or

presence-absence count was considerably low, with a high rate of false-negative detection specifically for species with scarce representation in the community sample. From the 45 morphologically identified taxa (mixed taxonomic-level), 44 species were detected by metabarcoding, with 35 species collapsed into 11 groups matching the morphologically identified taxa. However, abundance-based detection proved to be efficient, with 92% of the individuals correctly demonstrated. This is supported by the significant positive correlation between the logged depth (number of reads) and abundance of morphological taxa. The positive correlation between the abundance of morphological taxa and sequence read abundance of the metabarcoding taxa among the merged taxonomic groups suggests that metabarcoding could be used to quantify relative species abundance based on depth information. We also evaluated the ability of the morphological and metabarcoding approaches to assess the relationship between physicochemical characteristics and macroinvertebrate community composition. Both models were significant, but the metabarcoding data set explained a relatively higher percentage of variation between the environmental variables and community composition. Although both approaches presented almost similar responses for each predictor variable, only DNA metabarcoding data explained statistically significant variability between the environmental variables and community composition. With the comparability of DNA metabarcoding against morphology-based identification, and its ability to identify macroinvertebrates at higher taxonomic resolutions from community samples, we present that this method can be a useful tool for more reliable assessments.

Our next goal was to employ DNA metabarcoding on small-scale restoration monitoring programs to provide consistently-observed information for answering routine concerns from environmental managers of dam-impacted river ecosystems. First, we assessed the diversity and composition of benthic macroinvertebrates to better our understanding of the ecological consequences of sediment bypass tunnel (SBT) operations on the Alpine rivers in Switzerland. SBTs are guiding structures used to reduce sediment accumulation in reservoirs during high flows by transporting sediments to downstream reaches during operation. We used DNA metabarcoding analysis to assess the influence of SBT operations on macroinvertebrates downstream of SBT outlets by estimating species diversity and pairwise community dissimilarity between upstream and downstream locations in dam-fragmented rivers with operational SBTs in comparison to dam-fragmented (i.e., no SBTs) and free-flowing rivers (i.e., no dam). We report that the macroinvertebrate community dissimilarity decreases with increasing operation time and frequency of SBTs. These factors of SBTs operation influence changes in riverbed features, e.g., sediment relations, that subsequently affect the recovery of downstream macroinvertebrate communities to their respective upstream communities. In addition, SBT operation conditions, frequency, and duration influence changes in riverbed conditions, i.e., channel incision and riverbed armoring that subsequently restore natural geomorphic processes in the downstream reach. Riverbed conditions and macroinvertebrate communities in residual channels improved in the years after SBT operation due to increased bed mobility (high flows from SBTs), which is important for the reestablishment of more natural invertebrate communities. In addition, maximum SBT discharge, sediment released through the tunnel, and distance to the outlet mostly influenced hyphoreic properties, i.e., sediment respiration, organic matter content, and sediment size.

These factors may also influence assemblages and possible recovery of macroinvertebrate taxa downstream.

For another monitoring application, we evaluated benthic macroinvertebrate communities' response to the restoration or construction of gravel bars conducted in the dam-impacted Trinity River, with the non-dam influenced tributaries serving as the reference sites. DNA metabarcoding detected most of the taxa identified with morphological identification and provided higher taxonomic resolution. We also detected significant correlations between morphological sample abundance, biomass, and DNA metabarcoding read abundance. With a few exceptions, the alpha and beta diversity metrics calculated from morphological and DNA metabarcoding datasets were similar. We report significant differences in the community composition at the river scale between the dam-impacted Trinity River and its pristine tributary rivers. Also, a significant difference in community composition between the gravel bar and the free-flowing sites at the reach scale was observed. These results are supported by previous studies that reported biodiversity improvement of restoration in dam-influenced rivers. For one, most of the gravel bars in the Trinity River are constructed via fluvial deposition of locally added sediments or by mechanical construction of gravel islands and bars, whereas the tributaries assessed have naturally created gravel bars. The ability of rivers to move substratum changes alongside hydrological regime alterations, which in turn influence the way in which water moves around and over the instream structures, i.e., gravel bars.

Understanding the influence of pre-processing sediment samples for community analysis would be vital for conceptualizing appropriate study designs for sediment-associated microbial community profiling through molecular methods. We examined if there would be method-driven differences between non- and pre-processed sediment samples (represented by the collection filter) by sequential membrane filtration for microbial community profiling through DNA metabarcoding. We found no significant difference in the quality and quantity of extracted DNA and PCR amplicons between the two methods. Although we found a significant difference in raw and quality-filtered reads, read abundance after bioinformatics processing (i.e., denoising and the chimeric-read filtering steps) were not significantly different. These results suggest that read abundance after these read processing steps were not influenced by sediment processing or lack thereof. Our observations from this study highlighted the feasibility of pre-processing sediment samples for community analysis and the need to further assess sampling strategies to help conceptualize appropriate study designs for sediment-associated microbial community profiling.

In addition, we employed DNA metabarcoding of the 16s rDNA gene to profile the microbial communities from the same focal sites of the Trinity River. Comprehensive restoration programs implementing gravel augmentation and ecological flow restoration for recreating instream gravel features are expected to influence the diversity and composition of sediment microbial communities in accordance with changes attributed to the restoration of habitat characteristics in the dam-impacted channel. We report that the river restoration activities in the Trinity River significantly influenced the diversity and composition of the sediment microbial communities brought about by the restoration of habitat characteristics. Gravel bar recreation and restoration contributed to the increased microbial

biodiversity through the restoration of environmental heterogeneity at the river scale. The significant positive correlation between the functional diversity of the identified microbial taxa and beta diversity suggests that differences in the detected metabolic functions were closely related to dissimilarities in community composition. The methodology and results of this study have implications on the impact of construction and restoration of gravel bars in a dam-impacted river on environmental heterogeneity and how this influences the taxonomic and functional diversities of microbial communities in the gravel bars.

The development and evaluation of DNA metabarcoding protocols for haplotype level resolution require attention, specifically for basic population genetic applications, i.e., analysis to allow genetic diversity estimations and dispersal abilities of the species present in the bulk community samples. We assessed the ability to infer haplotype information of freshwater macroinvertebrate species from DNA metabarcoding community sequence. Using single-species mock samples with known Sanger-sequenced haplotypes, we assayed the effects of PCR cycle and denoising parameters for the detection and reduction of spurious haplotypes obtained from DNA metabarcoding. Lastly, we provided an example of population-based assessment for environmental monitoring, in this case, on agriculture insect pest invasions. We employed DNA barcoding to assess the population genetic structure and demography of the outbreak populations of the coconut scale insect, *Aspidiotus rigidus* in the Philippines. Our results provided an initial important genetic basis and information for designing and implementing biological control strategies against the invasive coconut scale insect pest *A. rigidus* in the Philippines.

To conclude, I am fortunate to be able to contribute to the increasing literature on DNA metabarcoding, where not only the people in the field of ecology would benefit from, but as well as those working in the areas of engineering and environmental management, and the policy-makers whose responsible for the translation of our studies into legislation and implementation for the betterment of the freshwater ecosystems that we highly rely on.