The impact of DNA extract homogenization and replication on marine sediment metabarcoding diversity and heterogeneity

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Abstract

Metabarcoding of environmental DNA (eDNA) is an attractive complement to morphological methods for characterizing marine sediment benthic communities. However, incomplete sampling is a major concern when inferring community composition, and metabarcoding results are heavily dependent on methodology. Using 18S V1-V2 and COI markers, we investigated the effect on observed alpha- and beta diversity of (A) homogenization intensity during sediment DNA extraction, (B) extraction replicates vs larger sediment extraction volume, and (C) pre- and post-PCR extract pooling. We show that an intermediate Precellys homogenizer program for DNA extraction can significantly improve sediment metabarcoding results in terms of captured diversity and inter-replicate homogeneity compared to vortexing only. This effect was stronger than that of increased sediment extract volume. Pre-PCR pooling of DNA extraction replicates increased observed rarefied richness compared to single extract medians, but not to the extent of amplifying or sequencing extraction replicates individually before pooling, i.e. post-PCR, or in silico pooling, respectively. We argue that this discrepancy was due to both an increased number of PCR artifacts and reduced PCR drift. Inter-sample heterogeneity was considerably higher for the COI metazoan dataset, compared to the total eukaryotic 18S dataset, likely due to a combination of metazoan eDNA distribution, stochastic effects due to less conserved primer sites, and a high degree of COI non-target amplification. Based on our results, extraction replicates of smaller sediment volumes, in combination with firm but intermediate homogenization and pre-PCR pooling, is a cost-effective way of maximizing sediment eDNA metabarcoding sample coverage, compared to increased extraction volume.

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