Metabarcoding on the deep seafloor: optimizing multigene approaches and sampling methods for large-scale biodiversity assessments.

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The deep sea, the largest and most poorly known biome on Earth, is under increasing threat from human-induced ecological impacts. Improved baseline knowledge and environmental impact assessment protocols are required to be able to alleviate potential changes in ecosystem diversity and functioning in the deep-sea. Metabarcoding of environmental DNA (eDNA) enables broader and faster biodiversity assessments, and is increasingly used to study eukaryote and prokaryote diversity. Whether metabarcoding provides reliable diversity inventories that meet the quality standards for accurate baseline data and biomonitoring is still uncertain in the deep-sea benthos, the latter being associated with specific taxonomic and sampling challenges. In particular, it is crucial to develop multigene metabarcoding protocols targeting living organisms and not extracellular, archived DNA.

Before launching a large-scale project for the reassessment of deep-sea biodiversity, we addressed these technical challenges using bathyal and abyssal sediments sampled in the Mediterranean and central Atlantic. Our aim was to setup optimized protocols and evaluate the strengths and limitations of multigene metabarcoding in the deep sea by 1) comparing eDNA-based with traditional morphology-based diversity inventories and 2) assessing the accuracy and/or bias associated with distinct sample processing methods, including RNA and size-selected DNA extracts lacking short (extracellular) DNA fragments.