OCB Small Group Activity Proposal: Intercomparing metabarcoding of marine microbial communities

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Background and Rationale. Marine microorganisms form the foundation of marine food webs and drive biogeochemical cycles in the ocean. In particular, cyanobacteria and eukaryotic phytoplankton transform inorganic carbon into organic matter, some of which is sequestered in the deep ocean via the biological pump. In turn, this organic matter may be consumed by heterotrophic protists or other zooplankton and support higher trophic levels or remineralized by heterotrophic prokaryotes. As a result, quantifying the abundances and diversity of marine microorganisms across all three domains of life is critical for understanding and modeling carbon cycling from estuarine to open ocean ecosystems including the biological carbon pump. Furthermore, examining their abundances and distributions is necessary to understand and predict marine ecosystem change.

Metabarcoding, or amplicon sequencing, has emerged as a widely used and relatively low-cost approach to examine the diversity of microbial communities, primarily using the 16S and 18S rRNA genes for prokaryotes and eukaryotes respectively. Its use has led to numerous insights into drivers of microbial community structure on global scales (Ibarbalz et al. 2019, Zakem et al. 2025), regional scales (James et al. 2022, Kolody et al. 2025), and with temporal variability (Raes et al. 2024). It has also increased our understanding and ability to predict biogeochemical rate processes such as carbon export (Kramer et al. 2025) and bacterial production (Connors et al. 2024). Due to its relatively low cost and complexity, it will likely remain the most widely used 'omics measurement in marine systems.

Despite its widespread use, efforts towards standardization and intercalibration remain limited in both size and scope, having focused on few parts of a metabarcoding workflow (Fig. 1) and with few participants (Berube et al. 2022). Variability in metabarcoding workflows can occur at several stages, including sample collection and storage, DNA extraction, PCR amplification, sequencing, and bioinformatic processing. As highlighted by the 2020 OCB workshop on standardization of 'omics measurements, metabarcoding intercalibration is necessary to enhance data quality and integrate data from past and future field programs, such as BioGeoSCAPES, which seeks to combine 'omics and biogeochemical measurements to understand ocean metabolism (Berube et al. 2022, Saito et al. 2024). Furthermore, increased understanding and confidence in translating metabarcoding data into estimates of biomass (e.g. cells or carbon) by adding known quantities of foreign DNA, or internal standards, may significantly improve our knowledge of microbial standing stocks and inform the development of Earth system models.

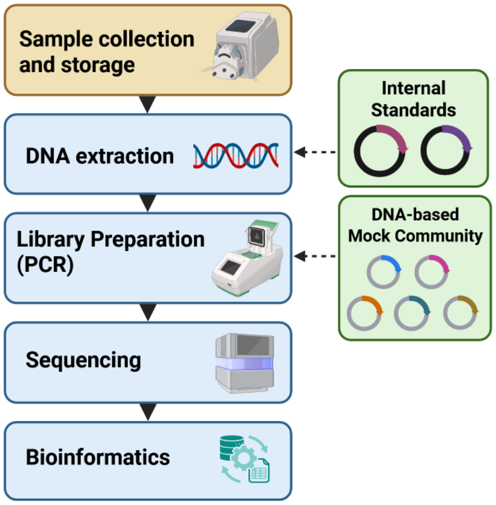

Fig. 1. Metabarcoding workflow. DNA collection and storage (orange) will not be compared. Resources such as internal standards and mock communities (green) will be provided.

Thus, we propose an OCB-led activity for intercomparing and developing best practices for metabarcoding of marine microbial communities that will be open and inclusive to the broader community. In addition to publishing our results, this activity will invest in community and capacity building through webinars, the development of training materials and open protocols, and the inclusion of early-career researchers without prior metabarcoding experience. To expand international reach and capacity-building efforts, a complementary proposal is being submitted for currently available BioGeoSCAPES Funds.

Proposed Activity. The primary objective is to publish results from a detailed metabarcoding intercomparison and guidance on metabarcoding best practices in a peer-reviewed journal. With currently available support to the organizers, filters will be collected at the Bermuda Atlantic Time Series site from two depths representing the euphotic and mesopelagic zones. A coastal sample set will also be collected from Scripps Pier, CA. Measurements of biomass (e.g. flow cytometry, HPLC pigments, microscopy, IFCB) will be collected alongside filter material to draw comparisons between metabarcoding results and other measures of abundance and diversity.

Subsamples will be supplied to participating labs along with internal standards, mock communities, and PCR primers to isolate and understand variability within different parts of a metabarcoding workflow post-sample collection (Fig. 1). Internal standards and mock communities (synthetic DNA-based mixtures providing samples with known quantities) will be provided from existing material (Lampe et al. in press, Lampe et al. 2025), and the provided primers enable amplification of an identical gene region that captures all three domains of life (515F-Y/926R; Parada et al. 2016, Priest et al. 2025). Participating labs will extract DNA and amplify the gene targets in triplicate using their preferred methodologies. Both the extracted DNA and PCR products will be returned to one of the organizing labs for further processing, sequencing, and analysis with a consistent approach. Additionally, participating labs will sequence replicates at their respective sequencing facilities. Following sequencing, data will be distributed to compare bioinformatic approaches among groups. The recovery of internal standards will enable further comparison between the metabarcoding results and other measures of abundances and diversity among groups (Jones-Kellet et al. 2024, Lampe et al. in press). Results will then be synthesized at multiple virtual meetings and one in-person meeting to publish our findings.

Assembly of group: Participation in the group will be open to the community and seek to maximize participation within the final budget. International participation will be expanded through the complementary proposal to BioGeoSCAPES. The activity will be advertised through OCB, the organizing committee's networks, BioGeoSCAPES, social media platforms, and the 2026 Ocean Sciences Meeting. Due to the widespread use of metabarcoding and recognition of the need for standardization, we anticipate there will be significant interest in participating. Participants will be required to commit to completing the necessary tasks and complete a short application that will be scored based on a rubric developed from the committee. A select number of applicants without prior metabarcoding experience will also be included and receive training. Participants will be selected to maximize diversity across geographic regions, career stages, metabarcoding methods, and organismal foci.

Additional activities: This activity will further engage in other community and capacity building activities. Specifically, we will *foster broader dialogue* by hosting bi-monthly webinars that will be open to the public and uploaded to OCB's YouTube channel. Each webinar will highlight studies using metabarcoding, particularly where it has further enabled understanding the carbon cycle and predicting ecosystem change. These may include but are not limited to global and regional insights into microbial community structure, the use of metabarcoding to support ecosystem models, leveraging metabarcoding to inform our understanding of the carbon cycle, or metabarcoding methods. Speakers will be invited by the organizing committee as well as selected through a separate public nomination process.

Alongside hands-on training in metabarcoding for a select number of participants, the group will also collectively generate training materials. These will include instructional materials on metabarcoding workflows, providing a resource for foundational knowledge. Participant groups will all be expected to provide detailed peer-reviewed protocols on protocols.io within a dedicated workspace for the activity, enabling others to easily find and replicate methods. Bioinformatic methods will also be required to be detailed on GitHub. These will be organized and either hosted or linked to on the OCB website, making them easily findable and accessible.

Although additional funding from BioGeoSCAPES is being requested, support from OCB is vital for the success of this activity. OCB funding is required for the proposed in-person synthesis meeting. Additionally, the digital infrastructure and logistical expertise provided by the OCB office is integral for coordinating the meetings and serving as a central hub. Rather than supporting the core activities, the funding being requested from BioGeoSCAPES serves to expand participation in this activity, particularly for international researchers with an emphasis on those from the Global South.

Proposed Timeline

Jan 2026	Advertise activity and application across communication channels.
Feb 2026	Announce the activity at the Ocean Sciences Meeting (OSM) BioGeoSCAPES Town Hall in Glasgow, Scotland. Host an informal kick-off meeting at OSM with the organizers and prospective participants to address questions and solicit feedback. Virtual meetings will be available for individuals not in attendance.
Spring 2026	Application closes and participants are selected. Environmental samples collected at BATS and Scripps Pier. Bi-monthly OCB-hosted webinars begin.
Summer 2026	Materials sent to participating labs. Labs will extract DNA, amplify gene targets, and send extracts and PCR products to the organizing lab. Organizers will host monthly check in meetings with the participants to discuss and troubleshoot issues leading up to synthesis meeting.
2027	In-person synthesis meeting in association with the ASLO Aquatic Sciences or a BioGeoSCAPES meeting in planning. Following, monthly meetings will be held to continue synthesis and develop a manuscript for publication.

Planned Outcomes and Benefits to Broader Community. Several *tangible outcomes* will emerge from this activity. The intercomparison effort will result in a publication that both describes the results of the effort and recommends best practices for performing metabarcoding analyses based on those results. Educational and training materials will also be generated that explain metabarcoding workflows and provide researchers with detailed protocols allowing methods to be easily replicated.

Standardization of metabarcoding data will also improve our ability to integrate and compare across datasets, including long-term time series data. Several of the organizers represent on-going long-term study sites (SPOT, NSF LTERs), and this activity would facilitate cross-site community building and integration of those data along with data from other participants. Community building will be further developed through webinars and the expansion of the group through open applications. Educational materials and inclusion of researchers without prior experience will further disseminate knowledge, making metabarcoding more accessible. Moreover, the results will have broader applicability to other microbiomes such as those from freshwater, benthic, soil, and animal environments.

Logistical Needs (OCB-specific items underlined)**:**

- One-day meeting: hotel and food for participants, A/V support for hybrid capabilities. Support for international participants is separate and requested from BioGeoSCAPES (NSF AccelNET).
- Organizational support coordinating bimonthly webinars.
- Collection of intercalibration materials (supported by BATS / BIOS-SCOPE / NSF CCE-LTER)
- Shipping of materials (domestic only). International shipping requested from BioGeoSCAPES.
- Research costs will be covered by participating labs. For any lab that routinely performs metabarcoding, costs for additional samples are extremely minimal. Cost of materials for groups in the Global South is being requested from BioGeoSCAPES.
- Publication fees for one journal article

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