

Title: High spatial resolution global ocean metagenomes from Bio-GO-SHIP repeat hydrography transects

Authors

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Abstract

Detailed descriptions of microbial communities have lagged far behind physical and chemical measurements in the marine environment. Here, we present 720 globally distributed surface ocean metagenomes collected at high spatio-temporal resolution. Our low-cost metagenomic sequencing protocol produced 2.75 terabases of data, where the median number of base pairs per sample was 3.48 billion. The median distance between sampling stations was 26 km. The metagenomic libraries described here were collected as a part of a biological initiative for the Global Ocean Ship-based Hydrographic Investigations Program, or “Bio-GO-SHIP.” One of the primary aims of GO-SHIP is to produce high spatial and vertical resolution measurements of key state variables to directly quantify climate change impacts on ocean environments. By similarly collecting marine metagenomes at high spatiotemporal resolution, we expect that this dataset will help answer questions about the link between microbial communities and biogeochemical fluxes in a changing ocean.

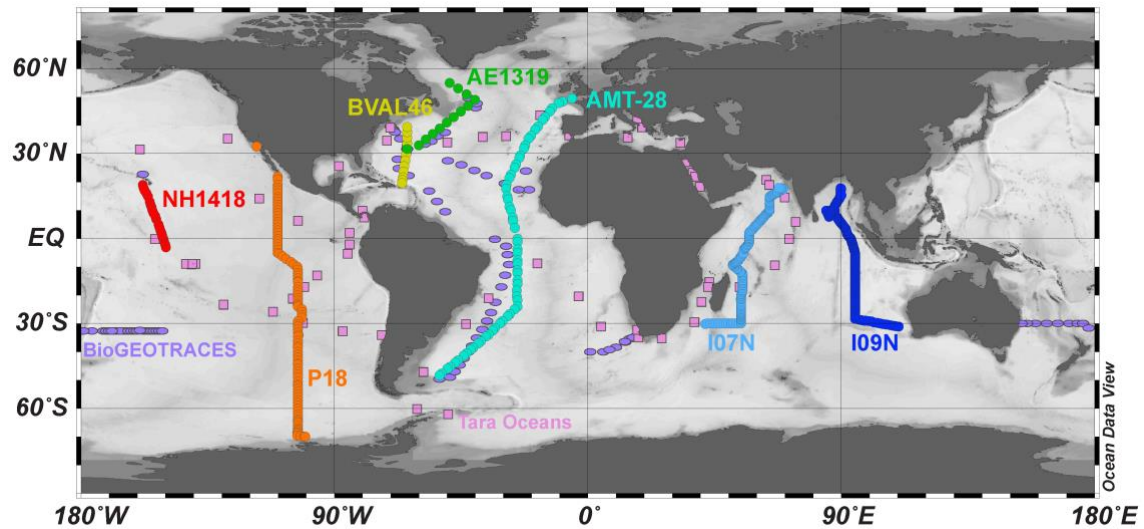
40 **Background & Summary**

41 A growing list of coordinated scientific efforts have produced deep metagenomic
42 libraries of the surface ocean. Projects such as the Global Ocean Survey, Tara Oceans, and
43 bioGEOTRACES¹⁻³ have significantly advanced our understanding of marine microbial
44 biogeography and biodiversity. However, this ever-increasing abundance of metagenomic
45 data raises the question of how do we move beyond analyses of biodiversity to linking
46 microbial traits with ecosystem function and elemental fluxes⁴. In oceanography, it has been
47 widely acknowledged that sparse sampling results in high noise and error rates that in turn
48 prevent the characterization of dynamic chemical balances and limit biogeochemical models⁵.
49 Thus, we propose that an increased emphasis on high resolution spatiotemporal sampling of
50 marine microbial communities would allow for a more mechanistic understanding of the
51 relationship between microbes and ocean biogeochemistry.

52 The Global Ocean Ship-based Hydrographic Investigations Program (GO-SHIP) seeks to
53 produce high spatial and vertical resolution measurements of physical, chemical, and
54 biological parameters over the full water column. This internationally-organized program
55 coordinates a network of sustained hydrographic sections that are repeatedly measured on
56 an approximately decadal time scale. Compared to autonomous programs such as Argo, which
57 has significantly increased the spatial and temporal resolution of ocean observations⁶, ship-
58 based programs have the advantage of a much broader range of biogeochemical
59 measurement capabilities. To date, repeat hydrography programs have largely focused on
60 physical (light, currents, water column thermohaline structure, etc.) and chemical (nutrients,
61 oxygen, dissolved organic and inorganic carbon, pH, etc.) state variables. This work has
62 significantly improved our understanding of the response of oxygen⁷, pH⁸, calcium carbonate
63 saturation depth⁹, and sea level rise¹⁰ to global warming and anthropogenic carbon
64 accumulation¹¹. By comparison, systematic and sustained biological measurements of the
65 microbial component of ocean ecosystems has lagged far behind.

66 Here, we present a dataset of 720 ocean surface water metagenomes collected at high
67 spatiotemporal resolution in an effort to more mechanistically link marine microbial traits and
68 biodiversity to both chemical and hydrodynamic ecosystem fluxes as a part of a novel Bio-GO-
69 SHIP sampling program. Samples were collected in the Atlantic, Pacific, and Indian Ocean
70 basins (Fig 1, Table 1). This effort has been supported by GO-SHIP, the Plymouth Marine
71 Laboratory Atlantic Meridional Transect (PML AMT), and three National Science Foundation
72 (NSF) Dimensions of Biodiversity funded cruises (AE1319, BVAL46, and NH1418) (Table 2).
73 Whereas the median distance between Tara Oceans sampling stations was 709 km and the
74 median distance between bioGEOTRACES sampling stations was 191 km, the median distance
75 between sampling stations in the current Bio-GO-SHIP dataset is 26 km (Fig 2). In addition, the
76 majority of Bio-GO-SHIP samples were collected every 4-6 hours, allowing for analysis of diel
77 fluctuations in microbial composition and gene content¹². We anticipate that our high-
78 resolution sampling scheme will allow for a more detailed examination of the relationship
79 between the broad range of geochemical parameters measured across the various cruises
80 (Table 2) and microbial diversity and traits.

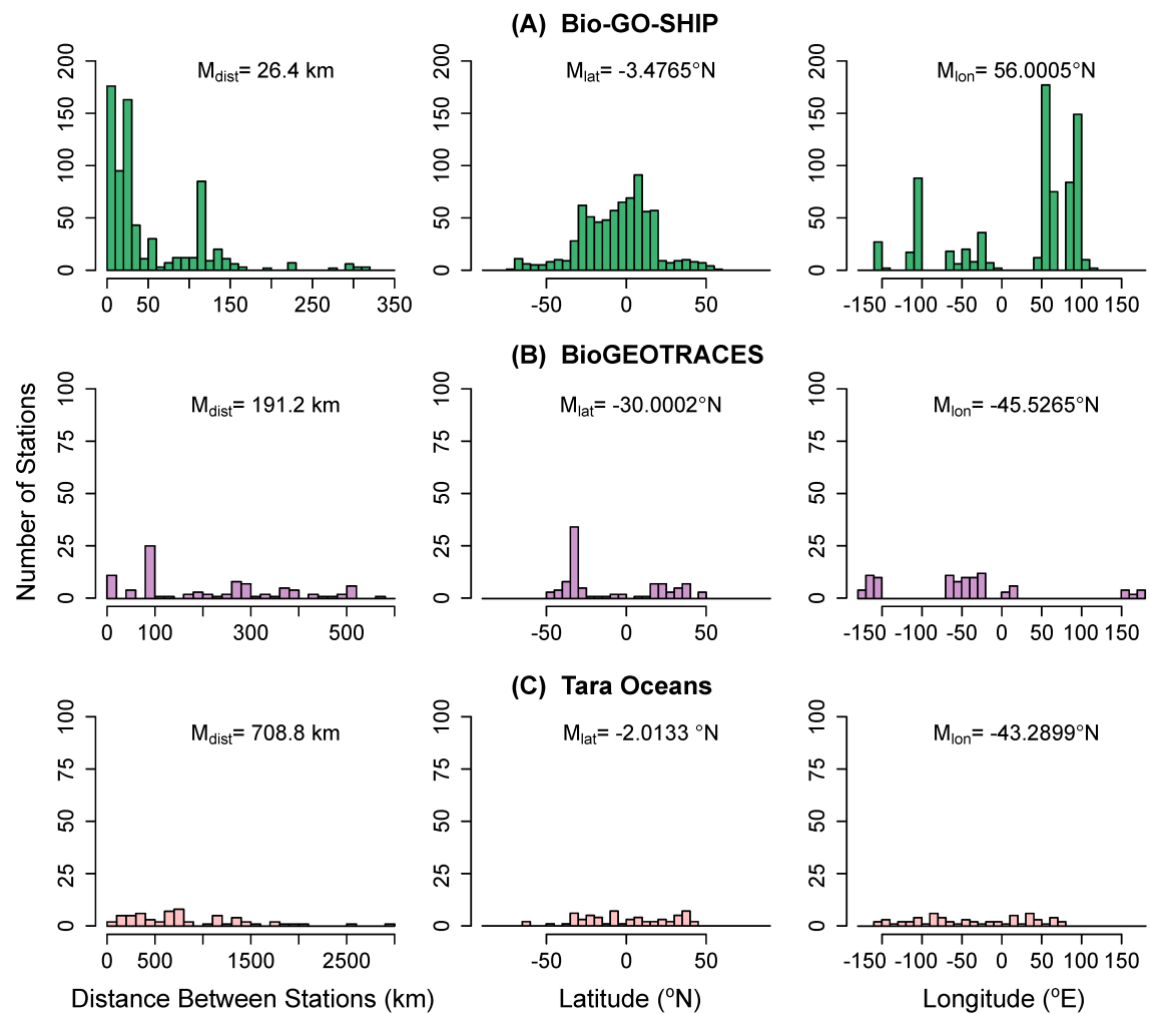
81 Due to their rapid generation times and high diversity, microbial genomes integrate
82 the impact of environmental change¹³ and can be used a 'biosensor' of subtle biogeochemical
83 regimes that cannot be identified from physical parameters alone^{12, 14-16}. Thus, the fields of
84 microbial ecology and oceanography would benefit from coordinated, high resolution
85 measurements of marine 'omics products (i.e., metagenomes, metatranscriptomes,
86 metaproteomes, etc.). This dataset provides an important example of the benefits of a high
87 spatial and temporal resolution sampling regime. Specifically, our data highlights the need for
88 increased sampling of marine metagenomes in the Central and Western Pacific Ocean (Fig 1),
89 areas above 50°N and 50°S (Fig 2), and below the euphotic zone. We hope and expect that
90 these challenges will be addressed by the scientific community in the coming decade.



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Figure 1: Distribution of global surface microbial metagenomes from Bio-GO-SHIP (circles) in comparison to Tara Oceans (squares) and bioGEO-TRACES (ovals).

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Figure 2: Comparison of the distance between stations, station latitudes, and station longitudes for global surface ocean metagenomes. Individual station locations from (A) Bio-GO-SHIP, (B) bioGEO-TRACES and (C) Tara Oceans were examined. Plots are labelled with the median value, M . Station distance was calculated as the distance to the nearest station.

94

95 **Table 1:** Sampling protocols and read counts for global Bio-GO-SHIP surface ocean
96 metagenomes.

Cruise	DNA Collection	DNA Volume	Station Count	Total Reads	Total Bases	Median Bases Per Sample	Range of Bases Per Sample
I09N	Underway	10 L	242	5.73x10 ⁹	8.64x10 ¹¹	3.10x10 ⁹	4.71x10 ⁸ – 1.22x10 ¹⁰
I07N	Underway, CTD	4 L, 2-4 L	248	6.20x10 ⁹	9.36x10 ¹¹	3.27x10 ⁹	2.47x10 ⁸ – 1.42x10 ¹⁰
P18	CTD	2 L	104	3.22x10 ⁹	4.86x10 ¹¹	4.46x10 ⁹	6.14x10 ⁷ – 1.77x10 ¹⁰
AMT-28	CTD	2 L	63	2.18x10 ⁹	3.29x10 ¹¹	4.95x10 ⁹	1.62x10 ⁹ – 1.22x10 ¹⁰
BVAL46	CTD	2 L	12	2.01x10 ⁸	3.04x10 ¹⁰	2.73x10 ⁹	2.33x10 ⁹ – 4.88x10 ⁹
AE1319	CTD	2 L	13	2.01x10 ⁸	3.03x10 ¹⁰	4.69x10 ⁹	2.15x10 ⁹ – 7.43x10 ⁹
NH1418	CTD	2 L	23	5.41x10 ⁸	8.17x10 ¹⁰	3.03x10 ⁹	2.42x10 ⁹ – 1.08x10 ¹⁰

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Methods

100 On all cruises, whole (i.e., no size fractionation) surface water was collected via either
101 the Niskin rosette system (depth ~3-5m) or the ship's circulating seawater system (depth
102 ~7m). Between 2-10 L of surface water (Table 1) was collected in triple-rinsed containers and
103 gently filtered through a 0.22 µm pore size Sterivex filter (Millipore, Darmstadt, Germany)
104 using sterilized tubing and a Masterflex peristaltic pump (Cole-Parmer, Vernon Hills, IL). DNA
105 was preserved with 1620 µl of lysis buffer (4 mM NaCl, 750 µM sucrose, 50 mM Tris-HCl, 20
106 mM EDTA) and stored at -20°C before extraction.

107 To extract DNA (modified from Bostrom et al. 2004)¹⁷ Sterivex filters were incubated
108 with 180 µl lysozyme (3.5 nM) at 37°C for 30 minutes followed by an overnight 55°C incubation
109 with 180 µl Proteinase K (0.35 nM) and 100 µl 10% SDS buffer. DNA was extracted from the
110 Sterivex with 1000 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA), precipitated in an ice-cold
111 solution of 500 µl isopropanol (100%) and 1980 µl sodium acetate (3 mM, pH 5.2), pelleted via
112 centrifuge for 30 mins at 4°C, and resuspended in TE buffer in a 37°C water bath for 30 min.
113 Next, DNA was purified using a genomic DNA Clean and Concentrator kit (Zymo Research
114 Corp., Irvine, CA). Finally, DNA concentrations were quantified using a Qubit dsDNA HS Assay
115 kit and Qubit fluorometer (ThermoFisher, Waltham, MA).

116 A total of 720 metagenomic libraries were prepared using Illumina-specific Nextera
117 DNA transposase adapters and a Tagment DNA Enzyme and Buffer Kit (Illumina, San Diego,
118 CA, cat. no. 20034197) (modified from Baym et al. 2015)¹⁸⁻²⁰. Nextera adapter sequences to
119 be used for bioinformatic quality trimming are: 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG
120 AGA CAG-3' and 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G-3'. Custom Nextera
121 DNA-style 8bp unique dual index (UDI) barcodes I7 (5'-CAA GCA GAA GAC GGC ATA CGA GAT
122 [NNN NNN NN]G TCT CGT GGG CTC GG-3') and I5 (5'-AAT GAT ACG GCG ACC ACC GAG ATC
123 TAC AC[N NNN NNN N]TC GTC GGC AGC GTC-3') were used to multiplex the metagenomic
124 libraries. A total of 1 µl of 2 ng/µl DNA was added to 1.5 µl tagmentation reactions (1.25 µl TD
125 buffer, 0.25 µl TDE1) and incubated at 55°C for 10 minutes. After tagmentation, product (2.5
126 µl) was immediately added to 22 µl reactions (1.02 µM per UDI barcode, 204 µM dNTPs,
127 0.0204 U Phusion High Fidelity DNA polymerase and 1.02X Phusion HF Buffer [ThermoFisher,
128 Waltham, MA] final concentration). Barcodes were annealed to tagmented products using the
129 following polymerase chain reaction (PCR): 72°C for 2 min., 98°C for 30 s., followed by 13
130 cycles of 98°C 10 s., 63°C 30 s., 72°C 30 s., and a final extension step of 72°C for 5 min.

131 To quality control tagmentation products, dimers that were less than 150 nucleotides
132 long were removed using a buffered solution (1 M NaCl, 1 mM EDTA, 10 mM Tris-HCl, 44.4 M
133 PEG-8000, 0.055% Tween-20 final concentration) of Sera-mag SpeedBeads (ThermoFisher,
134 Waltham, MA). Metagenomic libraries were quantified using a Qubit dsDNA HS Assay kit

135 (ThermoFisher, Waltham, MA) and a Synergy 2 Microplate Reader (BioTek, Winooski, VT).
136 Libraries were then pooled at equimolar concentrations. Pooled library concentration was
137 verified using a KAPA qPCR platform (Roche, Basel, Switzerland). Finally, dimer removal as well
138 as read size distribution were checked using a 2100 Bioanalyzer high sensitivity DNA trace
139 (Agilent, Santa Clara, CA).

140 54 samples were sequenced on two Illumina HiSeq 4000 lanes using 150 bp paired-
141 end chemistry with 300 cycles (Illumina, San Diego, CA). All remaining samples were
142 sequenced on three Illumina NovaSeq lanes using S4 150 bp paired-end chemistry with 300
143 cycles. The sequencing strategy produced a total of 1.83×10^{10} reads, or 2.75×10^{12} bp. The
144 median number of bases per sample was 3.48 billion (range: 61,400,000 – 17.7 billion). The
145 sequencing cost per bp in US dollars was $\$8.2 \times 10^{-9}$.

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148 **Data Records**

149 The majority of the samples here were collected under the auspices of the international GO-
150 SHIP program and the national programs that contribute to it²¹⁻²⁴. A comprehensive data
151 directory of metadata resources is available at <https://www.go-ship.org/>. Bottle data and
152 cruise report links are provided in Table 1.

153

154 Metadata variables from the AMT-28 cruise (<https://www.amt-uk.org/>) are hosted by the
155 British Oceanographic Data Centre, and may be requested through the following URL:
156 <https://www.bodc.ac.uk/>. Select metadata are also available through GO-SHIP²⁴.

157

158 The BVAL46, AE1319, and NH1418 cruises were collected as a part of the “Biological Controls
159 on the Ocean C:N:P Ratios” project funded by the NSF Division of Ocean Sciences²⁵⁻²⁸. Data
160 associated with these deployments are hosted by the NSF Biological and Chemical
161 Oceanography Data Management Office (BCO-DMO). A comprehensive list of metadata
162 resources is available at <https://www.bco-dmo.org/project/2178>.

163

164 All sequencing products associated with the Bio-GO-SHIP program can be found under
165 BioProject ID PRJNA656268 hosted by the National Center for Biotechnology Information
166 Sequence Read Archive (SRA)²⁹. SRA accession numbers associated with each metagenome file
167 are provided in Supplementary Table 1.

168 **Table 2:** Complete list of metadata variables collected on Bio-GO-SHIP cruises

Campaign	Metadata Access	Metadata Variables
I09N / GO-SHIP	https://cchdo.ucsd.edu/cruise/33RR20160321	Temperature, Dissolved O ₂ , Conductivity, Density, Salinity, Nutrients (NO ₃ , NO ₂ , NH ₄ , PO ₄ , SiO ₄), Chlorofluorocarbons (CFCs) /SF ₆ , Dissolved Inorganic Carbon (DIC), ¹³ C and ¹⁴ C of DIC, Total pH, Total Alkalinity, Stable gases (N ₂ , N ₂ O, Ar), ¹⁸ O, Chromophoric Dissolved Organic Matter (CDOM), Pigment HPLC, variable chlorophyll fluorescence, Dissolved Organic Carbon, underway Particulate Organic C N and P, underway pCO ₂ , Lowered Acoustic Doppler Current Profiler, Chipods, Dissolved/ particulate/ cellular P and Fe, N P and Fe uptake rates, <i>Prochlorococcus</i> / <i>Synechococcus</i> / Picoeukaryotes/ Nanoeukaryotes cell counts
I07N / GO-SHIP	https://cchdo.ucsd.edu/cruise/33RO20180423	Temperature, Dissolved O ₂ , Conductivity, Density, Salinity, Chlorophyll, Nutrients (NO ₃ , NO ₂ , PO ₄ , SiO ₄), Dissolved Inorganic Carbon (DIC), Chlorofluorocarbons (CFCs) /SF ₆ , ¹⁴ C of DIC, Dissolved Organic Carbon, Black Carbon, DO ¹⁴ C, Total pH, Total Alkalinity, Calcium, Dissolved Organics, Biomarkers, underway Particulate Organic C N and P, underway pCO ₂
P18 / GO-SHIP	https://cchdo.ucsd.edu/cruise/33RO20161119	Temperature, Dissolved O ₂ , Conductivity, Density, Salinity, Nutrients (NO ₃ , NO ₂ , PO ₄ , SiO ₄), Chlorofluorocarbons (CFCs) /N ₂ O/SF ₆ , Helium isotopes and noble gases (Ne, Ar, Kr, and Xe), Dissolved Inorganic Carbon (DIC), ¹³ C and ¹⁴ C of DIC, Total pH, Total Alkalinity, Stable gases (N ₂ , O ₂ , Ar), Dissolved Organic Carbon / Total Dissolved Nitrogen, Tritium, Black Carbon, DO ¹⁴ C/DO ¹⁴ C, Dissolved Organics, Biomarkers, underway Particulate Organic C N and P, underway pCO ₂ , Wind speed, Wind direction, Air temperature
AMT-28 / PML AMT / GO-SHIP	https://www.bodc.ac.uk/data/hosted_data_systems/amt/ https://amt-uk.org/Cruises/AMT28 https://cchdo.ucsd.edu/cruise/74JC20180923	Temperature, Dissolved O ₂ , Conductivity, Salinity, Nutrients (NO ₃ , NO ₂ , PO ₄ , SiO ₄), biogenic silica/silicon uptake, Total pH, Total Alkalinity, Pigment HPLC, Chlorophyll a, Dissolved Organic C N and P, <i>Prochlorococcus</i> / <i>Synechococcus</i> / Picoeukaryote/ Nanoeukaryote/ Heterotrophic bacteria cell counts, FlowCAM, 15N/13C, Respiration (total, bacterial, size-fractionated), Bacterial production, underway Particulate Organic C N and P, underway wave radar (Cband), Sky Infrared Brightness temperature, Hyperspectral radiance/irradiance, Wind speed, Wind direction, Aerosol size/composition
BVAL46 / NSF / BATS	https://www.bco-dmo.org/project/2178	Temperature, Dissolved O ₂ , Conductivity, Salinity, PAR irradiance, Chlorophyll a, NO ₃ + NO ₂ , NO ₂ , PO ₄ , SiO ₄ , Soluble Reactive Phosphorus (SRP), Particulate Organic C N and P, P uptake (max. uptake, half saturation conc.), <i>Prochlorococcus</i> / <i>Synechococcus</i> / Picoeukaryote/ Nanoeukaryote cell counts
AE1319 / NSF	https://www.bco-dmo.org/project/2178	Temperature, Dissolved O ₂ , Conductivity, Salinity, PAR irradiance, Chlorophyll a, NO ₃ + NO ₂ , NO ₂ , PO ₄ , SiO ₄ , Soluble Reactive Phosphorus (SRP), Particulate Organic C N and P, P uptake (max. uptake, half saturation conc.), <i>Prochlorococcus</i> / <i>Synechococcus</i> / Picoeukaryote/ Nanoeukaryote cell counts
NH1418 / NSF	https://www.bco-dmo.org/project/2178	Temperature, Dissolved O ₂ , Conductivity, Salinity, Density, Fluorescence, PAR irradiance, Chlorophyll a, NO ₃ + NO ₂ , NO ₂ , Soluble Reactive Phosphorus (SRP), Particulate Organic C N and P, <i>Prochlorococcus</i> / <i>Synechococcus</i> / Picoeukaryote/ Nanoeukaryote / Crocospheera cell counts

169 **Technical Validation**

170 To ensure that no contamination of metagenomes occurred, negative controls were used. To
171 ensure optimum paired-end short read sequencing, a 2100 Bioanalyzer high sensitivity DNA
172 trace (Agilent, Santa Clara, CA) was used for each library to confirm that 90% of the sequence
173 fragments were above 250 bp and below 600 bp in length. Qubit (ThermoFisher, Waltham,
174 MA) and a KAPA qPCR platform (Roche, Basel, Switzerland) were used to ensure that all pooled
175 libraries were submitted for sequencing at a concentration >15 nM.

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177

178 **Usage Notes**

179 The genomic data described here have not been pre-screened or processed in any way. We
180 recommend quality control parameters. Prior to our sequence analysis in subsequent projects,
181 we removed adapter sequences, performed sequence quality control, and ensured there was
182 no contamination from common genomic add-ins such as Phi-X using the following code
183 parameters:

184

185 Trimmomatic (v0.35): PE ILLUMINACLIP:NexteraPE-PE.fa:2:30:10 SLIDINGWINDOW:4:15

186 MINLEN:36

187 BBMap (v37.50): bbduk.sh -Xmx1g ref=/BBMap/37.50/resources/phix174_ill.ref.fa.gz k=31

188 hdist=1

189

190

191 **Code Availability**

192 Custom scripts were not used to generate or process this dataset. Software versions and non-
193 default parameters used have been appropriately specified where required.

194

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208

209 **Author contributions**

210 A.A.L. wrote the manuscript, coordinated sample collection, collected/processed samples,
211 designed protocols, performed metagenomic sequencing, and compiled metadata.
212 C.A.G. coordinated sample collection, collected/processed samples, performed metagenomic
213 sequencing, and compiled metadata.
214 M.L.B performed metagenomic sequencing.
215 J.A.L. collected/processed samples and performed metagenomic sequencing.
216 N.G. coordinated sample collection and collected samples.
217 L.J.U. processed samples and compiled metadata.
218 L.B, B.G.C., R.E.S., L.T., and D.L.V. coordinated GO-SHIP collection and collaboration efforts.
219 G.T. coordinated PML AMT/GO-SHIP collection and collaboration efforts.
220 A.C.M. designed and supervised the study, secured funding, and coordinated GO-SHIP
221 collection.
222 All authors contributed to manuscript editing and revision.
223

224 **Competing interests**

225 The authors declare no competing interests.
226

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228

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