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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection No computer code or algorithm was used in this study to collect data

Data analysis For bioinformatic analysis the following information is reported in the method section:
 "For each barcode, primers and adapter sequences were removed from raw reads with Cutadapt v. 4.5 (Martin, 2011). Paired-end reads were then imported and analyzed in RStudio v. 4.4.0 (RStudio Team, 2020) using the DADA2 package v. 1.32 (Callahan et al., 2016). Quality check and trimming of the reads were performed following the package instructions. Paired-end reads were subsequently grouped in amplicon sequence variants (ASVs); chimeric sequences were identified and removed from the dataset. Finally, taxonomy was assigned using a native implementation of the naive Bayesian classifier method against the SILVA database v138.1 (Quast et al., 2013). ASVs belonging to Eukarya, mitochondria, chloroplasts, and with frequency < 2 (singletons) were removed."
 For statistical analysis the following information is reported in the Method section:
 "The Shapiro-Wilk test was used to assess the normality of datasets. Significant differences in the datasets characterizing the different water masses were assessed through the Kruskal-Wallis ANOVA, followed by a post hoc 'Multiple comparisons of mean ranks for all groups' test, Bonferroni corrected (Statistica v.7, StatSoft).
 Non-metric multidimensional scaling (nMDS) was performed on the 16S rRNA bacterial gene dataset using a Jaccard dissimilarity matrix and average linkage approach and plotted with the ggplot2 package (v. 3.5.1) ref.80 in RStudio v. 4.4.0 ref.77. Water mass-based partitioning of 16S data was tested with PERMANOVA, using the same dissimilarity distance built for the nMDS ordination and computed through the function adonis2 of the vegan package (v. 2.7-1)ref. 81.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Data availability:

All the physical, chemical and microbiological data are available in Supplementary Table 1 and at <https://doi.org/10.13120/9vk3-0t31>. All DNA sequences have been submitted to the SRA Sequence Read Archive at NCBI (BioProject Accession no. PRJNA1184697, PRJNA1184714, PRJNA1196803).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	In this work we discuss 62 incubation experiments performed during two summertime cruises to the western Ross Sea which aimed at understanding the processing of inorganic and organic C pools in meso- and bathypelagic layers. Raw seawater samples were incubated at the in situ temperature in the dark. According to the tested parameters different replicated schemes were applied: DIC uptake: 1 blank, 2 replicates; heterotrophic production: 1 blank, 3 replicates. Cell abundance: 1 seawater sample -> 3 analytical replicates for microscopy, 1 analytical replicate for flow cytometry. Respiration (ETS) 1 seawater sample, 2 analytical blanks and two analytical replicates. DNA metabarcoding analysis was run in one replicate. Supplementary Table 1 reports all data as mean values among replicates and the standard deviation (when applicable)
Research sample	The collection of samples was carried out aiming to detect specific water masses. An even number of samples was collected in high salinity shelf water and circumpolar deep water; due to a higher degree of variability in its dynamics, a lower number of samples was collected in antarctic bottom water
Sampling strategy	The sampling scheme was designed in order to gain a comparable number of samples collected in the three water masses. As stated above a lower number of samples was collected in antarctic bottom water. All samples were collected by means of Niskin bottles
Data collection	Potential temperature and salinity data were acquired by means of a multiparametric probe. These data were collected and elaborated by the appointed technicians onboard. All the other data were generated at the home laboratories by specialized personnel.
Timing and spatial scale	Samples were collected in the western sector of the Ross Sea between 2014-01-11 and 2016-02-15, during two cruises. Since a small number of experiments was carried out during the first cruise we had the opportunity to repeat the same experimental design with a higher number of samples during the second cruise.
Data exclusions	No data was excluded from the study

Reproducibility	Reproducibility of experiments is witnessed by the generation of similar data during two different years. The method section reports the (standard) details to repeat the experiments
Randomization	The groups of samples (water masses) were established a priori to highlight potential differences among them. Samples within specific water masses (groups) are treated 'randomly' in the sense that they are presented in descriptive statistics (average and st. dev. in the text; box and whiskers plots in figures) regardless of the area or the depth at which they were collected
Blinding	Due to the novelty of the study, all sewer samples were collected without knowing the final outcomes. Our overarching hypothesis was that in the oldest, oxygen-poor water mass (CDW) chemoautotrophy could help supply microbial communities with freshly-produced organic carbon, whereas in the youngest, POM-rich HSSW heterotrophic processes would predominate.
Did the study involve field work?	☒ Yes ☐ No

Field work, collection and transport

Field conditions	Sampling was performed when sea conditions were favourable (wave height < 1.5 m). Atmospheric features are not relevant for seawater collected at 140m depth and below (well below the mixing depth)
Location	Samples were collected in the area comprised between 70.7655°S; 164.6848°E and 77.1704°A; 176.6214°W; Depth ranged between 140 and 2148 m.
Access & import/export	Between 2013 and 2016, the Italian National Antarctic Research Program (PNRA) conducted its oceanographic campaigns in the Ross Sea under the framework of several international regulations and agreements: 1) Antarctic Treaty System (ATS) -> Antarctic Treaty (1959) and the Madrid Protocol on Environmental Protection (1991, entered into force in 1998), with its Annexes governing environmental impact assessment, waste management, prevention of marine pollution, and the protection of flora, fauna, and specially protected areas. These obligations applied to all Italian Antarctic activities during that period. 2) Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR, 1980) -> PNRA campaigns in the Ross Sea complied with CCAMLR conservation measures regulating scientific research, marine ecosystem protection, and the management of fishing activities. Note: the Ross Sea region Marine Protected Area (RSrMPA) was adopted only in October 2016, entering into force in December 2017—so it did not yet apply during most of the 2013–2016 period, but Italian projects already operated in line with CCAMLR requirements. 3) International Maritime Organization (IMO) framework -> MARPOL Convention (Antarctic as a “Special Area” under Annex I/II/V, with strict restrictions on oil, noxious liquids, and garbage discharges). SOLAS Convention requirements for safety of life at sea. The Polar Code was adopted in 2014, but became mandatory only from January 2017; thus, during 2013–2016 PNRA vessels operated under MARPOL and SOLAS provisions, without yet being bound by the Polar Code certification regime. 4) United Nations Convention on the Law of the Sea (UNCLOS, 1982) -> The Ross Sea is largely high seas; therefore, Italian oceanographic campaigns relied on the freedom of marine scientific research (UNCLOS Articles 87, 238, 257), subject to the obligation of “due regard” for other applicable regimes such as the ATS and CCAMLR.
Disturbance	As a standard procedure PNRA follows the guidelines from the SCAR and from the Antarctic treaty. In particular: - Environmental Code of Conduct for Geosciences Field Research Activities in Antarctica - Environmental Code of Conduct for Terrestrial Scientific Field Research - Code of Conduct for the Use of Animals for Scientific Purposes in Antarctica - Code of Conduct for Activity with Terrestrial Geothermal Environments in Antarctica - Code of Conduct for the Exploration and Research of Subglacial Aquatic Environments No relevant disturbance to environmental elements other than plankton was generated

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Plants

Seed stocks	No plant material was used in this study
Novel plant genotypes	No novel plant genotype was generated in this study
Authentication	No authentication for seed stocks or novel plant genotype was necessary in this study

Flow Cytometry

Plots

Confirm that:

- ☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Prokaryotic cell abundance was estimated as follows. 1.7 mL aliquots were fixed with 0.5% (final concentration, f.c.) glutaraldehyde solution (Grade I for EM analyses, Sigma Aldrich). Fixed samples were kept at 4 °C for approximately 15 min and then stored at -80 °C until analysis (Brussaard, 2004). Prior to enumeration, samples were thawed at room temperature and diluted 1:10 with 0.2 µm-filtered Tris-EDTA buffer 1× (Sigma Aldrich). Then samples were stained with 1× (f.c.) SYBR Green I nucleic acid dye (Life Technologies) for 10 min in the dark at room temperature, according to Marie et al. (1999)
Instrument	FACSCanto II (Becton Dickinson)
Software	FACSDiva (Becton Dickinson). Gating was performed in the SSC vs green fluorescence according to standard procedures (Marie et al 1999)
Cell population abundance	Diluted samples were run for 2 minutes at a flow rate of (average) 60 µL min ⁻¹ , using green fluorescence as a trigger. Events in the gated population ranged between 2678 and 20019.
Gating strategy	A first gating is performed in the Red vs Green fluorescence cytogram. Then, a subpopulation is selected in the side scatter vs green fluorescence plot. Due to the standard nature of the analyses, NO PLOT IS PRESENTED IN THE MANUSCRIPT
☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.	