Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

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**
- □ 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 degrees of freedom and P value noted. Give exact P values wherever possible
- □ 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

The R Package DADA2 (v1.36) is used to process ITS sequences. BLASTn was used to analyze the ITS sequences and FlowJo 10.5.3 to analyze the flow cytometry data.

Data analysis

The codes to estimate autotrophic growth rates and to run individual-based simulations are available at Github with DOI 10.5281/zenodo.3944234 (https://github.com/zenodo/Prochlorococcus_Mixotrophy) with detailed instructions under MIT license.

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

The oceanographic measurements (CTD data, nutrient concentrations and cell counts) have been submitted to the BCO-DMO database under acronym HAFRA (dataset name “EUM photic zone”). Raw ITS sequences are available from GenBank under BioProject PRJNA802353, accessions SASN2555516-SASN2555524
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

Ecological, evolutionary & environmental sciences study design

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

**Study description**
Estimation of the amount of organic carbon taken up and utilized by Prochlorococcus, using a combination of field analyses and mathematical modeling. Field analysis included oceanographic measurements from the Eastern Mediterranean, including flow cytometry, genetic and nanoSIMS analyses (detailed below).

**Research sample**
An oceanographic profile was taken during August 2017 from the Eastern Mediterranean (station N1200, 32.45°N, 34.37°E). Discrete samples from 11 depths were used for cell counts using flow cytometry and for genetic analyses of the picoplankton population structure by amplicon sequencing of the ITS. An in-situ bottle incubation was performed using 13C-bicarbonate and 15N-ammonium. Prochlorococcus cells from two of the samples were FACS-sorted and isotopic enrichment was measured using nanoSIMS. The samples for nanoSIMS analysis were chosen based on their depth and (for the 115m sample) the presence of multiple Prochlorococcus populations.

**Sampling strategy**
Sampling was performed using Niskin bottles. Sample size (number of cells for nanoSIMS analysis) depended on the number of sorted cells available for nanoSIMS analysis (multiple fields, n=45-55 cells/sample). No sample size calculation was performed.

**Data collection**
CTD data were collected on-board and analyzed post-hoc by DS. During the cruise, a log was maintained by DS and made available as a scanned pdf. Cruise log contains detailed description of time of sample collection for each depth, time to sample fixation, etc. Genetic data were obtained by DA as described in the materials and methods. FACS-sorting was performed by DA, DRR and TLK, nanoSIMS acquisition was performed by AV and cells were analyzed by DA, DRR, TLK with the assistance of AV, FE and MV. Modelling was performed by ZW and MJF, and reanalysis of published time series was performed by DS.

**Timing and spatial scale**
Sampling was performed on a single cruise on August 7th, 2017. All relevant data are recorded in the manuscript. Sampling location was 32.45°N, 34.37°E.

**Data exclusions**
No data were excluded from the analysis

**Reproducibility**
Multiple depths were analyzed to verify reproducibility of nanoSIMS measurements. Attempts to perform flow-sorting of cells from shallower depths were unsuccessful due to limit of sensitivity of FACS sorter. Results of oceanographic cruises (e.g. flow cytometry, ITS sequencing, nanoSIMS) represent a "snap-shot" of a single timepoint, but were consistent with other studies as described in the text.

**Randomization**
Not relevant for this study (no distinct study groups).

**Blinding**
Blinding was not relevant to the study during data acquisition. Analysis of nanoSIMS ROIs was performed blind to the sample.

**Did the study involve field work?**
- Yes
- No

Field work, collection and transport

**Field conditions**
Field work was performed during summer in the oligotrophic Eastern Mediterranean. The water column was stratified and nutrients were depleted. These information are included in the manuscript.

**Location**
The sampling station was n-1200, 2.45°N, 3.37°E. A full description of the sampling location is found in Roth Rosenberg et al, Environmental Microbiology 2021, doi: 10.1111/1462-2920.15611

**Access & import/export**
Samples were collected on oceanographic cruise as per common practices. The location sampled is not protected (e.g. not a nature reserve), and no permits were required. Samples for nanoSIMS analysis were sent from Israel to Germany as fixed cells in accordance with all regulations.

**Disturbance**
The study resulted in no disturbance.

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.
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: Seawater samples were collected and fixed on board using glutaraldehyde, as described in the text.

Instrument: Flow cytometry was performed using a BD FACSCanto, and FACS using a BD FACSAria.

Software: Data were analyzed using FlowJo.

Cell population abundance: Post-sorting cell abundances in test runs to validate sorting purity were 2,120 high vs 240 low fluorescence cells in the high population, and 12C high vs 88C low fluorescence cells in the low fluorescence cells.

Gating strategy: Gating of phytoplankton cells for figure 1 was based on common procedure - chlorophyll autofluorescence (PerCP, Cy5) vs. forward scatter. Prochlorococcus were defined based on higher PerCP and FSC, and Synechococcus were defined based on phycocerythrin. The gating for flow sorting Prochlorococcus cells for nanoSIMS was based on the same criteria. A figure for the gating and sorting strategy is provided in the Supplementary Information.

[ ] Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.