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## Reporting Summary

Nature Research 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 Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

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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
	$oxed{x}$ 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. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
×	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
	$oxed{x}$ Estimates of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated
	Our web collection on <b>statistics for biologists</b> contains articles on many of the points above.

#### Software and code

Policy information about availability of computer code

Data collection

Data was collected using q2-clawback version 0.0.2 (https://github.com/BenKaehler/q2-clawback) and redbiom version 0.1.0 (https://github.com/biocore/redbiom). Data was also downloaded from NCBI and the Human Microbiome Project websites. Please see references and details in the manuscript.

Data analysis

Analysis was performed using q2-clawback version 0.0.5 (https://github.com/BenKaehler/q2-clawback) (also available through conda and pip) and paycheck version 0.0.3 (https://github.com/BenKaehler/paycheck).

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All of the raw data is available from https://doi.org/10.5281/zenodo.2548899 and https://doi.org/10.5281/zenodo.2549777.

### Field-specific reporting

### Life sciences study design

All studies must disclose on these points even when the disclosure is negative. All available data was downloaded from Qiita (https://qiita.ucsd.edu/) for the 14 EMPO 3 habitat types included in the study. For the HMP Sample size samples that were analysed, all samples for which shotgun and amplicon data were available were analysed. The three EMPO 1 control EMPO 3 habitat types were excluded, as well as Hypersaline (saline), Aerosol (non-saline), and Plant surface, which Data exclusions all had fewer than nine samples in the Qiita database. The smallest EMPO 3 data set that was included in the study had 152 samples. As 5-fold cross-validation was performed on the samples, it was determined that nine and fewer samples were too few for meaningful results. In no instance did results influence our decision to exclude data. Findings were similar across the 14 different EMPO 3 habitat types. Differences between the results for each EMPO 3 habitat type are Replication analysed in some detail in the manuscript, including relationships with other properties of the data. Randomization For each EMPO 3 habitat type, samples were randomly allocated into five folds for cross validation. The 16S reference sequences were stratified by taxonomy and randomly allocated into five folds for cross validation. Details are given in the Online Methods.

# Reporting for specific materials, systems and methods

was no opportunity for investigators to alter the outcomes between experiments.

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.

This work is a comparison of methods for taxonomic classification. Each method was tested using the same code on the same data sets. There

Materials & experimental systems	Methods	
n/a Involved in the study	n/a Involved in the study	
X Antibodies	ChIP-seq	
<b>✗</b> ☐ Eukaryotic cell lines	Flow cytometry	
<b>▼</b> Palaeontology	MRI-based neuroimaging	
Animals and other organisms	·	
Human research participants		
🗷 🔲 Clinical data		

#### Animals and other organisms

Blinding

Policy information about <u>studies involving animals</u>; <u>ARRIVE guidelines</u> recommended for reporting animal research The study did not involve laboratory animals. Laboratory animals Wild animals The study did not involve and wild animals. Field-collected samples The study only used microbial data that is freely available in online databases. Where those samples were field-collected, details are available in those studies or in those databases. Details are provided in the manuscript. This study only used microbial data that is freely available in online databases. No ethics oversight was required. Ethics oversight

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