QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science

Evan Bolyen[1,*], Jai Ram Rideout[1,*], Matthew R Dillon[1,*], Nicholas A Bokulich[1,*], Christian Abnet[2], Gabriel A Al-Ghalith[3], Harriet Alexander[4,5], Eric J Alm[6,7], Manimozhiyan Arumugam[8], Francesco Asnicar[9], Yang Bai[10,11,12], Jordan E Bisanz[13], Kyle Bittinger[14,15], Asker Brejnrod[16], Colin J Brislawn[17], C Titus Brown[5], Benjamin J Callahan[18,19], Andrés Mauricio Caraballo-Rodríguez[20], John Chase[1], Emily Cope[1,21], Ricardo Da Silva[20], Pieter C Dorrestein[20], Gavin M Douglas[22], Daniel M Durall[23], Claire Duvallet[6], Christian F Edwardson[24], Madeleine Ernst[20], Mehrbod Estaki[25], Jennifer Fouquier[26,27], Julia M Gauglitz[20], Deanna L Gibson[28,29], Antonio Gonzalez[30], Kestrel Gorlick[1], Jiarong Guo[31], Benjamin Hillmann[32], Susan Holmes[33], Hannes Holste[30,34], Curtis Huttenhower[35,36], Gavin A Huttley[37], Stefan Janssen[38], Alan K Jarmusch[20], Lingjing Jiang[39], Benjamin D Kaehler[37], Kyo Bin Kang[40,20], Christopher R Keefe[1], Paul Keim[1], Scott T Kelley[41], Dan Knights[42,32], Irina Koester[43,20], Tomasz Kosciolek[44], Jorden Kreps[1], Morgan GI Langille[45], Joslynn Lee[46], Ruth Ley[47,48], Yong-Xin Liu[10,11], Erikka Loftfield[2], Catherine Lozupone[49], Massoud Maher[50], Clarisse Marotz[30], Bryan Martin[51], Daniel McDonald[30], Lauren J McIver[35,36], Alexey V Melnik[20], Jessica L Metcalf[52], Sydney C Morgan[53], Jamie T Morton[30,50], Ahmad Turan Naimey[1], Jose A Navas-Molina[50,30,54], Louis Felix Nothias[20], Stephanie B Orchanian[55], Talima Pearson[1], Samuel L Peoples[56,57], Daniel Petras[20], Mary Lai Preuss[58], Elmar Pruesse[49], Lasse Buur Rasmussen[16], Adam Rivers[59], Michael S Robeson, II[60], Patrick Rosenthal[58], Nicola Segata[9], Michael Shaffer[49,61], Arron Shiffer[1], Rashmi Sinha[2], Se Jin Song[30], John R Spear[62], Austin D Swafford[55], Luke R Thompson[63,64], Pedro J Torres[65], Pauline Trinh[66], Anupriya Tripathi[20,30,67], Peter J Turnbaugh[68], Sabah Ul-Hasan[69], Justin JJ van der Hooft[70], Fernando Vargas[67], Yoshiki Vázquez-Baeza[30], Emily Vogtmann[2], Max von Hippel[71], William Walters[47], Yunhu Wan[2], Mingxun Wang[20], Jonathan Warren[72], Kyle C Weber[59,73], Chase HD Williamson[1], Amy D Willis[74], Zhenjiang Zech Xu[30], Jesse R Zaneveld[75], Yilong Zhang[76], Rob Knight[30,77,55], and J Gregory Caporaso[1,21,+]

* These authors contributed equally to this work.

[+] Please address correspondence about this document to gregcaporaso@gmail.com.

Author affiliations are provided following the Main Text References.

To get help with QIIME 2, visit https://forum.qiime2.org.

Abstract

We present QIIME 2, an open-source microbiome data science platform accessible to users spanning the microbiome research ecosystem, from scientists and engineers to clinicians and policy makers. QIIME 2 provides new features that will drive the next generation of microbiome research. These include interactive spatial and temporal analysis and visualization tools, support for metabolomics and shotgun metagenomics analysis, and automated data provenance tracking to ensure reproducible, transparent microbiome data science.

Main text

Rapid advances in DNA sequencing and bioinformatics technologies in the past two decades have significantly improved our understanding of the microbial world. These include our growing understanding of the vast diversity of microorganisms; how our microbiota and microbiomes impact disease[1] and medical treatment[2]; how microorganisms impact the health of our planet[3]; and our nascent exploration of the medical[4], forensic[5], environmental[6], and agricultural[7] applications of microbiome biotechnology. Much of this work has been driven by marker gene surveys (e.g., bacterial/archaeal 16S rRNA genes, fungal ITS, eukaryal 18S rRNA genes), which profile microbiota with varying degrees of taxonomic specificity and phylogenetic information. The field is now transitioning to integrate other data types, such as metabolite[8] or metatranscriptome[9] profiles.

The QIIME 1 microbiome bioinformatics platform has supported many microbiome studies and gained a broad user and developer community. Interactions with QIIME 1 users in our online support forum, our workshops, and direct collaborations showed the potential to better serve an increasingly diverse array of microbiome researchers in academia, government, and industry. Here we present QIIME 2, a completely reengineered and rewritten system that will facilitate reproducible and modular analysis of microbiome data to enable the next generation of microbiome science.

QIIME 2 is developed based on a plugin architecture (Figure S1) that allows third-parties to contribute functionality (see https://library.qiime2.org). QIIME 2 plugins exist for latest-generation tools for sequence quality control from different sequencing platforms (DADA2[10] and Deblur[11]), taxonomy assignment[12], and phylogenetic insertion[13], that quantitatively improve results over QIIME 1 and other tools (detailed in the corresponding tool-specific publications). Plugins also support qualitatively new functionality including microbiome paired-sample and time-series analysis[14], critical for studying the impact of treatment on the microbiome, and for machine learning[15], including the ability to save trained models and apply them to new data and to interrogate models to identify important microbiome features. Several recently released plugins, including q2-cscs[16], q2-metabolomics[17], q2-shogun[18], q2-metaphlan2[19], and q2-picrust2[20], provide initial support for analysis of metabolomics and shotgun metagenomics data. This marks

the potential of QIIME 2 to serve not only as a marker gene analysis tool, but also a multi-dimensional and powerful data science platform that can be rapidly adapted to analyze diverse microbiome features.

QIIME 2 provides many new interactive visualization tools (Figure 1), facilitating exploratory analyses and result reporting. QIIME 2 View (https://view.qiime2.org) is a unique new service (see Online Methods) that allows users to securely share and interact with results without installing QIIME 2. The QIIME 2 visualizations presented in Figure 1 are provided in Supplementary File 1 for readers to interact with using QIIME 2 View.

Reproducibility, transparency, and clarity of microbiome data science are guiding principles in the QIIME 2 design. Toward this end, it includes a decentralized data provenance tracking system: details of all analysis steps with references to intermediate data are automatically stored in the results. Users can thus retrospectively determine exactly how any result was generated (Figure 2). QIIME 2 also detects corrupted results, indicating that provenance is no longer reliable and the results no longer contain information enabling reproducibility. Provenance of the visualizations presented in Figure 1 can be interactively reviewed by loading the contents of Supplementary File 1 with QIIME 2 View, providing far more detailed information than can typically be provided in Methods text. QIIME 2 results are additionally semantically typed (Figure 2) and actions indicate acceptable input types, clarifying the data that actions should be applied to and making complex workflows less error-prone.

Finally, QIIME 2 provides a software development kit (see https://dev.qiime2.org) that can be used to integrate it as a component of other systems (e.g., such as Qiita[21] or Illumina BaseSpace) and to develop interfaces targeted toward users with different levels of computational sophistication (Figure S2). QIIME 2 provides the QIIME 2 Studio graphical user interface and QIIME 2 View, interfaces designed for end-user biologists, clinicians, and policy makers; the QIIME 2 application programming interface, designed for data scientists who want to automate workflows or work interactively in Jupyter Notebooks; and q2cli and q2cwl, providing a command line interface and Common Workflow Language[22] wrappers for QIIME 2, designed for high-performance computing experts.

Advances in microbiome research promise to improve many aspects of our health and our world, and QIIME 2 will help drive those advances by enabling accessible, community-driven microbiome data science.

Figures and figure captions

Figure 1: QIIME 2 provides many interactive visualization tools. Interactive versions of these screen captures are available in Supplementary File 1 and at https://github.com/qiime2/paper1. Detailed descriptions and methods are included in Online Methods. (A) Unweighted UniFrac PCoA plot containing 37,680 samples, illustrating the scalability of QIIME 2. Colors indicate sample type as described by the Earth Microbiome Project ontology (EMPO). (B) A feature volatility plot illustrating change in Bifidobacterium abundance over time in breast-fed and formula-fed infants. Temporally interesting features can be interactively discovered with this visualization. (C) Interactive taxonomic composition bar plot illustrating phylum-level composition of microbial mat samples collected along a temperature gradient in Yellowstone National Park Hot Spring outflow channels (Steep Cone Geyser). The many interactive controls available in this plot vastly reduce the burden of exploratory analysis over QIIME 1. (D) Molecular cartography of the human skin surface. Colored spots represent the abundance of the small molecule cosmetic, sodium laureth sulfate, on the human skin. Sample data can be interactively visualized on 3D models, supporting the discovery of spatial patterns.

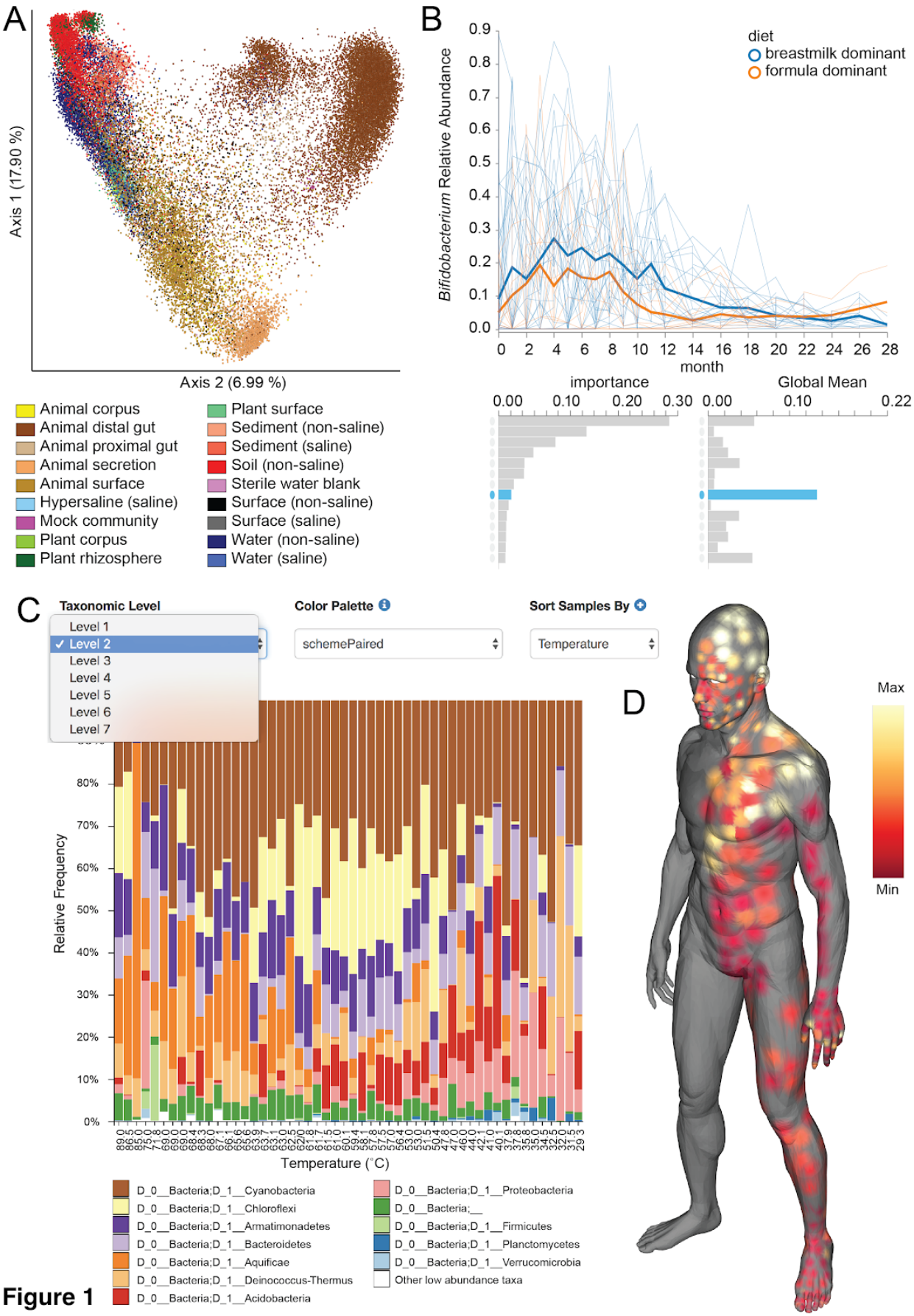
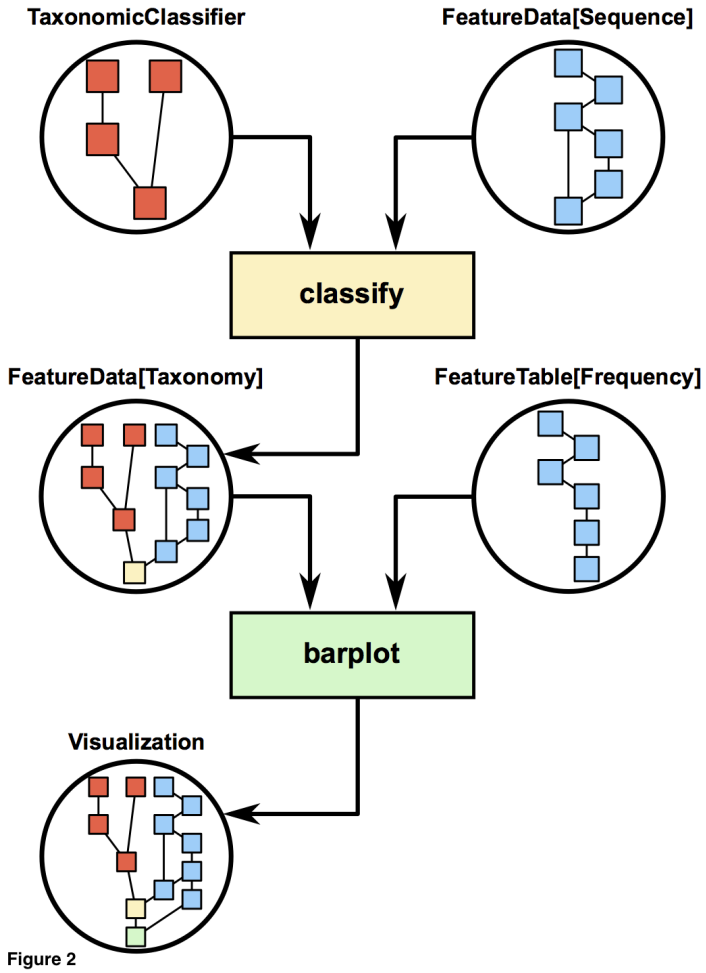

A
Axis 1 (17.90 %)
Axis 2 (6.99 %)
Animal corpus
Animal distal gut
Animal proximal gut
Animal secretion
Animal surface
Hypersaline (saline)
Mock community
Plant corpus
Plant rhizosphere
Plant surface
Sediment (non-saline)
Sediment (saline)
Soil (non-saline)
Sterile water blank
Surface (non-saline)
Surface (saline)
Water (non-saline)
Water (saline)
B
Bifidobacterium Relative Abundance
diet
breastmilk dominant
formula dominant
month
importance
Global Mean
C
Taxonomic Level
Level 1
Level 2
Level 3
Level 4
Level 5
Level 6
Level 7
Color Palette
schemePaired
Sort Samples By
Temperature
Relative Frequency
Temperature (˚C)
D_0__Bacteria;D_1__Cyanobacteria
D_0__Bacteria;D_1__Chloroflexi
D_0__Bacteria;D_1__Armatimonadetes
D_0__Bacteria;D_1__Bacteroidetes
D_0__Bacteria;D_1__Aquificae
D_0__Bacteria;D_1__Deinococcus-Thermus
D_0__Bacteria;D_1__Acidobacteria
D_0__Bacteria;D_1__Proteobacteria
D_0__Bacteria;__
D_0__Bacteria;D_1__Firmicutes
D_0__Bacteria;D_1__Planctomycetes
D_0__Bacteria;D_1__Verrucomicrobia
Other low abundance taxa
D
Max
Min

Figure 1

Figure 2: QIIME 2 iteratively records data provenance, ensuring bioinformatics reproducibility. This simplified diagram illustrates the automatically tracked information about the creation of the taxonomy barplot presented in Figure 1c. QIIME 2 results (circles) contain network diagrams illustrating the data provenance stored in the result. Actions (quadrilaterals) are applied to QIIME 2 results and generate new results. Arrows indicate flow of QIIME 2 results through actions. TaxonomicClassifier and FeatureData[Sequence] inputs contain independent provenance (red and blue, respectively) and are provided to a classify action (yellow), which taxonomically annotates sequences. The result of the classify action, a FeatureData[Taxonomy] result, integrates the provenance of both inputs with the classify action. This result is then provided to the barplot action with a FeatureTable[Frequency] input, which shares some provenance with the FeatureData[Sequence] input as they were generated from the same upstream analysis. The resulting Visualization (Figure 1c), has the complete data provenance and correctly identifies shared processing of inputs. An interactive and complete version of this provenance graph (as well as those for other Figures 1 panels) can be accessed through Supplementary File 1.



Code availability

QIIME 2 is open source and free for all use, including commercial. It is licensed under the BSD 3-clause license. Source code is available at https://github.com/qiime2.

Data availability

Data for the analyses presented in Figure 1 are available as follows: (a) Earth Microbiome Project data was obtained from ftp://ftp.microbio.me/emp/release1, and the American Gut Project (AGP) data was obtained from Qiita (http://qiita.microbio.me) study 10317. (b) Sequence data are available in Qiita under study id 10249 and EBI under accession number ERP016173. (c) Sequence data are available in Qiita under study id 925 and EBI under accession number ERP022167. (d) Data are available in the q2-ili GitHub repository (https://github.com/biocore/q2-ili). Interactive versions of the Figure 1 visualizations can be accessed at https://github.com/qiime2/paper1.

Acknowledgements

QIIME 2 development was primarily funded by NSF Award 1565100 to JGC and RK. Partial support was also provided from the following grants: NIH U54CA143925 (JGC, TP) and U54MD012388 (JGC, TP); grants from the Alfred P. Sloan Foundation (JGC, RK); ERC-STG project MetaPG (NS); Strategic Priority Research Program of the Chinese Academy of Sciences QYZDB-SSW-SMC021 (YB); from the Australian National Health and Medical Research Council APP1085372 (GAH, JGC, Von Bing Yap and RK); and from Natural Sciences and Engineering Research Council (NSERC) to DLG. Thanks to the Yellowstone Center for Resources for research permit #5664 to JRS for Yellowstone access and sample collection. We would like to thank the users of QIIME 1 and 2, whose invaluable feedback has shaped QIIME 2.

Author contributions

EB, JRR, MRD, NAB, YB, JEB, CJB, AMC, EC, RD, CFE, MEs, JMG, DLG, AKJ, KBK, STK, IK, TK, JL, YL, AVM, JLM, LFN, SBO, DP, AS, SJS, ADS, LRT, PJTo, PJTu, SU, FV, JW, RK, and JGC developed documentation, educational materials, and/or user/developer support content. EB, JRR, MRD, NAB, RK, and JGC wrote the manuscript; all authors assisted with revision of the manuscript. EB, JRR, MRD, NAB, and JGC designed and developed the QIIME 2 framework. DMD, RL, EL, SCM, RS, JRS, WW, CHDW, and RK contributed data used in the manuscript and/or testing of the QIIME 2. CA, CTB, EC, PCD, SH, PK, EL, TP, RS, EV, YW, and RK contributed to the design of analytic methods. EB, JRR, MRD, NAB, GAA, HA, EJA, MA, FA, KB, AB, BJC, JC, GMD, CD, MEr, JF, AG, KG, JG, BH, HH, CH, GH, SJ, LJ, BK, CRK, DK, JK, MGIL, CL, MM, CM, BM, DM, LJM, JM, ATN, JAN, SLP, MLP, EP, LBR, AR, MSR, PR, NS, MS,

PT, AT, JJJV, YV, MV, MW, KCW, ADW, ZZX, JRZ, YZ, and JGC contributed software to QIIME 2 plugins, interfaces, framework, and/or build and test systems.

Main text references

1. Smith, M.I. et al. Science **339**, 548–554 (2013).
2. Gopalakrishnan, V. et al. Science **359**, 97–103 (2018).
3. Gehring, C.A., Sthultz, C.M., Flores-Rentería, L., Whipple, A.V. & Whitham, T.G. Proc. Natl. Acad. Sci. U. S. A. **114**, 11169–11174 (2017).
4. Lee, K., Pletcher, S.D., Lynch, S.V., Goldberg, A.N. & Cope, E.K. Front. Cell. Infect. Microbiol. **8**, 168 (2018).
5. Metcalf, J.L. et al. Science **351**, 158–162 (2016).
6. Rubin, R.L. et al. Ecol. Appl. **28**, 1594–1605 (2018).
7. Pineda, A., Kaplan, I. & Bezemer, T.M. Trends Plant Sci. **22**, 770–778 (2017).
8. Kapono, C.A. et al. Sci. Rep. **8**, 3669 (2018).
9. Barr, T. et al. Gut Microbes 1–44 (2018).
10. Callahan, B.J. et al. Nat. Methods (2016).doi:10.1038/nmeth.3869
11. Amir, A. et al. mSystems **2**, (2017).
12. Bokulich, N.A. et al. Microbiome **6**, 90 (2018).
13. Janssen, S. et al. mSystems **3**, e00021–18 (2018).
14. Bokulich, N. et al. bioRxiv 223974 (2017).doi:10.1101/223974
15. Bokulich, N. et al. bioRxiv 306167 (2018).doi:10.1101/306167
16. Sedio, B.E., Rojas Echeverri, J.C., Boya P, C.A. & Wright, S.J. Ecology **98**, 616–623 (2017).
17. Wang, M. et al. Nat. Biotechnol. **34**, 828–837 (2016).
18. Hillmann, B. et al. bioRxiv 320986 (2018).doi:10.1101/320986
19. Truong, D.T. et al. Nat. Methods **12**, 902–903 (2015).
20. Langille, M.G.I. et al. Nat. Biotechnol. **31**, 814–821 (2013).
21. Gonzalez, A. et al. Nat. Methods **15**, 796–798 (2018).
22. Amstutz, P. et al. (2016).doi:10.6084/m9.figshare.3115156.v2

Author affiliations

[1]Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ, USA. [2]Metabolic Epidemiology Branch, National Cancer Institute, Rockville, MD, USA. [3]Department of Computer Science and Engineering, University of Minnesota, Minneapolis, Minnesota, USA. [4]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA, USA. [5]Department of Population Health and Reproduction, University of California, Davis, CA, USA. [6]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA. [7]Center for Microbiome Informatics and Therapeutics, Massachusetts Institute of Technology, Cambridge, MA, USA. [8]University of Copenhagen, Faculty of Health and Medical Sciences, Novo Nordisk Foundation Center for Basic Metabolic Research, Copenhagen, Denmark. [9]Centre for Integrative Biology, University of Trento, Trento, Italy. [10]State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China. [11]Centre of Excellence for Plant and Microbial Sciences (CEPAMS), Institute of

Genetics and Developmental Biology, Chinese Academy of Sciences & John Innes Centre, Beijing, China. [12]University of Chinese Academy of Sciences, Beijing, China. [13]Department of Microbiology and Immunology, University of California, San Francisco, CA, USA. [14]Division of Gastroenterology and Nutrition, Children's Hospital of Philadelphia, Philadelphia, PA, USA. [15]Hepatology, Children's Hospital of Philadelphia, Philadelphia, PA, USA. [16]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark. [17]Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA, USA. [18]Department of Population Health & Pathobiology, North Carolina State University, Raleigh, NC, USA. [19]Bioinformatics Research Center, North Carolina State University, Raleigh, NC, USA. [20]Collaborative mass spectrometry innovation center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, San Diego, CA, USA. [21]Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, USA. [22]Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada. [23]Irving K. Barber School of Arts and Sciences, University of British Columbia, Kelowna, British Columbia, Canada. [24]A. Watson Armour III Center for Animal Health and Welfare, Aquarium Microbiome Project, John G. Shedd Aquarium, Chicago, IL, USA. [25]Department of Biology, University of British Columbia Okanagan, Okanagan, BC, Canada. [26]Computational Bioscience Graduate Program, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado, USA. [27]Department of Medicine, Division of Biomedical Informatics and Personalized Medicine, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado, USA. [28]Irving K. Barber School of Arts and Sciences, Department of Biology, The University of British Columbia, Kelowna, BC, Canada. [29]Department of Medicine, The University of British Columbia, Kelowna, BC, Canada. [30]Department of Pediatrics, University of California San Diego, La Jolla, CA, USA. [31]Center for Microbial Ecology, Michigan State University, East Lansing, MI, USA. [32]Department of Computer Science and Engineering, University of Minnesota, Minneapolis, MN, USA. [33]Stanford University, Statistics Department, Palo Alto, CA, USA. [34]Department of Computer Science and Engineering, University of California San Diego, La Jolla, CA, USA. [35]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA. [36]Broad Institute of MIT and Harvard, Cambridge, MA, USA. [37]Research School of Biology, The Australian National University, Canberra, ACT, Australia. [38]Department of Pediatric Oncology, Hematology and Clinical Immunology, Heinrich-Heine University Dusseldorf, Dusseldorf, Germany. [39]Department of Family Medicine and Public Health, University of California San Diego, La Jolla, CA, USA. [40]College of Pharmacy, Sookmyung Women's University, Seoul, Republic of Korea. [41]San Diego State University, Department of Biology, San Diego, CA, USA. [42]Biotechnology Institute, University of Minnesota, Saint Paul, MN, USA. [43]Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, USA. [44]Department of Pediatrics, University of California San Diego, La Jolla, CA,. [45]Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada. [46]Science Education, Howard Hughes Medical Institute, Ashburn, VA, USA. [47]Department of Microbiome Science, Max Planck Institute for Developmental Biology, Tübingen, Germany. [48]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA. [49]Department of Medicine, Division of Biomedical Informatics and Personalized Medicine, University of Colorado Denver Anschutz Medical Campus, Aurona, CO, USA.

[50]Department of Computer Science & Engineering, University of California San Diego, La Jolla, CA, USA. [51]Department of Statistics, University of Washington, Seattle, WA, USA. [52]Department of Animal Science, Colorado State University, Fort Collins, CO, USA. [53]Irving K. Barber School of Arts and Sciences, Unit 2 (Biology), University of British Columbia, Kelowna, BC, Canada. [54]Mountain View, Google LLC, Mountain View, CA, USA. [55]Center for Microbiome Innovation, University of California San Diego, La Jolla, CA, USA. [56]School of Information Studies, Syracuse University, Syracuse, NY, USA. [57]School of STEM, University of Washington Bothell, Bothell, WA, USA. [58]Department of Biological Sciences, Webster University, St Louis, MO, USA. [59]Agricultural Research Service, Genomics and Bioinformatics Research Unit, United States Department of Agriculture, Gainesville, FL, USA. [60]College of Medicine, Department of Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, AR, USA. [61]Computational Bioscience Program, University of Colorado Denver Anschutz Medical Campus, Aurona, CO, USA. [62]Department of Civil and Environmental Engineering, Colorado School of Mines, Golden, CO, USA. [63]Department of Biological Sciences and Northern Gulf Institute, University of Southern Mississippi, Hattiesburg, Mississippi, USA. [64]Ocean Chemistry and Ecosystems Division, Atlantic Oceanographic and Meteorological Laboratory, National Oceanic and Atmospheric Administration, La Jolla, CA, USA. [65]Department of Biology, San Diego State University, San Diego, CA, USA. [66]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA. [67]Division of Biological Sciences, University of California San Diego, San Diego, CA, USA. [68]Department of Microbiology and Immunology, University of California San Francisco, San Francisco, CA, USA. [69]Quantitative and Systems Biology Graduate Program, University of California Merced, Merced, CA, USA. [70]Bioinformatics Group, Wageningen University, Wageningen, The Netherlands. [71]Department of Mathematics, University of Arizona, Tucson, AZ, USA. [72]National Laboratory Service, Environment Agency, Starcross, UK. [73]College of Agriculture and Life Sciences, University of Florida, Gainesville, FL, USA. [74]Department of Biostatistics, University of Washington, Seattle, WA, USA. [75]University of Washington Bothell, School of STEM, Division of Biological Sciences, Bothell, WA, USA. [76]Merck & Co. Inc., Kenilworth, NJ, USA. [77]Department of Computer Science and Engineering, University of California San Diego, La Jolla, California, USA

QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science

Online methods

Overview of QIIME 2

We provide a high-level overview of the QIIME 2 system. `Monospace font` is used to indicate literal terms, such as objects defined by QIIME 2. The most up-to-date information on these topics is available in the QIIME 2 developer documentation at https://dev.qiime2.org.

There are three core components of the QIIME 2 system architecture: the **framework**, the **interfaces**, and the **plugins** (Figure S1). **Interfaces** are responsible for turning user intent into action. **Plugins** define all domain-specific functionality. The most important restriction of the architecture, which is evident in Figure S1, is that interfaces and plugins do not communicate directly with one another -- that communication is always mediated by the **framework**. In other words, the domain-specific analytic functionality (defined in plugins) is entirely decoupled from how users interface with the system (defined in interfaces). This important constraint allows multiple kinds of interfaces to be dynamically generated, and as a result QIIME 2 can adapt its user interface to the audience and the task at hand (Figure S2).

Third-party developers can create and distribute both plugins and interfaces for QIIME 2 independently of the core QIIME 2 development group, which forms the basis for our goal of decentralized QIIME 2 development (see https://library.qiime2.org and https://dev.qiime2.org). By removing our team as a bottleneck in developers delivering their new methods to users through QIIME 2, microbiome research can advance more quickly by ensuring that QIIME 2 users can have access to the latest microbiome analytic methods as quickly as bioinformatics researchers and developers can distribute them. This model makes QIIME 2 (and tools that build on it, such as Qiita) a platform for microbiome data science, not only a tool for a specific type of analysis. Since plugins conform to requirements specified by the framework, framework features such as data provenance tracking and multiple interface support are available for all plugins without the plugin developers having to be aware of these features.

In the terminology of QIIME 2, an **`Action`** creates a **`Result`**, and a `Result` can be either an **`Artifact`** or a **`Visualization`**. An `Artifact` is data generated by one or more QIIME 2 `Actions` which can be used as the input to other QIIME 2 `Actions`. A `Visualization` on the other hand is a terminal output of QIIME 2, which could be an interactive visualization (as in the Figure 1 examples) or any other result that is intended to be consumed by humans (not by a QIIME 2 `Action`). QIIME 2 assigns version 4 universally unique identifiers (UUIDs) to each execution of an `Action`, and to all `Results`.

QIIME 2 stores information about the series of Actions that led to a Result, along with information about the environment (including versions of all QIIME 2 packages and other Python dependencies) where each Action was executed, and the data itself. We refer to this process as data provenance tracking, or simply provenance tracking. We did not want to create new bioinformatics file formats to support the storage of data provenance, so QIIME 2 Results are instead stored as zip files containing a data directory that contains only the data in a relevant format (e.g., fasta or fastq for sequence data, newick for phylogenetic trees, etc), plus QIIME-2-specific metadata in other directories (such as provenance). These files use the extension .qza (for QIIME zipped artifact) or .qzv (for QIIME zipped visualization), but they are standard zip files that could be unzipped using common tools such as unzip, WinZip, or 7-Zip. Additional motivations for the storage of QIIME 2 Results in these structured zip files include the ability to submit as supplementary material to journals (the extension can simply be changed to .zip if required by the journal); "future-proofing" of QIIME 2 Results (even if QIIME 2 weren't used anymore, Results could still be accessed by unzipping .qza or .qzv files - see Extracting data from QIIME 2 archives below); zip files contain an index, allowing them to be inspected for certain information without uncompressing them; and data are always compressed, facilitating data sharing. Because provenance is stored alongside data in .qza and .qzv files, provenance tracking is decentralized (no QIIME 2 server or database needs to be keeping track of this information) ensuring that information on how data was generated will not be lost as long as the data is intact. However, assignment of UUIDs to all QIIME 2 `Results` (as described above) lends itself to managing these data in a database if that is desired.

Another important component of QIIME 2 is its **semantic type system**. All `Artifacts` used in QIIME 2 are annotated with a semantic description of their type which conveys the meaning of the data. Semantic types differ from data types (how data is represented in memory) or file formats (how data is stored on disk), and allow QIIME 2 to constrain the composition of multiple actions to only those combinations which are semantically meaningful without needing to consider the specific file formats or data types. This also makes it possible to determine what Actions could be applied (and in what order) to generate a given Artifact from some set of input Artifacts. For example, phylogenetic trees in QIIME 2 can be either rooted or unrooted, and these two concepts are represented by the semantic types `Phylogeny[Rooted]` and `Phylogeny[Unrooted]`, respectively. QIIME 2 could support loading these into multiple different data types, including a scikit-bio `TreeNode` object or an ete3 `Tree` object. Both of these types are typically stored on disk in a newick-formatted file, but this format doesn't contain easily accessible information on whether the phylogeny is rooted or unrooted. Some QIIME 2 `Actions` can only generate a `Phylogeny[Unrooted]` (such as `fasttree`), and some other `Actions` only work on `Phylogeny[Rooted]`(such as `beta-phylogenetic`, which computes UniFrac distances). The semantic type system allows QIIME 2 to determine that the output of `fasttree` should not be directly provided as input to `beta-phylogenetic`, and to provide the user with that information prior to execution. This can help a researcher who is new to microbiome data science avoid using data incorrectly. This will also enable QIIME 2 to automatically assist users in identifying relevant workflows to generate desired data or further explore data they already have.

Due to recent advances in package management systems and bioinformatics package repositories (e.g., Anaconda, Bioconda[1], and Bioconductor[2]), QIIME 2 is straightforward to install.

QIIME 2 View

QIIME 2 View (https://view.qiime2.org) is a unique and novel contribution to the microbiome data science ecosystem that facilitates collaborative research. A user who has generated QIIME 2 visualizations can share those visualizations with a collaborator who can explore the results interactively without having QIIME 2 installed. QIIME 2 View achieves this simplified sharing of complex interactive visualizations through a novel combination of modern web browser APIs within a single-page application. It allows a user's browser to open and read `.qza` and `.qzv` files without the need to transfer the files over the network by utilizing a Service Worker to redirect HTTP requests directly into the archive which is retained on the user's computer. This approach of data unpackaging and local command execution makes QIIME 2 View well suited to cases where the results are unpublished or contain private information (that information will not be stored on any remote server). It is also possible to create "smart" URLs which automatically fetch content from a CORS-enabled web-server (for example, see the links in the README.md file at https://github.com/qiime2/paper1). This makes it very simple to share a single link with a collaborator that will be resolved into a fully interactive visualization on a user's computer automatically. The structured nature of the archive format (Figure S3) also allows QIIME 2 View to generate a dynamic provenance visualization, summarizing the entire provenance of the archive in question.

Extracting data from QIIME 2 archives

QIIME 2 `.qza` and `.qzv` files are zip file containers with a defined internal directory structure. It's very easy to get data out in the canonical formats (Figure S3). If QIIME 2 and the q2cli command line interface are installed, this can be achieved using the `qiime tools export` command. If QIIME 2 is not installed, this can be achieved using standard decompression utilities such as `unzip`, WinZip, or 7-zip. We illustrate how this can be achieved using `unzip` on macOS. This can similarly be achieved on Windows or Linux. We illustrate this here to further future-proof QIIME 2 `Results` - even if the QIIME 2 documentation were no longer accessible, users could follow these steps to access QIIME 2 `Results`.

First, obtain a `.qza` file. Here we use the `FeatureData[Sequence]` artifact generated during the QIIME 2 Moving Pictures tutorial.

```
$ wget https://docs.qiime2.org/2018.8/data/tutorials/moving-pictures/rep-seqs.qza
```

Next, unzip that file with the macOS (or Linux) `unzip` program. This will create a new directory. The name of that directory will be the UUID of the artifact being unzipped, in this case `8dc793b8-7284-462a-8578-6370ffccebdc`.

```
$ unzip rep-seqs.qza
Archive:  rep-seqs.qza
  inflating: 8dc793b8-7284-462a-8578-6370ffccebdc/metadata.yaml
  inflating: 8dc793b8-7284-462a-8578-6370ffccebdc/VERSION
  inflating: 8dc793b8-7284-462a-8578-6370ffccebdc/provenance/metadata.yaml
  inflating: 8dc793b8-7284-462a-8578-6370ffccebdc/provenance/citations.bib
  inflating: 8dc793b8-7284-462a-8578-6370ffccebdc/provenance/VERSION
  inflating:
8dc793b8-7284-462a-8578-6370ffccebdc/provenance/artifacts/bdaa3214-f883-4c8b-8db3-f6ea4910d724/me
tadata.yaml
  inflating:
8dc793b8-7284-462a-8578-6370ffccebdc/provenance/artifacts/bdaa3214-f883-4c8b-8db3-f6ea4910d724/ci
tations.bib
  inflating:
8dc793b8-7284-462a-8578-6370ffccebdc/provenance/artifacts/bdaa3214-f883-4c8b-8db3-f6ea4910d724/VE
RSION
  inflating:
8dc793b8-7284-462a-8578-6370ffccebdc/provenance/artifacts/bdaa3214-f883-4c8b-8db3-f6ea4910d724/ac
tion/action.yaml
  inflating:
8dc793b8-7284-462a-8578-6370ffccebdc/provenance/artifacts/7097fc98-ad5f-4b9d-a33e-39cd36857a0d/me
tadata.yaml
  inflating:
8dc793b8-7284-462a-8578-6370ffccebdc/provenance/artifacts/7097fc98-ad5f-4b9d-a33e-39cd36857a0d/ci
tations.bib
  inflating:
8dc793b8-7284-462a-8578-6370ffccebdc/provenance/artifacts/7097fc98-ad5f-4b9d-a33e-39cd36857a0d/VE
RSION
  inflating:
8dc793b8-7284-462a-8578-6370ffccebdc/provenance/artifacts/7097fc98-ad5f-4b9d-a33e-39cd36857a0d/ac
tion/action.yaml
  inflating:
8dc793b8-7284-462a-8578-6370ffccebdc/provenance/artifacts/7097fc98-ad5f-4b9d-a33e-39cd36857a0d/ac
tion/barcodes.tsv
  inflating: 8dc793b8-7284-462a-8578-6370ffccebdc/provenance/action/action.yaml
  inflating: 8dc793b8-7284-462a-8578-6370ffccebdc/data/dna-sequences.fasta
```

The last entry that is unzipped in this example is `data/dna-sequences.fasta`. All other directories and files are QIIME 2 specific metadata (such as information about the semantic type of the artifact and the data provenance). If you're only interested in the sequence data, you can safely ignore all of that information. The `data/dna-sequences.fasta` file is a typical fasta file containing sequence identifiers and sequences. The first four lines of this file can be viewed as follows:

```
$ head -4 8dc793b8-7284-462a-8578-6370ffccebdc/data/dna-sequences.fasta
>f352c1f1efecf483511c2270aabd0ae6
TACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTTGTAAGACAGAGGTGAAATCCCCGGGCT
CAACCTGGGAACTGCCTTTGTGACTGCAAGGCTG
>82e72255267397b777a1afd44ea22755
TACGGAGGATCCAAGCGTTATCCGGAATCATTGGGTTTAAAGGGTCCGTAGGCGGTTTAGTAAGTCAGTGGTGAAAGCCCATCGCT
CAACGGTGGAACGGCCATTGATACTGCTAGACTT
```

436

QIIME 2 user and developer community

QIIME 2 officially succeeded QIIME 1 (http://www.qiime.org)[3] in January of 2018, and has
438 developed an engaged user base and community. As of this writing there are over 1980 active users (users who have performed an action, such as creating or liking a post) on the QIIME 2
440 Forum; over 3000 monthly downloads of QIIME 2 from Anaconda; over 8000 unique visitors to the QIIME 2 Forum according to Google Analytics; and our multi-day workshops are frequently
442 filled to capacity (https://workshops.qiime2.org). QIIME 2 is also being adopted by third-party bioinformatics developers who are choosing to make their software accessible through plugins,
444 and who are motivated to develop for QIIME 2 by access to its integrated provenance tracking, multiple interfaces, standardization of data types provided by the semantic type system, large
446 user community, and supportive developer community.

A core goal of QIIME 2 is to cultivate a diverse and inclusive community of scientists, software
448 engineers, statisticians, educators, students, and other microbiome stakeholders who are openly sharing methods, data, and knowledge to advance microbiome research.

Supplementary figures, files, and captions

Figure S1. Schematic diagram of the QIIME 2 system. Interfaces define how users interact with the system; plugins define all domain-specific functionality; and the framework mediates communication between plugins and interfaces, and performs core functionality such as provenance tracking. Arrows indicate dependencies. Interfaces interact only with the `qiime2.sdk` submodule, while plugins interact only with the `qiime2.plugin` submodule. This design has led to a system that is readily extended by third-party plugin and interface developers.

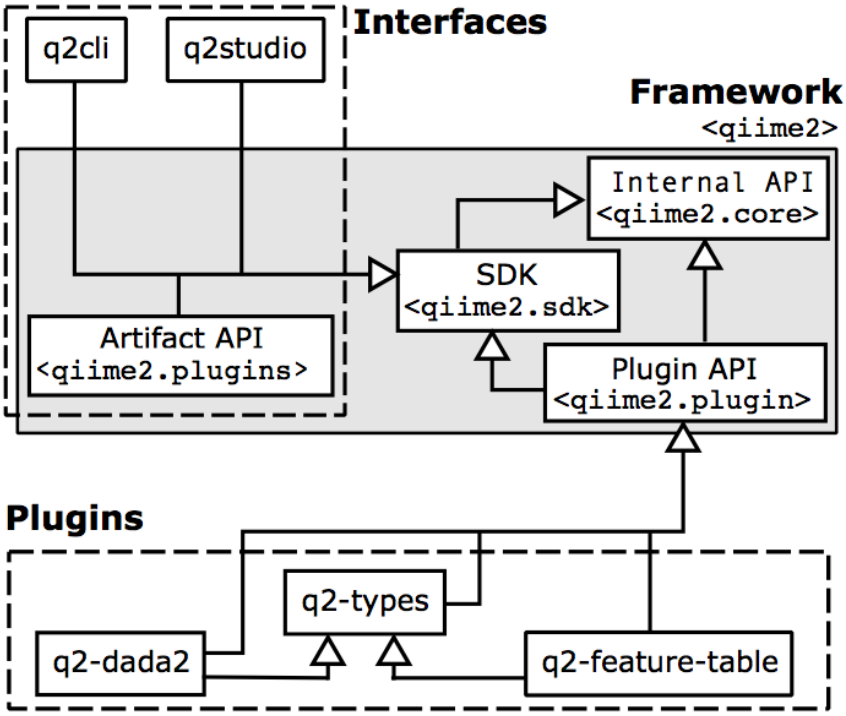


Figure S2. QIIME 2 is interface agnostic. The full suite of QIIME 2 functionality is useful to and usable by researchers ranging widely in their computational sophistication, a major advantage over technologies such as QIIME 1 that provide a single interface. (a) Users wanting to view QIIME 2 results or data provenance can use QIIME 2 View without installing QIIME 2, which is convenient for lead investigators, clinicians, or policy makers who may want to explore interactive visualizations generated by others. (b) Researchers who prefer graphical interfaces can use QIIME 2 Studio, our prototype graphical interface. This is convenient for users without command line or programming skills. (c) Power users (e.g., who are comfortable with the Linux command line and/or regularly work on institutional computer clusters), can use QIIME 2 through the command line interface, q2cli. (d) "Data scientists" (e.g., users who are programmers, who work in Jupyter Notebooks, or who are interested in automating QIIME 2 workflows), can use QIIME 2 through the Python 3 "artifact API".

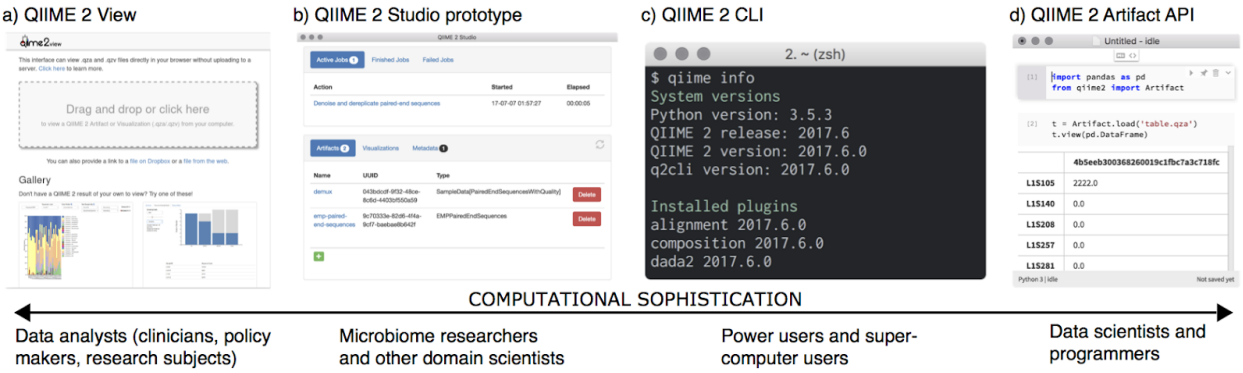
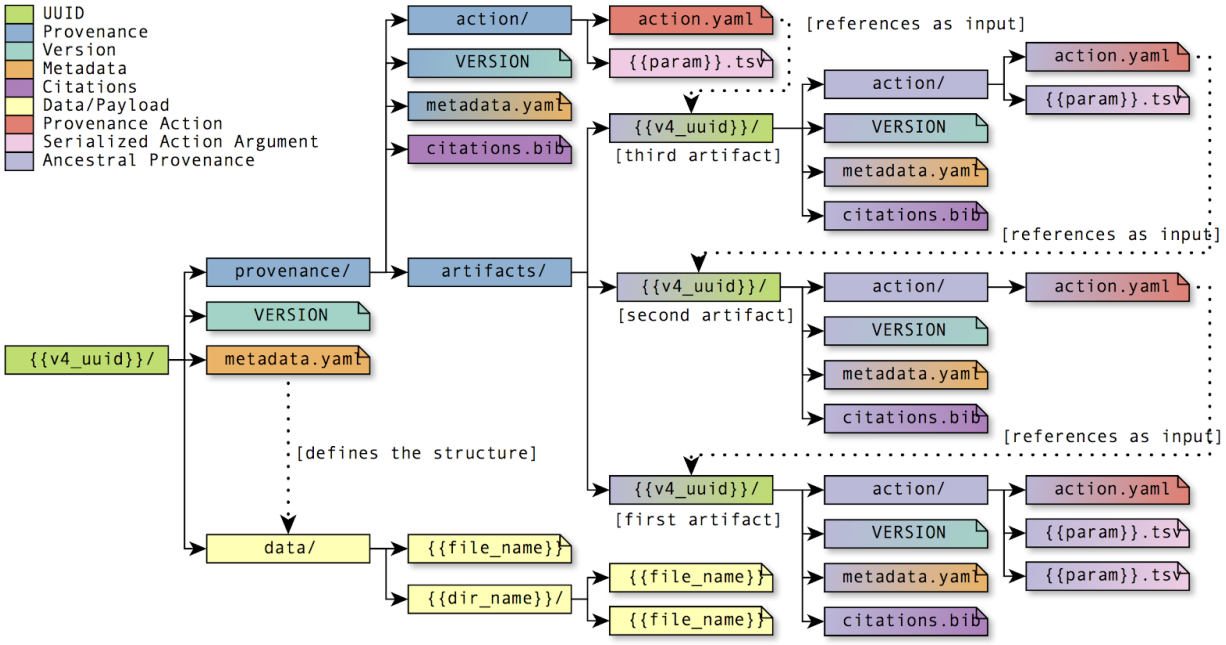


Figure S3. Anatomy of a QIIME 2 `Archive` (i.e., .qza or .qzv file). QIIME 2 stores data in a directory structure called an `Archive`. These archives are zipped to make moving data convenient. The directory structure has a single root directory named with a UUID which serves as the identity of the archive.



Supplementary File 1 contains the QIIME 2 `.qzv` files corresponding to **Figure 1a-d**. These are also accessible at https://github.com/qiime2/paper1 and can be viewed using QIIME 2 View (https://view.qiime2.org) where readers can interact with the results, and explore the methods used to generate them (see the Provenance tab after loading a `.qzv` file with QIIME 2 View). We describe the methods used to generate each of these visualizations here, and this information can be compared to the data provenance which contains far more detail that is possible or desirable to include in supplementary methods text.

a-pcoa.qzv: Emperor PCoA plot presenting a meta-analysis of the first release of the Earth Microbiome Project (EMP)[4] and the first release of the American Gut Project (AGP)[5]. The EMP data was obtained from ftp://ftp.microbio.me/emp/release1, and the AGP data was obtained from Qiita study 10317 for the set of samples used in its publication (samples described in the AGP supplemental data accession table). Both projects were downloaded and imported into QIIME 2 as BIOM tables[6]. The contingency matrices were combined, filtered for blooms[7], rarefied at an even depth (1000 sequences per sample), and compared using the unweighted UniFrac[8] metric. Lastly the samples were projected into a small dimensional space using principal coordinates analysis and visualized using Emperor[9]. The samples were colored according to the Earth Microbiome Project Ontology[4].

b-feature-volatility.qzv: Data were generated on five sequencing runs of V4 16S rRNA gene amplicons from the ECAM study[10]. Forward reads were imported separately in `EMPSingleEndDirFmt` format, demultiplexed with q2-demux's `emp_single` method, and denoised using q2-dada2's `denoise_single` method (`trunc_len`=150, other parameters used default values)[11]. Denoised feature tables and sequences were merged using q2-feature-table's `merge` and `merge-seqs` methods, respectively. q2-feature-table's `filter-samples` method was used to remove samples with fewer than 2000 sequences, and to perform metadata-based filtering to retain only children's samples. A naive Bayes taxonomy classifier was trained on the Greengenes[12] reference sequences (clustered at 99% similarity) using q2-feature-classifier's `fit-classifier-naive-bayes` method[13]. This classifier was used to taxonomically classify the ECAM ASVs using q2-feature-classifier's `classify-sklearn` method[13]. ASVs were collapsed based on genus-level taxonomy using q2-taxa's collapse method. Temporally predictive features were identified using q2-longitudinal's `feature-volatility` pipeline[14] using default parameters. Data contained in this artifact have been described in a previous publication[14].

c-taxa-barplot.qzv: Data were imported into QIIME 2 as multiplexed 2x150 MiSeq reads and demultiplexed. DADA2[11] was applied to single-end reads (as approximately 30% of reads failed to join due to the relatively short sequence length) with no trimming of reads. Taxonomy was assigned to the resulting amplicon sequence variants (ASVs) against the Silva version 132 99% OTUs (trimmed to the 515F/806R region of the 16S) using q2-feature-classifier's `classify-sklearn` method[13].

d-ili-plot.qzv: The primary files for this visualization are a stereolithography file (STL) and a sample metadata file with a mapping between samples and the spatial coordinates (`x`, `y` and `z`). Both files were obtained from `ili's GitHub page[15,16]. The comma-separated file was converted into a to a tab-separated format (to make it compatible with QIIME 2).

Online methods references

1. Grüning, B. et al. Nat. Methods **15**, 475–476 (2018).
2. Huber, W. et al. Nat. Methods **12**, 115–121 (2015).
3. Caporaso, J.G. et al. Nat. Methods **7**, 335–336 (2010).
4. Thompson, L.R. et al. Nature **551**, 457–463 (2017).
5. McDonald, D. et al. mSystems **3**, e00031–18 (2018).
6. McDonald, D. et al. Gigascience **1**, 7 (2012).
7. Amir, A. et al. mSystems **2**, (2017).
8. Lozupone, C. & Knight, R. Appl. Environ. Microbiol. **71**, 8228–8235 (2005).
9. Vázquez-Baeza, Y., Pirrung, M., Gonzalez, A. & Knight, R. Gigascience **2**, 16 (2013).
10. Bokulich, N.A. et al. Sci. Transl. Med. **8**, 343ra82 (2016).
11. Callahan, B.J. et al. Nat. Methods (2016).doi:10.1038/nmeth.3869
12. McDonald, D. et al. ISME J. **6**, 610–618 (2012).
13. Bokulich, N.A. et al. Microbiome **6**, 90 (2018).
14. Bokulich, N. et al. bioRxiv 223974 (2017).doi:10.1101/223974
15. Protsyuk, I. et al. Nat. Protoc. **13**, 134–154 (2018).
16. Bouslimani, A. et al. Proc. Natl. Acad. Sci. U. S. A. **112**, E2120–9 (2015).