DISCo-microbe: Design of an identifiable synthetic community of microbes

Dana L Carper Corresp., 1 , **Travis J Lawrence** 1 , **Alyssa A Carrell** 1, 2 , **Dale A Pelletier** 1 , **David J Weston** Corresp. 1

[1] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, United States

[2] Bredesen Center for Interdisciplinary Research and Graduate Education, University of Tennessee - Knoxville, Knoxville, Tennessee, United States

Corresponding Authors: Dana L Carper, David J Weston
Email address: carperdl@ornl.gov, westondj@ornl.gov

Background

Microbiomes are extremely important for their host organisms, providing many vital functions and extending their hosts' phenotypes. Natural studies of host-associated microbiomes can be difficult to interpret due to the high complexity of microbial communities, which hinders our ability to track and identify individual members along with the many factors that structure or perturb those communities. For this reason, researchers have turned to synthetic or constructed communities in which the identities of all members are known. However, due to the lack of tracking methods and the difficulty of creating a more diverse and identifiable community that can be distinguished through next-generation sequencing, most such *in vivo* studies have used only a few strains.

Results

To address this issue, we developed DISCo-microbe, a program for the design of an identifiable synthetic community of microbes for use in *in vivo* experimentation. The program is composed of two modules; (1) create, which allows the user to generate a highly diverse community list from an input DNA sequence alignment using a custom nucleotide distance algorithm, and (2) subsample, which subsamples the community list to either represent a number of grouping variables, including taxonomic proportions, or to reach a user-specified maximum number of community members. As an example, we demonstrate the generation of a synthetic microbial community that can be distinguished through amplicon sequencing. The synthetic microbial community in this example consisted of 2340 members from a starting DNA sequence alignment of 10,000 16S rRNA sequences from the Ribosomal Database Project. We then subsampled the community list using taxonomic proportions to mimic a natural plant host–associated microbiome, ultimately yielding a diverse community of 853 members.

Conclusions

DISCo-microbe can create a highly diverse community list of microbes that can be distinguished through 16S rRNA gene sequencing, and has the ability to subsample (i.e., design) the community for the desired number of members and taxonomic proportions. Although developed for bacteria, the program allows for any alignment input from any taxonomic group, making it broadly applicable. The software and data are freely available from GitHub (https://github.com/dlcarper/DISCo-microbe) and Python Package Index (PYPI).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27898v1 | CC0 Open Access | rec: 13 Aug 2019, publ: 13 Aug 2019

DISCo-microbe: Design of an identifiable synthetic community of microbes

Authors: Dana L. Carper[1], Travis J. Lawrence[1], Alyssa A. Carrell[1,2], Dale A. Pelletier[1], and David J. Weston[1]

[1] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge TN, USA

[2] Bredesen Center for Interdisciplinary Research and Graduate Education, University of Tennessee, Knoxville, TN, USA

This manuscript has been authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (http://energy.gov/downloads/doe-public-access-plan).

Word count – Abstract 345; Background 741; Implementation 1600; Results and Discussion 802; Conclusion 154; Acknowledgement 51; Total excluding references 3652

Corresponding authors:

Dana L. Carper, Email: carperdl@ornl.gov, David J. Weston, Email: westondj@ornl.gov

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27898v1 | CC0 Open Access | rec: 13 Aug 2019, publ: 13 Aug 2019

Keywords: constructed community, microbiome, 16S rRNA, synthetic community, taxonomic profiling, *in vivo* experimentation

Abstract

Background

Microbiomes are extremely important for their host organisms, providing many vital functions and extending their hosts' phenotypes. Natural studies of host-associated microbiomes can be difficult to interpret due to the high complexity of microbial communities, which hinders our ability to track and identify individual members along with the many factors that structure or perturb those communities. For this reason, researchers have turned to synthetic or constructed communities in which the identities of all members are known. However, due to the lack of tracking methods and the difficulty of creating a more diverse and identifiable community that can be distinguished through next-generation sequencing, most such *in vivo* studies have used only a few strains.

Results

To address this issue, we developed DISCo-microbe, a program for the design of an identifiable synthetic community of microbes for use in *in vivo* experimentation. The program is composed of two modules; (1) create, which allows the user to generate a highly diverse community list from an input DNA sequence alignment using a custom nucleotide distance algorithm, and (2) subsample, which subsamples the community list to either represent a number of grouping variables, including taxonomic proportions, or to reach a user-specified maximum number of community members. As an example, we demonstrate the generation of a synthetic microbial community that can be distinguished through amplicon sequencing. The synthetic microbial community in this example consisted of 2340 members from a starting DNA sequence alignment

of 10,000 16S rRNA sequences from the Ribosomal Database Project. We then subsampled the community list using taxonomic proportions to mimic a natural plant host–associated microbiome, ultimately yielding a diverse community of 853 members.

Conclusions

DISCo-microbe can create a highly diverse community list of microbes that can be distinguished through 16S rRNA gene sequencing, and has the ability to subsample (i.e., design) the community for the desired number of members and taxonomic proportions. Although developed for bacteria, the program allows for any alignment input from any taxonomic group, making it broadly applicable. The software and data are freely available from GitHub (https://github.com/dlcarper/DISCo-microbe) and Python Package Index (PYPI).

Background

Multicellular eukaryotes live in association with complex communities of microorganisms (Zilber-Rosenberg & Rosenberg, 2008; Bordenstein & Theis, 2015; Rosenberg & Zilber-Rosenberg, 2016) that play important roles in host health and function (Huttenhower et al., 2012; Schlaeppi & Bulgarelli, 2015; Engel et al., 2016). Given the complexity of these systems and our inability to track and identify all members, it is often difficult to disentangle the factors influencing the structure and interactions among host-associated microbiomes. The development of synthetic model communities is a key strategy for addressing this issue (Busby et al., 2017). Next-generation sequencing of marker genes has demonstrated that both abiotic and biotic factors structure host-associated microbiomes (Spor, Koren & Ley, 2011; Huttenhower et al., 2012; Ofek-Lalzar et al., 2014; Adair & Douglas, 2017); however, the marker genes commonly used in these studies provide low taxonomic resolution, making it difficult to identify all microbes present in the community (Caporaso et al., 2011). Metagenomics studies provide insight into potential microbial function, but are not feasible for microbiomes within host tissues due to the presence of excess host DNA (Jiao et al., 2006; Feehery et al., 2013; Thoendel et al., 2016; Marotz et al., 2018). Accordingly, recent studies have utilized synthetic or simplified microbiome approaches to examine the drivers of host-associated microbiome assembly, interactions, and function (Bodenhausen et al., 2014; Lebeis et al., 2015; Niu et al., 2017). This approach involves adding previously characterized microbial strains to an axenic host organism, allowing for the investigation of colonization, shifts in community structure (Bodenhausen et al., 2014), microbe–microbe interactions, and host–microbe interactions. When such data are paired with genomic information, it becomes feasible to infer microbial strain metabolic potential.

Despite the increased use and prioritization of synthetic systems by the research community (Busby et al., 2017), we currently lack adequate methods for systematically designing a microbial community that is identifiable by common sequencing techniques.

Until now, synthetic communities have been constructed from a functional perspective or with limited strains. For example, some researchers have focused on functional assets (characteristics) of microbes to create a specific metabolic output, often by combining a few bacterial (Shong, Jimenez Diaz & Collins, 2012; Mee et al., 2014; Shi et al., 2017) or fungal strains (Minty et al., 2013; Hu et al., 2017). Although useful for bio-engineering purposes, this approach is not as applicable to studies of microbiomes, in which diversity is much greater. Host-associated synthetic communities have also been restricted to a few strains, with confirmation through re-isolation, limiting researchers' ability to extrapolate to more diverse communities (Bodenhausen et al., 2014; Niu et al., 2017; Herrera Paredes et al., 2018). Recent studies have linked host-associated microbiome function to microbial diversity (Turnbaugh et al., 2008; Laforest-Lapointe et al., 2017), requiring the incorporation of phylogenetic distance into synthetic community design. The design of phylogenetically diverse communities is associated with at least two major challenges: (1) creating a diverse community that can easily be distinguished through common high-throughput sequencing technologies, and (2) ensuring that community members possess the desired attributes (e.g., taxonomic composition and metabolic potential). Without advanced computational abilities, overcoming these challenges is formidable and time-consuming. Furthermore, manual bioinformatic workflows are difficult to document and error-prone, costing additional time and decreasing reproducibility.

In this paper, we describe an easy-to-use command-line program, Design of an Identifiable Synthetic Community of Microbes (DISCo-microbe), for creation of diverse communities of organisms that can be distinguished through next-generation sequencing technology for use in *in vivo* experiments. DISCo-microbe consists of two modules, create and subsample. The create module constructs a highly diverse community at a specified sequence difference from an input of aligned DNA/RNA sequences, e.g., 16S sequence. The module can either design a *de novo* community or design a community that includes targeted organisms. create solves problem (1) by easily generating a diverse community of members through an easily documentable method, ensuring reproducibility. The subsample module provides options for dividing the community into subsets, according to either the number of members or the proportions of a grouping variable, both of which can be specified by the user. subsample module solves problem (2) by allowing the user to subsample an already distinguishable community of members based on attributes of interest. Although this software was designed for construction of microbial communities, any DNA/RNA alignment can be used as input; consequently, users are not restricted to any particular organismal group or marker gene. This program is implemented in Python and is available through GitHub and PYPI.

Implementation

DISCo-microbe is a command-line program written in Python and requires Biopython (Cock et al., 2009), which is automatically installed along with the program. DISCo-microbe consists of two modules, create and subsample. The program has extensive documentation following the principles outlined in (Seemann, 2013; Karimzadeh & Hoffman, 2018). We included a quick tutorial that walks users through all commands, illustrating the ease of use and reproducibility of DISCo-microbe.

Workflow

create module

The create module has two required arguments, an alignment of DNA or RNA sequences in FASTA format (--i-alignment) and a user-specified minimum sequence distance between community members (--p-editdistance). The module uses a greedy algorithm to construct a community with the maximum number of members at the user-specified sequence distance. The optional arguments for the create module include: i) a community starter list (--p-include-strains), containing members the user would like to be included in the community; ii) a seed number (--p-seed), for reproducibility; iii) a metadata file (--i-metadata) for combination with the final community; iv) an option to output the FASTA file (--o-fasta) of the final community and; v) an option to import a sequence distance database (--i-distance-dictionary; described below). Because alignment gaps are counted in the distance calculation, we recommend that the user perform a reference-based alignment (if available) to ensure reproducibility of the gapped sites.

The create module operates in two distinct phases. The first phase creates a database of all pairwise sequence distances from the input alignment, calculated using a modified Hamming distance. The Hamming distance is a coding theory metric that measures the number of positions at which two sequences of equal length differ. Because the Hamming distance does not consider the nature of the differences, it can be problematic to determine the distance between molecular sequences, in which nucleotide ambiguities can be common; such ambiguities artificially inflate the number of differences between sequences, possibly causing the final community to be less distinguishable than expected (Fig 1). To deal with IUPAC nucleotide ambiguities, we created a custom Hamming distance, termed the nucleotide Hamming distance, which accommodates nucleotide ambiguities and adjusts the distance value accordingly (Fig 1). Furthermore, this metric can mitigate sequence errors introduced by PCR and sequencing technologies (Pfeiffer et al., 2018; Filges et al., 2019), allowing the identification of sequences containing up to $d - 1$ errors, where d is the user-specified minimum sequence distance. Lastly, due to the potentially long running time of the nucleotide Hamming distance calculation, we included an export option for the distance database. This option saves time when a user wishes to construct a new community with a few more sequences added; in those circumstances, the user can load the database of already calculated differences, so that only new comparisons must be calculated. Furthermore, the distance database is updated in real-time as distances are calculated, acting as a checkpoint to resume calculations with minimal lost time in the event that DISCo-microbe quits unexpectedly.

The second phase of the create module runs a greedy algorithm to construct a community. To initiate the community-building algorithm, the user can specify a starting community, which will be validated to determine that all pairwise distances meet the minimum requirement indicated by --p-editdistance. If the starting community is not valid at the indicated sequence distance, an error message with the conflicting sequence identifiers will be displayed. If a starting community is not specified, the individual with the fewest connections at the user-specified sequence distance (--p-editdistance) will be used to initiate the community (Fig 2). If there is tie for the fewest connections, one individual is selected at random. Once an initial community is established, the algorithm will iteratively add new members to the community by creating a list of possible members that meet two requirements. First, the individual must not already be in the community. Second, the individual must meet the minimum sequence distance to any of the existing members; for example, if the user has specified a distance of 2, the module will check if the individual is at a distance of 0,1 or 2 from any existing members. If these two requirements are met, the individual is added to the list of potential community members. Next, the individual in the list with the fewest connections at the specified sequence distance (Fig 2 inset) will be added to the community. Ties for the fewest connections are broken by randomly selecting an individual. The module will continue the process as described until there are no more individuals that meet the requirements for addition to the potential community member list. Once the community list is complete, the program will output a tab-delimited text file of community members. The community list can be combined with metadata information (optional), such as taxonomic information, which is recommended if the user will be using the 'subsample by proportions' option later. A FASTA file of the community list can also be created if desired.

subsample module

The subsample module is designed to take the final output community from the create module and provide a subsample of the community. The module has multiple subsampling procedures. The first method is a random sampling (option: --p-num-taxa) of the indicated number of members, n_{final}. The second method (option: --p-proportion) is for subsampling the specific proportions of a grouping variable. To illustrate the use of this option, we will refer to taxonomic information as the grouping variable; however, the user may provide any grouping variable for subsampling.

For this option, the user will input two files: the community file from the create module with taxonomic information combined, and a file of the taxonomic groupings with desired proportions. DISCo-microbe will then generate a subsampling of the original community that is optimized to reflect the desired proportions. The optimization is accomplished through a greedy minimization of the sum of differences, $\sum_{t \in TG} f^{current}_{t} - f^{specified}_{t}$, for the set TG of taxonomic groups specified in file 2 (taxonomic proportions file). Here, $f^{current} = \langle f^{current}_{1},\ldots,f^{current}_{n} \rangle$ and $f^{specified} = \langle f^{specified}_{1},\ldots,f^{specified}_{n} \rangle$ are vectors of taxonomic group frequencies for the current and desired community, respectively, with $\sum_{t \in TG} f^{current}_{t} = 1$ and $\sum_{t \in TG} f^{specified}_{t} = 1$. The algorithm initializes $f^{current}$ as the vector f^{input} of taxonomic group frequencies of the community provided in file 1 (from create module) with members belonging to taxonomic groups in the set X, where groups not specified in file 2 are removed ($X \equiv \{x \in X \mid x \notin TG\}$), and f^{input} renormalized such that $\sum_{t \in TG} f^{current}_{t} = 1$. Next, the algorithm will continuously iterate the following three steps:

(1) Determine the taxonomic group with largest difference in taxonomic group frequencies, $t_{max} = \max_{t \in TG} (\{f^{current}_{t_1} - f^{specified}_{t_1}\},\ldots,\{f^{current}_{t_n} - f^{specified}_{t_n}\})$.

(2) If the number of members in the taxonomic group identified in step 1 is less than 2 ($n_{t_{max}} < 2$) break and output the current community; otherwise, randomly remove a member from t_{max}, resulting in $f^{current'}$.

(3) If $\sum_{t \in TG} f^{current'}_{t} - f^{specified}_{t} < \sum_{t \in TG} f^{current}_{t} - f^{specified}_{t}$, set $f^{current} = f^{current'}_{t}$, otherwise stop the module and output the current community.

The user can modify the behavior of the algorithm by specifying both the number of members and the taxonomic proportions (--p-num-taxa and --p-proportion). Providing both options will force the algorithm to continue until the total number of members in the community, n_{total}, is $\leq n_{final}$

(user-specified final number of members). Further, when both options are specified, step 2 of the greedy minimization is modified to not break iteration when $n_{t_{max}} < 2$, and instead removes a member from the taxonomic group with the next-largest difference in frequencies, t_{next}, where $n_{t_{next}} \geq 2$. Additionally, if the force number option (option: --p-taxa-num-enforce) is used along with --p-num-taxa and --p-proportion, the algorithm will stop iteration when $n_{total} = n_{final}$ regardless of whether the sum of frequency differences could be further minimized.

Benchmarking

The custom nucleotide Hamming distance calculation can be the most computationally intensive step of DISCo-microbe. Therefore, we focused on benchmarking the distance calculation using hyperfine (https://github.com/sharkdp/hyperfine). Benchmarking was performed on a MacBook Air with 1.3 GHz Intel Core i5 with 10 runs per benchmark. To accomplish the benchmarking, we wrote a Python script to generate datasets containing 50, 500, or 5000 random sequences with lengths of 100, 500, 1000, or 1500 bp and an average pairwise sequence distance of 72.1% (±2.4%) (Fig 3A). We benchmarked the time saved by importing a precalculated distance database by comparing the runtime of two 6,000 sequence (1,000 bp) data sets (Fig. 3B). In one of 6,000 sequences data sets, we imported a pre-calculated distance database of 5,000 sequences. We calculated statistical significance using the Wilcoxon rank–sum test implemented in the package ggpubr (Kassambara, 2017).

Test data set

The Ribosomal Database Project (Cole et al., 2014) file of 16S rRNA genes was downloaded (release 11.5, May 2019), and uncultured strains were filtered using fasgrep (Lawrence et al., 2015). The alignment was trimmed to the V4 region, which is commonly used region for next-generation sequencing of bacterial communities (Thompson et al., 2017). The initial file contained 239,244 sequences and was randomly subsampled to 10,000 sequences due to the computational intensity of building the community. A reference-based alignment against the SILVA database (v. 132 (Pruesse et al., 2007)) was created using the program SINA (Pruesse, Peplies & Glöckner, 2012). Alignment sites containing only gaps were removed using alncut (Lawrence et al., 2015). An additional 13 sequences were removed due to the failure to align properly, resulting in 9,987 sequences at a length of 502 bp. The 9,987-sequence alignment was used to create a highly diverse community at a minimum sequence distance of 3, with the seed set to 10 for reproducibility. Following construction, the subsample module was used to subsample the community list to mimic the taxonomic composition a plant-associated microbiome. The final alignment, with 9,987 sequences at a length of 502 bp, taxonomic proportion file, and commands used to create the community are available on GitHub for users to reproduce.

Results and Discussion

Microbial diversity is linked to function (Turnbaugh et al., 2008; Laforest-Lapointe et al., 2017), but understanding that diversity can be difficult due to the low resolution of taxonomic marker genes and the complexity of the microbial community, limiting our ability to identify and track individual community members. To tease apart the complex interactions within communities, there has been an increased demand for synthetic community systems (Busby et al., 2017).

However, the generation of complex communities of organisms that can be easily distinguished through high-throughput methods can be difficult without strong computational skills. In general, two challenges are associated with the design of a synthetic community: (1) creation of a distinguishable community through common sequencing methods and (2) development of a community with the desired traits. Additionally, manual creation can lead to a lack of reproducibility due to the difficulty of documenting the workflow. In this paper, we describe an easy to use command-line program, Design of an Identifiable Synthetic Community of Microbes (DISCo-microbe), for the creation of diverse communities of organisms that can be distinguished through next-generation sequencing technology during *in vivo* experiments. DISCo-microbe solves the two previously mentioned problems using two modules, create and subsample.

The create module allows the user to construct a diverse community that is identifiable using common sequencing methods, thus solving the first problem. The ability to specify a minimum sequence distance allows flexibility in the construction of the community due to its robustness to sequencing errors introduced through PCR and sequencing (Pfeiffer et al., 2018). For example, if the user sets the minimum sequence distance to 5, sequences containing up to 2 sequencing errors ($[^{d-1}]/2$) can be confidently assigned to the correct community member, sequences containing up to 4 errors ($d - 1$) can be identified, and it would take a minimum of 5 errors to assign a sequence to the incorrect community member. Usually, the smaller the minimum sequence distance, the more members will be included in the constructed community, potentially motivating users to set the minimum sequence distance to lowest setting of 1. However, at a minimum sequence distance of l, it only requires a single sequencing error to assign a sequence to the wrong community member. In order to implement the create module, we developed a custom nucleotide Hamming distance that accommodates nucleotide ambiguities. This is the first application of the Hamming distance algorithm incorporating IUPAC nucleotide ambiguity codes to measure distance between pairs of aligned sequences implemented in Python (see (Šošić & Šikić, 2017) for an implementation in C). Initially, we assumed that the most time-consuming step would be the creation of the distance database due to the number of calculations required [$n!/2(n-2)!$], motivating us to focus our benchmarking efforts on this function and implementing an export function for the distance database as a time-saving measure for adding new individuals to the community, re-running community construction at different minimum sequence distances, and restarting in the event DISCo-microbe crashes. As anticipated, runtime increased with sequence number and length, and importing a precomputed sequence database significantly decreased running time (Fig 3). However, during benchmarking of the example dataset (RDP), it became clear that average pairwise sequence distance (72.1±2.4% for benchmark datasets vs. 10.6 ± 3.6% for the RDP dataset),was a major determinant of the time required to calculate the distance database, with the community creation step being the most time-consuming step for the RDP dataset (Fig 3A).

The subsample module allows flexibility in the final constructed community. Specifically, it allows users to adapt the community to their experimental specifications, either by limiting the number of strains, specifying proportions of a grouping variable, or both. The subsample module eliminates major problem (2) by allowing users to tailor the already distinguishable community to include desired traits or proportions of members.

To demonstrate the applicability, usability, and ease of documenting workflows when using DISCo-microbe to construct identifiable diverse communities, we created and subsampled a community with a minimum sequence distance of 3 using 16S rRNA sequences from the RDP database. The initial sequence alignment contained the V4 region from 9,987 sequences with an average pairwise sequence distance of 10.6 ± 3.6%). Using the following create module command:

```
disco create --i-alignment RDP_aligned_sequences.fasta --p-editdistance 3 --p-seed 10 --i-metadata
RDP_Metadata_Taxonomy.txt --o-community-list RDP_Community_ED3_seed10.txt
```

we constructed a community of 2,340 members that could be distinguished through next-generation sequencing. The resultant community took 5.12 hours to construct. Using the following subsample module command:

```
disco subsample --i-input-community RDP_Community_ED3_seed10.txt --p-seed 10 --p-group-by Class --p-
proportion RDP_Class_Proportions_file.txt
```

the community was reduced to 853 community members with the approximate proportions of a plant–associated microbiome (Table 1; (Cregger et al., 2018)). The options for each module used above, along with the version of DISCo-microbe and Python, are the only documentation required to reliably reproduce the design of this extremely complex community.

Conclusions

DISCo-microbe is the first software designed for the construction of a diverse community of organisms that can be distinguished through low-cost, high-throughput amplicon sequencing for use in *in vivo* experiments. DISCo-microbe allows non-programmers to easily and reproducibly construct communities in which the members are identifiable through amplicon sequencing and the communities conform to user-specified attributes or numbers of members. DISCo-microbe is also the first software to implement a nucleotide specific Hamming distance in Python that takes into account nucleotide ambiguities in sequencing data. Although initially designed for construction of bacterial community construction, the input of a nucleotide sequence alignment from any region allows the software to be used with any group of organisms. DISCo-microbe is designed for easy expansion of utilities; planned future versions will include new algorithms for community construction as well as new modules for creating a suite of tools for the design of constructed communities and processing of the resulting data.

Availability and requirements

Project name: DISCo-microbe
Project home page: https://github.com/dlcarper/DISCo-microbe
Operating system(s): platform-independent
Programming language: Python ≥ 3.4
Other requirements: BioPython
License: GNU General Public License v3.0

Abbreviations

DNA: Deoxyribonucleic acid

RNA: Ribonucleic acid

rRNA: Ribosomal ribonucleic acid

FASTA: Fast-all (file format)

PYPI: Python Package Index

PCR: polymerase chain reaction

Declarations

Availability of data and material

All data generated for the example data set and benchmarking can be found at https://github.com/dlcarper/DISCo-microbe

Authors' contributions

DLC conceived and wrote most of the program code base and wrote the manuscript. TJL wrote a portion of the program code and contributed substantially to the writing of the manuscript. AAC wrote the documentation and performed testing of the program, as well as contributing to the writing of the manuscript. DAP and DJW contributed to the writing of the manuscript. All authors All authors read and approved the final manuscript.

Acknowledgements

Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725.

References

Adair KL, Douglas AE. 2017. Making a microbiome: the many determinants of host-associated microbial community composition. *Current Opinion in Microbiology* 35:23–29. DOI: 10.1016/j.mib.2016.11.002.

Bodenhausen N, Bortfeld-Miller M, Ackermann M, Vorholt JA. 2014. A synthetic community approach reveals plant genotypes affecting the phyllosphere microbiota. *PLoS Genetics* 10:e1004283. DOI: 10.1371/journal.pgen.1004283.

Bordenstein SR, Theis KR. 2015. Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLOS Biology* 13:e1002226. DOI: 10.1371/journal.pbio.1002226.

Busby PE, Soman C, Wagner MR, Friesen ML, Kremer J, Bennett A, Morsy M, Eisen JA, Leach JE, Dangl JL. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLOS Biology* 15:e2001793. DOI: 10.1371/journal.pbio.2001793.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences* 108:4516–4522. DOI: 10.1073/pnas.1000080107.

Cock PJA, Antao T, Chang JT, Chapman BA, Cox CJ, Dalke A, Friedberg I, Hamelryck T, Kauff F, Wilczynski B, de Hoon MJL. 2009. Biopython: freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics* 25:1422–1423. DOI: 10.1093/bioinformatics/btp163.

Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, Brown CT, Porras-Alfaro A, Kuske CR, Tiedje JM. 2014. Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Research* 42:D633–D642. DOI: 10.1093/nar/gkt1244.

Cregger MA, Veach AM, Yang ZK, Crouch MJ, Vilgalys R, Tuskan GA, Schadt CW. 2018. The *Populus* holobiont: dissecting the effects of plant niches and genotype on the microbiome. *Microbiome* 6:31. DOI: 10.1186/s40168-018-0413-8.

Engel P, Kwong WK, McFrederick Q, Anderson KE, Barribeau SM, Chandler JA, Cornman RS, Dainat J, de Miranda JR, Doublet V, Emery O, Evans JD, Farinelli L, Flenniken ML, Granberg F, Grasis JA, Gauthier L, Hayer J, Koch H, Kocher S, Martinson VG, Moran N, Munoz-Torres M, Newton I, Paxton RJ, Powell E, Sadd BM, Schmid-Hempel P, Schmid-Hempel R, Song SJ, Schwarz RS, VanEngelsdorp D, Dainat B. 2016. The bee microbiome: impact on bee health and model for evolution and ecology of host-microbe interactions. *mBio* 7:1–9. DOI: 10.1128/mBio.02164-15.

Feehery GR, Yigit E, Oyola SO, Langhorst BW, Schmidt VT, Stewart FJ, Dimalanta ET, Amaral-Zettler LA, Davis T, Quail MA, Pradhan S. 2013. A method for selectively enriching microbial DNA from contaminating vertebrate host DNA. *PLoS ONE* 8:e76096. DOI: 10.1371/journal.pone.0076096.

Filges S, Yamada E, Ståhlberg A, Godfrey TE. 2019. Impact of polymerase fidelity on background error rates in next-generation sequencing with unique molecular identifiers/barcodes. *Scientific Reports* 9:3503. DOI: 10.1038/s41598-019-39762-6.

Herrera Paredes S, Gao T, Law TF, Finkel OM, Mucyn T, Teixeira PJPL, Salas González I, Feltcher ME, Powers MJ, Shank EA, Jones CD, Jojic V, Dangl JL, Castrillo G. 2018. Design of synthetic bacterial communities for predictable plant phenotypes. *PLOS Biology* 16:e2003962. DOI: 10.1371/journal.pbio.2003962.

Hu J, Xue Y, Guo H, Gao M, Li J, Zhang S, Tsang YF. 2017. Design and composition of synthetic fungal-bacterial microbial consortia that improve lignocellulolytic enzyme activity. *Bioresource Technology* 227:247–255. DOI: 10.1016/j.biortech.2016.12.058.

Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, Creasy HH, Earl AM, Fitzgerald MG, Fulton RS, Giglio MG, Hallsworth-Pepin K, Lobos EA, Madupu R, Magrini V, Martin JC, Mitreva M, Muzny DM, Sodergren EJ, Versalovic J, Wollam AM, Worley KC, Wortman JR, Young SK, Zeng Q, Aagaard KM, Abolude OO, Allen-Vercoe E, Alm EJ, Alvarado L, Andersen GL, Anderson S, Appelbaum E, Arachchi HM, Armitage G, Arze CA, Ayvaz T, Baker CC, Begg L, Belachew T, Bhonagiri V, Bihan M, Blaser MJ, Bloom T, Bonazzi V, Paul Brooks J, Buck GA, Buhay CJ, Busam DA, Campbell JL, Canon SR, Cantarel BL, Chain PSG, Chen IMA, Chen L, Chhibba S, Chu K, Ciulla DM, Clemente JC, Clifton SW, Conlan S, Crabtree J, Cutting MA, Davidovics NJ, Davis CC, Desantis TZ, Deal C, Delehaunty KD, Dewhirst FE, Deych E, Ding Y, Dooling DJ, Dugan SP, Michael Dunne W, Scott Durkin A, Edgar RC, Erlich RL, Farmer CN, Farrell RM, Faust K, Feldgarden M, Felix VM, Fisher S, Fodor AA, Forney LJ, Foster L, Di Francesco V, Friedman J, Friedrich DC, Fronick CC, Fulton LL, Gao H, Garcia N, Giannoukos G, Giblin C, Giovanni MY, Goldberg JM, Goll J, Gonzalez A, Griggs A, Gujja S, Kinder Haake S, Haas BJ, Hamilton HA, Harris EL, Hepburn TA, Herter B, Hoffmann DE, Holder ME, Howarth C, Huang KH, Huse SM, Izard J, Jansson JK, Jiang H, Jordan C, Joshi V, Katancik JA, Keitel WA, Kelley ST, Kells C, King NB, Knights D, Kong HH, Koren O, Koren S, Kota KC, Kovar CL, Kyrpides NC, La Rosa PS, Lee SL, Lemon KP, Lennon N, Lewis CM, Lewis L, Ley RE, Li K, Liolios K, Liu B, Liu Y, Lo CC, Lozupone CA, Dwayne Lunsford R, Madden T, Mahurkar AA, Mannon PJ, Mardis ER, Markowitz VM, Mavromatis K, McCorrison JM, McDonald D, McEwen J, McGuire AL, McInnes P, Mehta T, Mihindukulasuriya KA, Miller JR, Minx PJ, Newsham I, Nusbaum C, Oglaughlin M, Orvis J, Pagani I, Palaniappan K, Patel SM, Pearson M, Peterson J, Podar M, Pohl C, Pollard KS, Pop M, Priest ME, Proctor LM, Qin X, Raes J, Ravel J, Reid JG, Rho M, Rhodes R, Riehle KP, Rivera MC, Rodriguez-Mueller B, Rogers YH, Ross MC, Russ C, Sanka RK, Sankar P, Fah Sathirapongsasuti J, Schloss JA, Schloss PD, Schmidt TM, Scholz M, Schriml L, Schubert AM, Segata N, Segre JA, Shannon WD, Sharp RR, Sharpton TJ, Shenoy N, Sheth NU, Simone GA, Singh I, Smillie CS, Sobel JD, Sommer DD, Spicer P, Sutton GG, Sykes SM, Tabbaa DG, Thiagarajan M, Tomlinson CM, Torralba M, Treangen TJ, Truty RM, Vishnivetskaya TA, Walker J, Wang L, Wang Z, Ward D V., Warren W, Watson MA, Wellington C, Wetterstrand KA, White JR, Wilczek-Boney K, Wu Y, Wylie KM, Wylie T, Yandava C, Ye L, Ye Y, Yooseph S, Youmans BP, Zhang L, Zhou Y, Zhu Y, Zoloth L, Zucker JD, Birren BW, Gibbs RA, Highlander SK, Methé BA, Nelson KE, Petrosino JF, Weinstock GM, Wilson RK, White O. 2012. Structure, function and diversity of the healthy human microbiome. *Nature* 486:207–214. DOI: 10.1038/nature11234.

Jiao J-Y, Wang H-X, Zeng Y, Shen Y-M. 2006. Enrichment for microbes living in association with plant tissues. *Journal of Applied Microbiology* 100:830–837. DOI: 10.1111/j.1365-2672.2006.02830.x.

Karimzadeh M, Hoffman MM. 2018. Top considerations for creating bioinformatics software documentation. *Briefings in Bioinformatics* 19:693–699. DOI: 10.1093/bib/bbw134.

Kassambara A. 2017. ggpubr: “ggplot2” based publication ready plots.

Laforest-Lapointe I, Paquette A, Messier C, Kembel SW. 2017. Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. *Nature* 546:145–147. DOI: 10.1038/nature22399.

Lawrence TJ, Kauffman KT, Amrine KCH, Carper DL, Lee RS, Becich PJ, Canales CJ, Ardell DH. 2015. FAST: FAST Analysis of Sequences Toolbox. *Frontiers in Genetics* 6. DOI: 10.3389/fgene.2015.00172.

Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, Malfatti S, Glavina del Rio T, Jones CD, Tringe SG, Dangl JL. 2015. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* 349:860–864. DOI: 10.1126/science.aaa8764.

Marotz CA, Sanders JG, Zuniga C, Zaramela LS, Knight R, Zengler K. 2018. Improving saliva shotgun metagenomics by chemical host DNA depletion. *Microbiome* 6:42. DOI: 10.1186/s40168-018-0426-3.

Mee MT, Collins JJ, Church GM, Wang HH. 2014. Syntrophic exchange in synthetic microbial communities. *Proceedings of the National Academy of Sciences* 111:E2149–E2156. DOI: 10.1073/pnas.1405641111.

Minty JJ, Singer ME, Scholz SA, Bae C-H, Ahn J-H, Foster CE, Liao JC, Lin XN. 2013. Design and characterization of synthetic fungal-bacterial consortia for direct production of isobutanol from cellulosic biomass. *Proceedings of the National Academy of Sciences* 110:14592–14597. DOI: 10.1073/pnas.1218447110.

Niu B, Paulson JN, Zheng X, Kolter R. 2017. Simplified and representative bacterial community of maize roots. *Proceedings of the National Academy of Sciences* 114:E2450–E2459. DOI: 10.1073/pnas.1616148114.

Ofek-Lalzar M, Sela N, Goldman-Voronov M, Green SJ, Hadar Y, Minz D. 2014. Niche and host-associated functional signatures of the root surface microbiome. *Nature Communications* 5:4950. DOI: 10.1038/ncomms5950.

Pfeiffer F, Gröber C, Blank M, Händler K, Beyer M, Schultze JL, Mayer G. 2018. Systematic evaluation of error rates and causes in short samples in next-generation sequencing. *Scientific Reports* 8:10950. DOI: 10.1038/s41598-018-29325-6.

Pruesse E, Peplies J, Glöckner FO. 2012. SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28:1823–1829. DOI: 10.1093/bioinformatics/bts252.

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glockner FO. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* 35:7188–7196. DOI: 10.1093/nar/gkm864.

Rosenberg E, Zilber-Rosenberg I. 2016. Microbes drive evolution of animals and plants: the hologenome concept. *mBio* 7:1–8. DOI: 10.1128/mBio.01395-15.

Schlaeppi K, Bulgarelli D. 2015. The plant microbiome at work. *Molecular Plant-Microbe Interactions* 28:212–217. DOI: 10.1094/MPMI-10-14-0334-FI.

Seemann T. 2013. Ten recommendations for creating usable bioinformatics command line software. *GigaScience* 2:15. DOI: 10.1186/2047-217X-2-15.

Shi Y, Pan C, Wang K, Chen X, Wu X, Chen C-TA, Wu B. 2017. Synthetic multispecies microbial communities reveals shifts in secondary metabolism and facilitates cryptic natural product discovery. *Environmental Microbiology* 19:3606–3618. DOI: 10.1111/1462-2920.13858.

Shong J, Jimenez Diaz MR, Collins CH. 2012. Towards synthetic microbial consortia for bioprocessing. *Current Opinion in Biotechnology* 23:798–802. DOI: 10.1016/j.copbio.2012.02.001.

Šošić M, Šikić M. 2017. Edlib: a C/C ++ library for fast, exact sequence alignment using edit distance. *Bioinformatics* 33:1394–1395. DOI: 10.1093/bioinformatics/btw753.

Spor A, Koren O, Ley R. 2011. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews Microbiology* 9:279–290. DOI: 10.1038/nrmicro2540.

Thoendel M, Jeraldo PR, Greenwood-Quaintance KE, Yao JZ, Chia N, Hanssen AD, Abdel MP, Patel R. 2016. Comparison of microbial DNA enrichment tools for metagenomic whole genome sequencing. *Journal of Microbiological Methods* 127:141–145. DOI: 10.1016/j.mimet.2016.05.022.

Thompson LR, Sanders JG, McDonald D, Amir A, Ladau J, Locey KJ, Prill RJ, Tripathi A, Gibbons SM, Ackermann G, Navas-Molina JA, Janssen S, Kopylova E, Vázquez-Baeza Y, González A, Morton JT, Mirarab S, Zech Xu Z, Jiang L, Haroon MF, Kanbar J, Zhu Q, Jin Song S, Kosciolek T, Bokulich NA, Lefler J, Brislawn CJ, Humphrey G, Owens SM, Hampton-Marcell J, Berg-Lyons D, McKenzie V, Fierer N, Fuhrman JA, Clauset A, Stevens RL, Shade A, Pollard KS, Goodwin KD, Jansson JK, Gilbert JA, Knight R. 2017. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* 551:457–463. DOI: 10.1038/nature24621.

Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. 2008. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host & Microbe* 3:213–223. DOI: 10.1016/j.chom.2008.02.015.

Zilber-Rosenberg I, Rosenberg E. 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews* 32:723–735. DOI: 10.1111/j.1574-6976.2008.00123.x.

Figure 1

Demonstration of custom nucleotide Hamming distance

Demonstration of Python Hamming distance and custom nucleotide Hamming distance, which takes into account nucleotide ambiguities

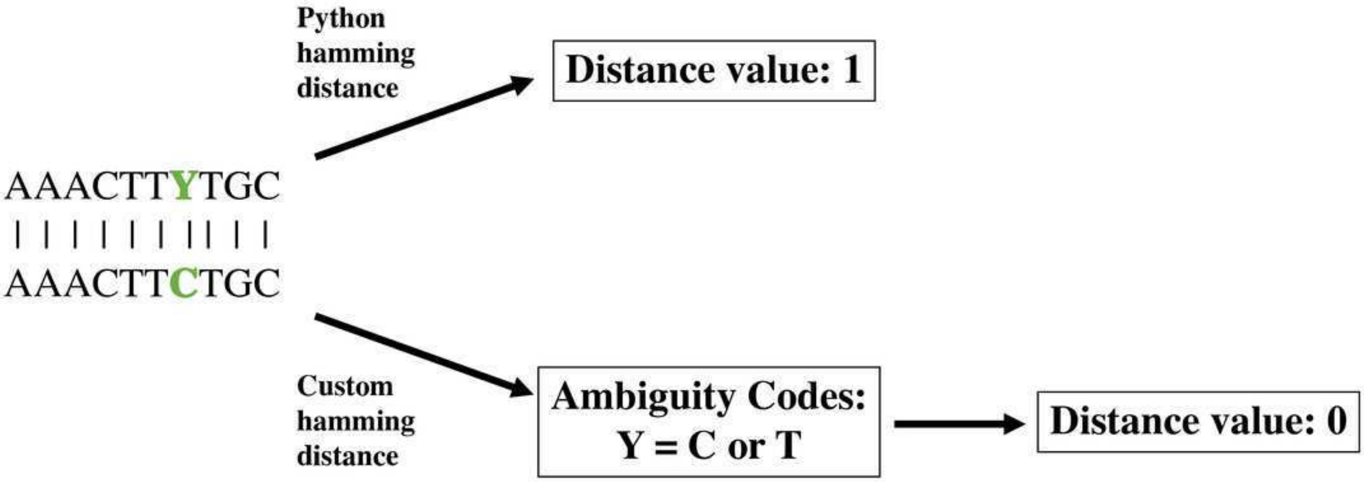
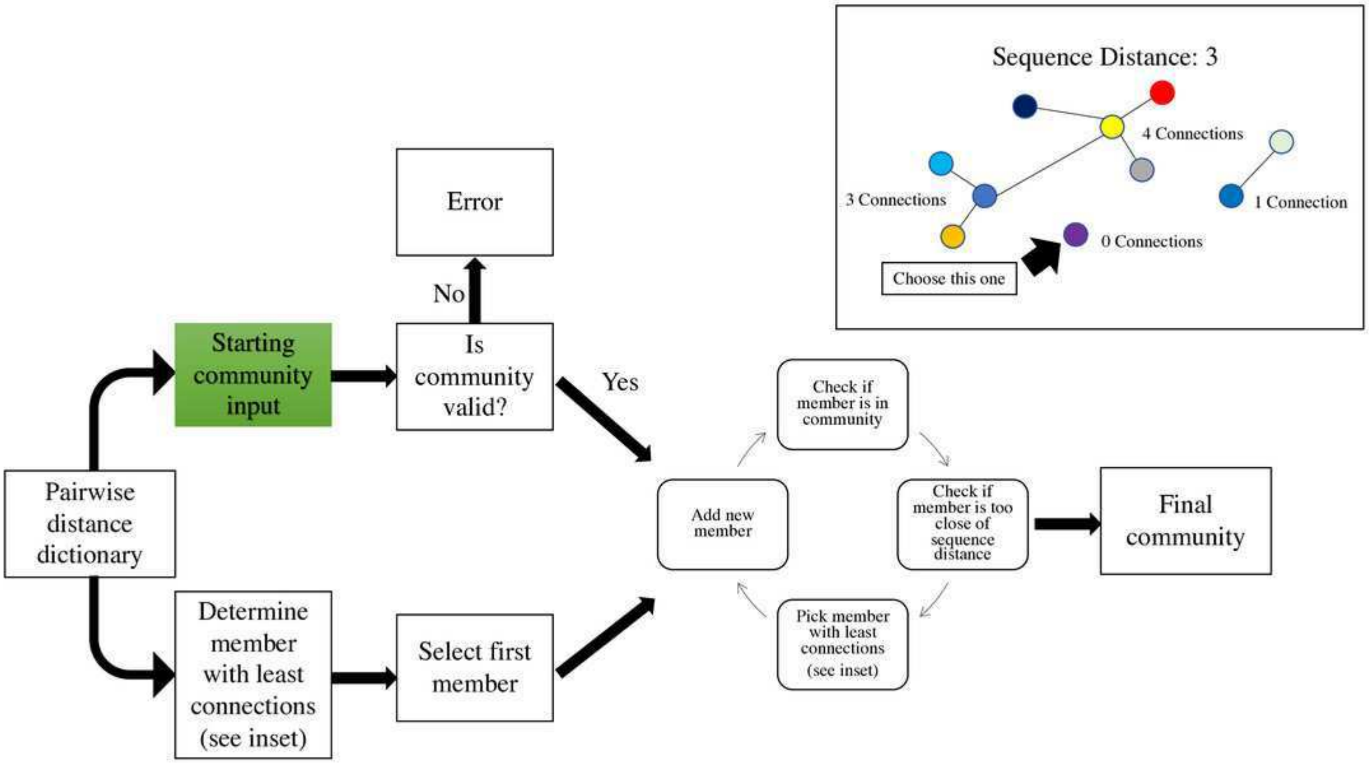


Figure 2

Workflow schematic of the loop that adds new members to the community, starting with the pairwise distance dictionary

Inset: Schematic of adding members with fewest connections at a specified DNA distance. Circles represent individuals, and lines indicate that the connected individuals are at a sequence distance of 3. Green indicates the user of file.


Sequence Distance: 3
4 Connections
3 Connections
1 Connection
0 Connections
Choose this one
Error
No
Starting community input
Is community valid?
Yes
Check if member is in community
Pairwise distance dictionary
Add new member
Check if member is too close of sequence distance
Final community
Pick member with least connections (see inset)
Determine member with least connections (see inset)
Select first member

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27898v1 | CC0 Open Access | rec: 13 Aug 2019, publ: 13 Aug 2019

Figure 3

Benchmarking of test data sets

A) Benchmarking of custom nucleotide Hamming distance function for DNA at various sequence lengths and numbers of sequences. The point in green shows Ribosomal Database Project sequences. B) T-test comparison of benchmark times of custom nucleotide Hamming distance with dictionary import function in use vs. no input dictionary. ns = not significant, * p<=0.05, ** p<=0.01, ***p<=0.001.

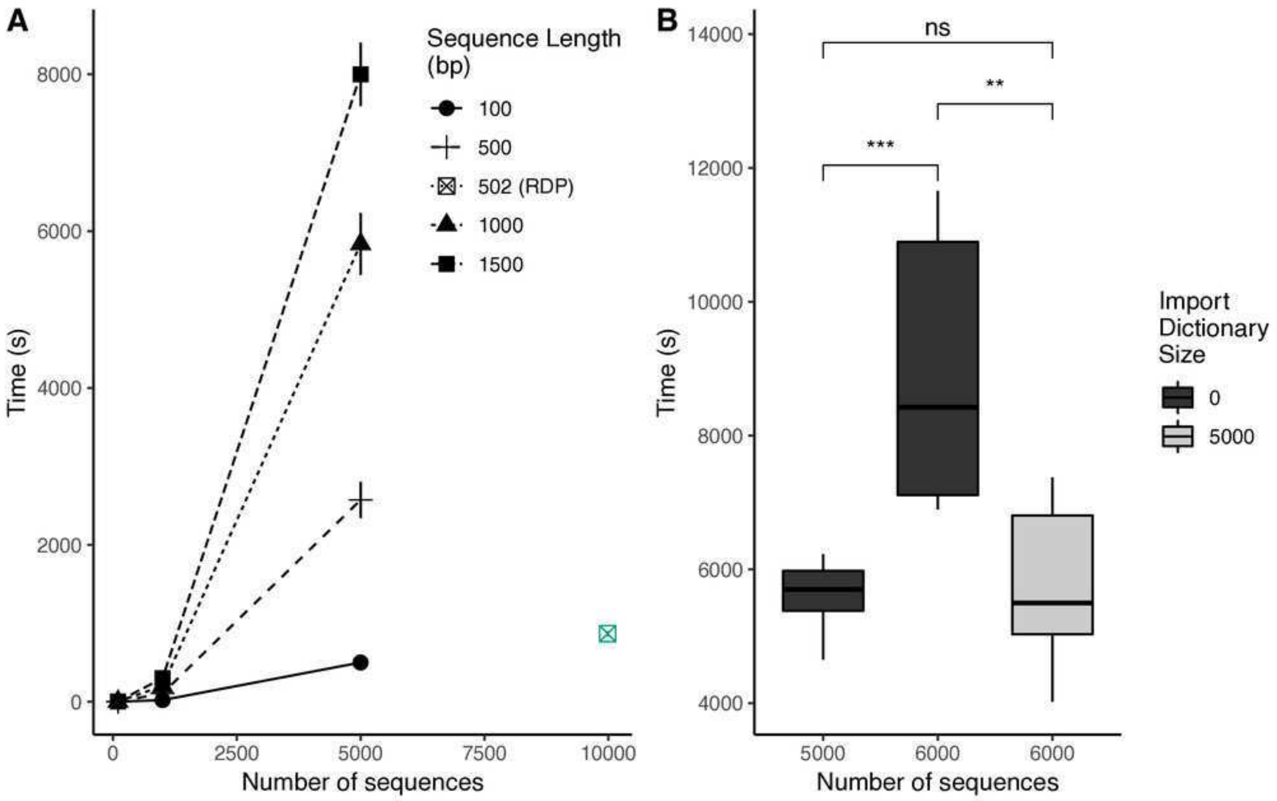


Table 1(on next page)

Subsampled bacterial class proportions

Bacterial class proportions used to subsample the community generated from the Ribosomal Database Project database and the actualized proportions of the resultant community

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27898v1 | CC0 Open Access | rec: 13 Aug 2019, publ: 13 Aug 2019

Bacterial class	Input Proportions	Actualized Proportions
Actinobacteria	0.0885	0.0903
Alphaproteobacteria	0.1857	0.1876
Anaerolineae	0.004	0.0012
Aquificae	0.0003	0.0012
Bacteroidia	0.1	0.0996
Betaproteobacteria	0.1286	0.1301
Chitinivibrionia	0.004	0.0012
Chloroflexia	0.005	0.0047
Deferribacteres	0.0003	0.0023
Deinococci	0.0003	0.0023
Deltaproteobacteria	0.0418	0.0434
Fibrobacteria	0.0004	0.0023
Fusobacteriia	0.0003	0.0023
Gammaproteobacteria	0.4112	0.4127
Gemmatimonadetes	0.0073	0.0023
Ktedonobacteria	0.0097	0.0012
Nitrospira	0.0036	0.0047
Planctomycetia	0.009	0.0106