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Analyzing Multiplexed Paired-End FASTQ data Using
DADA2



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RESEARCH ARTICLE
Applied and Environmental Science



Significant Impacts of Increasing Aridity on the Arid Soil Microbiome

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ABSTRACT Global deserts occupy one-third of the Earth's surface and contribute significantly to organic carbon storage, a process at risk in dryland ecosystems that are highly vulnerable to climate-driven ecosystem degradation. The forces controlling desert ecosystem degradation rates are poorly understood, particularly with respect to the relevance of the arid-soil microbiome. Here we document correlations between increasing aridity and soil bacterial and archaeal microbiome composition along arid to hyperarid transects traversing the Atacama Desert, Chile. A meta-analysis reveals that Atacama soil microbiomes exhibit a gradient in composition, are distinct from a broad cross-section of nondesert soils, and yet are similar to three deserts from different continents. Community richness and diversity were significantly positively correlated with soil relative humidity (SoilRH). Phylogenetic composition was strongly correlated with SoilRH, temperature, and electrical conductivity. The strongest and most significant correlations between SoilRH and phylum relative abundance were observed for *Acidobacteria*, *Proteobacteria*, *Planctomycetes*, *Verrucomicrobia*, and *Euryarchaeota* (Spearman's rank correlation $r_s = >0.81$; false-discovery rate $[q] = \leq 0.005$), characterized by 10- to 300-fold decreases in the relative abundance of each taxon. In addition, network analysis revealed a deterioration in the density of significant associations between taxa along the arid to hyperarid gradient, a pattern that may compromise the resilience of hyperarid communities

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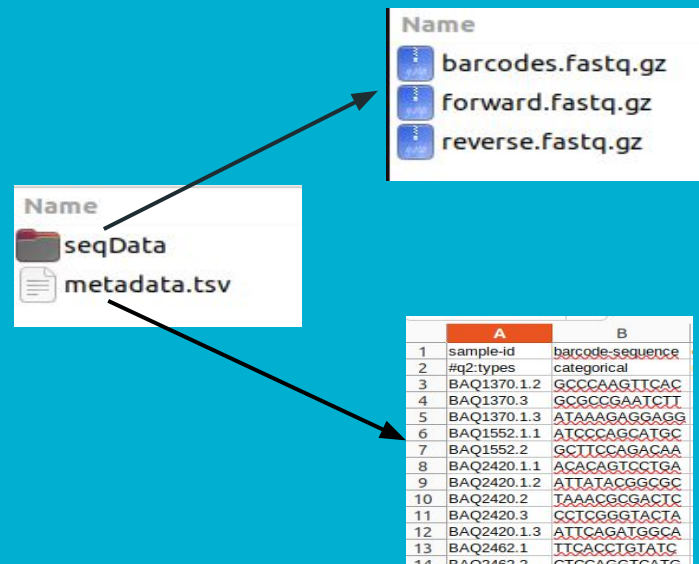
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 Increased aridity significantly affects the diversity, community connectivity, and phylogenetic composition of the arid soil microbiome.

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AAAAFBFBFFFGGGGGEFFFAEG2E?EHFHDfBAEHSDGEGGEEGHHCEGEEFHFF1EE?F33GFHFEG4BGHHGHGHGHGFHGE@?EGHFHDFGFF3BGHHEGFHHGHHHHACC/CHGGFD//@@HFFHFGHC?CCFHFFFFHH
@M00176:65:000000000-A41FR:1:2114:3733:19005 2:N:0:0
CCTGTCTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTACAGGTCCCGGGGATTGCCTTCGCCATCGGTGTTCTCTCCGCATATCTACGCATTTCACTGCTACACGCGGAATTCATCCCCCTCTACCGTACTCTCGCTATGCAGTCCCAAC
+
>A11>1D1B3FFGGBGAEEAAFE0BAGHF11A00A/EFBA2DBBGH0A//E/B0BFD1FBEF/BBE>B??EDHHHH/E@EEFBF2FFG/?EGHEH21B1>F1B/>/</?GFFDGDGCGGGF?1?G?/><<>G1.<<.<DF0DGC0<<G.
@M00176:65:000000000-A41FR:1:2114:16055:19007 2:N:0:0
TGATTGCTACTGACCGCTCTCGTGCTCGCTGCGTTGAGGCTTGCGTTTATGGTACGCTGGACTTTGTAGGATACCTCGCTTTCCTGCTCTGTTGAGTTTATTGCTGCCGTCATTGCTTATTATGTTTATCCCGTCAACATTCAAA
+
AAAAADFFFFFAFGFCA1E0FAEFFH0EG0AEGFCGGGHHCEHHGGCEAEHFGHDD/EEAFFHHCGEGGHGFHGFHFGGEAEFFHFFBGHCHDGD12BBGHHHHEH2GBFCEGCFHDFGG2FHHFGGHG2F@GHCCCEA?<?>D<DG

TACGTAGGCAGCGAGCGTTGTTCCGAGTTACTGGCGCTAAAGAGTGCGTAGGCCGTTTTCTAAGTTTGGTGTGAATCTCCCGCTTAAGTGGGAGGTCGCCGAAAACCTGGAAGCCTTCGATATGGGAGAGGTAAGCGGAATTCTGG
+
CCCCBFFF@BFABGGFFFEFGGGHDFDEEHGHHHHHGEGGHGHHDFGEFEGGHGGGGCGHHDFFFHHHGGGHGDGFHHHHHHGGE>EGGHFHGGGGCGFFGGGGGGGGHHFHCGGGGHHFGEHHHHHHGFGAEGHFDGCGG@@EHHHHHH
@M00176:65:00000000-A41FR:1:2114:19441:15539 1:N:0:0
TACGAAGGGGCTAGCGTTGCTCGGAATTACTGGCGCTAAAGGGCGCTAGGCCGATATTTAAGTTGGGGTGAAATCCCGGGCTCAACCTCGGAATTGCCTTCAAACCTGGATATCTTGAGTTCGGGAGAGGTGAGTGGAACCTCCGAGT
+
CCCCBCCCCCCBGGGGGGGGGGHGGGGHGGGGGHHHHHHGCGGGGHHGGGGGGGGGGGGGGGGGGHHHHHHHHHHGGGGGGHHHHHHHHGGGGC?GGHHHHGGGGHHHHHHHHHHHHHHHHHHHHHHGGGGGGGGGGGGGGGGGGGG
@M00176:65:00000000-A41FR:1:2114:14657:15542 1:N:0:0
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```
/home/hkim/Downloads/Practical/input/fqMuBoPe-project_
emp1/seqData/barcodes.fastq.gz
```

```

GTTAAGCCCGAT
+
CCCCCFFD@B
@M00176:65:000000000-A41FR:1:2114:19441:15539 1:N:0:0
CTTCAGTTCGCC
+
BBCBCFFFFDDEE
@M00176:65:000000000-A41FR:1:2114:14657:15542 1:N:0:0
TCAGTACGAGGC
+
BBBAABFBABBB
@M00176:65:000000000-A41FR:1:2114:13030:15552 1:N:0:0
TTCTCACCTTTC
+
AA>AAFFFFFBB

```

Import into QIIME2

```
qiime tools import \  
  --type EMPPairedEndSequences \  
  --input-path /home/hkim/Downloads/Practical/input/fqMuBoPe-project_emp1/seqData \  
  --output-path emp-paired-end-sequences.qza
```

Demultiplexing = q2 - demux

```
qiime demux emp-paired \  
  --i-seqs emp-paired-end-sequences.qza \  
  --m-barcodes-file /home/hkim/Downloads/Practical/input/fqMuBoPe-project_emp1/metadata.tsv \  
  --m-barcodes-column barcode-sequence \  
  --o-per-sample-sequences demux.qza \  
  --o-error-correction-details demux-details.qza \  
  --p-rev-comp-barcodes \  
  --p-rev-comp-mapping-barcodes
```

Denoise Sequences

```
qiime dada2 denoise-paired \  
  --i-demultiplexed-seqs demux.qza \  
  --p-trunc-len-f 0 \  
  --p-trunc-len-r 0 \  
  --o-table table.qza \  
  --o-representative-sequences rep-seqs.qza \  
  --o-denoising-stats denoising-stats.qza  
  
qiime metadata tabulate \  
  --m-input-file denoising-stats.qza \  
  --o-visualization denoising-stats.qzv
```

Summarize the Feature Table and Feature Data

```
qiime feature-table summarize \  
  --i-table table.qza \  
  --m-sample-metadata-file /home/hkim/Downloads/Practical/input/fqMuBoPe-project_emp1/metadata.tsv \  
  --o-visualization table.qzv
```

```
qiime feature-table tabulate-seqs \  
  --i-data rep-seqs.qza \  
  --o-visualization rep-seqs.qzv
```

```
qiime feature-table tabulate-seqs \  
  --i-data table.qza \  
  --o-visualization featuretable.qzv
```



We are currently beta testing a new version of q2view. If you would like to use the old version [click here](#).

This interface can view .qza and .qzv files directly in your browser without uploading to a server. [Click here to learn more](#).

Drag and drop or click here

to view a QIIME 2 Artifact or Visualization (.qza/.qzv) from your computer.

You can also provide a link to a [file on Dropbox](#), a [file on Zenodo](#), or a [file from the web](#).

<https://view.qiime2.org/>

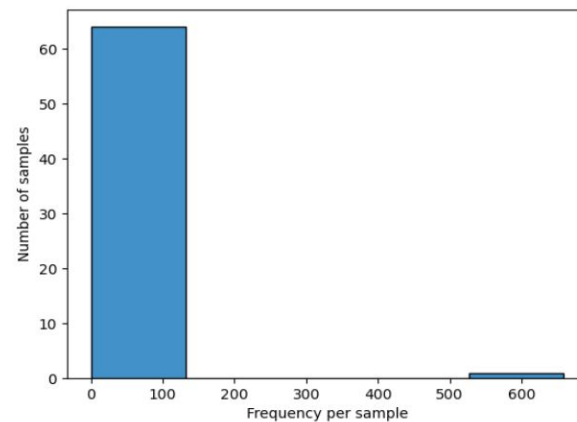
[Overview](#)[Interactive Sample Detail](#)[Feature Detail](#)

Table summary

Summary Statistic	Value
Number of samples	65
Number of features	22
Total frequency	663

Frequency per sample

	Frequency
Minimum frequency	0
1st quartile	0
Median frequency	0
3rd quartile	0
Maximum frequency	659
Mean frequency	10.2



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PLOS NEGLECTED TROPICAL DISEASES

OPEN ACCESS RESEARCH ARTICLE

A Comparison between Transcriptome Sequencing and 16S Metagenomics for Detection of Bacterial Pathogens in Wildlife

Maria Razzauti, Maxime Galin, Maria Bernard, Sarah Maman, Christophe Kopp, Nathalie Chabonnet, Muriel Vayssier-Tanis, Marc Eloit, Jean-François Cossou

Published: August 18, 2015 • <https://doi.org/10.1371/journal.pntd.0003929>

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Abstract

Background

Rodents are major reservoirs of pathogens responsible for numerous zoonotic diseases in humans and livestock. Assessing their microbial diversity at both the individual and population level is crucial for monitoring endemic infections and revealing microbial association patterns within reservoirs. Recently, NGS approaches have been employed to characterize microbial communities of different ecosystems. Yet, their relative efficacy has not been assessed. Here, we compared two NGS approaches, RNA-Sequencing (RNA-Seq) and 16S metagenomics, assessing their ability to survey neglected zoonotic bacteria in rodent populations.

Methodology/Principal Findings

We first extracted nucleic acids from the spleens of 150 voles collected in France. RNA-extracts were pooled, randomly retro-transcribed, then RNA-Seq was performed using HiSeq.

	A	B	C	D	E	F	G
1	Sample ID	Plate	Well	Index1(I7)	I7 sequence	Index2 (I5)	I5 sequence
2	MgArd0001	1A01	SA701	AACTCTCG	SA501	ATCGTACG	
3	MgArd0002	1B01	SA701	AACTCTCG	SA502	ACTATCTG	
4	MgArd0003	1C01	SA701	AACTCTCG	SA503	TAGCGAGT	
5	MgArd0004	1D01	SA701	AACTCTCG	SA504	CTGCCGTG	
6	MgArd0005	1E01	SA701	AACTCTCG	SA505	TCATCGAG	
7	MgArd0006	1F01	SA701	AACTCTCG	SA506	CGTGAGTG	
8	MgArd0007	1G01	SA701	AACTCTCG	SA507	GGATATCT	
9	MgArd0008	1H01	SA701	AACTCTCG	SA508	GACACCGT	
10	MgArd0009	1A02	SA702	ACTATGTC	SA501	ATCGTACG	
11	MgArd0010	1B02	SA702	ACTATGTC	SA502	ACTATCTG	
12	MgArd0011	1C02	SA702	ACTATGTC	SA503	TAGCGAGT	
13	MgArd0012	1D02	SA702	ACTATGTC	SA504	CTGCCGTG	
14	MgArd0013	1E02	SA702	ACTATGTC	SA505	TCATCGAG	
15	MgArd0014	1F02	SA702	ACTATGTC	SA506	CGTGAGTG	
16	MgArd0015	1G02	SA702	ACTATGTC	SA507	GGATATCT	
17	MgArd0017	1A03	SA703	AGTAGCGT	SA501	ATCGTACG	
18	MgArd0021	1D03	SA703	AGTAGCGT	SA504	CTGCCGTG	
19	MgArd0024	1G03	SA703	AGTAGCGT	SA507	GGATATCT	
20	MgArd0029	1A04	SA704	CAGTGAGT	SA501	ATCGTACG	
21	MgArd0030	1B04	SA704	CAGTGAGT	SA502	ACTATCTG	
22	MgArd0032	1C04	SA704	CAGTGAGT	SA503	TAGCGAGT	
23	MgArd0035	1F04	SA704	CAGTGAGT	SA506	CGTGAGTG	
24	MgArd0036	1G04	SA704	CAGTGAGT	SA507	GGATATCT	
25	MgArd0038	1A05	SA705	CGTACTCA	SA501	ATCGTACG	
26	MgArd0040	1C05	SA705	CGTACTCA	SA503	TAGCGAGT	
27	MgArd0041	1D05	SA705	CGTACTCA	SA504	CTGCCGTG	
28	MgArd0042	1E05	SA705	CGTACTCA	SA505	TCATCGAG	

<https://doi.org/10.1371/journal.pntd.0003929>