Contents

Introduction **3**

1 Overview **5**

1.1 Diversity 5

2 Explore Your Microbiome **9**

2.1 Getting Started 9

2.2 Microbes to watch 12

2.3 How to Use the uBiome Taxonomy Files 21

2.4 Exploratory Data Analysis 30

Methods **45**

Introduction

This is a short book about my personal experiments with the microbiome.

During the period from 2014 until late 2017, I sequenced over 500 samples of my microbiome. Inspired by the experiment in David et al. (2014), during most of that time I also carefully tracked the food I ate, my sleep, and other variables like activity or location. Most of my near-daily samples were of my gut, but I also regularly tested my skin, nose, and mouth. Since I'm generally healthy, I didn't have a specific goal in mind other than to try to understand better what these microbes are doing, so many of my tests were taken while undergoing simple experiments, like eating a specific type of food or visiting a new location. While not necessarily up to the rigorous standards of a formal scientific trial, these "n of 1" studies on myself helped me discover several new interesting facts about my own microbiome, many of which appear to contradict other published studies. In addition, hundreds of people sent me their own test results, letting me compare many different microbiomes. And of course, I also followed the latest developments in scientific publications and the general press as I eagerly tried to learn more.

What follows is a brief overview of some of the key things I learned.

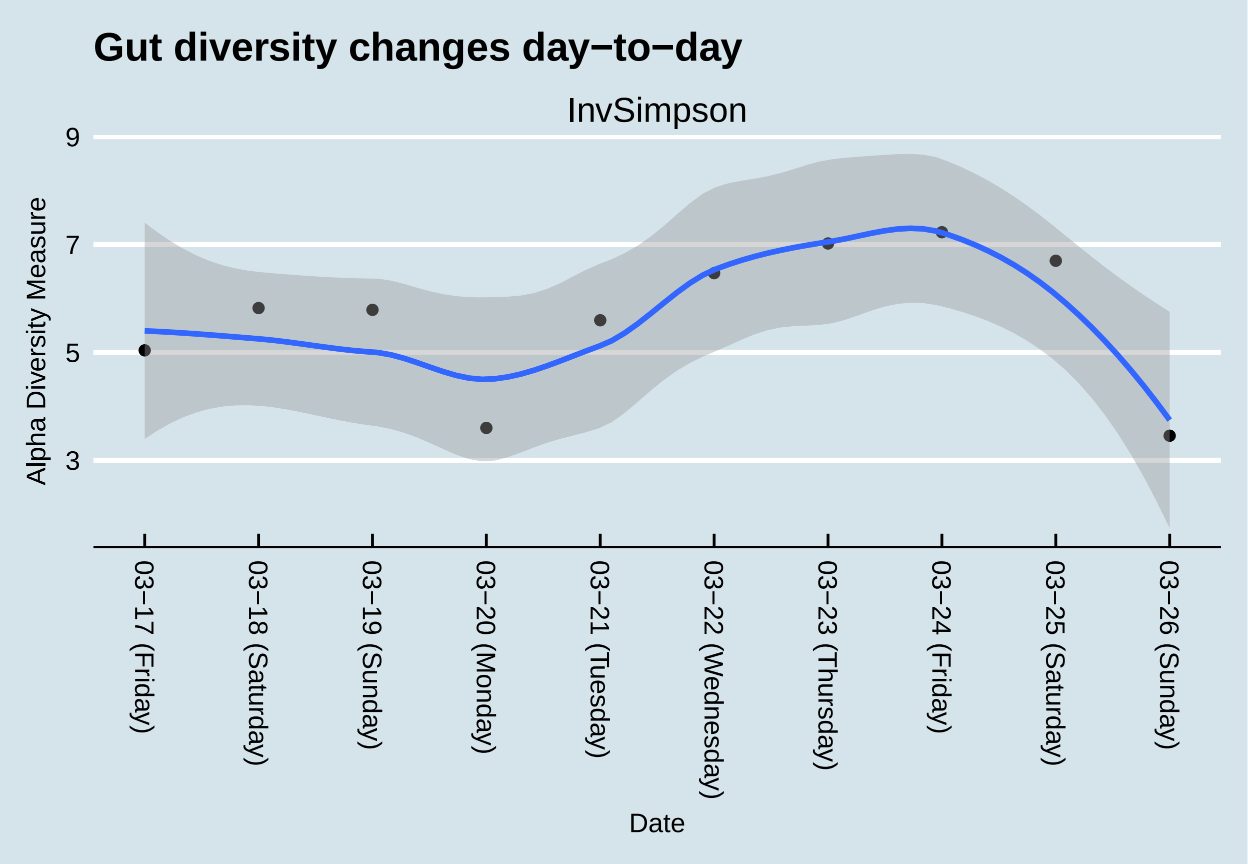
- The microbiome is highly variable from day to day, often moving in ways that appear indistinguishable from random.
- Broad trends *are* there if you look closely. I found many intriguing new results.
- It *is* possible to change your microbiome in specific circumstances.
- People's microbiomes are frustratingly different from one another. An aspect that seems to be true about one person may not apply to another.

Chapter 1

Overview

1.1 Diversity

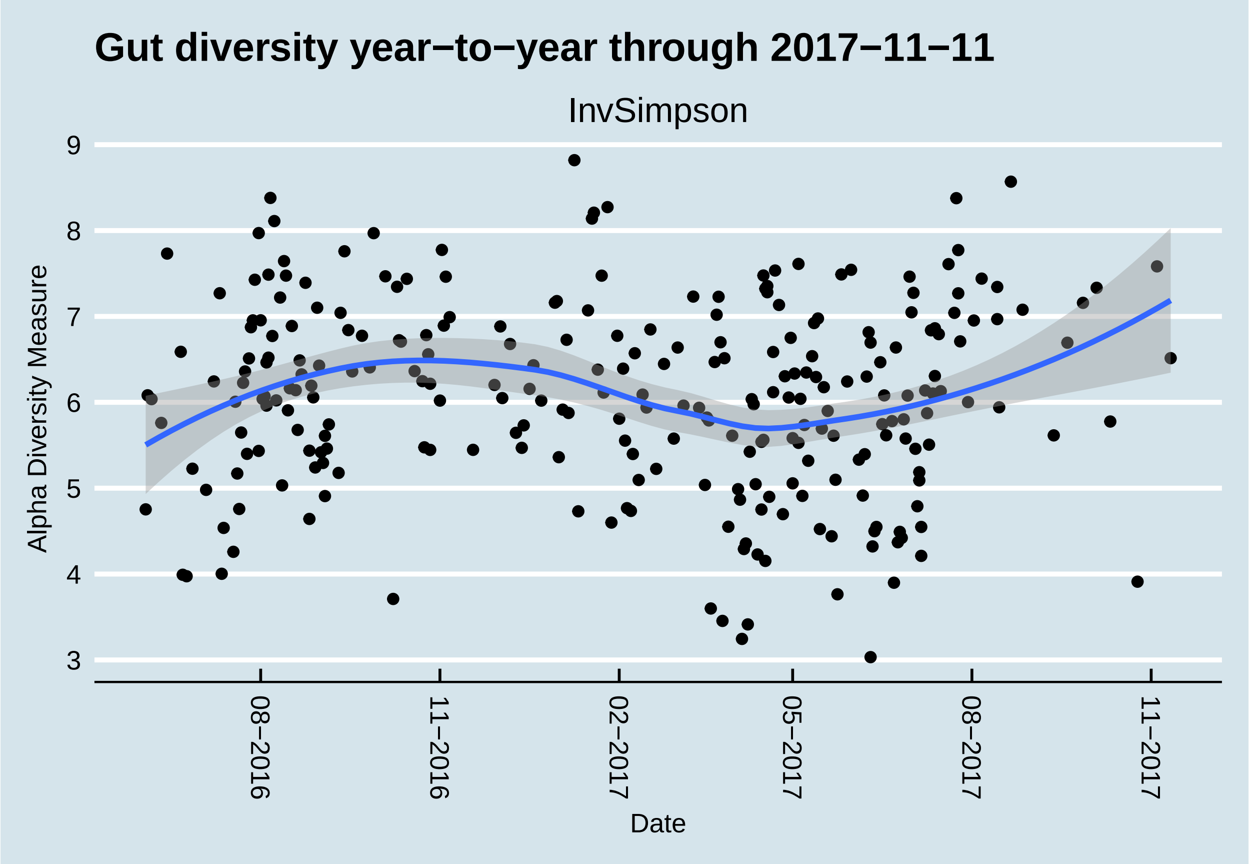
The general consensus is that diversity is good: a greater variety of microbes ensures more resillience against the daily threat of invaders. Many people, after taking just one test, often feel either reassured that their diversity is “good” or disappointed that it’s “bad”. But I find that day-to-day variability is great enough that it’s almost never useful to use a single result. For example, here’s my diversity during a typical week:



If I had only tested on Monday, I may have been disappointed with my 0.38 score. Wait another day or two and, with no significant changes in diet, I was up to 0.72 – before plunging to 0.37 by the weekend. Moral:

don't take a single result too seriously.

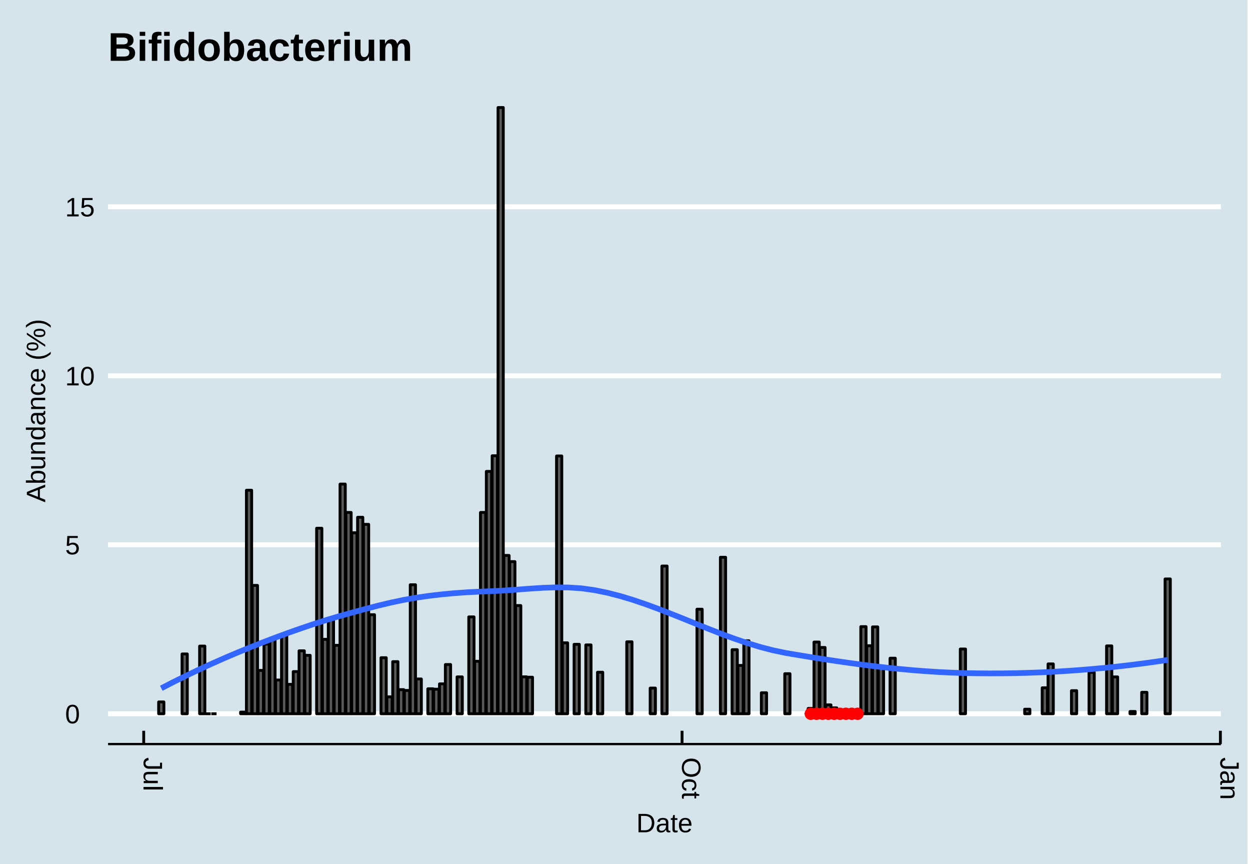
To get a sense of how much diversity can vary over a year:



Although the blue moving-average line shows apparent stability, there are many days that are far above and below the average. Yes, at various times during this period I was eating different types of food, often in a deliberate attempt to influence my microbiome, but believe me: that is not the reason for the wild changes up and down. I also studied the mathematics behind how diversity is measured, hoping to find something more "accurate", but ultimately I concluded that, like many attempts to summarize the microbiome in a single number, the whole concept of diversity is a mirage. Everything depends.

Bifidobacterium is a key component of virtually all popular probiotic supplements, partly because it is so easy to manufacture, but also due to its proven association with sleep and other aspects of health. A six month picture of my levels shows some dramatic ups and downs[1].

[1] Important note: in all of these charts, I don't distinguish between a day when the tested abundance was zero, and a day when I have no reliable test results. Besides the daily variability in the microbiome, the test results themselves often come with wild differences in quality. This is nothing to complain about: the ability to see these microbes in the first place is a true miracle of technology, and even the most careful lab can't get around the myriad of technical issues that come with such a complex process.



Incidentally, the red dots indicate days when I was taking a powerful probiotic supplement that contained *Bifidobacterium*. And that big spike in September? That was during a trip to New Orleans, when I ate a lot of red beans and rice. At least for me, food seems to work better than taking supplements.

Chapter 2

Explore Your Microbiome

This chapter assumes you have already sequenced your microbiome. You'll have a breakdown of the types of microbes found in your sample, and depending on the lab, you'll have some simple web tools to explore your sample. I have a complete review of the various microbiome sequencing services, but for most people the best service is uBiome, a low-cost (under $100) lab that will give you results in a few weeks. Although their web interface is limited and a bit confusing, uBiome shines at letting you download the raw data, either as an easy-to-process JSON or CSV file, or as the original FASTQ file – the same format used by nearly all microbiome scientists. The powerful ability to study your sample in high-level tools like Python, R, or even Excel, makes this the best way for serious microbiome hackers to understand their results. But even if you don't want to build tools for yourself, there are plenty of pre-built tools (like the ones on this site) that can do more analysis for you.

But first, let's look at the built-in uBiome web tools. Although this description is specific to uBiome, the general principles apply to just about any site that offers basic microbiome analysis.

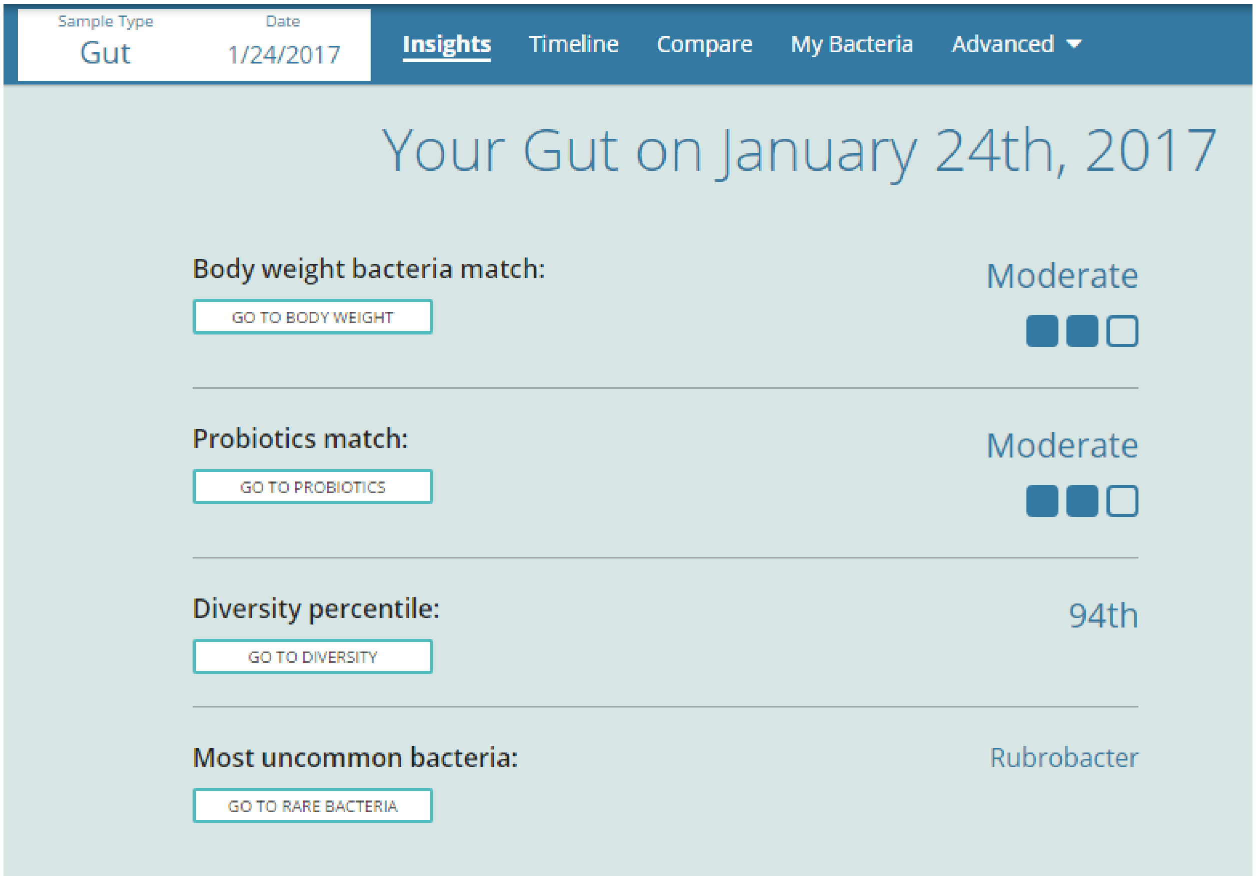
2.1 Getting Started

You just received an email that uBiome has finished processing your sample. Now what do you do?

The first stop is the uBiome Explorer web dashboard, where you'll see an overview of your results. The instructions there are self-explanatory and you'll learn most of what you need to know by simply clicking around. The following explanation will help you dig deeper.

If you only have one sample, your page will look something like Figure 2.1.

That's the Insights pane, designed to tell you the highlights. If this is your first time here, go ahead and breeze through this page, but don't get lost in the details. As we'll learn, scientists have barely scratched the surface of what we will eventually understand about the microbiome, so think of this page as a teaser – a hint of the potential. If your results show something you didn't expect, say on diversity or body weight, don't worry: it almost certainly just means that the Insights pane is wrong about you. Later we'll see how to pull out far more interesting and useful results.



Sample Type: Gut

Date: 1/24/2017

Your Gut on January 24th, 2017

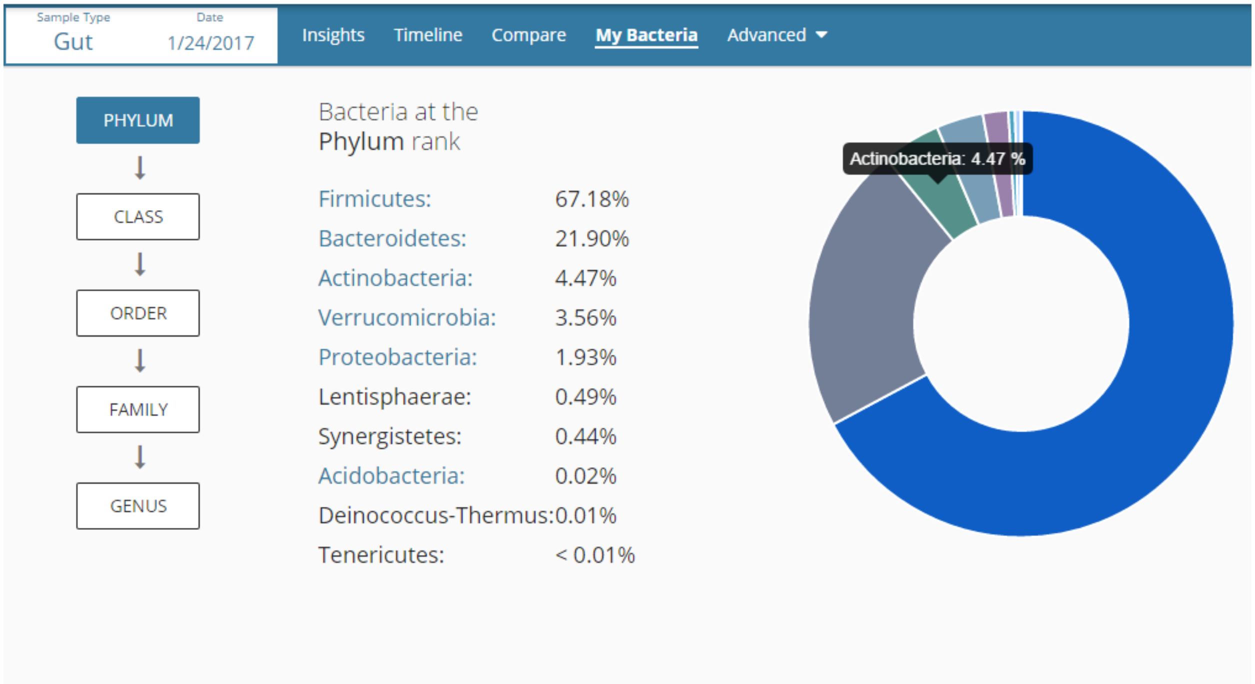
Body weight bacteria match: Moderate ■■□

Probiotics match: Moderate ■■□

Diversity percentile: 94th

Most uncommon bacteria: Rubrobacter

Figure 2.1: uBiome Explorer Insights Overview



Bacteria at the
Phylum rank

Firmicutes:	67.18%
Bacteroidetes:	21.90%
Actinobacteria:	4.47%
Verrucomicrobia:	3.56%
Proteobacteria:	1.93%
Lentisphaerae:	0.49%
Synergistetes:	0.44%
Acidobacteria:	0.02%
Deinococcus-Thermus:	0.01%
Tenericutes:	< 0.01%

Click next on the "My Bacteria" tab. The colorful pie chart there is your first high-level glimpse of the bacteria found in your sample. Hover over it with your mouse (or tap with your finger if you're on a phone or iPad), to see a pop-up view of the approximate percentages.

You're seeing a *phyla*-based look at your microbiome. As we'll learn in more detail later, this is a very high level view, a bit like how a Martian might see life on earth from far in outer space. Keep in mind that by "very high level", we mean *very* high, the equivalent of seeing life only at the level of "vertebrate" and "invertebrate". The incomprehensible diversity of bacterial life means that at this level, you don't even bother separating fish from one another or for that matter, from land animals: just lump all of them together. At the phyla level, mammals, reptiles, fish – they're all the same. Similarly, when you see the phylum *Firmicutes*, for example, think of it as having as encompassing as much variation as all the vertebrates, from those on land, in the ocean, big, small, harmless, dangerous and everything in between. In other words, this first approximation is pretty crude.

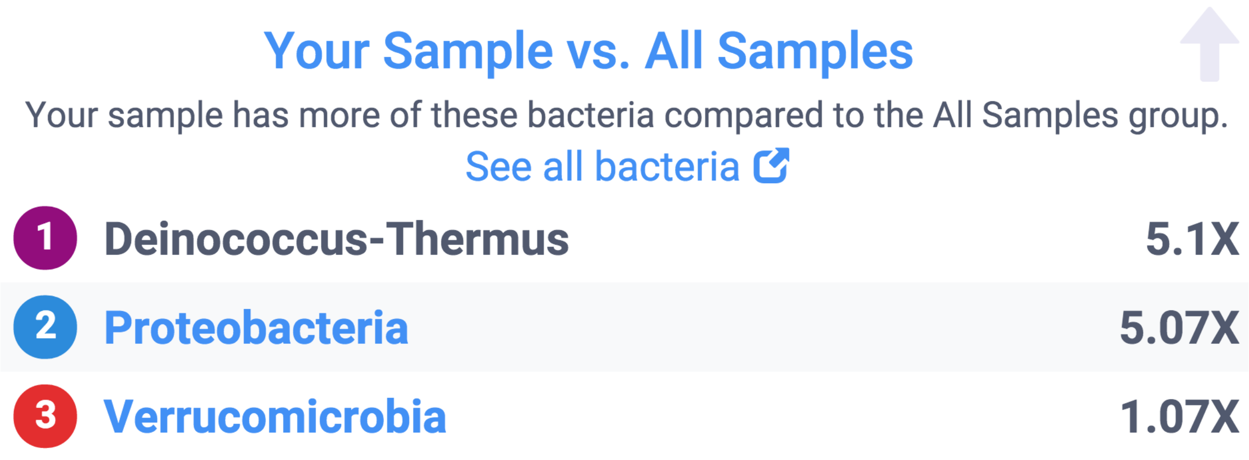
This time the various phyla *are* represented as percentages of the whole, so some of them are relegated to tiny slices of the pie. That doesn't necessarily mean they're unimportant, though, because in the world of life, even some tiny populations can exert an outsized influence. A wolf is just one of a gazillion different creatures in Yellowstone National Park, but that is little comfort to a rabbit.

Remember too that so far we're treating each individual organism as if it's the same size as the others, but that's an oversimplification too, just like it would be a mistake to assume that elephants matter more because they're bigger than humans.

All we know at the phylum level is that proportionately, certain large classes of bacteria are more well-represented than others. That may not sound like much to go on, but as we'll see, scientists have been able to learn quite a bit from tiny clues like this.

2.1.1 How are you different?

Since so little about the microbiome is well-understood, most of the time it will be hard to interpret your results by themselves. This is where the power of uBiome's citizen science can help, because the web page lets you easily compare your results with other people.



Your Sample vs. All Samples

Your sample has more of these bacteria compared to the All Samples group.

See all bacteria

1	Deinococcus-Thermus	5.1X
2	Proteobacteria	5.07X
3	Verrucomicrobia	1.07X

That first bacteria, *Deinococcus-Thermus* is an extremophile, usually only found in harsh environments, like the impossibly acidic human stomach. Why would I have so many more than other people like me?

One clue is the similarly-high levels of Proteobacteria, which as we just noted is often high in people with gastro-intestinal issues. Maybe I was exposed to something that released a bit of stomach acid, and its extremophile *Deinococcus* into my colon.

Verrucomicrobia is elevated too, though not by much. This is a Phylum that is generally associated with soil microbes, though it's not unusual to find it in the gut as well.

I looked back at my notes during the period of a few days before this sample, and interestingly I had been doing work in my garden at the time. We have deer and other animals that come near our house... is it possible that I was exposed to some animal excrement, maybe some of which somehow made it into my gut?

It's just a theory, and perhaps an unlikely one, but I wonder if my body was fighting some type of pathogen as a result of my outdoor activities. Now before we get too excited, keep in mind that I never felt the slightest bit ill during this period. Can I thank my microbiome? Perhaps I was exposed to something nasty, the kind of bug that under other circumstances might have wreaked havoc and left me terribly sick for days. Now I have no way of knowing for sure, but this tidbit makes me wonder if maybe my microbiome acted as a guardian angel, protecting me from something much worse.

Similarly, your own results comparing to others may lead you to clues about aspects of your environment you didn't otherwise suspect.

Incidentally, since this test I've sent dozens of additional samples to uBiome and never saw that *Deinococcus* thing again. Good riddance? or maybe I should be thankful for the protection, and hope that little bits of it may still be present, undetectably in my stomach, waiting to fight another external enemy the next time I come in contact with something in the soil.

2.2 Microbes to watch

If you're brand new to the microbiome, the variety of microbes can be pretty daunting. Let's look through specific microbes you're likely to see and compare them to the ones that were in my samples.

In this first step, we'll just consider the most common microbes and the overall consensus on what they do. Later, in the chapter on experiments, we'll show more about how you can *manipulate* them.

What you're really wondering is how does your sample compare to others? Do you have an unusual abundance (or lack) of a particular taxa? Is there something that might indicate a greater or lesser similarity between

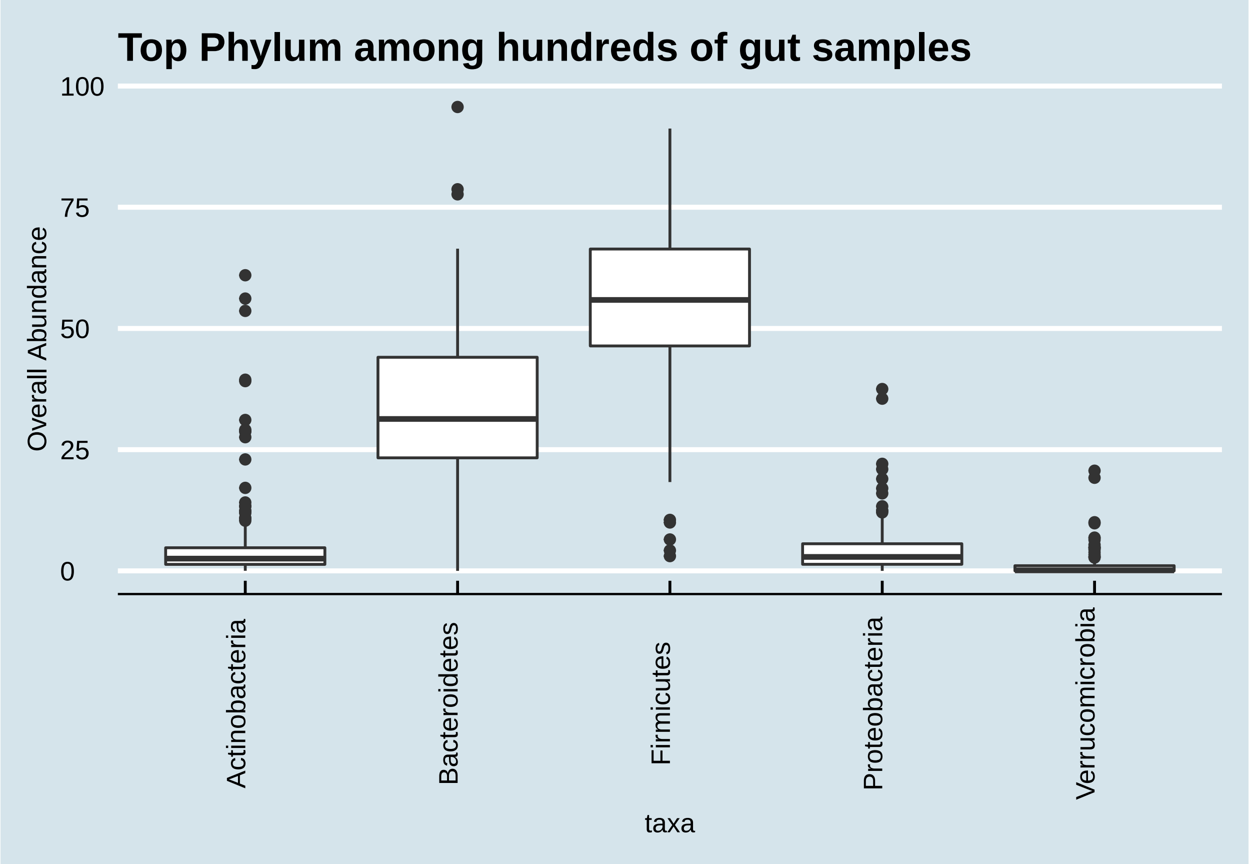


Figure 2.2: Relative abundance of the most common phylum across hundreds of gut samples.

your sample and certain other types of people? That is a very difficult question which we'll address over and over in this book, but for now let's just look at the overall abundances among the hundreds of random people who have sent me their samples over the years.

This is a "box and whisker" chart where the white boxes show where the vast majority of samples occur. The line through the middle of the white box is the median, and the other dots are outliers that fall outside the majority.

2.2.1 The most common phyla

Most westerners will find their sample dominated by two phyla: *Firmicutes* and *Bacteroidetes.* These are so common in healthy people that it's tempting to assume this is "natural" or "normal", but like much else with the microbiome, the situation is different outside the western world, a clue that it's difficult to summarize a single individual's microbiome as "good" or "bad." It all depends.

Prevotella is associated with carbohydrates, and you might think they're more common in people who eat lots of

Proteobacteria If you're an otherwise normal omnivore and see double-digit amounts of this it can be a sign that your body was recently under attack. That's because the Proteobacteria includes many pathogens like *E. Coli* and *Shigella.* For example, in one of my tests I had 14% Proteobacteria, which often is elevated in people with gastric disorders like Crohn's disease. That doesn't mean that Proteobacteria is *bad*; in fact, it could mean the opposite, that we need more of these to fight back invaders. In my case the unusually high amount came right after a trip to Central America, during which my body was exposed to many new and possibly pathogenic microbes. My Proteobacteria fell soon after I returned to the U.S. and my normal diet.

Proteobacteria: come back quickly after antibiotics ("a weed") QuantifiedBody

Does Proteobacteria play a role?

TS: Only 52 of the 4200 compounds found in normal mice blood were identified in germ-free mice. The implication is that most of the chemicals in our blood are synthesized by microbes.

Using examples from my own sampling, can I say something about what to look for?

2.2.2 Firmicutes

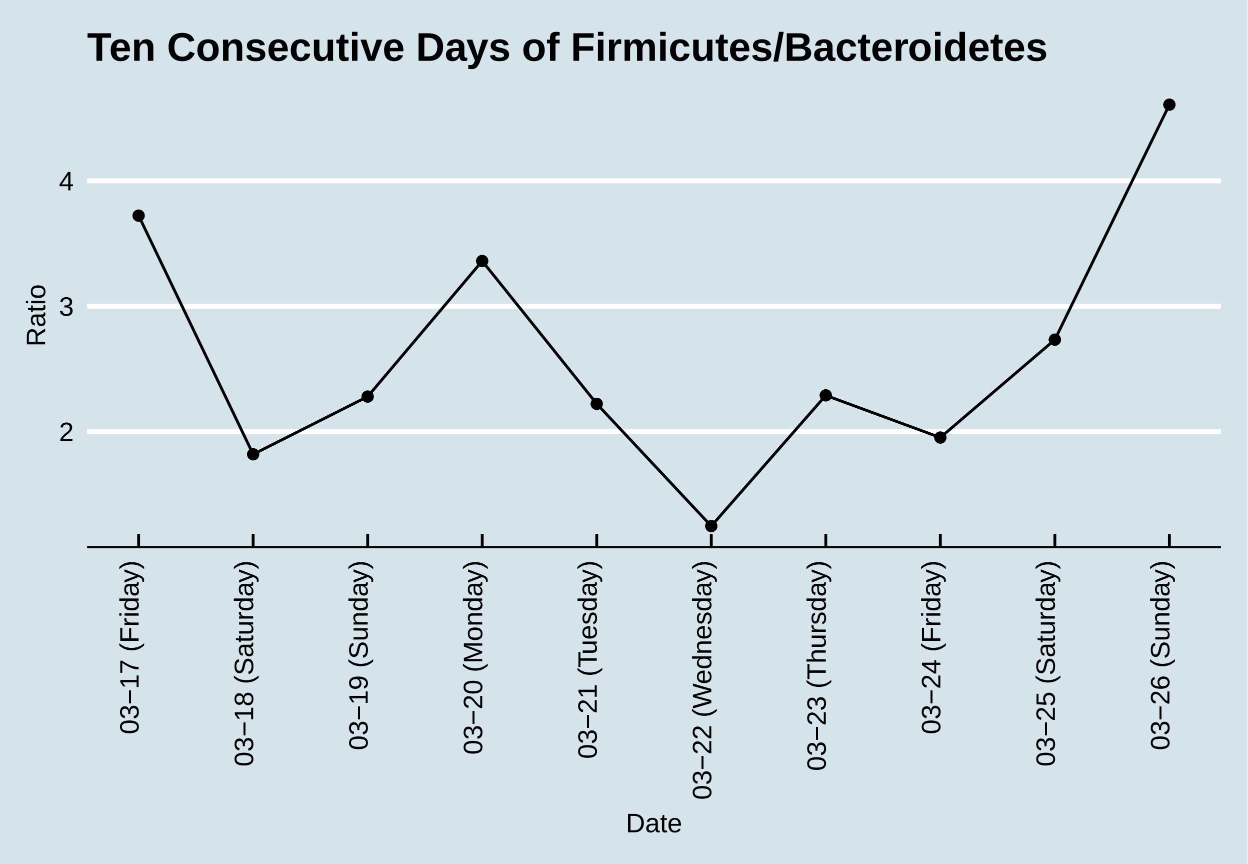
Firmicutes is usually the most abundant in uBiome tests of modern, urban people, and for good reason: it's very good at sucking energy out of the types of high-fat, highly-processed food we eat in industrialized societies. For that reason, some scientists have claimed it might associate with obesity, since its presence might indicate a gut that is highly-optimized to pull as many calories as possible from food. On the other hand, it's not hard to find skinny people with lots of Firmicutes, so this theory doesn't always hold. I've seen it lower in vegetarians, and I suspect that maybe it reflects diet more than weight.

2.2.3 Bacteroides

Bacteroidetes is not as good at digesting fat, so it sticks to high-fiber foods like those in vegetables, which is why vegetarians tend to have high levels of this phylum. It's often most prominent outside the industrialized world, too, where people eat less meat. Often, though not always, when I see a sample with high *Bacteroidetes* (i.e. higher than *Firmicutes*) it'll turn out the person is a vegetarian. Incidentally, don't confuse this phylum with the similar-sounding genus *Bacteroides* [see below].

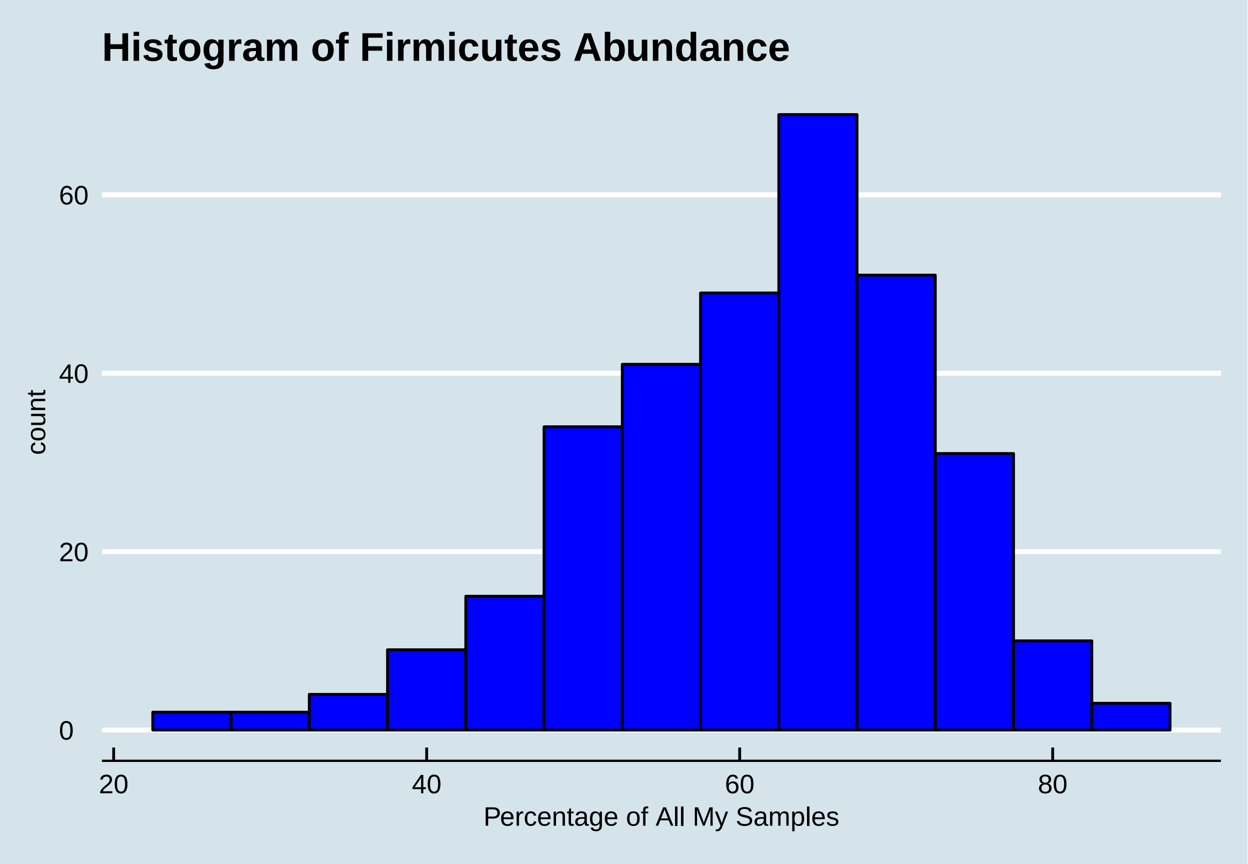
Although many of the species in this genus are pathogenic, you're much more likely to encounter one of the "mutualist" versions that are co-dependent on humans, providing us with many important features we have not had to evolve, while our gut gives back nutrients and more that *they* need to survive. Read much more in (Wexler 2007).

In general I find too much day-to-day variability to rely on a single test. Here's how my levels of the two most common phyla change on a daily basis:



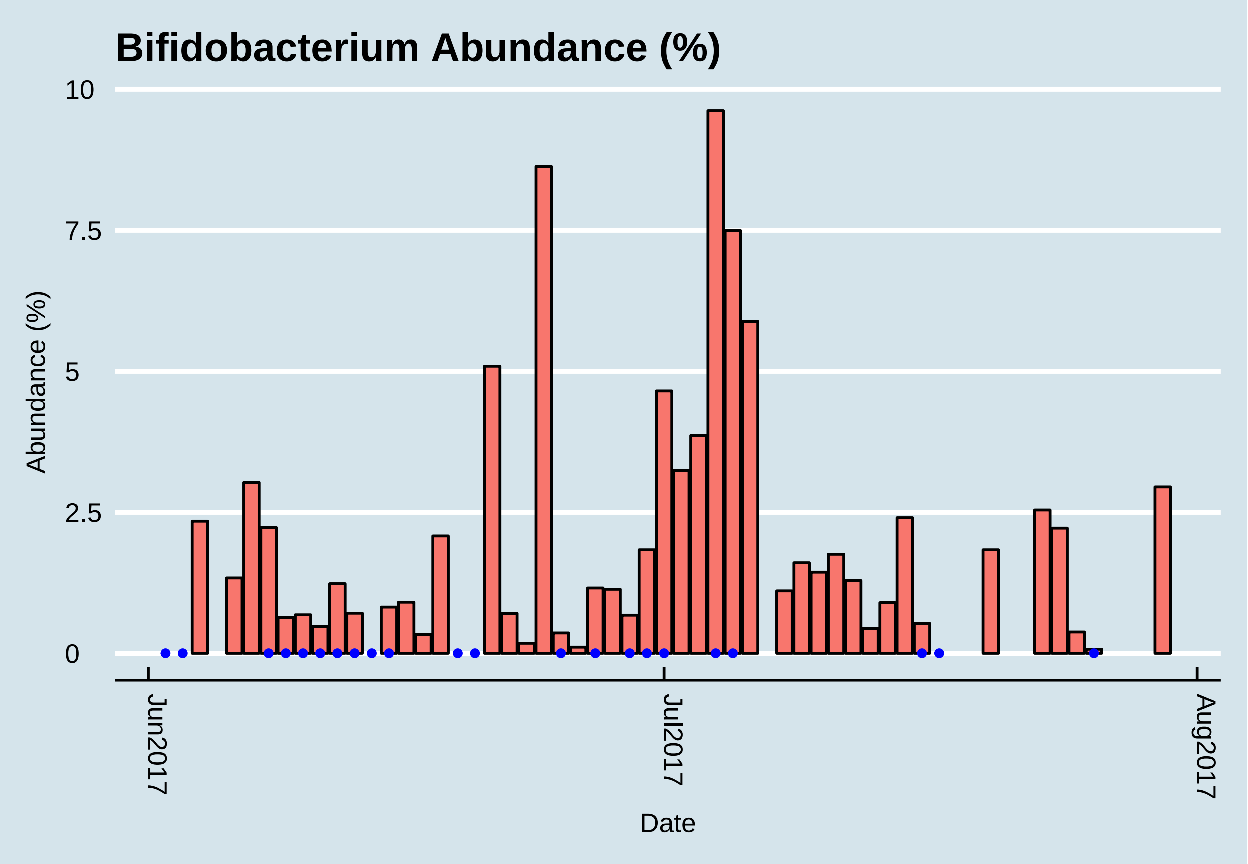
If you sampled on a Monday, you could well get a different answer than if you sampled on a Tuesday.

In fact, although there *does* seem to be an average where my *Firmicutes* is most abundant, there are many days where the amounts drop well below half.



2.2.4 Bifidobacterium

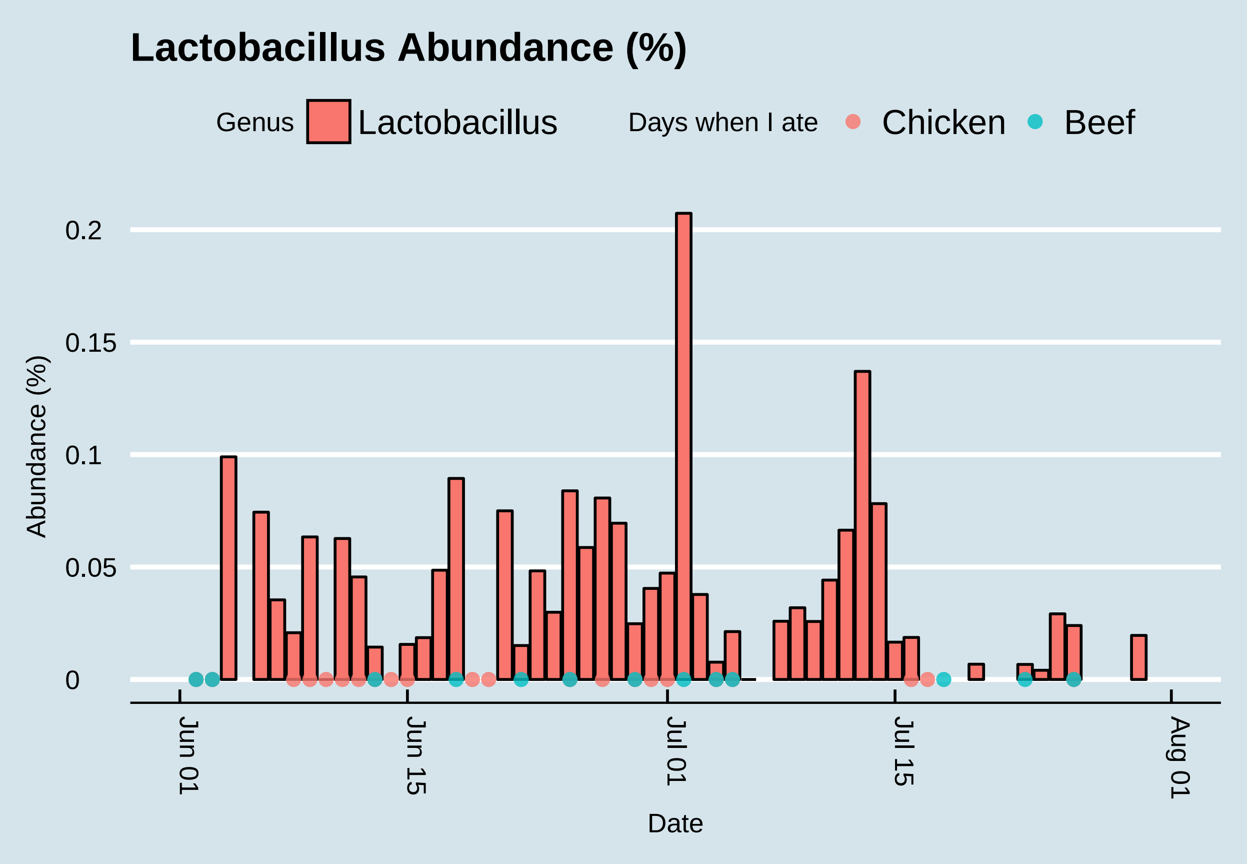
The blue dots indicate days on which I ate some beans.



2.2.5 Lactobacillus

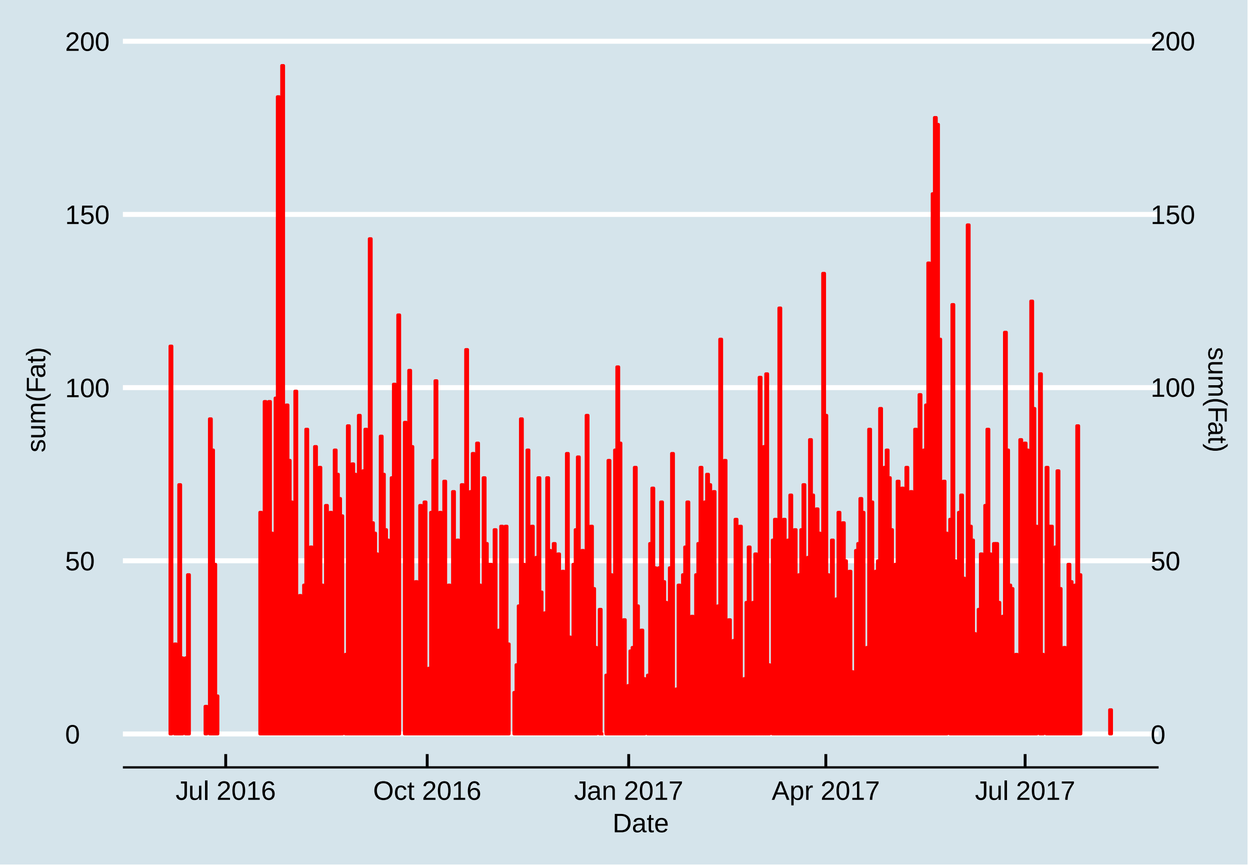
A 2017 careful study of rats revealed higher levels of *Lactobacillus* on a diet of chicken (versus beef or soy)[1]. The blue dots represent days on which I ate poultry (chicken or turkey). Green dots are days I ate beef.

[1] Zhu et al. (2017)



2.2.6 Fat and my levels of Lactobacillus

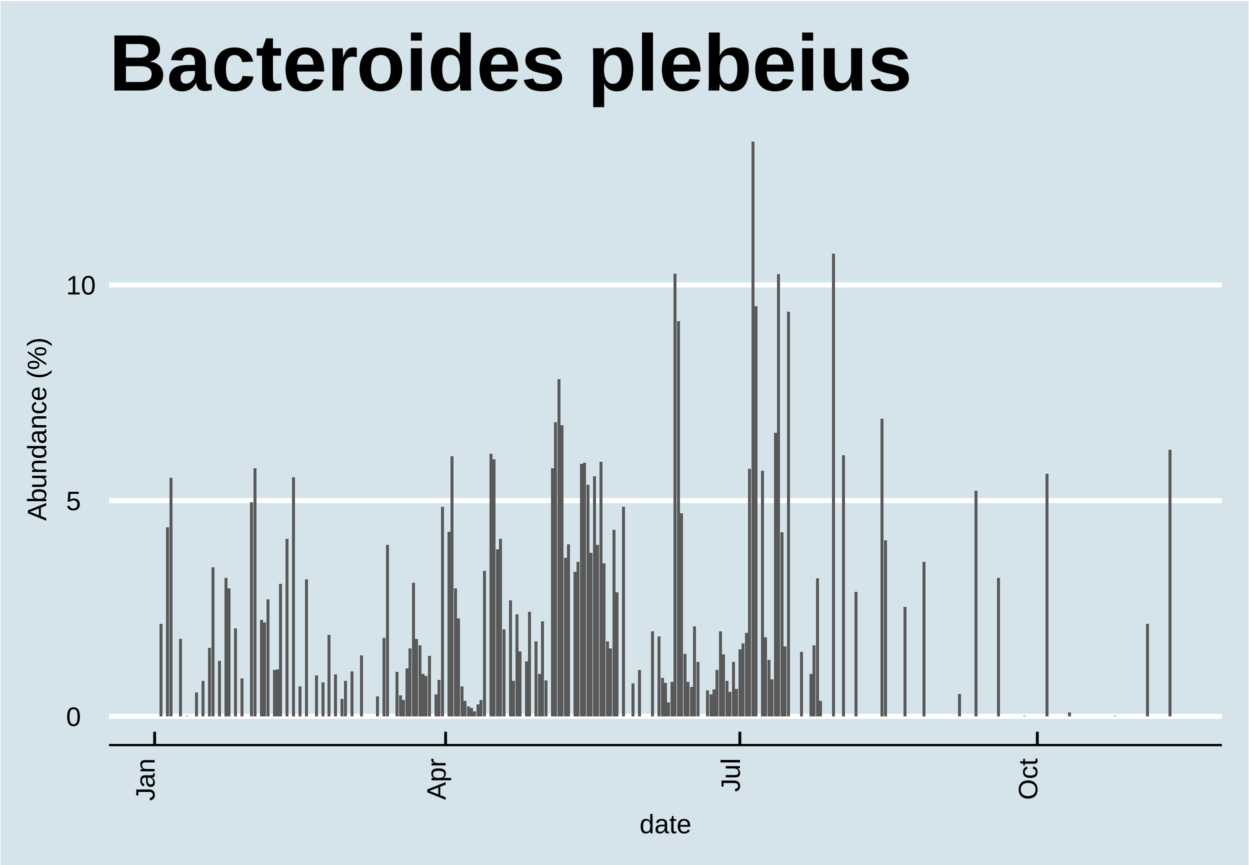
Here's how much fat I eat daily. Does this correlate with my levels of *Lactobacillus*?



2.2.7 Other microbes and digestion

Bacteroides plebeius : Japanese version can digest seaweed, thanks to genes stolen from Zobellia galactanivorans, that lives on the seaweed. Hehemann et al. (2010)

Notice that I seem to have quite a bit of this taxa:



```
##  [1] "Bacteroides fragilis"            "Bacteroides thetaiotaomicron"
##  [3] "Bacteroides uniformis"           "Bacteroides vulgatus"
##  [5] "Bacteroides caccae"              "Bacteroides acidifaciens"
##  [7] "Bacteroides massiliensis"        "Bacteroides salyersiae"
##  [9] "Bacteroides plebeius"            "Bacteroides finegoldii"
## [11] "Bacteroides chinchillae"         "Bacteroides sp. TP-5"
## [13] "Bacteroides stercorirosoris"     "Bacteroides coprocola"
## [15] "Bacteroides eggerthii"           "Bacteroides clarus"
## [17] "Bacteroides nordii"              "Bacteroides ovatus"
## [19] "Bacteroides sp. AR20"            "Bacteroides sp. AR29"
## [21] "Bacteroides sp. XB12B"           "Bacteroides sp. D22"
## [23] "Bacteroides sp. SLC1-38"         "Bacteroides sp. XB44A"
## [25] "Bacteroides sp. 35BE35"          "Bacteroides sp. 35AE37"
## [27] "Bacteroides sp. 31SF15"          "Bacteroides intestinalis"
## [29] "Bacteroides dorei"               "Bacteroides sp. EBA5-17"
## [31] "Bacteroides oleiciplenus"        "Bacteroides faecis"
## [33] "Bacteroides sp. J1511"           "Bacteroides sp. DJF_B097"
## [35] "Bacteroides sp. 14(A)"           "Bacteroides faecichinchillae"
## [37] "Bacteroides stercoris"           "Bacteroides sp."
## [39] "Bacteroides sp. 2_2_4"           "Bacteroides barnesiae"
## [41] "Bacteroides sp. dnLKV9"          "Bacteroides sp. S-17"
## [43] "Bacteroides sartorii"            "Bacteroides sp. 3_1_40A"
## [45] "Bacteroides sp. R6"              "Bacteroides coprophilus"
## [47] "Bacteroides sp. Smarlab 3301643" "Bacteroides sp. 4072"
## [49] "Bacteroides sp. D20"             "Bacteroides xylanisolvens"
```

Why? and is it a coincidence that very few others I've tested have this taxa? Is it also strange that I lived in

Japan for about 10 years? Could all that living in Japan have caused this microbe to take hold in me but not in other North Americans.

Renouf J Nutr 2011 says it's Bacteroides uniformis that breaks down the soy (and why Asians find soy healthier than americans)

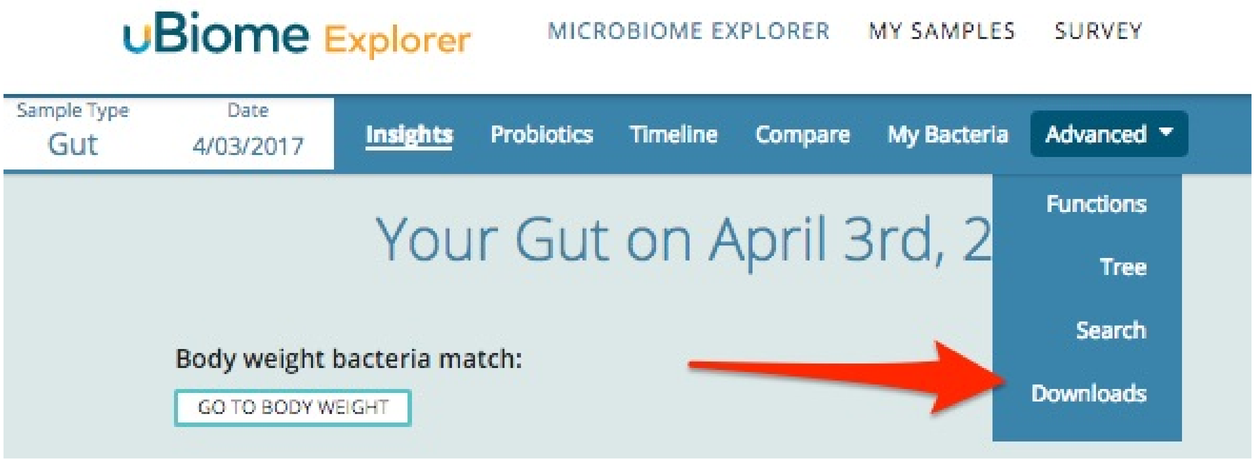
2.3 How to Use the uBiome Taxonomy Files

The information you see in the uBiome web app is a greatly simplified view of the data torrent that comes from the gene sequencing machines that crunched your sample. Your raw data can be thousands of lines long, so to be more useful, uBiome provides a much more concise version. It's called a "taxonomy" file, which is generally only a few hundred lines showing just the organisms that the uBiome computer algorithms think they found in your sample.

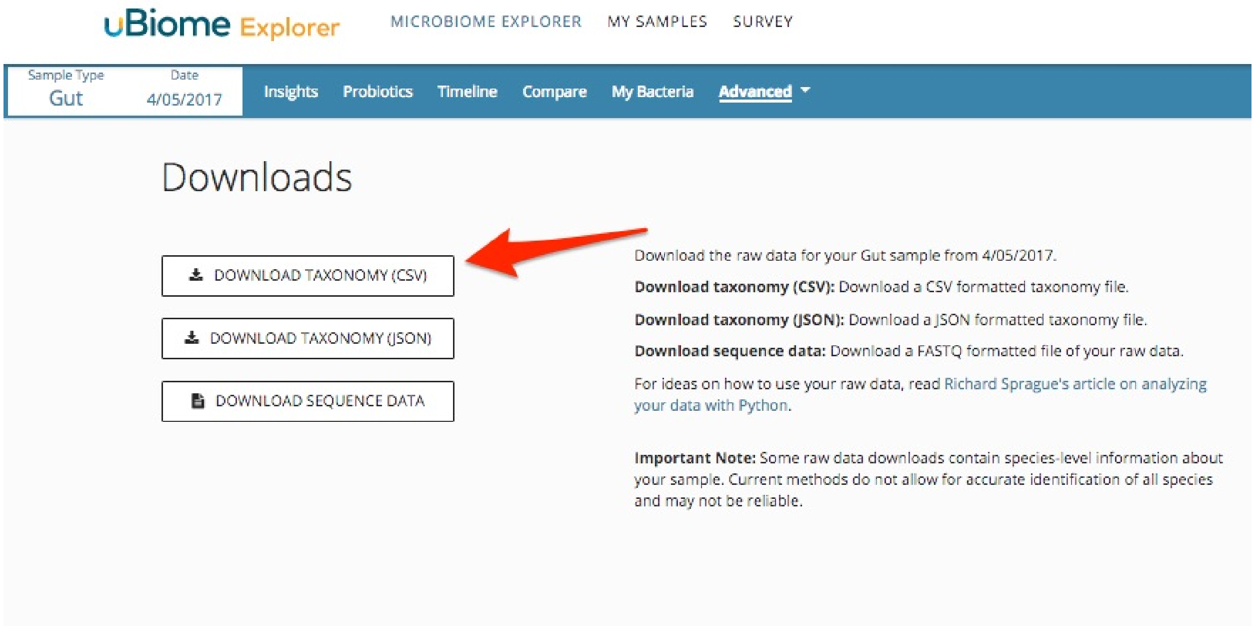
Because uBiome JSON files are well-structured, it's easy to process them with other software, including Excel. That's our next step: bring this data into Excel.

2.3.1 Study your results in Excel

The uBiome web interface includes an easy way to download your data as a CSV (comma-separated value) file, which can be read into a spreadsheet like Excel. Just click the 'downloads' in the 'Advanced' tab (Figure ??)



Then select "Download Taxonomy CSV" (Figure ??)



Downloads

DOWNLOAD TAXONOMY (CSV)

DOWNLOAD TAXONOMY (JSON)

DOWNLOAD SEQUENCE DATA

Download the raw data for your Gut sample from 4/05/2017.

Download taxonomy (CSV): Download a CSV formatted taxonomy file.

Download taxonomy (JSON): Download a JSON formatted taxonomy file.

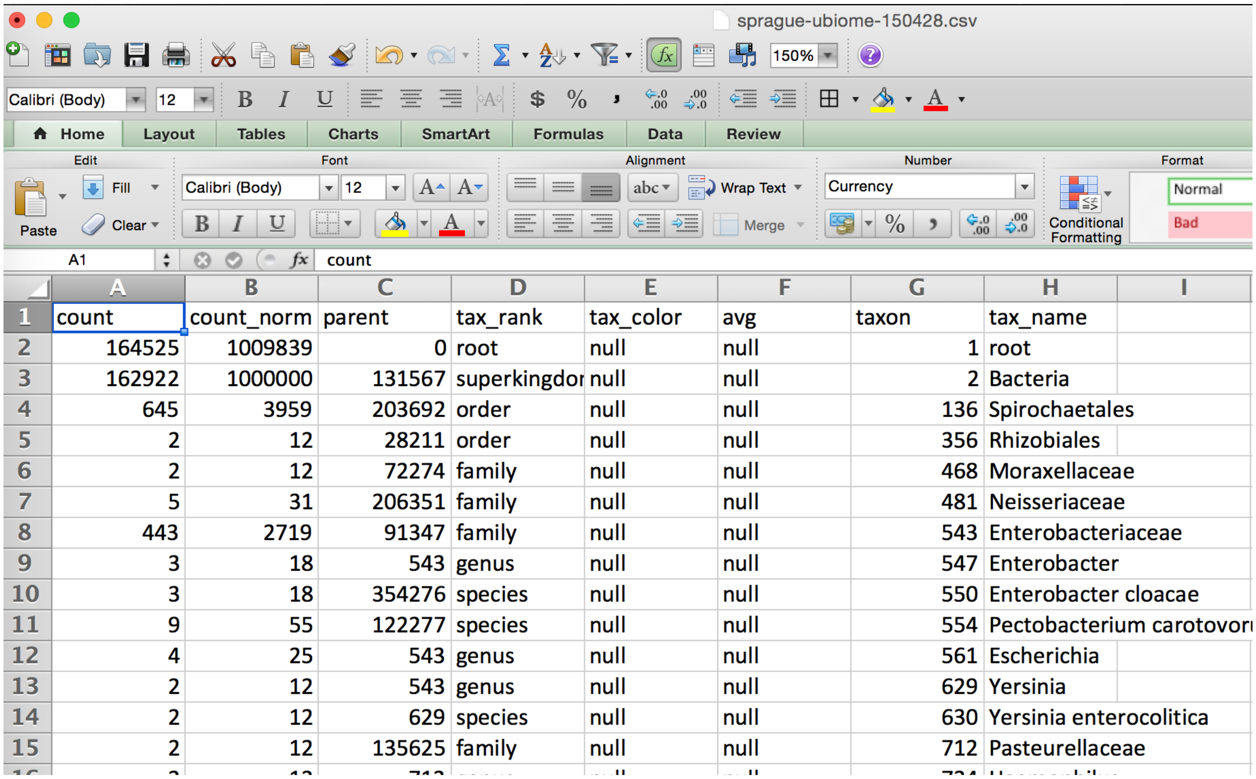
Download sequence data: Download a FASTQ formatted file of your raw data.

For ideas on how to use your raw data, read Richard Sprague's article on analyzing your data with Python.

Important Note: Some raw data downloads contain species-level information about your sample. Current methods do not allow for accurate identification of all species and may not be reliable.

and a CSV copy of your results will be downloaded to your computer.

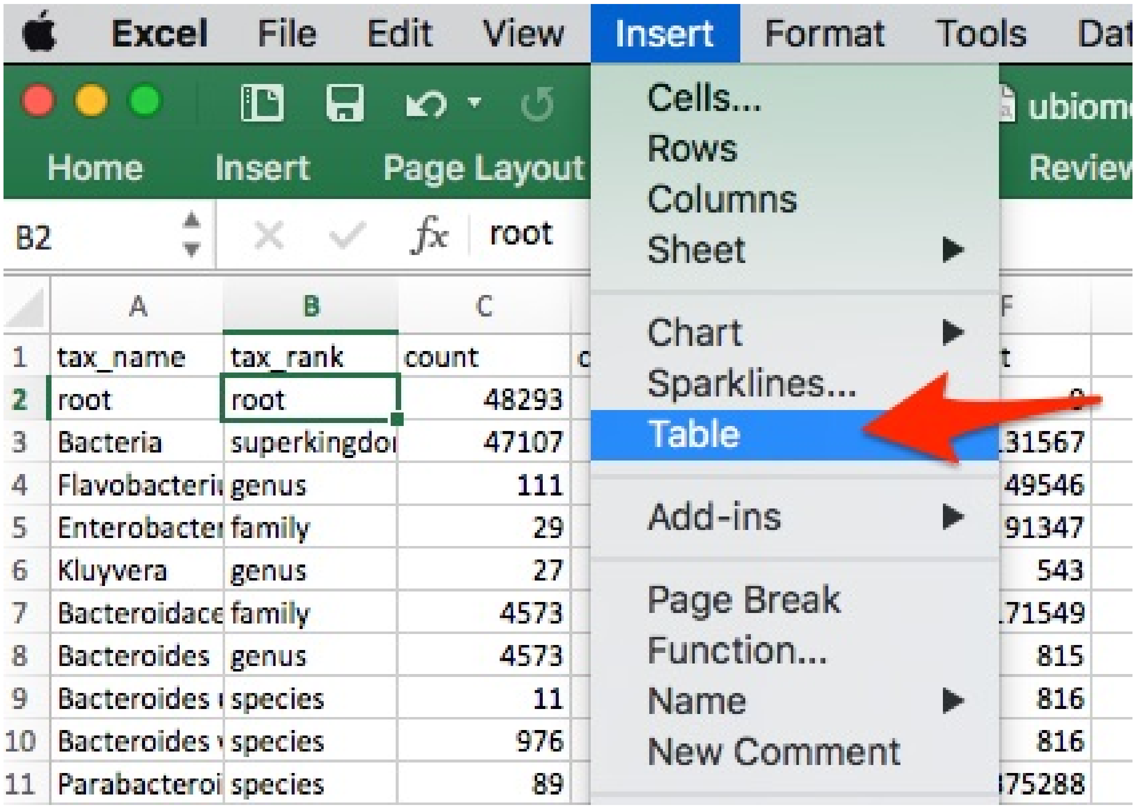
Once the data is in Excel, you can work with it just as you would with any Excel data sheet. Here's how one of my recent uBiome taxonomy files looks in Mac Excel (Figure **??**)



A1 count

	A	B	C	D	E	F	G	H	I
1	count	count_norm	parent	tax_rank	tax_color	avg	taxon	tax_name	
2	164525	1009839	0	root	null	null	1	root	
3	162922	1000000	131567	superkingdor	null	null	2	Bacteria	
4	645	3959	203692	order	null	null	136	Spirochaetales	
5	2	12	28211	order	null	null	356	Rhizobiales	
6	2	12	72274	family	null	null	468	Moraxellaceae	
7	5	31	206351	family	null	null	481	Neisseriaceae	
8	443	2719	91347	family	null	null	543	Enterobacteriaceae	
9	3	18	543	genus	null	null	547	Enterobacter	
10	3	18	354276	species	null	null	550	Enterobacter cloacae	
11	9	55	122277	species	null	null	554	Pectobacterium carotovor	
12	4	25	543	genus	null	null	561	Escherichia	
13	2	12	543	genus	null	null	629	Yersinia	
14	2	12	629	species	null	null	630	Yersinia enterocolitica	
15	2	12	135625	family	null	null	712	Pasteurellaceae	

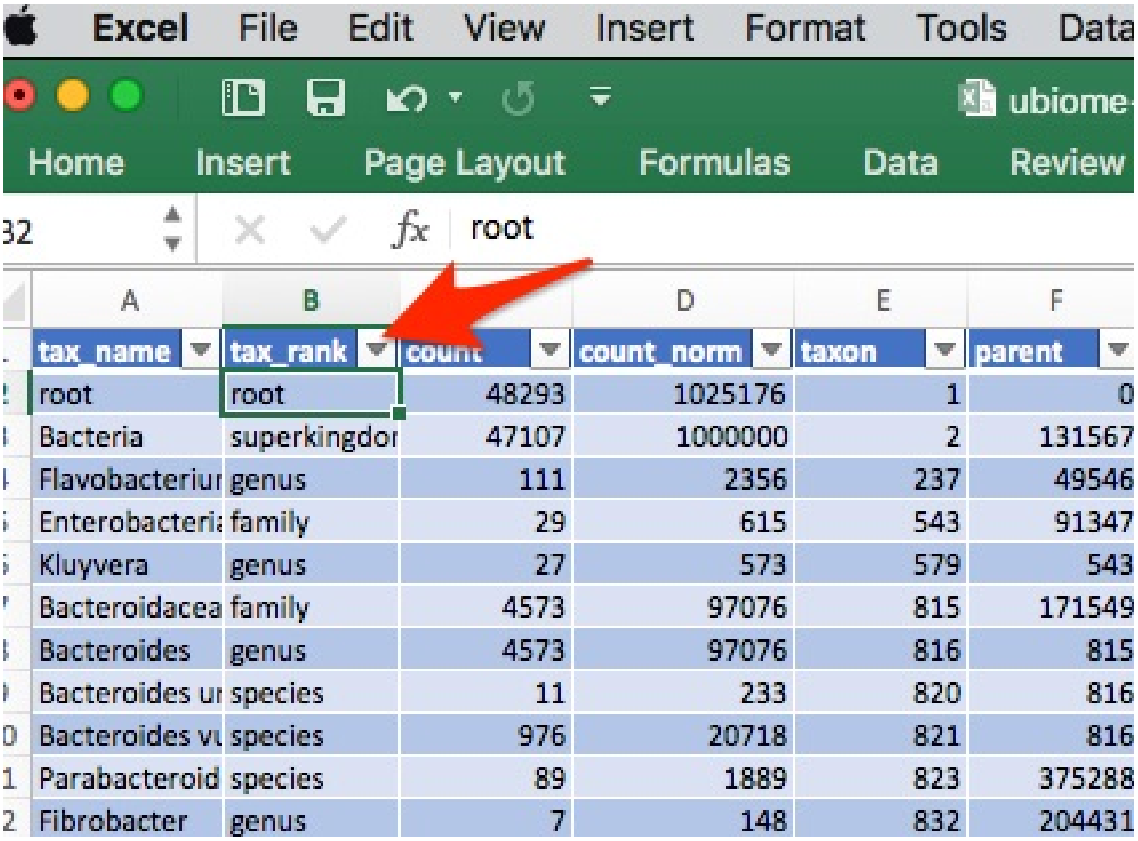
At this point, we can now apply the full power of your Excel skills to do the analysis. I usually start by turning the sheet into a "table", to make it easier to do filtering and sorting later. Most popular spreadsheets have an easy way to do this. On Mac Excel, I do that from the "insert" menu:



B2 fx root

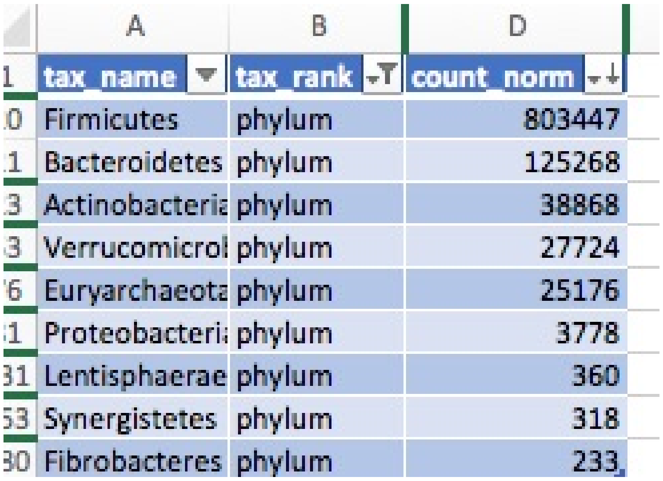
	A	B	C	F
1	tax_name	tax_rank	count	t
2	root	root	48293	0
3	Bacteria	superkingdoı	47107	.31567
4	Flavobacteri	genus	111	49546
5	Enterobacteı	family	29	91347
6	Kluyvera	genus	27	543
7	Bacteroidace	family	4573	.71549
8	Bacteroides	genus	4573	815
9	Bacteroides (	species	11	816
10	Bacteroides \	species	976	816
11	Parabacteroi	species	89	75288

Once I have a table, then it's easy to apply a filter. For now I just want to look at the Phylum level, so I click the tiny triangle to the right of the "tax_rank" label, like this:


Excel File Edit View Insert Format Tools Data
ubiome
Home Insert Page Layout Formulas Data Review
32
fx root
A B D E F

tax_name	tax_rank	count	count_norm	taxon	parent
root	root	48293	1025176	1	0
Bacteria	superkingdor	47107	1000000	2	131567
Flavobacteriur	genus	111	2356	237	49546
Enterobacteri:	family	29	615	543	91347
Kluyvera	genus	27	573	579	543
Bacteroidacea	family	4573	97076	815	171549
Bacteroides	genus	4573	97076	816	815
Bacteroides ur	species	11	233	820	816
Bacteroides vu	species	976	20718	821	816
Parabacteroid	species	89	1889	823	375288
Fibrobacter	genus	7	148	832	204431

And now I can look just at the Phylum rank. I won't bother showing each step after this, but after filtering and sorting, I can quickly end up with something like this:



tax_name	tax_rank	count_norm
Firmicutes	phylum	803447
Bacteroidetes	phylum	125268
Actinobacteri:	phylum	38868
Verrucomicrol	phylum	27724
Euryarchaeota	phylum	25176
Proteobacteri:	phylum	3778
Lentisphaerae	phylum	360
Synergistetes	phylum	318
Fibrobacteres	phylum	233

Looks like there is about 80% Firmicutes in this sample, with Bacteroidetes at about 12%, and a few other phyla under about 4%. We'll go into more detail about what this means, but first let's talk a little about the uBiome taxonomy file format.

2.3.2 uBiome JSON taxonomy files

Excel is great for a quick overview of your sample, but to really take advantage of the raw data it helps to understand the raw JSON format.

After uBiome's lab completes the sequencing of your sample, the data generated goes into a bioinformatics pipeline where powerful algorithms use the zillions of DNA letters to find names for the specific organisms that inhabit you. One result, after many hours of computation, is a list of the organisms (taxa) found, along with their taxonomical rank and abundance. The result is stored in a simple structured text format called JSON (JavaScript Object Notation), commonly used across the web. Although it's not as convenient for people as the rest of the uBiome web site, programmers refer to JSON as "human readable", because if you squint enough you can sorta tell what it means without a computer program. Here's a sample:

```
{
  "download_time_utc": "2016-07-30T02:04:44Z",
  "sequencing_revision": "72126",
  "site": "gut",
  "sampling_time": "2014-05-16T07:56:00.000Z",
  "notes": "Before starting probiotics experiment",
  "ubiome_bacteriacounts": [
    {
      "taxon": 1,
      "parent": 0,
      "count": 57603,
      "count_norm": 1001617,
      "tax_name": "root",
      "tax_rank": "root"
    },
    {
      "taxon": 2,
      "parent": 131567,
      "count": 57510,
      "count_norm": 1000000,
      "tax_name": "Bacteria",
      "tax_rank": "superkingdom"
    },
```

JSON is just structured data. That's it: a big bunch of lookup keys and values. The keys are unique identifiers and the values are their values. The whole file looks that way. The only requirement of a JSON file is that it be precisely consistent with this pattern, because after all it will need to be read by a computer eventually.

The uBiome Taxonomy JSON files include the key `uBiome_bacteriacounts` which is mapped to a bunch of fields, each representing a particular organism found in your sample. Look at the figure above to get the basic idea.

2.3.3 The uBiome data tags

Let's review the meaning of each of the columns, which are derived from the following uBiome JSON keys:

download_time_utc: The time and date when this JSON file was downloaded. Think of this as a version number for the data format for this sample. uBiome can and sometimes does update its reference database, which can affect your results, so when comparing two different results you'll need to know whether the underlying data is comparable. If you are comparing results where the download times are significantly different (e.g. a year apart or more), you may want to download the data again for a fresher version.

sequencing_revision: A unique identifer for this sample, often abbreviated "SSR". A sample is given a new SSR each time it is run through the gene sequencer. If the sequencing fails, then the SSR is simply thrown away. If the same sample is then run again, it will have a brand new SSR. This means that, while it is possible for the same sample to have multiple SSRs, the reverse is never true; an SSR can never refer to more than one sample. Incidentally, since SSRs are generated incrementally as new samples enter the machine, the numeric “distance” between SSRs represents the number of other samples that uBiome processed between them.

sampling_time : The time and date when the sample was collected. By default, this field is set to the time and date when the user registers the sample. The user has the option to change the date, but not the time. Be careful how you use this field. Dates are represented internally in Universal time (UTC aka GMT), but the time zone is set to Pacific time of the moment the sample was collected, so if the sample was collected at another time zone, you may need to adjust the dates accordingly. Either way, don’t rely on the time of day.

site : The part of the body where the sample was collected. Valid values are “gut”, “skin”, “mouth”, “nose”, and “custom”. Occasionally uBiome may add new site values for specific partner experiments, so for example you may encounter “forehead”, for skin samples collected using the Radiolab giveaway. The default value for each is set when the sample vial is created, but users are able to edit the value later. Because the information is self-reported, if you encounter a new sample, you should double-check that this value is correct. People who submit non-standard samples, like those from pets, often don’t bother to change this field, so it’s always best to confirm.

notes : The user can enter an optional text field when submitting a sample. This is useful if you want to know more details about how it was collecte, or if it was part of another experiment.

count: an absolute measure of number of organisms found in the sample. Commonly known among bioinformaticians as “read count” or “reads”, it tells the number of DNA letters that were successfully read for that organism at a specific taxonomical rank. Without knowing the size of the sample, or how many times the DNA inside was processed through PCR amplification, this number doesn’t mean much except in relation to other counts at the same taxonomical rank. The **count** for the taxon “root” tells the total number of reads found in the entire sample, a useful measure of the quality of that sample. For consumer-grade sampling, uBiome reports samples with greater than 3,000 reads; for clinical samples, all results are greater than 10,000. A sample with fewer than these total reads will be rejected.

count_norm: a “normalized” version of the count, created by simply dividing the count on a given row by the count found on the row with tax_name = Bacteria and then multiplying by one million. It’s easier to just think of it as parts per million: each unit is 1 / 10,000th of a percent. For example, if you see a row with count_norm = 500,000, you can just think of that as 50% of the sample.

tax_name: this is the classification of the organism based on the level of its taxonomy. If you were looking at a human being, for example, you would see homo sapiens if you selected tax_rank = species, but you’d see mammalia if you selected tax_rank = class.

tax_rank: tells the level of the taxonomy. In daily conversation about animals or plants, we usually refer to the species (e.g. homo sapiens), but sometimes it’s more useful to talk about bigger groupings of related organisms. For example, humans are members of the class mammalia, along with tigers and horses. If you were counting organisms at the level of class mammalia, the count_norm would almost certainly be bigger than the count_norm for humans alone, unless humans were the only type of mammal found in the sample.

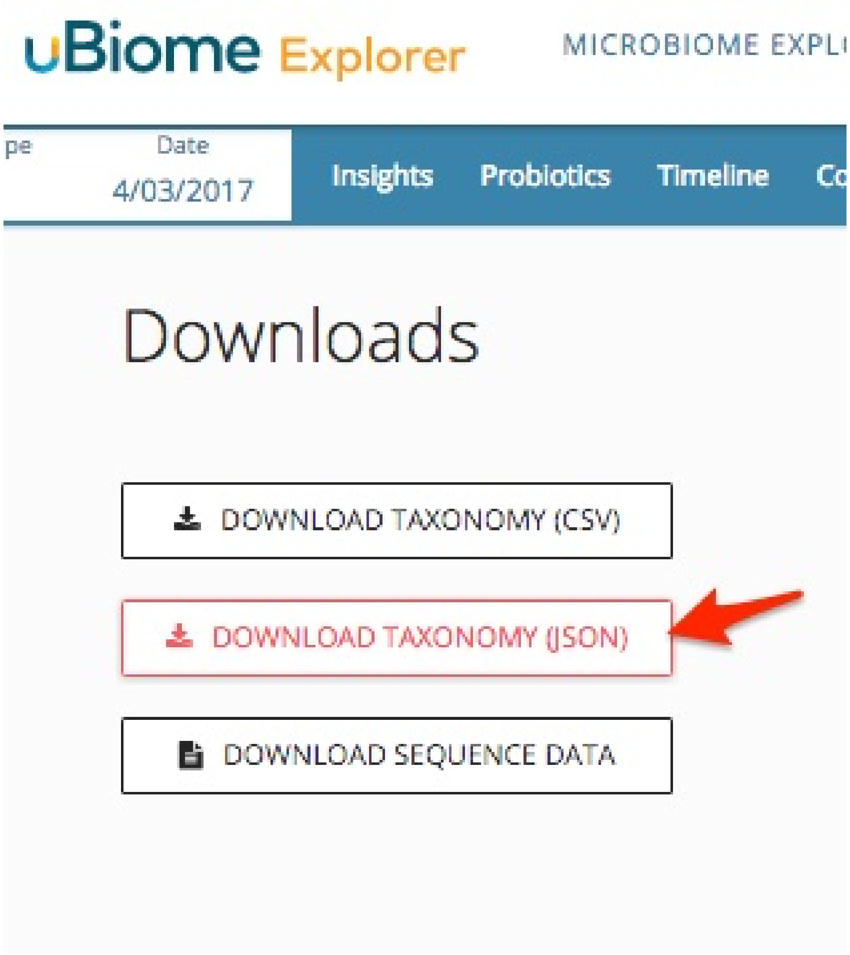
taxon and **parent**: these help identify the ranking in a more precise way by pointing out which tax_ranks are subsets of which. For example, Bacteroidia above has a parent = 976, meaning that it is a subset of the taxon 976, Bacteroidetes. When you follow the various taxons and parents up the chain, you’ll see they all end in the superkingdom Bacteria, which has a taxon of 2. The values for these numbers, incidentally, are taxonomical numbers from the curated database at NCBI, the national bioinformatics center run by the U.S. government. Enter the number into the taxonomy browser at the NCBI Taxonomy Browser and you can learn as much as you want about that organism.

tax_color doesn’t matter for this anaysis, but uBiome software uses this to colorize their pretty graphs to make them more readable.

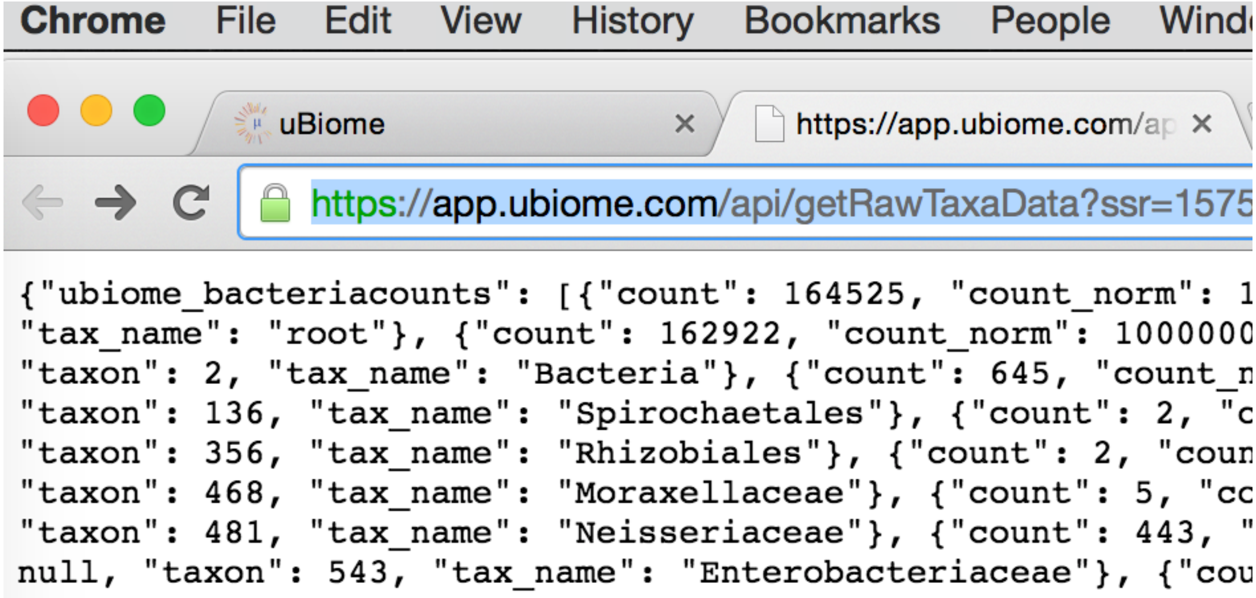
Of these fields, the most important ones are **tax_name**, **tax_rank**, and **count_norm**.

2.3.4 Converting uBiome taxonomy JSON to Excel

As already noted, it's easy to bring uBiome taxonomy data into Excel, and that's fine for just one or two samples. But if you want more powerful analysis, you'll need the raw JSON. Go to the uBiome Explorer page and click on the section labeled "Advanced" and then "Downloads":



You'll see a page of JSON representing what uBiome found in your sample.



https://app.ubiome.com/api/getRawTaxaData?ssr=1575

```
{"ubiome_bacteriacounts": [{"count": 164525, "count_norm": 1
"tax_name": "root"}, {"count": 162922, "count_norm": 1000000
"taxon": 2, "tax_name": "Bacteria"}, {"count": 645, "count_n
"taxon": 136, "tax_name": "Spirochaetales"}, {"count": 2, "c
"taxon": 356, "tax_name": "Rhizobiales"}, {"count": 2, "coun
"taxon": 468, "tax_name": "Moraxellaceae"}, {"count": 5, "cc
"taxon": 481, "tax_name": "Neisseriaceae"}, {"count": 443, "
null, "taxon": 543, "tax_name": "Enterobacteriaceae"}, {"cou
```

On a desktop computer, if you select-all (press control-A or command-A) then you can copy this data to the clipboard and then into an email to share with a friend.

If you like, you can even convert the JSON directly into Excel. Google "Json to Excel" or "Json to CSV" and you'll find one. Just paste your data into one of those sites to convert it for either Excel's native XLS or XLSX format, or the more universal CSV format, readable by Excel. Here are a few of the sites I've tested:

http://www.convertcsv.com/json-to-csv.htm

http://www.json-xls.com/json2xls

It's nice to know that we can always go back to a spreadsheet version when needed. But the real power of JSON comes when you want to handle more than just one or two samples.

2.3.5 Comparing samples

There are two main things you'll want to measure when comparing two samples:

Uniqueness: which organisms are found in only one sample and not the other? If you are comparing two samples from the same individual (e.g. Yourself), then uniqueness is another way of talking about either extinctions (when a taxon has disappeared entirely in a later sample) and appearances (when a new taxon magically shows up). In other words, you care a lot about the order in which the samples were taken.

On the other hand, if you're comparing two separate individuals, then the ordering of the samples doesn't matter. Uniqueness just tells which organisms are unique to a specific person.

Relative abundance: which sample has more of which organism? There are two senses in which we care about the relative frequency of an organism's occurrence in a sample. You might care, among all the taxons found, which ones are more highly represented in absolute amounts? Or you might care on a relative basis. To help understand why this matters, it may help to think of the following specific example:

In many Americans the phylum Firmicutes makes up a majority of the sample, often reaching 60% or more. You might find two people, one who has 60% Firmicutes and the other with 30% — only half as much. The relative difference is 2x and the absolute difference is 30 percentage points.

On the other hand, you might find Bifidobacterium makes up 10% of the first sample and 5% of the second. In this case, the relative difference is 2x — just like the Firmicutes case — but the absolute difference is only 5 percentage points, much less than in the first example.

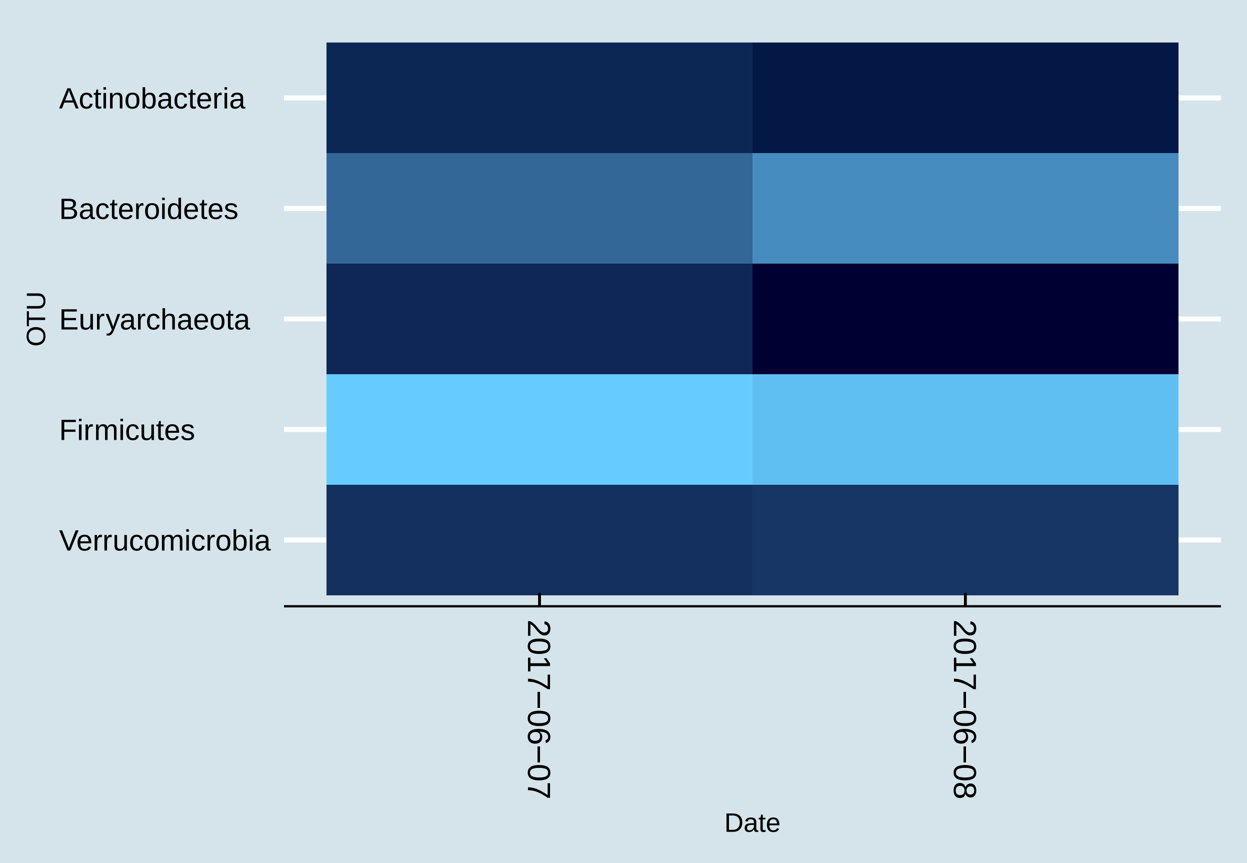
The built-in uBiome web tools look for relative differences. A taxon might be a minuscule component of both samples, but when you start from a small base, even a few additional organisms can make up a big percentage change. In the extreme case, a sample with a count_norm of 100 compared to another sample with a count_norm of 300 will show a 3X increase — very high in aBiome terms, even though the absolute difference is only 300 - 100 = 200 organisms.

Compare that with a an organism with a count_norm of 50,000 in one sample and 51,000 in another sample. UBiome's algorithms will treat these as a virtual tie, even though the absolute number of organisms in the second sample outnumber the first by 1,000 organisms.

Which method of measuring is better? It depends on what you care more about. Some species need only a tiny representation in a sample to make a big difference in health outcomes.

I prefer to use both methods of measurement: absolute number changes for the most popular taxons, and relative amounts more for the less-popular ones.

Here's an example showing two samples I took, one day after another.



	2017-06-07	2017-06-08
Firmicutes	72.04	59.75
Bacteroidetes	15.47	28.05
Verrucomicrobia	6.09	6.73
Euryarchaeota	5.19	2.79
Actinobacteria	5.15	3.89
Proteobacteria	1.03	1.12

The Abundance shown is a graphical heatmap that makes it easier for the eye to see changes, especially when looking at large amounts of data. In this chart, darker squares are *lower* in abundance than the lighter ones, so we can see that while *Firmicutes* stayed at a high percentage both days, *Bacteroidetes* dropped a bit and I saw the brand new appearance of both *Euryarchaeota* and *Verrucomicrobia*.

2.4 Exploratory Data Analysis

If you feel comfortable writing simple programming scripts to analyze your data, the following sections will show you how to do more detailed analysis.

2.4.1 Study your data in R

2.4.1.1 Introduction

Phyloseq is an open source microbiome manipulation package in the BioConductor toolkit for R[2]. Originally described in a 2013 PLOS paper by McMurdie and Holmes[3], it turns your microbiome data into an object-oriented representation that can be easily imported or exported to other common formats, and it supports many analysis techniques including calibration, filtering, subsetting, agglomeration, multi-table comparisons, diversity analysis, parallelized Fast UniFrac, ordination methods, and production of publication-quality graphics.

The combination of R, with RStudio, and Phyloseq is a powerful environment for microbiome investigation. Providing easy, interactive access to nearly every state-of-the-art algorithm in statistics, machine and deep learning, it can let uBiome researchers study our data quickly and iteratively, with exceptional plotting features that set the standard for publication-quality work.

Although the R language, compared to Python, can be more daunting for non-programmers, the package presented here should greatly simplify the process for scientists who already know the types of microbiome-related questions they want answered. If you are already familiar with the basic terms for how uBiome stores data in CSV files, you should be able to easily perform some powerful analysis and graphing by simply copying the environment and examples in this demo.

2.4.1.2 Loading the package

If this is your first time to use Phyloseq, you will need to load a few prerequisites:

```
> install.packages(c(""ggplot2","dplyr", "tidyr", "readxl"))
> source("<https://bioconductor.org/biocLite.R>")
> biocLite("phyloseq")
```

Once these are installed, you'll need the `actino` package, which you can download from this repo using the devtools package:

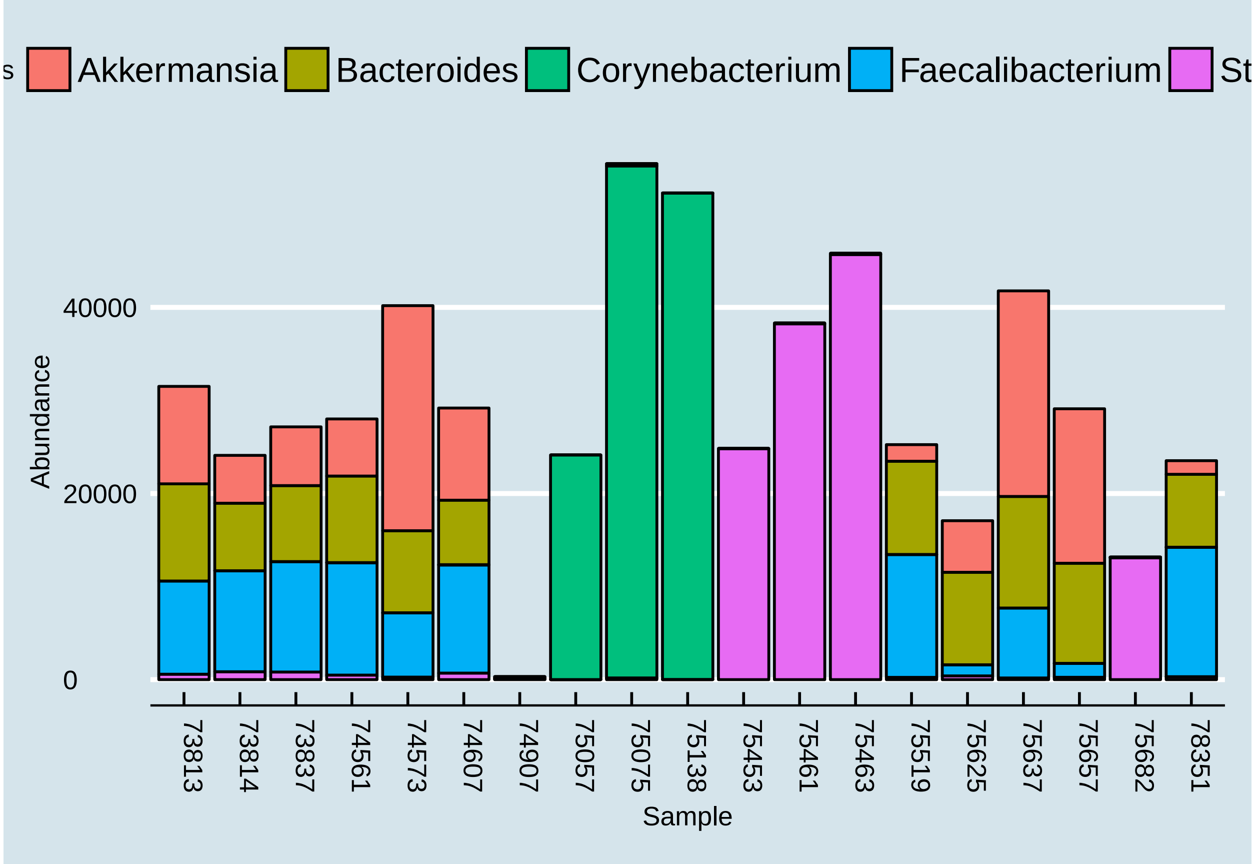
```
> library(devtools)
> install_github("richardsprague/actino")
```

You may find that you lack a number of prerequisites, such as the Phyloseq package and a few common packages like `dplyr` and `readxl`. The system will warn you, and then you should install them yourself in the manner in which you are accustomed.

[2] Read this beginner's guide: http://joey711.github.io/phyloseq-demo/phyloseq-demo.html

[3] McMurdie, Paul J., and Susan Holmes. "Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data." Edited by Michael Watson. PLoS ONE 8, no. 4 (April 22, 2013): e61217. doi:10.1371/journal.pone.0061217.

Actino includes a few built-in phyloseq objects, including `kombucha.genus`. The following commands will plot the abundances:



To load a new phyloseq object from a JSON file you will need a mapfile, which is an Excel file containing a row for each sample, and several columns including “SSR”, “Date”, “Label” and more.

```
> experiment_to_phyloseq("myCSVfile.csv",mapfile)
```

from a JSON file:

```
> phyloseq_from_JSON_at_rank("myJSONfile.json",mapfile,rank="genus")
```

Either of these functions will generate a valid phyloseq object.

The package includes some sample data, which you can load like this:

```
##              tax_name     tax_rank count count_norm taxon parent ssr
## 1                root         root 77768    1035401     1      0   0
## 2            Bacteria superkingdom 75109    1000000     2 131567   0
## 3        Campylobacter        genus     2         26   194  72294   0
## 4      Flavobacterium        genus    60        798   237  49546   0
## 5      Alcaligenaceae       family    38        505   506  80840   0
## 6 Enterobacteriaceae       family    87       1158   543  91347   0
```

The data, loaded into the variable `kombucha.csv` is a dataframe created from a CSV file with three columns: `tax_name`, `ssr`, and `count`. It represents the SSRs associated with a week-long experiment of a user (me!) who drank several liters of kombucha each day to see the effect on his microbiome.

This is the raw data. To be useful for Phyloseq, it needs an accompanying file, called a mapfile, that maps attributes to each sample. The mapfile is a dataframe where the columns correspond to attributes of the data. You can have as many columns as you like, but one of the columns (usually the first one) must stand for the name of the sample. In most uBiome situations, the samples will be referred to by their SSR, and the `actino` package will expect a column named `ssr` in its mapfile. (Note: lowercase)

Let's create a very simple mapfile for `sprague.kombucha.csv`:

```
## [1] 0
```

In this simple example, our CSV file contains only a single SSR, because it results from a single sample. Let's construct a very simple map file.

The first column represents the sample name (aka SSR). We'll add a second column that represents the date of the sample. Finally, let's add a third column `color` that we'll pretend is associated with some feature of the samples. Color of the stool? Color of the kombucha drink that day? Okay, if you don't like my example features, so go ahead and add some other columns if you like.

```
##   ssr       date color
## 1   0 2017-12-11 white
```

Although this example is a bit contrived, the result for Phyloseq is an entirely valid mapfile. Alternatively you could have made the map file manually too; Remember that in R it's easy to create any dataframe from a CSV or Excel file. If you create one by hand, simply read it into R using the `read.csv` command. Although in this demo, we are loading some pre-existing data, you could just as easily have loaded straight from the CSV file of your choice like this:

```
kombucha <- read.csv("myKombuchaDataFile.csv")
mapfile <- read.csv("myKombuchaMapFile.csv")
```

Once your data is available as an R dataframe, use the function `actino::experiment_to_phyloseq` to convert it to Phyloseq:

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 97 taxa and 1 samples ]
## sample_data() Sample Data:       [ 1 samples by 3 sample variables ]
## tax_table()   Taxonomy Table:    [ 97 taxa by 1 taxonomic ranks ]
```

And that's it! You now have a perfectly good Phyloseq object.

To run Phyloseq commands, you'll need to load the Phyloseq library, and after that everything's super-easy. In this example, we'll use another of the `actino` built-in data objects:

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 193 taxa and 19 samples ]
## sample_data() Sample Data:       [ 19 samples by 7 sample variables ]
## tax_table()   Taxonomy Table:    [ 193 taxa by 1 taxonomic ranks ]
```

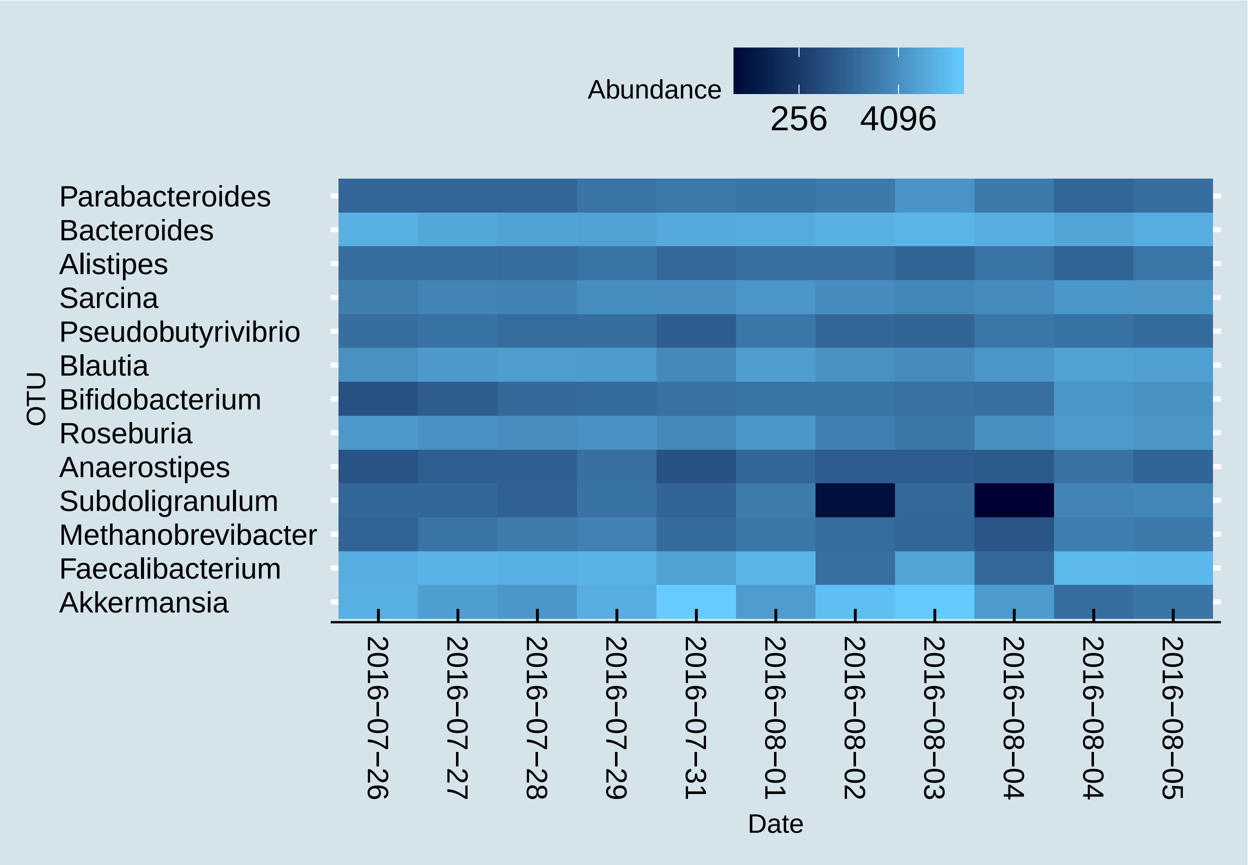
This is a series of 19 samples taken from an experiment where I drank kombucha and tested my microbiome daily to see the effect. To generate this object, I downloaded my uBiome JSON data as separate files which I read one-by-one using `actino::phyloseq_from_JSON_at_rank()`. Two phyloseq objects can be combined using the function `phyloseq::merge_phyloseq()`. A series of these pairwise merges later, and I had all 19 samples into a single phyloseq object. The mapfile contained the following meta-data:

```
## [1] "Username" "Date"     "SSR"      "Site"     "Notes"    "Geo"
## [7] "Label"
```

The first five samples and their key metadata:

```
##             Date  Site       Label
## 73813 2016-07-26   gut Burger King
## 73837 2016-07-27   gut Crab salmon
## 73814 2016-07-28   gut    Kombucha
## 75453 2016-07-28 mouth    Kombucha
## 75057 2016-07-28  nose    Kombucha
```

Because `kombucha.genus` is a full-blown phyloseq object, I can do any of the standard phyloseq analysis on it, and like any other R command, the functions can be combined. For example, this single command generates a heatmap of those genera that make up more than 1% of the samples (i.e. 10,000 (out of 1 Million) normalized reads).



In real life, of course, you'll want to reorder the axes and perform some other transformations of the data. Phyloseq makes that trivially easy with some other useful commands:

```
head(get_taxa_unique(kombucha.ps, taxonomic.rank = "Genus"),10) # which are the unique taxa at Genus le
```

```
##  [1] "Acetitomaculum"    "Acidaminococcus"   "Actinomyces"
##  [4] "Adlercreutzia"     "Akkermansia"       "Alistipes"
##  [7] "Allisonella"       "Anaerococcus"      "Anaerofilum"
## [10] "Anaerosporobacter"
```

```
#Note: if our data included other tax_ranks, we could have specified that.

ntaxa(kombucha.ps) # how many unique taxa were in the sample?
```

```
## [1] 97
```

```
names(sort(taxa_sums(kombucha.ps),TRUE)[1:10]) # the ten most common genera
```

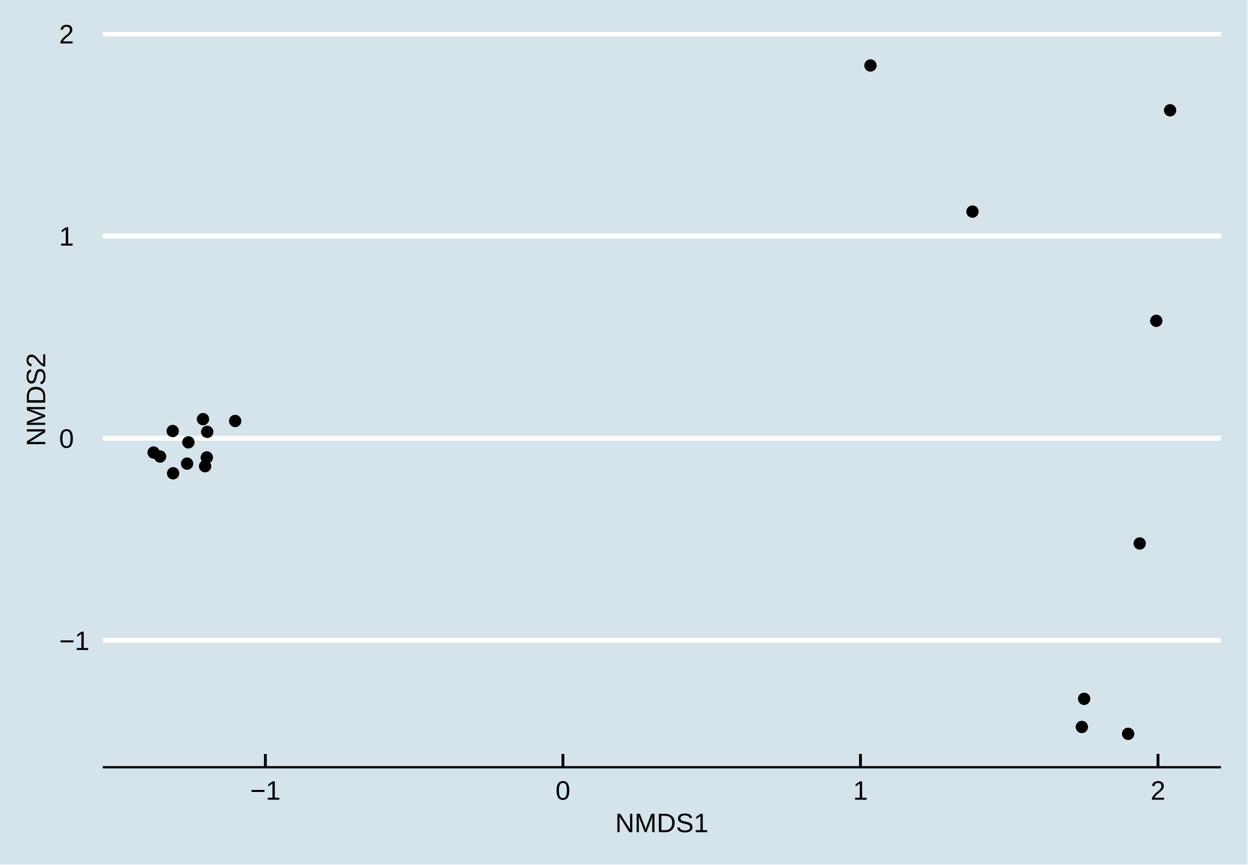
```
##  [1] "g__Faecalibacterium"   "g__Akkermansia"
##  [3] "g__Bacteroides"        "g__Blautia"
##  [5] "g__Roseburia"          "g__Sarcina"
##  [7] "g__Methanobrevibacter" "g__Parabacteroides"
##  [9] "g__Alistipes"          "g__Subdoligranulum"
```

```
sample_data(subset_samples(kombucha.genus,grepl("[P|p]otato",sample_data(kombucha.genus)$Notes)))[,c("Da
```

```
##             Date              Label
## 75625 2016-08-04    Potatoes lobster
## 75682 2016-08-04 potatoes bad breath
```

Clustering and PCA

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.04082374
## Run 1 stress 0.1034517
## Run 2 stress 0.08529348
## Run 3 stress 0.08534112
## Run 4 stress 0.04082373
## ... New best solution
## ... Procrustes: rmse 0.00003184833  max resid 0.00007828272
## ... Similar to previous best
## Run 5 stress 0.08527205
## Run 6 stress 0.08534199
## Run 7 stress 0.04082373
## ... Procrustes: rmse 0.000007196812  max resid 0.00002006098
## ... Similar to previous best
## Run 8 stress 0.04097072
## ... Procrustes: rmse 0.004826611  max resid 0.01929056
## Run 9 stress 0.04097072
## ... Procrustes: rmse 0.004826458  max resid 0.01929107
## Run 10 stress 0.04082373
## ... Procrustes: rmse 0.000006858887  max resid 0.00001734217
## ... Similar to previous best
## Run 11 stress 0.04097072
## ... Procrustes: rmse 0.004827218  max resid 0.01929372
## Run 12 stress 0.04082374
## ... Procrustes: rmse 0.00001870345  max resid 0.0000421544
## ... Similar to previous best
## Run 13 stress 0.04097072
## ... Procrustes: rmse 0.004824758  max resid 0.01928375
## Run 14 stress 0.08559959
## Run 15 stress 0.04097072
## ... Procrustes: rmse 0.004832709  max resid 0.01931653
## Run 16 stress 0.04082373
## ... Procrustes: rmse 0.00001467021  max resid 0.00003411114
## ... Similar to previous best
## Run 17 stress 0.04082373
## ... Procrustes: rmse 0.00001518355  max resid 0.00003563875
## ... Similar to previous best
## Run 18 stress 0.1181168
## Run 19 stress 0.04097072
## ... Procrustes: rmse 0.004826244  max resid 0.01929093
## Run 20 stress 0.04082375
## ... Procrustes: rmse 0.00003601862  max resid 0.00009126253
## ... Similar to previous best
## *** Solution reached
```



2.4.1.3 More advanced commands

Phyloseq comes with zillions of additional and useful commands. Here are a few.

Rarefaction

Here's a simple example where we rarefy all samples to an even depth. Again, because the counts are already so low, this is a bit of a contrived example, but you can see how rarefaction *did* change the number of taxa in the samples.

```
##          tax_name before after
## 1 Flavobacterium      5   254
## 2       Kluyvera      3   204
## 3    Bacteroides      2 33628
## 4    Fibrobacter     98    31
## 5  Porphyromonas   9902  1969
## 6      Roseburia   1692 14992
```

2.4.1.4 References and useful ways to get started

The paper that announced it to the world: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0061217

Here is an excellent beginners guide:

http://joey711.github.io/phyloseq-demo/phyloseq-demo.html

How to import from other data formats: http://joey711.github.io/phyloseq/import-data.html

Don't forget to check the Phyloseq online manual: https://rdrr.io/bioc/phyloseq/

FAQ: https://www.bioconductor.org/packages/release/bioc/vignettes/phyloseq/inst/doc/phyloseq-FAQ.html#other-issues-related-the-biom-format

2.4.2 Analyze your uBiome Results in Python

The charts and tables you see in the uBiome Explorer are generated from the same data file that you can download for yourself in the Advanced tab. If you're comfortable with programming, you can pull that data into your favorite software tools for even more analysis.

To get started, look at the open source uBiome Tools Repository which contains source code that will let you:

- Compare two samples to find the unique organisms in each.
- Compare two samples and show the differences in counts for each.
- Merge more than one sample into a single file.

For Python programmers, the code is available either in the official uBiome library or as an easy-to-install library hosted on the Python Package Index and installable through PIP. Just type this from the console application on your computer (either Terminal on Mac, or Powershell on Windows).

```
$ pip install ubiome
```

The uBiome package is designed for either Python 2 or 3, and once installed you can use it as a console script, for a new system command like this:

```
> ubiome -h

usage: ubiome.py [-h] [-c COMPARE] [-u UNIQUE] [-d DEBUG] sample1 sample2

positional arguments:
  sample1               filename for a valid uBiome JSON taxonomy file
  sample2               sample you are comparing to

optional arguments:
  -h, --help            show this help message and exit
  -c COMPARE, --compare COMPARE
                        Compare sample1 with with sample2
  -u UNIQUE, --unique UNIQUE
                        Find items in sample1 not in sample2
  -d DEBUG, --debug DEBUG
                        turn debug mode to run tests
```

If you have two uBiome-formatted JSON files in the current directory, try this:

```
$ ubiome -c sample1.json sample2.json
```

It will compare the two samples and output a comma-separated (CSV) dump of the differences between them. Similarly, the following command returns the parts of a sample that are unique:

```
$ ubiome -u sample1.json sample2.json > uniques.csv
```

Now you'll have a new file in your directory, "uniques.csv", that you can load into any spreadsheet program. The file will have a series of rows representing each taxa of bacteria that are unique, i.e. only found in "sample1.json".

You'll find both the compare and unique commands useful for understanding the differences between two of your own samples. If you have a sample of yourself before taking probiotics, and another afterwards, for example, this can help you tell what organisms changed and by how much.

The uBiome package can of course be imported into Python as the basis of your own scripts and modules. To see how, run the following series of commands in your Python 2+ or 3+ console:

```
$ python

>>> from ubiome import *
>>> x1 = UbiomeSample("sample1.json")
>>> x2 = UbiomeSample("sample2.json")
>>> x = UbiomeMultiSample(x1)
>>> x.merge(x2)
>>> # continue to merge as many additional samples as you like
>>> x.write("x.csv")
```

Now your directory will have a new file 'x.csv' with all samples merged. The first row is all the taxons ever found in your samples, and the other columns are your different samples, with rows containing the **count_norm** value for every taxon, including zero values for those taxa that are missing in a particular test.

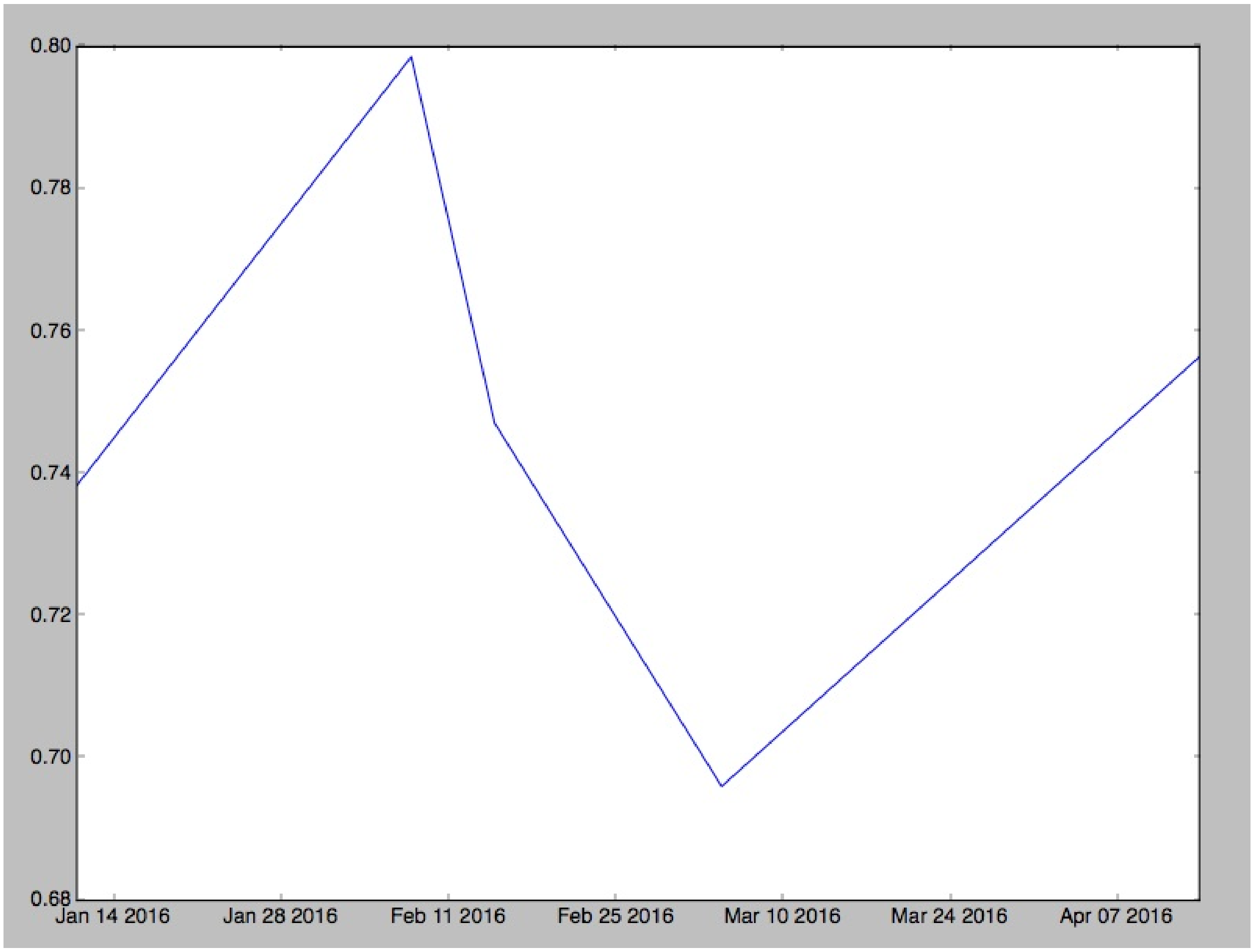
The merged form, called a **UbiomeMultiSample**, can do even more. For example, continue to merge a few more samples in the example above, and then run this code:

```
samples = x.originalSampleObjects # just the UbiomeSample objects of the merged items

diversity = [sample.diversity() for sample in samples]

dates = [sample.date for sample in samples]
```

If you have matplotlib on your system and run this:

```
plot(dates,diversity)
```

You will see this chart that plots the diversity of a series of samples over time:



See the script ubiome_example.py for a complete example. There are also several user-supplied JSON files for you to try if you want to test your own code on real samples.

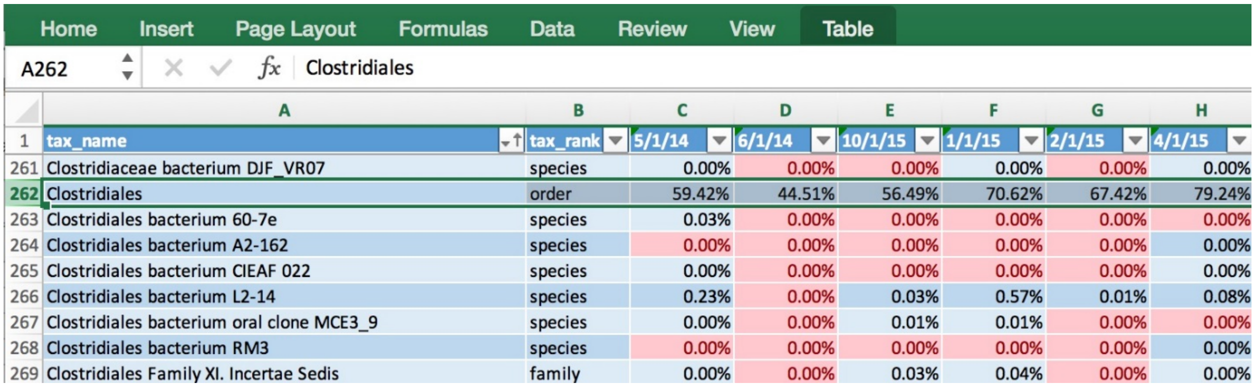
2.4.3 Make a single sheet with all your samples

I often read news about a fresh scientific discovery involving the microbiome and immediately wonder if the discovery applies to me. For example, I recently saw a study from Oregon State University[4] that seemed to find a link between high sugar diets and "cognitive flexibility", i.e. your ability to adapt and adjust to changing circumstances. The study's author, Kathy Magnusson, a professor in the OSU College of Veterinary Medicine, found that mice who eat lots of sugar have elevated levels of Clostridiales bacteria, and that this seemed to relate to a slower ability to solve a maze. Hmmm, I thought — how much Clostridiales do I have?

If you have just one uBiome result, that's easy: log into http://explorer.ubiome.com and search for it in the section "All My Bacteria". But in my experience a single result doesn't tell you much. You really need at least two and hopefully several uBiome results to see what might be actionable. In my case, I want to know how my Clostridiales may have changed over time.

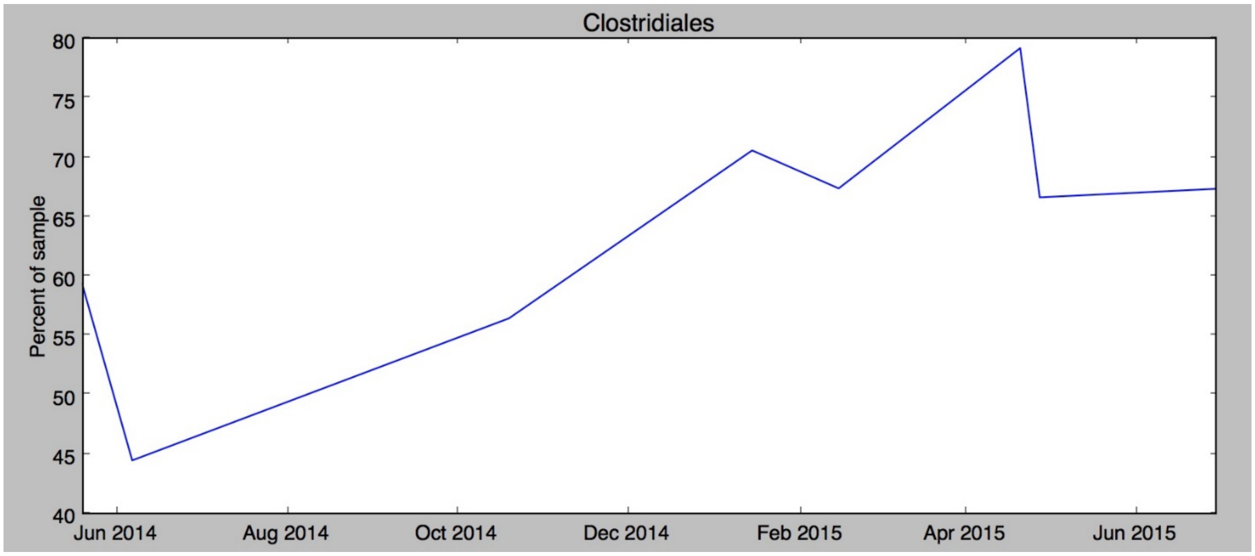
If you are comfortable with programming, uBiome has an opensource Python script to generate a single Excel table that combines multiple separate JSON files. Something like this:

[4] http://www.sciencedaily.com/releases/2015/06/150622182034.htm



	A	B	C	D	E	F	G	H
1	tax_name	tax_rank	5/1/14	6/1/14	10/1/15	1/1/15	2/1/15	4/1/15
261	Clostridiaceae bacterium DJF_VR07	species	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
262	Clostridiales	order	59.42%	44.51%	56.49%	70.62%	67.42%	79.24%
263	Clostridiales bacterium 60-7e	species	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%
264	Clostridiales bacterium A2-162	species	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
265	Clostridiales bacterium CIEAF 022	species	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
266	Clostridiales bacterium L2-14	species	0.23%	0.00%	0.03%	0.57%	0.01%	0.08%
267	Clostridiales bacterium oral clone MCE3_9	species	0.00%	0.00%	0.01%	0.01%	0.00%	0.00%
268	Clostridiales bacterium RM3	species	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
269	Clostridiales Family XI. Incertae Sedis	family	0.00%	0.00%	0.03%	0.04%	0.00%	0.00%

The data makes it easy to generate a chart showing how my Clostridiales changes over time:



Hmmm, in my case it looks like something happened since last fall to increase my Clostridiales levels. Maybe it was the potato starch I tried in order to hack my sleep? Was it my trip to Central America in February? And of course the biggest question: has the increase affected my cognitive flexibility? I'm not really sure. Whatever happened, the level of Clostridiales seems to have stabilized in the past couple of months.

uBiome has identified more than 900 unique taxa (groups of organisms) in the half-dozen samples I've submitted over the past year, and after running this script I have them all laid out on a single page. Now, armed with this one spreadsheet I can search anytime for a new microbe and quickly see if I have it now, or if it's ever been detected in a previous test. Reading news about microbiome has taken on a whole new personal meaning when I can see if the discovery relates to me.

Next we'll show you how to make your own charts.

2.4.4 Working with FASTQ files

The uBiome JSON taxonomy information will be more than enough for most people new to biology. But for anyone interested in expanding their bioinformatics skills, the raw data files are the place to look.

FASTQ files are the text-based results of a run at your sample using one of Illumina's high-end gene sequencers.

For example, the first line in my data is:

```
@NS500457:29:H2HLGAFXX:1:11101:16760:1033 1:N:0:ACTTACA+NTGCGAA
```

This contains information about the gene sequencing hardware uBiome used for this sample. The other lines in the raw text file contain the actual base pairs detected by the sequencer, along with quality information to indicate the certainty of a particular read. In my case, the entire file is over 170,000 lines long, and that's just one of the eight files in the FASTQ archive. That's a lot of data!

How sequencing works

Each strand of DNA will be read from two directions (called 3-Prime and 5-Prime). This means that a given DNA letter will be read twice (once forward, once backward), so the `count` field of your JSON file will never show a number less than 2. But 2 is a tiny number that might just be there by chance. For that reason, careful scientists will avoid using any data with such small abundances.

uBiome will not release samples with under 3000 reads, so the minimum abundance you should bother with is 2/3000 = 0.07%. Most samples will have far more than 3000 reads (my average is closer to 60,000), so if you want to compare small abundances, you shouldn't bother with anything less than 0.07%.

Because it's hard to compare low-abundance taxa across many samples that may have wide variations in total read counts, I discount anything with under about 12 reads if you are comparing across samples that are all greater than 10,000 reads, and 42 if you are comparing against those of 3,000 reads. Why those numbers? Because 2/10000 = 0.0002 multiplied by my average of 60000 reads is 12; meanwhile, 0.000.007 % of 60,000 is about 42 ^[You can do this with the following Phyloseq command:

```
prune_taxa(taxa_sums(gut.norm)>42, gut.norm)
```

]. These are just rough guidelines – a more thorough calculation would take into account the exact number of reads for all samples. But a conservative scientific approach says it's better to throw away information that might be low quality.

Quality of your FASTQ

Here's how to check the quality of your overall FASTQ file.

Sum(count of the number of lines in the R1 files / 4)

In a Terminal session on OSX, you can check this with the following command.

```
$ gzcat *R1*.fastq.gz | echo $((`wc -l`/4))
```

A typical result for one of my files is `0.375`.

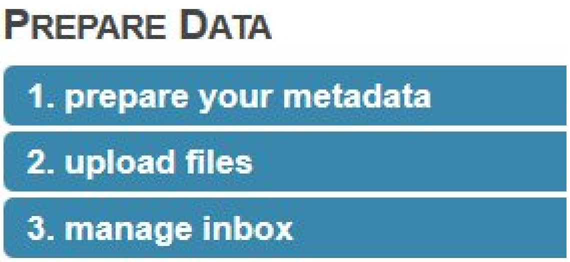
Study your FASTQ online

Although it's possible to analyze this data on my own in a tool like BioPython, it's much easier to submit the raw data to a public gene processing server, such as the Metagenomics Analysis Server (MG RAST) hosted by Argonne National Labs at this site:

http://metagenomics.anl.gov/

Note: You must use the Firefox browser (not Chrome, IE, or Safari), and before submitting any jobs you must apply for a (free) login ID, which you receive by email in a day or two.

After logging in with your new account ID, click the "upload" icon on the home page and follow the process to prepare and submit your data:



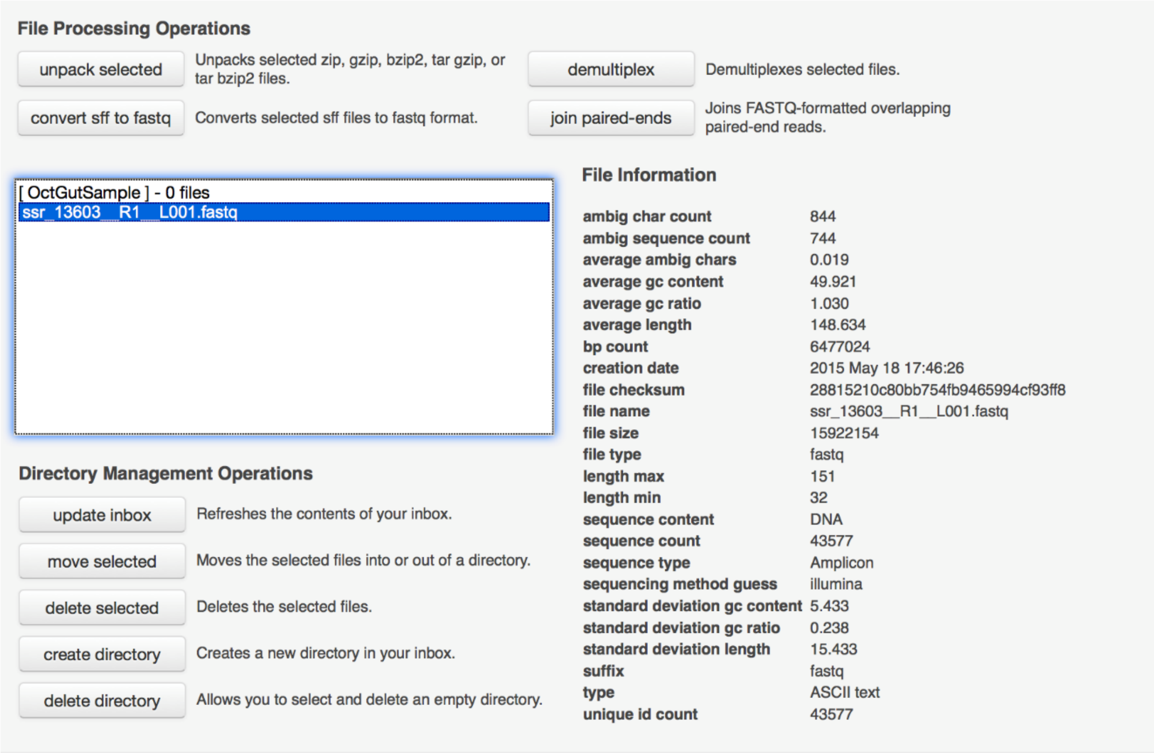
1. Ignore the part about preparing the meta data; you can add it later.

2. Upload files. The site offers to let you upload from the web browser, but I've had better luck using their api and the following curl command:

```
curl -H "auth: webkey" -X POST -F "upload=@/path\_to\_file/metagenome.fasta" "<http://api.metagenomics.a
```

I just type this from Terminal on my Mac. Note that you'll need to get your own webkey (hit the 'view webkey' button on the submission page) which you will substitute into the curl command line above. Also change the filename part of the "upload=@" section to point to the correct file. Note that uBiome fastq files are compressed, but it's okay to upload them as-is. You'll uncompress them on the server after the upload.

1. Manage your inbox. Once the file has successfully uploaded, you'll see it in your inbox. At this point, you should "unpack selected" if you uploaded a compressed file. Note depending on how busy the server is, it may be minutes or even hours of waiting, and pressing "update inbox" before you see the File Information shown below.


File Processing Operations
[OctGutSample] - 0 files
ssr_13603__R1__L001.fastq
Directory Management Operations

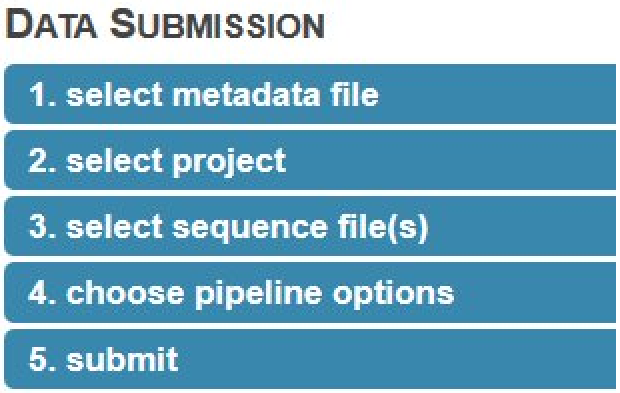
unpack selected	Unpacks selected zip, gzip, bzip2, tar gzip, or tar bzip2 files.
demultiplex	Demultiplexes selected files.
convert sff to fastq	Converts selected sff files to fastq format.
join paired-ends	Joins FASTQ-formatted overlapping paired-end reads.

File Information

ambig char count	844
ambig sequence count	744
average ambig chars	0.019
average gc content	49.921
average gc ratio	1.030
average length	148.634
bp count	6477024
creation date	2015 May 18 17:46:26
file checksum	28815210c80bb754fb9465994cf93ff8
file name	ssr_13603__R1__L001.fastq
file size	15922154
file type	fastq
length max	151
length min	32
sequence content	DNA
sequence count	43577
sequence type	Amplicon
sequencing method guess	illumina
standard deviation gc content	5.433
standard deviation gc ratio	0.238
standard deviation length	15.433
suffix	fastq
type	ASCII text
unique id count	43577

update inbox	Refreshes the contents of your inbox.
move selected	Moves the selected files into or out of a directory.
delete selected	Deletes the selected files.
create directory	Creates a new directory in your inbox.
delete directory	Allows you to select and delete an empty directory.

Next you're ready to submit the data



Data Submission

1. select metadata file
2. select project
3. select sequence file(s)
4. choose pipeline options
5. submit

Here are my suggestions for this section

1. Check the box to say you won't supply metadata.

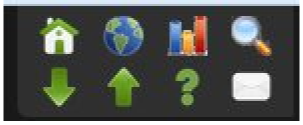
2. Create a new project and select it.

3. Select the files you uploaded

4. Use the default values to choose pipeline options

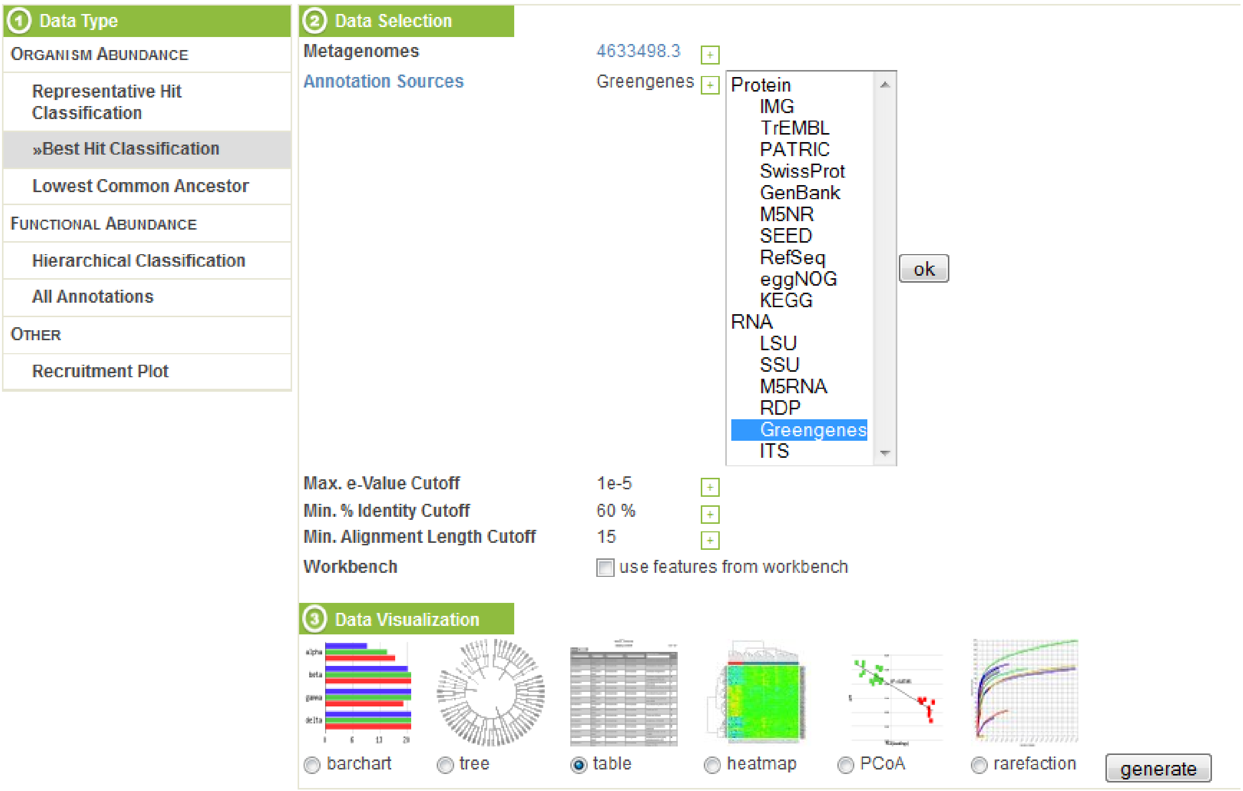
5. Submit the job, being sure to make the data publicly accessible immediately, to increase the priority.

There you go! Now a job has been submitted and you wait for it to complete. It usually takes a day or two to complete, after which you'll receive an email notification. After that, click the bar chart icon on the upper right of the page:



This brings you to an analysis page where you can study your data in much more detail.

When I select my data under "Metagenomes" on the analysis page, I'm offered several options for Annotation Sources. Since the uBiome fastq data comes from 16S ribosomal RNA, I choose to compare my data against one of the large databases of known RNA. The popular Greengenes database is one of them, so I select it, and then ask to generate a table of the results.



① Data Type

ORGANISM ABUNDANCE
- Representative Hit Classification
- »Best Hit Classification
- Lowest Common Ancestor

FUNCTIONAL ABUNDANCE
- Hierarchical Classification
- All Annotations

OTHER
- Recruitment Plot

② Data Selection

Metagenomes: 4633498.3

Annotation Sources: Greengenes

Protein: IMG, TrEMBL, PATRIC, SwissProt, GenBank, M5NR, SEED, RefSeq, eggNOG, KEGG

RNA: LSU, SSU, M5RNA, RDP, Greengenes, ITS

ok

Max. e-Value Cutoff: 1e-5

Min. % Identity Cutoff: 60 %

Min. Alignment Length Cutoff: 15

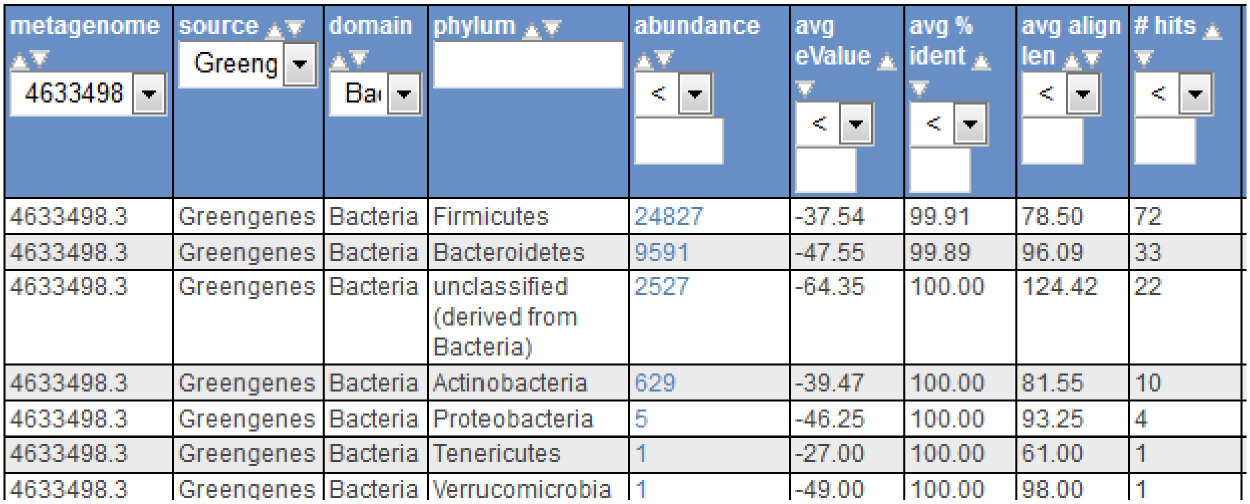
Workbench: □ use features from workbench

③ Data Visualization

○ barchart ○ tree ● table ○ heatmap ○ PCoA ○ rarefaction

generate

The table, sorted by abundance and filtered to phylum, looks like this:



metagenome	source	domain	phylum	abundance	avg eValue	avg % ident	avg align len	# hits
4633498	Greeng	Ba		<	<	<	<	<
4633498.3	Greengenes	Bacteria	Firmicutes	24827	-37.54	99.91	78.50	72
4633498.3	Greengenes	Bacteria	Bacteroidetes	9591	-47.55	99.89	96.09	33
4633498.3	Greengenes	Bacteria	unclassified (derived from Bacteria)	2527	-64.35	100.00	124.42	22
4633498.3	Greengenes	Bacteria	Actinobacteria	629	-39.47	100.00	81.55	10
4633498.3	Greengenes	Bacteria	Proteobacteria	5	-46.25	100.00	93.25	4
4633498.3	Greengenes	Bacteria	Tenericutes	1	-27.00	100.00	61.00	1
4633498.3	Greengenes	Bacteria	Verrucomicrobia	1	-49.00	100.00	98.00	1

The results roughly conform to what we see from uBiome: the most common organisms are the same in each case, and although the scales are different, the abundance computed corresponds roughly to what we see from the uBiome count field.

Other MG RAST tools let you compare your data against other databases of bacteria and proteins, graph the results, and perform sophisticated statistical analysis to determine other unique features of your sample. You can also compare your sample to microbiomes that have been uploaded by others, enabling you to study your results in much more detail.

Methods

This chapter will go into more detail about methods, building on the Explore Your Microbiome chapter to show more precisely how I measured myself and how I used the tools needed to build this book.

David, Lawrence A., Arne C. Materna, Jonathan Friedman, Maria I. Campos-Baptista, Matthew C. Blackburn, Allison Perrotta, Susan E. Erdman, and Eric J. Alm. 2014. "Host Lifestyle Affects Human Microbiota on Daily Timescales." *Genome Biology* 15 (7): R89. doi:10.1186/gb-2014-15-7-r89.

Hehemann, Jan-Hendrik, Gaëlle Correc, Tristan Barbeyron, William Helbert, Mirjam Czjzek, and Gurvan Michel. 2010. "Transfer of Carbohydrate-Active Enzymes from Marine Bacteria to Japanese Gut Microbiota." *Nature* 464 (7290): 908–12. doi:10.1038/nature08937.

Wexler, H. M. 2007. "Bacteroides: The Good, the Bad, and the Nitty-Gritty." *Clinical Microbiology Reviews* 20 (4): 593–621. doi:10.1128/CMR.00008-07.

Zhu, Yingying, Xuebin Shi, Xisha Lin, Keping Ye, Xinglian Xu, Chunbao Li, and Guanghong Zhou. 2017. "Beef, Chicken, and Soy Proteins in Diets Induce Different Gut Microbiota and Metabolites in Rats." *Frontiers in Microbiology* 8 (July). doi:10.3389/fmicb.2017.01395.